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NEURONS IN ACTION

6th CONFERENCE

2025

NENCKI INSTITUTE OF EXPERIMENTAL BIOLOGY, WARSAW, POLAND

6TH NEURONS IN ACTION CONFERENCE

NENCKI INSTITUTE OF EXPERIMENTAL BIOLOGY, WARSAW, POLAND, 27TH – 30TH MAY 2025

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PROGRAM

TUESDAY, 27.05.2025
KONORSKI HALL, NENCKI INSTITUTE

10:00 **Pre-conference workshop with Nencki OpenLab: 3D printing, day 1**

WEDNESDAY, 28.05.2025
KONORSKI HALL, NENCKI INSTITUTE

10:00–12:30 **Pre-conference workshop with Nencki OpenLab: 3D printing, day 2**
Conference Hall, Nencki Institute

12:30–13:15 **Opening Ceremony**

13:15–14:15 **Keynote lecture**
Who and what: How neuroimaging and AI inform the treatment of Major Depression
W. Edward Craighead, Emory University, Atlanta, USA

14:15–15:45 **Symposium I: Regulation of Brain Plasticity by Activity-Regulated Transcription Factors and Chromatin Modifiers**
Chairs: Katarzyna Kalita & Adriana Magalska

Experience-Driven Regulation of Neuronal Gene Programs
Angel Barco, Instituto de Neurociencias, Universidad Miguel Hernández, Alicante, ES

All IEGs Are not Created Equal – Diverse Roles of Activity-dependent Transcription Factors in Neural Circuit Plasticity
Yingxi Lin, University of Texas Southwestern Medical Center, Dallas, TX, USA

Jacob: a synapto-nuclear messenger protein linking NMDAR activation to CREB-dependent gene expression
Anna Karpova, Leibniz Institute for Neurobiology, Magdeburg, DE

15:45–16:15 **Coffee break**

16:15–18:00 **Symposium II: Thalamus in action**
Chair: Kasia Radwańska, Nencki Institute

Structural and Functional Specialization in Thalamic Reticular Nucleus Subnetworks: Implications for Sensory Processing and Salience Detection
Zhanyan Fu, Stanley Center for Psychiatric Research, Broad Institute of MIT and Harvard, USA

Thalamic energy metabolism and autism-like deficits in a mouse model of the TCF7L2-related neurodevelopmental disorder
Marta B. Wisniewska, CeNT, Warsaw, PL

Memory Capacity Beyond Limits and Sex-Specific Regulation
Elvira de Leonibus, Institute of Biochemistry and Cellular Biology (IBBC), National Research Council of Italy (CNR), Monterotondo (Rome), IT

Contribution of thalamic projections to the hippocampus to memory processes
Kasia Radwanska, Laboratory of Molecular Basis of Behavior, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, PL

18:00–20:00 **Poster session & snacks**

THURSDAY, 29.05.2025
CONFERENCE HALL, NENCKI INSTITUTE

9:00–10:00 **Keynote lecture**
Beyond dopamine: VTA to VP GABA signaling in reward valuation
Marina Picciotto, Yale School of Medicine, USA

10:00–11:30 **Symposium III: GABAergic system in health and disease**
Chair: Joanna Urban-Ciećko, Nencki Institute

Investigating the function of GABAB receptors in human cortex, in comparison to rodents
Sam A Booker, Centre for Discovery Brain Sciences University of Edinburgh, UK

Dopaminergic modulation of GABAergic synaptic plasticity in mouse hippocampus
Jerzy W. Mozrzymas, Medical University, Wroclaw, PL

GABA transporter subtypes 1 and 3 as targets for novel memory-improving drugs
Kinga Sałat, Chair of Pharmacodynamics, Faculty of Pharmacy, Jagiellonian University Medical College, Krakow, PL

11:30–12:00 **Coffee break**

12:00–13:30 **Symposium IV: Fundamental mechanisms underlying social behavior**
Chair: Ewelina Knapska, Nencki Institute

Have we met? Hippocampal circuits for social memories
Rebecca Ann Piskorowski, Sorbonne University, Institut Biologie Paris Seine, Neurscience Paris Seine, FR

Cortical and Hippocampal Circuits for Discriminating Positive Emotions and Social Learning in Mice
Ewelina Knapska, Nencki Institute of Experimental Biology PAS, Warsaw, PL

13:30–14:30 **Lunch break**

14:30–16:00 **Symposium V: The heterogeneity of protein lipid modifications in the brain**
Chairs: Tomasz Wójtowicz & Magda Czekalska, Nencki Institute

Palmitoylation-dependent signaling in and from distal axons
Gareth Thomas, Cellular and Molecular Neuroscience Lab Temple University School of Medicine in Philadelphia, USA

Spatiotemporal methods to chart the cell states of brain development
Gioele La Manno, Laboratory of Brain Development and Biological Data Science, Swiss Federal Technology Institute of Lausanne, CH

Protein palmitoylation in synaptic plasticity and spatial learning
Tomasz Wójtowicz, Nencki Institute, Warsaw, PL

16:00–16:30 **Coffee Break**

16:30–18:00 **Symposium VI: Spatial memory - mapping the territory**
Chair: Rafał Czajkowski, Nencki Institute

My place *vs.* your place - what can we say about the hippocampus and territoriality?
Dori Derdikman, Technion - Israel Institute of Technology, Haifa, IL

Neural segmentation of space through active vision
Sylvia Wirth, CNRS, Paris, FR

The Naming of Nonhuman Primates: Vocal Labeling of Others by Nonhuman Primates
David Omer, The Hebrew University of Jerusalem, IL

FRIDAY, 30.05.2025
CONFERENCE HALL, NENCKI INSTITUTE

9:30–11:00 **Symposium VII: Targets and therapy mechanisms in neuropsychiatric disorders**
Chairs: Marek Wypych & Jakub Wlodarczyk, Nencki Institute

Could symptoms of Compulsive Sexual Behavior Disorder be cured? Insights from neuroimaging studies applied to the clinical trial intervention
Malgorzata Draps, Institute of Psychology PAS, Warsaw, PL

Palmitoylation of the glucocerebrosidase receptor LIMP2: therapeutic target for Parkinson disease?
Gary Ho, Harvard Medical School, Brigham and Women's Hospital, Boston, USA

Navigating Astro-Neuro Dynamics: The Impact of Glial Wnt Signaling on Neuronal Development and Function
Łukasz M. Szewczyk, University of Warsaw, Centre of New Technologies, Warsaw, PL

11:00–11:30 **Coffee break**

11:30–13:00 **Symposium VIII: Toward understating the neuronal mechanism of Working Memory**
Chair: Jan Kamiński, Nencki Institute

Memory in Sequence: Prefrontal and Medial Temporal Neurons Encode Order of Events in Humans
Jie Zheng, University of California, Davis, USA

Redundant, weakly connected prefrontal hemispheres balance precision and capacity in spatial working memory
Joao Barbosa, Institut de Neuromodulation and Neurospin, Paris, FR

The role of concept neurons in the human medial temporal lobe for working and long-term memory
Florian Mormann, University of Bonn, Bonn, DE

13:00–14:00 **Lunch break**

14:00–15:30 **Symposium IX: Imaging the brain with light and sound**
Chairs: Anna Beroun & Marzena Stefaniuk, Nencki Institute

Cell vibes: how collicular cell-types contribute to guiding mice innate behaviors
Anna Chrzanowska, ICM Paris, FR

Optical and computational tools to explore brain-wide behavior-specific circuits
Ludovico Silvestri, University of Florence, Florence, IT

Listening to light and seeing sound in the brain
Daniel Razansky, Institute for Biomedical Engineering, University and ETH Zurich, CH

15:30–16:00 **Coffee Break**

16:30–18:15 **Symposium X: New Insights into Human Brain Development**
Chairs: Bogna Badyra & Aleksandra Pękowska, Nencki Institute

Epigenetic and cellular regulation of cortex expansion and folding
Victor Borrell, Universidad Miguel Hernández, Sant Joan d'Alacant, ES

Choosing to be different. Cell identity and fate choice in the developing brain
Elena Taverna, Human Technopole, Milan, IT

The role of NEUROG2 T149 phosphorylation site in the developing human neocortex
Julien Pigeon, Institut du Cerveau-Paris Brain Institute, Sorbonne Université, Paris, FR

Investigating Evolutionary Expansion of the Human Cerebellum Using Cross-Species Cerebellar Organoids
Luca Guglielmi, MRC Laboratory of Molecular Biology (LMB), Cambridge, University of Cambridge, UK

18:15–18:30 **Closing remarks & awards**

19:30 **Integration party**

KEYNOTE SPEAKERS

WHO AND WHAT: HOW NEUROIMAGING AND AI INFORM THE TREATMENT OF MAJOR DEPRESSION

W. Edward Craighead
Emory University, Atlanta, USA

The presentation will begin with a brief description of Major Depressive Disorder (MDD) including its prevalence and consequences for individuals and society. This will be followed by a description of the largest (344 MDD patients), single-site treatment study (PReDICT) of clinical and neuroimaging-based differential moderators and mechanisms of remission of MDD when randomly assigned to treatments including CBT or SSRI (escitalopram) or SNRI (duloxetine). Knowing which patient will remit to which treatment answers the most pressing question regarding a personalized medicine approach to treating MDD; unfortunately, the answer largely remains trial and error. A pattern of neural connectivity at baseline differentially predicted which patients remitted with CBT and the antidepressants (ADT) whose prediction did not differ from each other. CBT and ADT had shared and unique mechanisms of neural connectivity changes associated with remission; each will be discussed in terms of the hypothesized theories of decreased MDD. The clinical implications of each of the preceding findings will be presented, including the finding that not all MDD patients respond to very high-quality CBT nor to appropriately administered ADT. Finally, data will be presented to demonstrate how machine learning and AI can be employed to enhance the preceding moderators and mechanisms of change. The discussion will include information on how matching treatments *via* moderators enhances remission rates. It will be suggested that adolescent prevention programs may be working by adaptive development of neural functioning rendering the "at risk" adolescent more resistant to developing MDD. The ultimate objective of this research program is the development and identification of a psychometrically sound self-report instrument that is both related to neural connectivity moderators of change and can also be used in clinical practice in any location to identify which patients will respond to CBT and which will respond to ADT.

BEYOND DOPAMINE: VTA TO VP GABA SIGNALING IN REWARD VALUATION

Marina Picciotto
Yale School of Medicine, New Haven, USA

Dopamine (DA) signaling from the ventral tegmental area (VTA) plays critical roles in reward-related behaviors, but less is known about the functions of VTA GABA projection neurons. We have used a genetically-encoded calcium sensor *in vivo*, to evaluate the firing pattern of VTA-to-ventral pallidum (VP) GABA neurons during performance of reward-relevant tasks along with chemogenetic and optogenetic stimulation to evaluate the effects of pathway stimulation on VP dynamics and tasks relevant to reward value. We found that activity of VTA-to-VP-projecting GABA neurons correlated consistently with size and palatability of reward and did not change following cue-learning, providing a direct and unvarying measure of reward value. Response of these neurons varied with satiety, suggesting that this pathway provides information about current reward value. Stimulation of this GABA projection increased activity of a subset of VP neurons that are active while mice seek reward, improved performance in cue-reward tasks and altered strategy in a probabilistic reward task. We conclude that this VTA GABA projection provides information about reward value directly to the VP that is distinct from the prediction error signal carried by VTA dopamine neurons. These data show that VTA GABA neurons maintain a stable representation of reward value that does not shift with habituation or learning and that activity of this pathway is critical for making appropriate choices for differentially rewarded outcomes.

SYMPOSIA SPEAKERS

REDUNDANT, WEAKLY CONNECTED PREFRONTAL HEMISPHERES BALANCE PRECISION AND CAPACITY IN SPATIAL WORKING MEMORY

Joao Barbosa

Institut de Neuromodulation and Neurospin, Paris, France

How the prefrontal hemispheres coordinate to adapt to spatial working memory (WM) demands remains an open question. Recently, two models have been proposed: A specialized model, where each hemisphere governs contralateral behavior, and a redundant model, where both hemispheres equally guide behavior in the full visual space. To explore these alternatives, we analyzed simultaneous bilateral prefrontal cortex recordings from three macaque monkeys performing a visuo-spatial WM task. Each hemisphere represented targets across the full visual field and equally predicted behavioral imprecisions. Furthermore, memory errors were weakly correlated between hemispheres, suggesting that redundant, weakly coupled prefrontal hemispheres support spatial WM. Attractor model simulations showed that the hemispheric redundancy improved precision in simple tasks, whereas weak inter-hemispheric coupling allowed for specialized hemispheres in complex tasks. This interhemispheric architecture reconciles previous findings thought to support distinct models into a unified architecture, providing a versatile interhemispheric architecture that adapts to varying cognitive demands.

EXPERIENCE-DRIVEN REGULATION OF NEURONAL GENE PROGRAMS

Ángel Barco

Instituto de Neurociencias, Universidad Miguel Hernández, Alicante, Spain

Transcriptional and epigenetic mechanisms provide a molecular framework through which environmental influences and life experiences exert lasting effects on the brain. In this talk, we will examine two examples that illustrate the interplay between genomic regulation and neuronal plasticity. First, we will discuss how environmental enrichment and deprivation shape cognitive function through specific gene expression programs. Second, we will explore the transcriptional signatures associated with memory encoding and recall in the hippocampus.

EPIGENETIC AND CELLULAR REGULATION OF CORTEX EXPANSION AND FOLDING

Víctor Borrell

Institute of Neuroscience, CSIC-UMH, San Juan de Alicante, Spain

One of the most prominent features of the human brain is the fabulous size of the cerebral cortex and its intricate folding, both of which emerge during development. Over the last few years we have shown that cortex folding depends on high rates of neurogenesis and abundance of a particular type of basal progenitor, basal Radial Glia Cells (bRGCs). bRGCs profusely populate the Outer Subventricular Zone (OSVZ), and modify the organization of the radial fiber scaffold used by migrating neurons, hence driving cortex folding. The formation of the OSVZ along development, and of the highly stereotyped patterns of cortex folding, are linked to spatial-temporal patterns of progenitor cell proliferation, which are defined by a spatial-temporal protomap of gene expression within germinal layers. I will present recent findings from my laboratory revealing novel cellular and genetic mechanisms that regulate cortex expansion and folding. We have uncovered the contribution of epigenetic regulation to the establishment of the cortex folding protomap, modulating the expression levels of key transcription factors that control progenitor cell proliferation and cortex folding. At the single cell level, we have identified an unprecedented diversity of cortical progenitor cell classes in the ferret and human embryonic cortex. These are differentially enriched in gyrus *versus* sulcus regions and establish parallel cell lineages, not observed in mouse. Neurons born in gyrus *versus* sulcus are also transcriptomically distinct, especially related to human cortical malformation genes. Our findings show that genetic and epigenetic mechanisms in gyrencephalic species diversify cortical progenitor cell types and implement parallel cell linages, driving the expansion of neurogenesis and patterning cerebral cortex folds.

INVESTIGATING THE FUNCTION OF GABAB RECEPTORS IN HUMAN CORTEX, IN COMPARISON TO RODENTS

Sam A Booker

Simons Initiative for the Developing Brain ESAT; Centre for Discovery Brain Sciences University of Edinburgh, Edinburgh, UK

How circuits of neurons balance input to output transformations relies on precisely timed inhibitory signaling. Fast GABAA receptors regulate signal propagation on short timescales, meanwhile GABAB receptors operate over longer, behaviorally relevant timescales to control excitatory and inhibitory neurotransmission. Despite much being known of the role of GABAB receptors in rodent circuit motifs, little is known of how this crucial receptor regulates neuron and circuit function in the living human cortex. This talk will discuss our recent work to define the functional role of GABABRs in human neocortex and how this relates to neuropathology, such as seizure disorders.

CELL VIBES: HOW COLLICULAR CELL-TYPES CONTRIBUTE TO GUIDING MICE INNATE BEHAVIORS

Anna Chrzanowska

Neuro-Electronics Research Flanders, Leuven, Belgium; Paris Brain Institute (ICM), Paris, France

Neuronal cell types are organized into brain-wide circuits that guide behavior. Using the mouse superior colliculus as a model, we first demonstrated how optogenetic activation of a set of genetically targetable collicular cell types each leads to various defensive behaviors, engaging distinct but overlapping brain-wide dynamics captured with functional ultrasound imaging (fUSI). Each cell type was functionally connected to at least 82 brain areas, including some not previously considered part of these networks. Since optogenetic activation differs from natural stimulus-driven activity, we next examined how specific pathways enable behavioral responses to ecologically relevant stimuli. We focused on wide- and narrow-field neurons and their responses to predator-like visual stimuli (e.g., sweeping and looming discs). Behavioral tests with chemogenetic inhibition of these populations showed that disrupting their activity impaired appropriate reactions to potential threats. Our findings highlight the role of neuronal cell types as fundamental units of brain function and raise questions about how their coordinated activity shapes behavior.

MEMORY CAPACITY BEYOND LIMITS AND SEX-SPECIFIC REGULATION

Elvira De Leonibus

Institute of Biochemistry and Cellular Biology (IBBC), National Research Council of Italy, (CNR), Monterotondo (Rome), Italy

Memory capacity—the number of items we can retain over a short time interval—is constrained by both temporal and informational load, and its regulation depends on dynamic interactions between cortical and subcortical circuits. Using behavioral, molecular, and circuit-level approaches in mice, we dissect the neural substrates underlying incidental memory capacity. In this presentation, we will address how the brain encodes high *versus* low memory load during incidental encoding, whether and how memory capacity can be expanded beyond its physiological limit, and how many of the items encoded in short-term memory are ultimately consolidated into long-term memory. The latter is made possible by the discovery that male and female mice engage distinct cortico-subcortical circuits under high memory load, with dorsal hippocampus activation in males and ventral midline thalamus recruitment in females. As memory capacity underlies fluid intelligence and is often compromised in neuropsychiatric and neurodegenerative conditions, these findings provide a foundation for developing targeted cognitive-enhancing treatments.

MY PLACE *VS.* YOUR PLACE – WHAT CAN WE SAY ABOUT THE HIPPOCAMPUS AND TERRITORIALITY?

Dori Derdikman

Israel Institute of Technology – Technion, Haifa, Israel

Territoriality involves an understanding of the cognitive map, as animals must distinguish between areas they consider their own and those they do not. Accordingly, we expect the hippocampus to play a role in representing territoriality. We recorded neural activity from the dentate gyrus of a mouse in a resident-intruder paradigm, introducing an intruder into a resident mouse's territory over several consecutive days. We found that, after repeated exposures, dentate activity increased by an order of magnitude, suggesting a strong plasticity event that may underlie the learning of aggressive behavior by the resident. This provides an example of how a significant life event—such as the first appearance of an intruder—can drive substantial plastic changes in the hippocampus.

COULD SYMPTOMS OF COMPULSIVE SEXUAL BEHAVIOR DISORDER BE CURED? INSIGHTS FROM NEUROIMAGING STUDIES APPLIED TO THE CLINICAL TRIAL INTERVENTION

Małgorzata Draps

Clinical Neuroscience Laboratory, Institute of Psychology, Polish Academy of Sciences, Warsaw, Poland

In 2019 Compulsive Sexual Behavior Disorder (CSBD) has been included into the International Classification of Diseases 11th revision (WHO, 2019), however there is still ongoing discussion on theory that explains the mechanisms underlying this disorder. In the context of treatment an important research aim seems to be to identify key processes maintaining the symptoms and then to apply targeted therapies. Recent studies show beneficial effects of pharmacological interventions with SSRIs or naltrexone for individuals with CSBD. Studies using comprehensive measurement methods, including neuroimaging tools shows similarity between CSBD and addictions, thus pointing to the importance of sensitization processes. In this context a functional magnetic resonance imaging (fMRI) study examined brain reactivity towards erotic and monetary stimuli among 73 heterosexual CSBD male patients who were admitted for a 20-week double-blind and placebo-controlled randomized clinical trial were carried out. Clinical trial results from patients using paroxetine (20 mg/day) or naltrexone (50 mg/day) or placebo shows a significant effect of time during treatment on: severity of CSBD symptoms measured by self-report questionnaires, frequency of pornography consumption and sexual craving measured using ecological momentary assessment tool across all conditions. On neuronal level we analyze responses to erotic and monetary stimuli in the brain regions identified in previous research as differentiating males with CSBD from healthy controls. Results showed that in paroxetine and naltrexone groups a decrease of BOLD response in the brain regions which were initially hyperactive for erotic stimuli, while in the placebo group the increase of BOLD response to the monetary stimuli was pronounced. Based on analyses of neuronal data, the importance of hyperactivity to erotic stimuli, understood as sensitization, has been shown. Moreover, the presented data show that these processes can be reversible as an effective pharmacological strategy for treating symptoms of CSBD.

STRUCTURAL AND FUNCTIONAL SPECIALIZATION IN THALAMIC RETICULAR NUCLEUS SUBNETWORKS: IMPLICATIONS FOR SENSORY PROCESSING AND SALIENCE DETECTION

Zhanyan Fu

Stanley Center for Psychiatric Research, Broad Institute of Massachusetts Institute of Technology and Harvard, Cambridge, USA

The thalamic reticular nucleus (TRN), the primary source of thalamic inhibition, plays a crucial role in regulating sensation, action, and cognition. Despite its well-established function in modulating thalamocortical interactions, the organizational principles underlying its diverse functions remain incompletely understood. Here, we present an integrative framework for understanding the structural and functional specialization of TRN subnetworks and their implications for sensory processing and salience detection. Using a combination of single-cell transcriptomics, electrophysiology, and whole-brain circuit mapping, we identify two molecularly distinct TRN subpopulations that exhibit core- or shell-like anatomical organization and possess distinct electrophysiological properties. The TRN subpopulations form functionally segregated

subnetworks by making differential connections with first-order and higher-order thalamic nuclei. We thus develop Cre mouse lines that selectively label the two genetically segregated populations of TRN. Comprehensive mapping of whole-brain afferent circuit connectivity further reveals different biases in cortical and thalamic inputs to each subnetwork. Functional interrogation *via* inhibitory chemogenetic perturbation demonstrates that disruption of the TRN subnetworks leads to distinct EEG and sensory deficits reminiscent of phenotypes commonly observed in neuropsychiatric disorders, suggesting their potential involvement in disease pathophysiology. Together, our findings provide a multi-scale analysis of TRN subnetwork specialization, linking molecularly identity to the functional organization of thalamocortical circuits while highlighting the relevance of TRN dysfunction in neurodevelopmental and neuropsychiatric disorders.

INVESTIGATING EVOLUTIONARY EXPANSION OF THE HUMAN CEREBELLUM USING CROSS-SPECIES CEREBELLAR ORGANOIDS

Luca Guglielmi

MRC Laboratory of Molecular Biology (LMB), Cambridge, UK

The human cerebellum, which contains approximately 80–90% of the neurons in the adult brain, plays a central role in motor control and cognitive functions. During human evolution, cerebellar expansion significantly contributed to the unique size of the human brain and the emergence of complex behaviors, such as tool-making and language. Compared to other species, the human cerebellum is not only substantially larger but also occupies a greater proportion of total brain volume, underscoring its importance in shaping human-specific traits. To investigate the evolutionary mechanisms underlying differences in cerebellar size, we developed a cross-species cerebellar organoid model (CeOs). By employing minimal and conserved cerebellar determinants, we override the default telencephalic fate in unguided cerebral organoids and stabilize cerebellar identities, reproducing neuronal diversity alongside species-specific differences in cerebellar size. Furthermore, when cultured at the air-liquid interface, CeOs can be maintained for several months, allowing the development of mature cerebellar cell types and morphologies, including the formation of Purkinje cells with polarized dendritic arbors. Using this model, we interrogate cerebellar development in human and mouse CeOs and present preliminary data pointing towards species-specific differences in morphogen signaling as a contributing factor to cerebellar size variation across species.

PALMITOYLATION OF THE GLUCOCEREBROSIDASE RECEPTOR LIMP-2: THERAPEUTIC TARGET FOR PARKINSON'S DISEASE?

Gary Ho

Harvard Medical School, Brigham and Women's Hospital, Boston, USA

Disruption of vesicle and protein trafficking by the neuronal protein alpha-synuclein (αS) is a key pathophysiological mechanism in Parkinson's disease (PD). In contrast, palmitoylation, lipid modification of proteins at cysteines, has an essential role in trafficking in diverse cellular contexts, including in transport of lysosomal enzymes. We reasoned that enhancing palmitoylation may be a therapeutic strategy. Accordingly, we previously reported that increasing palmitoylation by inhibiting the depalmitoylase APT1 improved αS homeostasis and motor phenotypes in PD/DLB model mice, presumably by correcting vesicle trafficking. However, since the substrate(s) of APT1 and its selectivity are unknown, the pathway(s) underlying this improvement remained to be determined. We conducted an unbiased screen of APT1 substrates to identify specific proteins that could mediate these actions. Interestingly, we found LIMP-2 (lysosomal integral membrane protein 2) as a top "hit". This was a remarkable finding to us because LIMP-2 is the trafficking receptor for beta-glucocerebrosidase (GCase), encoded by the *GBA1* gene, the commonest genetic risk factor for PD. GCase is a lysosomal enzyme which hydrolyzes glucosylceramides into free ceramides and glucose. While homozygous mutations in GBA1 cause Gaucher's disease, heterozygous mutations significantly increase the risk of PD. This is likely due to GBA1 mutations causing misfolding and mis-targeting of GCase and subsequent lysosomal dysfunction. Since LIMP-2 binds and transports GCase to the lysosome, and because increased palmitoylation improves PD phenotypes, we investigated the role of LIMP-2 palmitoylation in regulating GCase function and αS homeostasis in the context of PD. LIMP-2 palmitoylation had not previously been

reported. Thus we confirmed that LIMP-2 is palmitoylated and identified the modified sites at cysteines 4, 5, and 458. Expression of LIMP-2-wt, but not the palmitoylation deficient mutant, restored GCase activity and αS homeostasis as measured by the level of physiological tetramers in GBA1 mutant patient-derived neurons. This finding links for the first time the fundamental cell biological process of palmitoylation to the central role of lysosomal function in PD and presents a potential novel therapeutic strategy.

JACOB: A SYNAPTO-NUCLEAR MESSENGER PROTEIN LINKING NMDAR ACTIVATION TO CREB-DEPENDENT GENE EXPRESSION

Anna Karpova

Leibniz Institute for Neurobiology, Magdeburg, Germany

Pyramidal neurons of the hippocampus possess a highly complex dendritic arbor, decorated with a vast array of spine synapses that receive excitatory input. These synaptic signals not only exert local effects but are also transmitted to the nucleus of the postsynaptic neuron. Nuclear Ca^{2+} waves, triggered by NMDAR and L-type voltage-gated Ca^{2+} channels, along with synapse-to-nucleus protein transport, play crucial roles in regulating plasticity-related gene expression. Jacob is a protein that translocates a signalosome from N-methyl-D-aspartate receptors (NMDAR) to the nucleus, where it docks the signalosome to the transcription factor CREB. Intriguingly, its residence time in the nucleoplasm closely correlates with the pattern of nuclear Ca^{2+} transients ($[Ca^{2+}]$) induced by neuronal activity, resulting in plasticity-dependent gene expression. In my talk, I will summarize the key findings regarding the "Jakobsweg" to the nucleus and the role of the protein messenger in plasticity and neurodegeneration.

CORTICAL AND HIPPOCAMPAL CIRCUITS FOR DISCRIMINATING POSITIVE EMOTIONS AND SOCIAL LEARNING IN MICE

Ewelina Knapska

Laboratory of Neurobiology of Emotions, Nencki-EMBL Partnership for Neural Plasticity and Brain Disorders–BRAINCITY, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland

How do social networks shape learning about rewards, and what neural circuits support this process? Despite its critical role in survival, the neural basis of socially transmitted reward learning remains poorly understood. To investigate this, we used Eco-HAB, an automated system for tracking group behavior in mice. We found that scent cues from rewarded individuals guide conspecifics' search behavior, with social rank modulating this effect. Prelimbic cortex plasticity is crucial for both maintaining social network stability and utilizing socially acquired reward information, while acute inhibition of the prelimbic cortex selectively disrupts responses to social cues, revealing distinct cortical contributions to social learning. Beyond reward-seeking, animals must also use socially transmitted information to navigate their environment. Can rodents learn food locations from their peers? To address this, we developed the Socially Transmitted Place Preference paradigm, demonstrating that mice and rats acquire food location knowledge through brief social interactions. Single-photon imaging reveals that hippocampal cells encoding these locations are reactivated during social interactions, suggesting a neural mechanism for the storage and retrieval of socially acquired spatial information. Together, our findings suggest that distinct cortical and hippocampal circuits support different aspects of social learning: prefrontal circuits regulate social influence on decision-making, while hippocampal activity encodes and replays socially transmitted spatial knowledge.

SPATIOTEMPORAL METHODS TO CHART THE CELL STATES OF BRAIN DEVELOPMENT

Gioele La Manno

Laboratory of Brain Development and Biological Data Science, Swiss Federal Technology Institute of Lausanne, Lausanne, Switzerland

The developing brain is like a complex chess game with millions of pieces – each belonging to one of hundreds of distinct cell types. While the single-cell revolution has revealed the foundation of this "game", becoming true "masters" is still ahead. With spatial transcriptomics, we aim to interpret each snapshot of

cells in the tissue, distinguish normal from pathological states, and predict "future moves". Here, we present two complementary approaches toward this dream. On the temporal front, VeloCycle, an advanced RNA velocity framework, tracks cell cycle-driven expression dynamics in real time to predict cellular trajectories. On the spatial front, PointillHist, a GNN-based mapper, reveals the organization of hundreds of distinct cell states across the embryonic brain. Applied to study folate deficiency, these tools uncover the differential susceptibility of radial-glial populations and a "catastrophic flipping" of patterned territories.

ALL IEGs ARE NOT CREATED EQUAL – DIVERSE ROLES OF ACTIVITY-DEPENDENT TRANSCRIPTION FACTORS IN NEURAL CIRCUIT PLASTICITY

Yingxi Lin

Department of Psychiatry, Psychiatry Neuroscience Research Division, UT Southwestern Medical Center, Dallas, US
Department of Neuroscience, O'Donnell Brain Institute, Dallas, US

Our research investigates the molecular and circuit mechanisms underlying neurodevelopment, memory formation, and neuropsychiatric disorders. Using a multidisciplinary approach that integrates genomic, molecular, synaptic, circuit, and behavioral analyses, we seek to understand how sensory and behavioral experiences reshape neural circuits to enable learning and memory. We focus on the mechanisms by which immediate-early genes (IEGs) direct distinct activity-dependent transcription pathways to regulate circuit plasticity and learned behavioral adaptation. This talk will highlight the diverse roles of individual IEGs in orchestrating circuit-specific transcriptional programs, shedding light on how activity-dependent transcription programs contribute to experience-dependent circuit reconfiguration across behavioral contexts.

THE ROLE OF CONCEPT NEURONS IN THE HUMAN MEDIAL TEMPORAL LOBE FOR WORKING AND LONG-TERM MEMORY

Florian Mormann

University of Bonn, Bonn, Germany

The human medial temporal lobe contains neurons that respond selectively to the semantic contents of a presented stimulus. These "concept cells" may respond to very different pictures of a given person and even to their written and spoken name. Their response latency is far longer than necessary for object recognition, they follow subjective, conscious perception, and they are found in brain regions that are crucial for declarative memory formation. It has thus been hypothesized that they may represent the semantic "building blocks" of episodic memories. In this talk I will present data from single unit recordings in the hippocampus, entorhinal cortex, parahippocampal cortex, amygdala, and piriform cortex during paradigms involving working and long-term memory in order to characterize the role of concept cells in these cognitive functions.

DOPAMINERGIC MODULATION OF GABAergic SYNAPTIC PLASTICITY IN MOUSE HIPPOCAMPUS

Jerzy W. Mozrzymas

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Dopamine is a major modulator of key brain functions such as memory and learning, and so far studies into underlying mechanisms have been largely focused on glutamatergic synapses and their plasticity. Little is known about the dopaminergic modulation of inhibitory plasticity at synapses formed by distinct GABAergic interneurons innervating different cells. Herein, we studied the role of D1-type dopamine receptors (D1Rs) in inhibitory plasticity at synaptic connections between interneurons (INs) and pyramidal cells (PCs), and also between INs in the CA1 region. Activation/blockade (with SKF/SCH) of D1Rs increased/reduced the mIPSCs amplitude (measured from PCs), while the decay kinetics was prolonged for SKF, indicating a postsynaptic mechanism. We also checked the D1Rs impact on heterosynaptic NMDA-induced inhibitory long-term potentiation (iLTP) measured at PCs. Blockade of D1Rs converted iLTP into inhibitory long-term depression (iLTD), while D1Rs activation slightly diminished the extent of iLTP. NMDA-induced iLTP in synapses formed by parvalbumin (PV)-positive INs on PCs was reduced to zero by SKF, while SCH converted iLTP to iLTD. In-

terestingly, both SKF and SCH reversed NMDA-evoked iLTP in the somatostatin (SST)-positive INs to iLTD, while these compounds were ineffective on baseline activity, and these effects were mirrored by changes in gephyrin clusters. Thus, the impact of D1Rs on inhibitory plasticity observed at the SST INs and PCs showed differences with respect to baseline activity, NMDA-induced plasticity, and the kinetics of synaptic currents. Altogether, we show that D1Rs modulate inhibitory long-term plasticity in a manner dependent on the presynaptic and target neurons.

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SPACE, TIME AND OTHERS IN THE HIPPOCAMPUS & THE NAMING OF NON-HUMAN PRIMATES

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I will first discuss our recent findings revealing the hippocampus's critical role in social cognition. By examining dorsal CA1 activity in mammals, we identified explicit neuronal representations of others' locations ("social place cells") and integrated space-time codes ("social time cells") for both self and others. These discoveries highlight how the hippocampus underpins the ability of social animals to synchronize behavior in space and time, essential for survival and reproduction. In the second part, I will present our recent work on non-human primates. Using machine learning to analyze spontaneous vocal interactions between marmoset monkeys (*Callithrix jacchus*), we discovered, for the first time, that these primates vocally label their conspecifics—a sophisticated cognitive function previously attributed only to humans and dolphins. These findings challenge long-held views of language evolution in humans and offer important perspectives on the evolutionary origins of language and underscore the need to elucidate the underlying neural basis of this ability.

THE ROLE OF NEUROG2 T149 PHOSPHORYLATION SITE IN THE DEVELOPING HUMAN NEOCORTEX

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Neocortical expansion throughout evolution has been responsible for higher-order cognitive abilities and relies on the increased proliferative capacities of cortical progenitors to increase neuronal production. Therefore, in gyrencephalic species such as humans and primates, where the neurogenic period is protracted, the regulation of the balance between progenitor maintenance and differentiation is of key importance for the right neuronal production. The control of this balance in the neocortex is mediated by feedback regulation between Notch signaling and the proneural transcription factor Neurogenin2 (NEUROG2). As the expression of NEUROG2 alone is sufficient to induce neurogenesis in the neocortex, its regulation at the gene level has been extensively studied in mice. However, recent findings highlight that regulation at the protein level through post-translational modifications can profoundly influence protein activity and stability. Indeed, the modulation of the conserved NEUROG2 T149 phosphorylation site in the developing mouse neocortex results in an altered pool of progenitors and number of neurons in the deep and upper layers. Nevertheless, it is not known how such post-translation modification regulates NEUROG2 activity in the development of the human neocortex under endogenous levels and its contribution to the development of the neocortex. We hypothesize that phosphorylation of NEUROG2 at T149 modulates the timing of cortical progenitor differentiation in humans. To test this, we used 3D cortical organoids generated from CRISPR/Cas9-engineered iPSC lines. Using live imaging of radial glial cell (RGC) clones, immunohistochemistry, machine learning-based cell quantification, transcriptional activation assays, stem cell reprogramming, and multi-omics (snRNA-seq and snATAC-seq), we observed that preventing T149 phosphorylation shifts RGC division from proliferative to neurogenic modes. This results in increased neuron production during mid- and late-stages of organoid cortical development. Mechanistically, we identified an opening of JUN binding sites in RGCs, enhancing the transition to intermediate progenitors (IPs) and subsequently increasing neuron generation. Thus, the phosphorylation at T149 acts as a regulatory rheostat, modulating NEUROG2-driven neurogenesis in human cortical development.

CONTRIBUTION OF THALAMIC PROJECTIONS TO THE HIPPOCAMPUS TO MEMORY PROCESSES

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The ability to extinguish contextual fear in a changing environment is crucial for animal survival. Recent data support the role of the thalamic nucleus reuniens (RE) and its projections to the dorsal hippocampal CA1 area (RE→dCA1) in this process. However, it remains poorly understood how RE impacts dCA1 neurons during contextual fear extinction (CFE). During my talk I will discuss our recent data demonstrating that the RE→dCA1 pathway contributes to extinction of contextual fear by affecting CFE-induced molecular remodeling of excitatory synapses in dCA1 stratum lacunosum-moleculare.

LISTENING TO LIGHT AND SEEING SOUND IN THE BRAIN

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Development of more efficient and less intrusive ways to alter and observe brain activity is instrumental towards tackling neurological diseases in an aging population and for advancing basic neuroscience research. Light- and ultrasound-based technologies are growingly used for brain interrogation, modulation of neural activity, and treatment of brain diseases. The talk focuses on our latest additions to the arsenal of multi-scale neuroimaging techniques, including large-field multifocal illumination microscopy, super-resolution fluorescence localization imaging, whole-brain functional optoacoustic imaging, localization optoacoustic tomography, multi-modal combinations with functional ultrasound, magnetic resonance imaging and more. The new methods enable transcranial large-scale recordings of neural and hemodynamic activity and molecular agents at penetration depths and spatio-temporal resolution scales not covered with the existing micro- and macro-scopic functional neuroimaging techniques. Examples of applications include large-scale monitoring of neurovascular coupling and neural activity indicators, tracking circulating cells and microrobots, targeted molecular imaging of Alzheimer's and Parkinson's, studying microcirculation in stroke. Our current efforts are also geared toward employing optical and optoacoustic techniques for monitoring the effects of transcranial ultrasound stimulation of the living brain. The marriage between light and sound thus brings together the highly complementary advantages of both modalities toward high precision interrogation, stimulation, and therapy of the brain with strong impact in the fields of neuromodulation, gene and drug delivery, or noninvasive treatments of neurological and neurodegenerative disorders.

GABA TRANSPORTER SUBTYPES 1 AND 3 AS TARGETS FOR NOVEL MEMORY-IMPROVING DRUGS

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Alzheimer's disease (AD) is a neurodegenerative disorder characterized by memory decline and accompanying behavioral and psychological symptoms of dementia (depression, anxiety). Considering that the available anti-AD therapies based on the enhancement of cholinergic neurotransmission are weakly effective in advanced stages of the disease, novel molecular targets for memory-improving drugs are being explored. GABAergic neurotransmission is found to be crucial for learning and memory both in humans and experimental animals. Several preclinical studies revealed that targeting GABAergic system holds potential in overcoming memory deficits in AD. Hence, GABAergic signaling presents a promising target for anti-AD drug development and recently GABA transporters (GAT) have become a subject of interest as a target for procognitive drugs. Previously, we focused on GAT1 inhibition and we found that tiagabine, a selective GAT1 inhibitor, in contrast to many available antiepileptic drugs, not only effectively reduces seizures but it has also potential to attenuate memory deficits and reduce behavioral symptoms of dementia (anxiety and depression) in animal models. Our present research is therefore focused on investigating therapeutic potential of compounds acting at other less explored GAT, namely GAT-3 (human nomenclature). Using a combination of crystallography and computational methods we developed a series of compounds among which the compound 6 demonstrated inhibition of GAT-1 (IC50=10.96 μM) and GAT-3 (IC50=7.76 μM), along with a favorable drug-like-

ness profile. Subsequent *in vivo* studies revealed the effectiveness of 6 in enhancing learning and memory retention and alleviating anxiety and depression symptoms in mouse models, while also proving safety and bioavailability for oral administration. The innovative ligand 6 offers a new approach to treat AD patients with symptoms of cognitive deficits and accompanying mood disorders.

OPTICAL AND COMPUTATIONAL TOOLS TO EXPLORE BRAIN-WIDE BEHAVIOR-SPECIFIC CIRCUITS

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Complex behaviors are the result of the coordinated activity of large populations of interconnected neurons across the entire brain. A detailed charting of this orchestrated flow of information would be fundamental for understanding brain function in healthy and disease states. However, the detailed organization of brain-wide behavior-specific circuits remain elusive, mainly for technical reasons. Indeed, most imaging methods suffer from either poor resolution – insufficient to disentangle single cells – or limited field of view – offering only a partial view of wider brain networks. In this scenario, light-sheet fluorescence microscopy (LSFM), coupled with chemical clearing of tissue, surged as a potential game changer allowing full volumetric reconstruction of entire organs with sub-cellular resolution. However, despite the great promise hold by this method, its routine use is still often limited to the production of a couple of fancy 3D renderings without any real biological insight. In this talk, I will analyze the optical and computational limitations of state-of-the-art LSFM, and discuss recent advances to achieve scalable, robust, and quantitative analysis of activation patterns in whole mouse brains. Finally, I will describe application of this "adaptive and smart" microscopy to the dissection of brain-wide circuits involved in fear memory, and discuss future directions of the field.

NAVIGATING ASTRO-NEURO DYNAMICS: THE IMPACT OF GLIAL Wnt SIGNALING ON NEURONAL DEVELOPMENT AND FUNCTION

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The Wnt/β-catenin pathway contains multiple high-confidence risk genes that are linked to neurodevelopmental disorders, including autism spectrum disorder. However, its ubiquitous roles across brain cell types and developmental stages have made it challenging to define its impact on neural circuit development and behavior. Here, we show that TCF7L2, which is a key transcriptional effector of the Wnt/β-catenin pathway, plays a cell-autonomous role in postnatal astrocyte maturation and impacts adult social behavior. TCF7L2 was the dominant Wnt effector that was expressed in both mouse and human astrocytes, with a peak during astrocyte maturation. The conditional knockout of TCF7L2 in postnatal astrocytes led to an enlargement of astrocytes with defective tiling and gap junction coupling. These mice also exhibited an increase in the number of cortical excitatory and inhibitory synapses and a marked increase in social interaction by adulthood. These data reveal an astrocytic role for developmental Wnt/β-catenin signaling in restricting excitatory synapse numbers and regulating adult social behavior.

CHOOSING TO BE DIFFERENT. CELL IDENTITY AND FATE CHOICE IN THE DEVELOPING BRAIN

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Neurons forming the neocortex are generated during embryonic development from two main classes of neural progenitor cells: apical and basal progenitors (APs and BPs, respectively). Our lab is interested in understanding the role of the Golgi apparatus and glycosylation in regulating neuronal stem cell behavior and fate choice during brain development. The Golgi apparatus is the main hub for glycosylation and defects in Golgi-associated glycosylation can lead to primary microcephaly, a neurodevelopmental defect associated with alterations in neural stem cell behavior and lineage progression. The cell biological mechanisms

linking defective Golgi glycosylation and neurodevelopmental manifestations are currently unknown. We want to fill this gap by investigating the influence of the Golgi apparatus on stem cell identity and fate transition during brain development. We show that the Golgi apparatus is rearranged during APs to BPs fate transition, in particular in relation to its association with the centrosome. In addition, pharmacological and genetic perturbation of GA integrity in mouse embryos and human brain organoids favors the APs to BPs fate transition. Our data suggest that the Golgi apparatus structure and function are linked to cell fate switch during brain development.

PALMITOYLATION-DEPENDENT SIGNALING IN AND FROM DISTAL AXONS

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Neuronal axons are thin, delicate projections whose integrity is critical for nervous system function. Many proteins that control the balance between axonal integrity and degeneration are covalently modified with the lipid palmitate. This process, palmitoylation, serves to target proteins to specific subcellular membranes. We and others have revealed critical roles for palmitoylated proteins that 'hitchhike' on axonal vesicles to convey responses from damaged or stressed axons back to neuronal cell bodies. These palmitoylated retrograde signaling proteins then drive pro-degenerative, or sometimes pro-regenerative, responses. Recently, we have investigated the potential of selectively preventing such axonal retrograde signaling as a novel neuroprotective strategy. We are also now revealing roles for palmitoylation in autonomously controlling the axo-degenerative process in distal axons themselves. These exciting findings provide new insights into axonal biology and may reveal new ways to lessen the impact of the many neuropathological conditions of which axon degeneration is a hallmark.

HOW PARIETAL CORTEX AND HIPPOCAMPUS CONTRIBUTE TO SPACE FOR ACTION

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Non-human primates and rodents can find their way in virtual environments, avoiding obstacles and planning trajectories to reach goals. How can a sense of space arise from visual-only stimulation? Here we show how cells in the parietal cortex and the hippocampus of macaques navigating a virtual space are driven by visual explorations, titling the space as a function of the animal's attention, expressed by saccades and fixations to paths and landmarks, salient elements of the virtual space. Further, we show how, both regions anticipated landmarks before they appeared in the field of view, suggesting a shared knowledge of the spatial layout. Yet, cells in the parietal cortex were sensitive to the side of appearance of the landmark while hippocampal ones weren't, expressing egocentric *versus* allocentric complementarity. In light of these findings, I will discuss the neural processes that make up place in primates, stemming from visual exploration of objects in space combined with memory-driven actions.

THALAMIC ENERGY METABOLISM AND AUTISM-LIKE DEFICITS IN A MOUSE MODEL OF THE TCF7L2-RELATED NEURODEVELOPMENTAL DISORDER

Marta Wiśniewska

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The *TCF7L2* gene, which encodes a transcription factor, is a recognized risk gene for psychiatric conditions. Its mutations lead to a recently identified rare neurodevelopmental disorder characterized, among other symptoms, by traits of autism spectrum disorder (ASD). TCF7L2 is an effector of Wnt signaling enriched in the brain, exhibiting particularly high expression in thalamic neurones. We investigated whether postnatal disruption of TCF7L2 in the thalamus contributes to ASD-like symptoms in mice. Our findings indicate that thalamic TCF7L2 regulates energy metabolism within thalamocortical circuitry, with its deficiency resulting in the dysregulation of metabolism-related genes in the thalamus and a considerable reduction in

pyruvate utilization efficiency in both the thalamus and cortex. Furthermore, the ketogenic diet restored brain energy metabolism and normalized social behavior, thereby linking metabolic deficits in thalamocortical circuitry to behavioral symptoms. These findings provide evidence that ASD may be directly associated with impaired brain energy metabolism and emphasise the role of thalamocortical circuit dysfunction in the pathogenesis of social deficits.

PROTEIN PALMITOYLATION IN SYNAPTIC PLASTICITY AND SPATIAL LEARNING

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Synaptic plasticity is a fundamental process underlying learning and memory. However, the molecular mechanisms governing this phenomenon remain incompletely understood. One emerging regulatory mechanism is S-palmitoylation – a reversible post-translational lipid modification that modulates the function of synaptic proteins by influencing their conformation, localization, trafficking, and molecular interactions. Over the past decade, S-palmitoylation (S-PALM) has been increasingly recognized as a key factor in the sorting and membrane localization of neuronal proteins. Despite numerous reports identifying S-PALM targets *in vitro*, the functional consequences of this modification on synaptic proteins and neural circuits remain largely unexplored. In this talk, I will present our most recent findings from several *in vitro* and *in vivo* models of neuronal plasticity in which S-PALM was manipulated pharmacologically. Our data support the view that both short- and long-term changes in synaptic strength, as well as increased neuronal spiking following network activation, require protein palmitoylation. We identified several pre-, post-, and intersynaptic proteins whose palmitoylation is dynamically regulated by synaptic activity. Furthermore, using mass spectrometry analysis of brain samples from rats exposed to a spatial learning paradigm, we identified a subset of synaptic proteins undergoing S-PALM *in vivo*. Notably, we found that S-PALM can occur locally at isolated excitatory synapses immediately after synaptic activity. Altogether, our findings highlight local and rapid protein-specific palmitoylation as a vital mechanism for synaptic plasticity, contributing to the dynamic regulation of neuronal network function and memory formation.

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MEMORY IN SEQUENCE: PREFRONTAL AND MEDIAL TEMPORAL NEURONS ENCODE ORDER OF EVENTS IN HUMANS

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Remembering the temporal order of events is critical for episodic memory. Previous research suggests that linking individual events into temporally associated memories relies on the medial temporal lobe and prefrontal cortex. Damage to these regions can disrupt the ability to recall stories and real-life events in the correct order. However, little is known about how brain encodes and retrieves temporal order information at the neural level. To investigate this, we designed an order memory task using video clips that mimic real-life experiences. Each clip contained four sequential everyday events of varying length, with visual cuts inserted at event transitions (i.e., event boundaries). Participants watched the clip and were then asked to recall the order of event sequence within each clip and identify familiar scenes. This study involved 23 patients with refractory epilepsy who had depth electrodes implanted for seizure monitoring, allowing us to record single neuron activity while they performed the task. Among the 1014 recorded neurons, we identified order selective neurons (OSNs) in the hippocampus, amygdala, and orbitofrontal cortex that selectively responded to specific event orders (i.e., preferred order), regardless of event content and the absolute time. Most of these OSNs exhibit transient theta phase precession following their preferred order during memory encoding and also when retrieving order memory involving their preferred order. Furthermore, the strength of theta phase precession in OSNs predicted participants' order memory performance (correct *versus* incorrect). These findings shed lights on how the brain weaves discrete episodic events into a coherent temporal narrative, advancing our understanding of human episodic memory.

POSTERS

WUTScope-FPM – A FOURIER PTYCHOGRAPHIC MICROSCOPE SYSTEM FOR ULTRA HIGH-RESOLUTION, LABEL-FREE WIDEFIELD BIOMEDICAL IMAGING

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Fourier ptychographic microscopy (FPM) is a computational imaging technique that uses multi-angle illumination to enhance imaging resolution. By employing synthetic aperture methods together with an iterative phase-retrieval algorithm, it reconstructs both amplitude and phase images with an effective numerical aperture far exceeding that of the objective lens. Thanks to these capabilities, FPM is a promising tool for biomedical imaging, combining high resolution with phase measurement over a wide field of view (FOV). In this work, we present the WUTScope-FPM – a microscope system, which uses a high-brightness programmable LED array as the illumination source instead of the traditionally used LED-condenser system. The developed system achieves an effective NA of 0.78 when using a 4×, NA 0.2 objective, corresponding to nearly a fourfold improvement in resolving power (transverse resolution<500 nm). WUTScope-FPM features an easy-to-use graphical interface (FPMapp), requiring only basic familiarity with optical microscopes, so no extensive optics expertise is needed. Presented WUTScope-FPM system enables high resolution, label-free high contrast imaging of neurons, organoids or tissue slices with sub-cellular resolution and FOV over 4 mm × 5 mm without image stitching or mosaicking.

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NEURAL ORGANOIDS AS A MODEL TO TRACK THE FORMATION AND MATURATION OF DENDRITIC SPINES

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Neural organoids provide a great tool to decipher human brain diseases at the molecular and physiological levels. The organoids are of great value in understanding neurodevelopmental diseases, as they can reflect changes occurring even in the prenatal period. Those diseases are often accompanied by aberrant changes in the formation of dendritic spines harboring excitatory synapses. Yet, none of the studies focused on detailed characterization of dendritic spines in organoids. Herein, we present the novel protocol to visualize and characterize single dendritic spines in matured organoids. Human induced pluripotent stem cells were differentiated to cortical spheroids. On subsequent stages of development organoids were evaluated by whole organoids' imaging and western blot analysis. Till day 200 the organoids were evaluated for proper differentiation: rosettes formation, cortex layering and glial/neuronal differentiation. After day 200 organoids were evaluated for their maturation properties. Live calcium imaging was performed to analyze the spontaneous activity of cells. Next, organoids older than one year were evaluated for dendritic spines formation. Spines were characterized using biolistic delivery of lipophilic dye combined with subsequent immunolabeling of pre- and postsynaptic markers. We show that organoids' maturation can be manifested by spontaneous activity of neurons with visible synchronization. This maturation is accompanied by changes in expression of repertoire of synaptic-related proteins (glutamate receptors, postsynaptic scaffolding proteins). Importantly, we were able to optimize protocol to successfully visualize dendritic spines in neurons within organoids. Furthermore, we were able to immunolabel dendritic spines with antibodies directed to proteins forming either pre- or postsynaptic compartments. This method enables a more detailed characterization of complex dendritic spine structure and function in human neurons in both health and disease.

THE IMPACT OF DONOR NERVE DEVELOPMENTAL STAGE ON MOTOR NEURON SURVIVAL AND MICROGLIAL ACTIVATION FOLLOWING SCIATIC NERVE GRAFTING IN NEWBORN RATS

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Peripheral nerves in adult mammals show considerable regenerative capacity. In contrast to adults, nerve injuries during the neonatal period are often followed by a limited regenerative response and motoneuron loss. The underlying mechanisms of regenerative failure in newborns are not yet fully understood. Interestingly, research has demonstrated that when a sciatic nerve fragment from a more mature rat pup (P5 or older) is grafted into a younger one (P3), axons can regenerate. This regenerative response does not occur if the graft is taken from a younger or age-matched donor. This study employed single-nucleus RNA sequencing (snRNA-seq) and immunohistochemistry to investigate changes occurring in the spinal cord following the transplantation of a sciatic nerve fragment derived from either 3-day-old (P3) or 6-day-old (P6) donor rats into 3-day-old recipients. Transplantation of the younger nerve segment resulted in a reduced number of choline acetyltransferase positive (ChAT+) motoneurons within the ipsilateral ventral horn, compared to the contralateral side as well as to the parallel structure of the spinal cord of rats receiving transplants from older donors. Up to 4 days post-injury, the number of microglial cells identified by ionized calcium-binding adaptor molecule 1 (Iba1+) immunostaining was also lower in the P3 transplant group. However, at later time points, an increase in microglial cell density was observed in the P3 group, whereas in the P6 group, the number of Iba1$^+$ cells gradually declined. These findings highlight the critical role of the donor nerve developmental stage in modulating spinal cord regenerative and inflammatory responses. Understanding what transplant-related factors and molecular mechanisms influence motor neuron survival may offer valuable insights for improving nerve repair strategies.

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mGluR-1 ACTIVATION REGULATES LONG-TERM POTENTIATION OF EXCITATORY SYNAPSES ON LAYERS 2/3 VIP-INs IN THE MOUSE SOMATOSENSORY CORTEX

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Long-term potentiation (LTP) is a type of cellular model of synaptic plasticity. The mechanisms of LTP induction and expression have been under intense investigations in respective to specific synapses and neuronal classes. In the present study, we aimed to uncover cellular mechanisms that elicit LTP on a subpopulation of neocortical GABAergic interneurons; vasoactive intestinal polypeptide-expressing interneurons (VIP-INs) in layers 2/3 of the mouse somatosensory cortex. VIP-INs contribute to one of three main inhibitory interneurons and target other interneurons to disinhibit pyramidal cells. This disinhibitory mechanism has been described as crucial for learning and memory formation. We implemented *in vitro* whole-cell patch-clamp technique to characterize electrophysiological properties underlying LTP induction in excitatory synapses onto L2/3 VIP-INs. To induce LTP, we used neighboring extracellular stimulation paired with postsynaptic membrane depolarization. First, we found that this protocol induces LTP and GABAaR antagonist enhances this form of plasticity. Furthermore, N-methyl-D-aspartate receptor (NMDAR) is not necessary for LTP induction on L2/3 VIP-INs. Next, pharmacological blockage of metabotropic glutamate receptor (mGluR)-1 but not mGluR-5, prevents LTP formation. Since mGluRs-1 mediate Ca2+ influx through number of signaling pathways, we targeted Src-family tyrosine kinase with the antagonist application, which led to LTP abolition. Summarizing, our research gives insights into molecular mechanism underlying synaptic plasticity of excitatory inputs onto VIP-INs in the neocortex.

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KETAMINE INDUCED REORGANIZATION OF BRAIN ACTIVITY: SPIKE ACTIVITY AND CHANGES IN GAMMA AND HIGH-FREQUENCY OSCILLATIONS

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Fast oscillations recorded in local field potentials, such as gamma oscillations (30–100 Hz) and high-frequency oscillations (HFOs), are thought to temporally coordinate spiking activity. Ketamine is a psychoactive compound currently under extensive investigation for its antidepressant potential. In rodents, the olfactory bulb exhibits large-amplitude HFOs following ketamine administration. Here, we examined the relationship between spontaneous gamma oscillations and spiking activity in the olfactory bulb before and after ketamine injection. Local field potentials from freely moving rats were analyzed and band-pass filtered for low-gamma (30–70 Hz), high-gamma (70–100 Hz), and HFO (130–180 Hz) bands. Spike activity were detected with Kilosort4 package. After ketamine administration, a decrease in both gamma bands power and a noticeable increase in HFO power were observed. Modulation analysis indicated that high-gamma activity was largely replaced by HFO, implying a reorganization of oscillatory dynamics. Phase Locking Value analysis demonstrated that high-gamma in baseline condition and post-ketamine HFO bursts were strongly phase-aligned. The peri-stimulus time histogram analysis identified two spike clusters: one showing synchronized activity with gamma oscillations during the baseline and another lacking of such synchronization. Interestingly, spike activity in both clusters coordinated with HFO after ketamine treatment. Spike-field coherence analysis verified increased spike-phase locking within the post-ketamine administration, indicating improved synchronization between spiking activity and HFO. Ketamine-induced brain oscillations may alter cognitive function by balancing gamma and HFO activity. The change from gamma synchronization to extensive HFO may lead to less specificity in brain communication, resulting in altered information processing.

NEURAL EFFECTS OF CBT FOR PROCRASTINATION: PRELIMINARY EEG FINDINGS FROM A RANDOMIZED CONTROLLED STUDY

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Procrastination – the voluntary delay of intended actions despite expecting negative consequences – is a widespread self-regulatory failure linked to psychological distress. Cognitive-behavioral therapy (CBT) has emerged as a promising intervention for reducing procrastination and related symptoms. However, longitudinal studies investigating the neural mechanisms underlying CBT-based treatment for procrastination are scarce. This research explored therapy-induced changes in brain activity using event-related potentials (ERPs). Seventy-nine university students (planned n=135) with high levels of procrastination participated in a randomized trial comparing a 5-week group CBT intervention (intervention group, IG) to a time-matched wait-list control (WL). The intervention resulted in significant reductions in procrastination, with large effect sizes (d=-1.24). EEG data were collected before and after the intervention during a Passive Viewing Task, where participants read words from three categories: procrastination-related, emotionally negative, and neutral. At baseline, the Late Positive Potential (LPP) differed significantly between stimulus types, with procrastination-related words eliciting higher amplitudes than emotionally negative words. From pre- to post-intervention, a significant main effect of time was observed for early posterior negativity amplitudes across all stimulus types. Exploratory *post-hoc* analyses suggested this increase was most pronounced in the IG. No significant time-related changes were found for the LPP or N400 components. These preliminary findings suggest that CBT for procrastination may enhance early automatic attentional processing during exposure to written material, independent of stimulus content. However, no significant changes were observed in later stages of emotional and semantic processing. Early attentional modulation may therefore represent a potential neural mechanism supporting improved self-regulation following CBT.

THE ROLE OF APOE IN ASTROCYTE-NEURON INTERPLAY

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Astrocytes feature remarkable evolutionary changes. Human astrocytes are significantly larger and more complex than their rodent counterparts, and primate astrocytes exhibit greater morphological diversity than rodent astrocytes. Therefore, the evolutionary changes in astrocytes may contribute to the expansion of the human brain's cognitive capacities. Numerous evolutionarily affected genes are highly expressed during fetal development, yet the molecular markup of fetal astrocyte evolution in primates remains poorly understood. In the past, using iPS cell-derived fetal-like human, chimpanzee, and macaque astrocytes (iAstrocytes), we uncovered a congruent, evolutionary up-regulation of genes related to long-distance intercellular communication. Remarkably, disease-related genes are often downregulated in evolution. *APOE*, a broadly studied gene associated with Alzheimer's disease, exemplifies this trend. Yet, the possible role of the transcriptional downregulation of APOE in human brain evolution and development has not been defined. In the brain, APOE is mainly produced by astrocytes. Using ELISA, we show reduced production of APOE by the human iAstrocytes as compared to the non-human primate cells. We find that the reduction of APOE protein is uncoupled from changes in the level of cholesterol secreted by astrocytes. This result suggests potential additional roles of APOE in regulating brain functions. We overexpressed APOE in the human iAstrocytes and measured the effect of the gain of APOE production on astrocytes and neurons. Transcriptomic analysis revealed the deregulation of expression of genes regulating neuronal development upon APOE overexpression in the human iAstrocytes. *Ex vivo* co-culture of rat neurons with human iAstrocytes showed that APOE dosage contributes to the regulation of neuronal development and activity. Therefore, we hypothesize that *APOE* is an evolutionarily affected gene, involved in maintaining proper temporal development of neurons.

UNPREDICTABLE CHRONIC MILD STRESS INDUCES CHANGES IN SERUM METABOLOME, VTA PROTEOME AND REDOX STATE IN THE MESOCORTICOLIMBIC PATHWAY

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Chronic stress is a well-established public health concern. Characterized by psychophysiological responses to challenging situations, it is linked to the development of various illnesses including mental disorders. Evaluate the anxiety-like behavior; serum metabolome, ventral tegmental area (VTA) proteome and brain redox state of rats submitted to two weeks of unpredictable chronic mild stress protocol (UCMS). Male Wistar rats (300-350 g) underwent two weeks of UCMS (adapted from Burstein et al., 2018) and were then tested behaviorally (EPM, dark/light box, open field, sucrose preference). After euthanasia, brain and blood were collected for analysis. Statistical analysis was performed using GraphPad Prism, with data assessed for normality (Shapiro-Wilk) and analyzed *via* Unpaired T or Mann-Whitney tests. Rats subjected to 2 weeks of UCMS showed in the EPM: 1) less time in open arms (CON 97.81±14.34, n=11 *vs.* UCMS 45.38±12.22; p=0.0115); 2) fewer entries in open arms (CON 4.909±0.5633, n=11 *vs.* UCMS 2.182±0.4635, n=11; p=0.0013); 3) more time in closed arms (CON 150.9±12.92 *vs.* UCMS 222.8±15.84; p=0.0022). The UCMS also significantly altered metabolites: glycine (CON 213.5±48.16, n=11 *vs.* UCMS 259.3±46.63, n=9; p=0.0460), myo-inositol (CON 67.90±16.07, n=11 *vs.* UCMS 52.69±10.71, n=9; p=0.0125), and uridine (CON 3.145±3.884, n=11 *vs.* UCMS 6.356±4.986, n=9; p=0.0321). VTA proteomics identified 4,172 proteins, with differential expression linked to phosphatidylinositol signaling and antigen processing. Redox state analysis showed increased SOD activity in the prefrontal cortex (CON 11.78±2.935, n=8 *vs.* UCMS 15.25±2.967, n=8; p=0.0341) and decreased SOD

in the hypothalamus (CON 11.95±1.650, n=8 *vs.* UCMS 9.547±2.399, n=8; p=0.0349). In the hippocampus, UCMS reduced catalase activity (CON 9.045±4.547, n=7 *vs.* UCMS 3.462±3.397, n=8; p=0.0176) and increased protein carbonyl content (CON 14.18±4.496, n=6 *vs.* UCMS 23.96±3.3927, n=6; p=0.0025). UCMS induces anxiety-like behavior in rats, which may be associated with alterations in the redox state across different brain regions. Proteomic analysis of the VTA indicates that UCMS triggers changes in the brain's inflammatory response, while serum metabolomics analysis suggests systemic metabolic reprogramming.

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TUBULOIDS – A NEW STRATEGY FOR GENERATING INNERVATED SKELETAL MUSCLES

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The formation of functional skeletal muscle is a complex, multistep process governed by the coordinated actions of diverse cell populations with varying differentiation potentials. Capturing this complexity *in vitro* requires advanced models that account for cellular heterogeneity and the spatial organization of muscle tissue – an unmet need in current research. In this project, we developed a novel three-dimensional (3D) culture system that combines a custom-engineered hydrogel platform with primitive neuromesodermal progenitors (NMPs), resulting in a new cellular model we term muscle tubuloids. These tubuloids mimic the formation of innervated, structured muscle fibers and provide a foundation for constructing a fully functional, potentially transplantable neuromuscular unit. We used NMPs (as the starting cell population) – bipotent stem cells that give rise to both the spinal cord and musculoskeletal system during embryonic development. NMPs were derived from human induced pluripotent stem cells (iPSCs). Using a 3D wet-spinning biofabrication system, we produced aligned hydrogel fibers loaded with NMPs and directed them toward neuromyogenic differentiation. Differentiation was monitored over a 30-day culture period. Within the hydrogel fibers, the NMPs formed elongated, tubular structures that contracted spontaneously around day 10. We also observed motoneuron-like structures on the surface of tubuloids. Gene expression analysis revealed robust induction of myogenic markers (Msx1, Pax7, MyoD1, MyoG) alongside neurogenic markers (Neurod1, Map2, Pax6). Our results demonstrate that culturing NMPs in 3D hydrogel fibers enables synchronized and spatially organized differentiation of muscle and nerve tissue, closely resembling native muscle architecture. We believe that proposed platform of muscle tubuloids will offer key insights into muscle biology, and will open new research avenues for curing difficult-to-treat neuromuscular pathologies.

CAFFEINE ATTENUATES CUE-INDUCED ALCOHOL SEEKING *VIA* AMYGDALAR GluA1 DOWNREGULATION IN FEMALE MICE

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Alcohol use disorder (AUD) is marked by compulsive alcohol seeking, impaired control over drinking, and increased relapse risk. Caffeine influences ethanol intake in a dose-, sex-, and context-dependent manner. As a non-selective adenosine receptor antagonist, caffeine likely interacts with glutamatergic and dopaminergic systems, influencing alcohol-related behaviors. However, the main mechanisms by which caffeine affects AUD-related behaviors remain poorly understood. This study investigated whether caffeine affects alcohol-seeking in adult female C57BL/6J mice using the IntelliCage system, which enables automated tracking of voluntary alcohol intake. In the first experiment, mice had free access to both 10% ethanol and caffeine (~20 mg/kg b.m.). Caffeine co-administration reduced alcohol-seeking, as shown by fewer visits to alcohol corners and a significant drop in nosepokes for ethanol. In the second experiment, Introducing caffeine after three months of alcohol access also reduced alcohol-seeking behavior during withdrawal and decreased persistent seeking and overall alcohol consumption. To assess underlying mechanisms, a third experiment involved intraperitoneal injection of caffeine (20 mg/kg) prior to re-exposure to alcohol-predicting cues. A single caffeine dose significantly reduced cue-relapse behavior, indicated by reduced visits and nosepokes at

alcohol-associated corners. Immunofluorescence analysis revealed decreased GluA1 expression in the lateral and medial regions of central amygdala, a brain region involved in cue relapse. Western blot confirmed 50% reduction in GluA1 protein levels in amygdala of caffeine-treated mice. These findings suggest that caffeine suppresses alcohol-seeking and cue-induced relapse *via* modulation of glutamatergic signaling in the amygdala, highlighting its potential therapeutic role in AUD.

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RETINAL SIGNAL TRANSDUCTION CASCADE MODULATION FOR VISION RECOVERY

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Vision is a crucial sense that enables individuals to perceive and navigate their surroundings. However, along with changes in the environment, lifestyle, and other factors, eye-related diseases expand tremendously among the modern population. As a result, extensive research has been dedicated to exploring potential solutions for restoring vision in those affected. Despite these efforts, there remains no method to fully reverse the already caused damage. The proposed strategy involves the use of viral vectors to deliver the therapeutic cargo to precisely targeted cells, potentially through the introduction of a light-sensitive channel. However, this alone may not be sufficient to achieve a functional effect. Therefore, the ultimate goal is to identify an appropriate response amplifier and convert the remaining retinal cells into synthetic photoreceptors, enhancing the vision restoration. Initial selection can be achieved through *in vitro* patch-clamp techniques, followed by virus production and *in vivo* studies. Voltage-clamp experiments in the first step identified a promising candidate. However, further evaluation is necessary to assess its application and functionality for vision restoration in blind animal models. To achieve efficient retinal infection, genetically modified animals will be injected subretinally. Following an appropriate period, electrophysiological measurements will be conducted to analyze the amplifying properties of the selected candidate and its potential for therapeutic application.

THE SWITCHBOARD TEST: A FLEXIBLE, LOW-COST SETUP FOR ASSESSING SPATIAL MEMORY IN RATS DURING SEQUENTIAL INSTRUMENTAL TASKS

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Animals often share environments where their actions have similar effects, and each agent can freely alter the state of the environment. In such contexts, social strategies directly affect success in acquiring resources, as lack of cooperation impedes each other's actions. Typical strategies involve cooperation, competition driven by conflicting interests, direct conflict, and spatial or temporal segregation. For the current study, we prepared an automated, interactive experimental environment in which rats perform a sequential spatial memory task. This open-field environment (64 × 64 cm) features a single reward dispensing area and nine equally spaced floor buttons (3 × 3 arrangement) that rats can press using a fraction of their body weight. The system operates using an Arduino controller and a PC to manage floor switches, a reward dispenser, an amplifier with a speaker for playback of feedback sounds, and an LED array that signals reward delivery. Rats (n=16) were individually trained to memorize a chosen sequence of button presses and became experts in the task. Expert rats were then introduced into the environment in pairs: 8 pairs of cagemate experts for 9 days, and all 112 possible unique intercage expert pairs for 3 days each. Additionally, we tested interactions in 28 pairs of experts with naive cagemates, and 28 pairs of experts with naive rats from different cages. Behavioral system data was combined with position tracking from video recordings processed using DeepLabCut, allowing us to

determine each rat's behavior. Our data suggest that, despite task proficiency, cooperation in a social context is a new skill that rats must learn and develop to succeed in an environment featuring a single reward-dispensing area. We observed different modes of operation, including cooperation, temporal segregation, and conflicts, which were resolved in various ways. We evaluated the effects of conflict on dynamic changes in social strategies and examined whether, over time, rats generalized this social skill to effectively perform the task with different partners, and whether this extended social experience affected their individual performance in the same task. Such testing may be useful for identifying individuals with reduced ability to operate in a social context during sequential instrumental tasks, and for studying social hierarchies in an interactive, dynamic environment.

mTOR DYSREGULATION DISRUPTS DENTATE GYRUS GATING AND HIPPOCAMPAL MEMORY ENGRAM IN A CONDITIONAL PTEN KNOCKOUT MODEL OF TEMPORAL LOBE EPILEPSY

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The mTOR pathway is a key regulator of neuronal function, and its hyperactivation is implicated in epilepsy, cognitive dysfunction, and network instability. PTEN loss leads to mTOR hyperactivity, a hallmark of several seizure-associated conditions, including temporal lobe epilepsy (TLE) and autism spectrum disorder (ASD). This study investigates how PTEN deletion in adult dentate gyrus (DG) granule cells (GCs) alters neuronal excitability, network remodeling, and behavior. Using a conditional (PTEN-cKO) mouse model, patch-clamp recordings revealed a progressive decline in GC excitability, potentially reflecting an mTOR-driven intrinsic dysfunction. Notably, some GCs failed to fire action potentials (APs) at the set –70mV resting potential (RP) for recording but regained excitability at depolarized RPs of –60mV and –50mV, suggesting a positive shift in AP threshold and potential ion channel dysfunction. Video EEG detected spontaneous seizures with behavioral automatisms, while histopathological analysis confirmed hippocampal sclerosis (HS), astrogliosis, and mossy fiber sprouting, consistent with TLE pathology. Crucially, PTEN-KO GCs not only exhibited intrinsic hyperactivity but also propagated mTOR dysregulation across the DG, suggesting non-cell-autonomous effects involved in epileptogenesis. Behaviorally, radial arm maze tasks revealed pattern separation deficits linked to widespread c-Fos memory engram failure, warranting direct assessment of DG/CA3 synaptic plasticity. These findings suggest that mTOR hyperactivity disrupts DG's gating function and impairs its computational role in hippocampal circuits, facilitating both seizures and cognitive dysfunction. Ongoing studies will directly assess DG/CA3 transmissions and plasticity to determine how mTOR-driven synaptic dysfunction destabilizes hippocampal networks. Understanding how PTEN loss reshapes DG circuits may provide insights into therapeutic targets for epilepsy and ASD-related cognitive impairments.

IDENTIFICATION OF CHANGES IN ASTROCYTES AND THE BASEMENT MEMBRANE OF BLOOD VESSELS IN ANIMAL MODELS OF RARE X-LINKED DISORDER

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Creatine transporter deficiency (CTD) is one of the leading causes of X-linked intellectual disability, associated with cognitive and language deficits, autistic-like behaviors, motor dysfunction, and seizures. Despite advances in genetic research, therapeutic progress remains limited by the lack of non-invasive and quantitative biomarkers of brain dysfunction. In this project, we investigated neurovascular alterations in a CTD KO mouse model. Previous findings suggest altered cerebral blood flow in CTD mice, implying underlying molecular changes that could affect the morphology and integrity of the blood-brain barrier. Given their crucial role in cell adhesion, extracellular matrix signaling, and vascular stability, integrins were selected as the

main focus of the study. The objective was to characterize potential changes in the vascular basement membrane of CTD mice in comparison to WT animals, with a particular focus on integrin expression, distribution, and potential dimerization. To achieve this, we conducted immunofluorescence staining for six integrin subunits, including the widely expressed β1 integrin, and co-stained for astrocytic and endothelial markers. 3D reconstructions of the stained regions were generated using Imaris software, allowing detailed visualization of cellular interactions and integrin localization in the neurovascular unit. Colocalization analyses were performed to assess integrin pairing, and electron microscopy was used to measure vessel diameter, astrocyte coverage, and pericyte distribution. The results did not reveal statistically significant differences in integrin expression levels or their spatial colocalization between CTD KO and WT mice. Similarly, ultrastructural analyses showed consistent vessel diameters and pericyte coverage across genotypes. Although astrocyte morphology displayed variability, no genotype-specific patterns were identified.

MAPPING THE HUMAN THALAMIC RETICULAR NUCLEUS BY MEANS OF AN ULTRA-HIGH FIELD 7T MRI: TOWARD A PROBABILISTIC ATLAS

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The thalamic reticular nucleus (TRN) is a thin layer of inhibitory GABAergic neurons surrounding the thalamus. It is thought to play several important roles, including regulating the flow of sensory information from the thalamus to the cortex, acting as an attentional gatekeeper, and contributing to the regulation of the sleep-wake cycle. Despite its relevance, the TRN remains understudied in the human brain due to its small size and deep anatomical location, presenting challenges for studying the TRN with conventional non-invasive imaging techniques. In the present study, we leverage the high spatial resolution provided by ultra-high field (UHF) magnetic resonance imaging (MRI) at 7 Tesla to assess the feasibility of reliably visualizing the TRN *in vivo*. We acquired quantitative T1 and T2* partial brain datasets targeting subcortical regions, at 0.35 × 0.35 × 0.35 mm3 voxel resolution3 from 11 healthy young subjects. The data underwent thermal noise reduction using tNORDIC4, followed by preprocessing3 and smoothing with the Segmentator Python package. Our preliminary findings show that the TRN is visible in 7 out of 11 subjects on the quantitative T2* images, demonstrating the feasibility of *in vivo* visualization at 7 Tesla. Datasets of the remaining 4 subjects were of lower quality, likely due to the incomplete nature of these datasets or motion artifacts. As next steps, we plan to manually segment the TRN, followed by quantitative assessment of inter- and intrasubject variability in its volume, surface area, and thickness. Our ultimate goal is to provide a probabilistic atlas of the human TRN, providing a foundation for future studies including clinical applications.

ARC PROTEIN UBIQUITINATION REGULATES ADDICTION-RELATED BEHAVIORS AND MEMORY

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The activity-regulated and cytoskeleton-associated protein (Arc) plays a crucial role in synaptic function, memory and addiction. The expression of Arc protein is a highly dynamic process, regulated by multiple factors including ubiquitination. Despite intensive research on the role of Arc in addiction, its specific involvement in addiction-related behaviors and mechanisms remains incompletely understood. The aim of the present study was to investigate the effects of Arc ubiquitination using Arc knock-in mice (ArcKR, where predominant Arc ubiquitination sites are mutated increasing protein half-life) in addiction-related behaviors and memory processes. We hypothesized that prolonged Arc protein expression in ArcKR mice will reduce alcohol-seeking behaviors and improve updating of other forms of memory. Addiction-related behaviors were investigated using the IntelliCage, while memory retrieval was assessed with a fear conditioning (FC) test. Our results confirmed that ArcKR mutation decreased alcohol seeking during alcohol withdrawal period and in response to alcohol predicting cues, whereas sucrose-seeking behavior was not significantly affected. Moreover, ArcKR mice showed significantly improved extinction of contextual and cued fear memory. Based on our re-

sults, we can conclude that Arc ubiquitination specifically impacts alcohol-related behaviors, with minimal effect on general reward processing. Furthermore, it contributes to learning and behavioral flexibility. Our data suggest that boosting Arc signaling is a potential avenue in AUD treatment.

NUCLEAR mTOR-Brg1 INTERACTIONS MEDIATE DISTINCT SYNAPTIC AND NETWORK EFFECTS IN THE CONTEXT OF TSC2 DEFICIENCY

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Brg1 is an ATP-dependent catalytic subunit of the BAF chromatin remodeling complex. It impacts gene expression, stimulates DNA repair, facilitates RNA processing, and contributes to neuron development and synaptic activity. Through mass spectrometry analysis, we identified Brg1 as one of the nuclear interactors of the mTOR. mTOR plays a pivotal role in cellular metabolism and growth across diverse cell types. In neurons, mTOR assumes critical importance for both neuronal development and plasticity. Dysregulation of mTOR has been implicated in mTORopathies, including TSC and epilepsy. Our study aimed to delve into the mTOR-Brg1 interaction within the nucleus and its implications for neuronal development and disease. Using *in vitro* cultured rat neurons, our data confirmed an increased nuclear mTOR-Brg1 interaction following kainic acid (KA) treatment, highlighting mTOR-induced phosphorylation of Brg1. We observed that modulation of mTOR and the proteasome influenced the Brg1 nuclear presence, suggesting proteasome-mediated degradation of Brg1 in the nucleus upon KA treatment. Consistent with these findings, the downregulation of Brg1 expression was noted upon TSC2 loss, resulting in mTOR hyperactivation in neurons. Ca^{2+} imaging and morphometric analyses revealed strong similarities between neurons lacking TSC2 and those deficient in Brg1. However, further investigation demonstrated that despite both conditions enhancing network activity, their synaptic parameters differed, suggesting that Brg1 loss does not directly contribute to TSC-associated synaptic phenotypes. Collectively, these findings provide new insights into the nuclear functions of mTOR in neurons, particularly in regulating Brg1 stability and activity.

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DYNAMIC CONTROL OF WORKING MEMORY: LINKING EEG AND SINGLE-NEURON ACTIVITY IN HUMANS

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Efficiency of the working memory system relies on the flexible allocation of attention between new inputs and internally maintained representations. To investigate the neural dynamics supporting this flexibility, we examined the transition from encoding to maintenance using human EEG and intracranial recordings of single-neuron activity. At the onset of the retention period, we observed a midfrontal ERP component whose amplitude decreased with increasing memory load and reflected prior trial context—pointing to a discrete state transition rather than a general attentional effect. This ERP was followed by an alpha-band power increase that emerged earlier at higher loads, suggesting anticipatory preparation for maintenance. Intracranial recordings from medial prefrontal cortex (mPFC), in regions identified *via* EEG source localization, revealed a complementary pattern: when retention onset was unpredictable, mPFC neurons showed increased firing; but when predictable, activity rose proactively. Together, these EEG and single-unit findings reveal coordinated neural signatures of encoding-to-maintenance transitions and shed light on the dynamic regulation of working memory states.

DYSREGULATED MITOCHONDRIAL METABOLISM IN THE BRAIN OF ASD-MOUSE MODEL, TRAP1 MUTANT MICE

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The brain represents the largest source of energy consumption in our body, most of the energy being primarily utilized at the synapses. Therefore, regulation of metabolite supply and energy metabolism is especially critical to the central nervous system and even subtle changes in energy production may lead to neurological diseases. Indeed, mitochondrial dysfunction was observed in a number of neurodevelopmental disorders. In an ASD patient whose identical twin was unaffected, we identified a postzygotic mosaic mutation p.Q639* in the *TRAP1* gene, which encodes a mitochondrial chaperone of the HSP90 family. Additional screening of 176 unrelated ASD probands revealed an identical TRAP1 variant in a male patient who had inherited it from a healthy mother. We generated knock-in Trap1 p.Q641* mice that revealed male-specific social behavior abnormalities accompanied by altered synaptic transmission and dendritic spine morphology. The functional mitochondrial phenotyping of synaptoneurosomes isolated from mouse brains (cortex and hippocampus) of male and female TRAP1 mice revealed differences in the use of the tricarboxylic acid cycle substrates in males but not in females. Next, targeted metabolomics was performed to assess levels of amino acids in the brains of mutant and WT mice. Our preliminary data suggests that the levels glutamate and GABA are decreased in the hippocampus of male mutant mice, but not in females. Finally, the levels of NAD/NADH and NADP/NADPH were assessed as a readout for the energetic state and cellular redox state in the hippocampi of WT and mutant mice.

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ULTRASTRUCTURAL MORPHOLOGY OF SYNAPTIC MITOCHONDRIA IN A MOUSE MODEL OF AUTISM-ASSOCIATED NEURODEVELOPMENTAL DISORDER, TRAP-1 MUTANT MICE

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Autism spectrum disorder (ASD) is a heterogeneous group of early-onset neurodevelopmental conditions characterized by persistent deficits in social communication and interaction, along with restricted and repetitive behaviors. With an estimated prevalence of approximately 1% globally, ASD presents a wide range of symptom severity and lifelong challenges. While the etiology is multifactorial, a strong genetic component and growing evidence of synaptic and mitochondrial dysfunction are central to its pathophysiology. Neurons rely heavily on mitochondrial function to meet the high energy demands of synaptic transmission and calcium homeostasis. TRAP1, a mitochondrial chaperone of the HSP90 family, plays a crucial role in regulating mitochondrial stress responses. Notably, TRAP1 mutant mice exhibit behavioral phenotypes reminiscent of ASD, including deficits in social interaction. To investigate the structural underpinnings of mitochondrial and synaptic alterations in ASD, we employed serial block-face scanning electron microscopy (SBF-SEM) to analyze brain tissue from TRAP1 mutant and wild-type mice. Three-dimensional reconstruction of mitochondrial morphology and volume was performed using RECONSTRUCT software. To further enhance spatial mapping, we developed a deep learning pipeline for the accurate identification of cellular compartments from electron microscopy images. This high-throughput approach provides precise structural insights into synaptic mitochondria, enabling a deeper understanding of how mitochondrial morphology and function may contribute to ASD pathogenesis.

TRAP1 MUTANT MICE, A NOVEL MODEL OF ASD, SHOW ALTERED MITOCHONDRIAL DYNAMICS IN THE HIPPOCAMPUS

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Neuronal cells depend on mitochondrial activity to maintain membrane excitability, neurotransmission and synaptic plasticity. The AMP-activated protein kinase (AMPK) signaling pathway is a key regulator of cellular energy homeostasis and has been implicated in mitochondrial dynamics. Dynamics of these organelles determine their morphology, allowing them to adapt to metabolic needs. Additionally, phosphorylation of the main protein involved in mitochondrial fission/fusion- DRP1 is essential for regulating mitochondrial dynamics, synapse maturation, synaptic transmission and plasticity. A mutation (p.Q639*) in the *TRAP1* gene encoding the mitochondrial chaperone was identified in an ASD patient whose monozygotic twin brother was unaffected. TRAP1 belongs to the HSP-90 family of proteins involved in protection against oxidative stress and regulation of the cell's metabolism. A novel model of ASD- Trap1 p.Q641* knock-in mouse model carrying the identical mutation was generated using CRISPR-Cas9. We observed sex-specific deficits in the sociability of Trap1 p.Q641* knock-in mice. Here, we aimed to investigate the mitochondrial dynamics of Trap1 male and female mice in the hippocampus of Trap1 (mutant and wild-type). To achieve this, we isolated mitochondria from the hippocampi of Trap1 mutant and WT mice and assessed the levels and phosphorylation status of proteins involved in fission and fusion in both sexes. In males, we observed downregulated levels of some proteins involved in fission, whereas the level of Mitofusin2 was increased. AMPK signaling connected with the regulation of mitochondrial dynamics was analysed.

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WHAT DRIVES VARIABLE RESPONSE TO STRESS? A STUDY USING DEEP PHENOTYPING, WHOLE BRAIN ACTIVITY AND PLASTICITY MAPPING

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The social defeat paradigm serves as an ethologically valid stress model, effectively inducing depression-like phenotype in rodents. This model allows to investigate individual variability in stress responses that results in distinguishable responding and non-responding populations. Understanding the pathways behind this would aid the scientific community to eventually diagnose the impact of, and provide treatment and care for stress associated disorders. We implemented a 10-day chronic social defeat paradigm to characterise responders *vs.* non-responders. Leveraging deep learning based social behavioral analysis tool DeepOF in conjunction with DeepLabCut, we comprehensively assessed social interaction behavior and found that responders exhibited pre-disposition in vigilance like behavior – one of the outputs of the supervised analysis approach. Next we delved deeper to understand changes in different brain regions in response to stress. To capture activity changes during the stress paradigm, we employed Manganese-Enhanced MRI (MEMRI), which is based on principle of Manganese uptake through calcium channels. MEMRI revealed highly significant changes in multiple brain regions amongst the groups including CA1 of the hippocampus, PAG and dorsal raphe nucleus (DR). Additionally, we conducted whole brain plasticity mapping by staining against c-FOS expression. Here also we found multiple regions showing changes across the groups including few that also exhibited changes in activity, like the DR, hippocampus among others. Interestingly, correlation matrices and module analysis revealed that non-responders exhibited a map that was completely different from controls and responders indicating multiple pathways acting in tandem to eventually manifest a differential and muted response to stress. Based on our analysis, we selected to target a DR-basolateral amygdala circuit using chemogenet-

ics. Acute inhibition of this circuit alleviated threat perception behavior as assessed by standard social avoidance test after social defeat stress – highlighting the importance of this relatively under studied pathway and the power of our network association analysis. We strongly believe that the dataset generated in this project will also be helpful for future endeavours to investigate neural circuitry and regions – even neuronal subtypes involved in stress response, aiding in more comprehensive knowledge on impact and treatment of stress disorders.

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GRAPHS AS TOOLS FOR DISENTANGLING THE MOUSE BRAIN FUNCTIONAL CONNECTOME

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Learning alters the molecular landscape of brain cells. Whole-brain imaging of c-Fos—the protein rapidly upregulated in neurons upon external excitation—enables a juxtaposition of these activation landscapes in response to different behavioral paradigms. Graphs—mathematical structures that remain underutilized in molecular neurobiology—are promising tools for describing functional networks, as evidenced by their widespread application in fMRI data analysis, and could therefore serve as a natural representation of global molecular expression patterns. In this study, mice were trained in the IntelliCage and exposed to either an appetitive (sucrose) or an aversive (quinine) stimulus, while control mice had access only to water. Subsequently, brains were collected and subjected to optical tissue clearing combined with immunostaining to detect c-Fos. Finally, brains were imaged using a light-sheet microscope, and c-Fos positive cells were aligned to the Allen Mouse Brain Atlas annotating them to the brain structures. Graphs were created from correlation matrices of c-Fos activation within experimental groups and across regions. Graph-theoretical approach was directly compared with conventional analytical methods to assess whether a network-based perspective can reveal deeper insights into the spatial and functional organization of brain activation. Graph features that differentiate experimental groups were defined and a bootstrap approach assessing the statistical significance of those differences was employed. Among the graph properties analyzed, betweenness centrality differentiated the experimental groups effectively and highlighted key nodes that act as critical connectors within the network. This work introduces easy-to-use, graph-oriented analytical pipelines and demonstrates that network-based metrics can robustly differentiate between molecular landscapes.

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DIVERGENT REGULATION OF mTOR AND RAPTOR IN MATURE HIPPOCAMPAL NEURONS DURING NUTRIENT STRESS AND SYNAPTIC ACTIVATION

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mTOR is a protein complex that helps neurons respond to nutrient levels and external signals, playing a key role in regulating cell growth, metabolism, and synaptic plasticity. While well studied in developing neurons, how mTOR behaves in mature neurons under metabolic stress—particularly within the nucleus—remains poorly understood. In this study, we used mature hippocampal neurons cultured from embryonic rat brains to examine how mTOR signaling responds to nutrient deprivation. Neurons were incubated in nutrient-poor Neurobasal medium (NB) for either 2 hours (short-term deprivation) or 6 hours (long-term deprivation). In some conditions, full medium was reintroduced for 20 minutes to assess recovery. Interestingly, mTOR responses depended on the duration of deprivation. After 2 hours in NB, the highest levels of mTOR and its active form, phospho-mTOR (P-mTOR), were observed after full medium was added—suggesting strong nutrient-sensitive activation. In contrast, after 6 hours of deprivation, mTOR and P-mTOR levels were already elevated and did not further increase following nutrient reintroduction. This may indicate that prolonged deprivation engages different cellular mechanisms or leads to a shift in mTOR regulation. We also examined Raptor,

a key component of the mTORC1 complex. In control neurons, Raptor formed distinct nuclear puncta. After kainic acid stimulation, which mimics neuronal activity, Raptor became more diffusely distributed within the nucleus. Raptor consistently localized to the nucleolus, as shown by co-labeling with nucleolin, whereas mTOR was excluded from this region regardless of condition. Our findings reveal a time-dependent mTOR response to nutrient stress and suggest that Raptor may have spatially distinct roles from mTOR in mature neurons, particularly within the nuclear compartment.

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GRADED NEURONAL RESPONSES TO EMOTIONAL STIMULI REVEALED BY SINGLE-CELL RECORDINGS IN HUMANS

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Processing emotional stimuli is a fundamental function of the human brain. Emotions influence our daily lives, shaping decisions, behaviors, and social interactions. Moreover, emotional processing plays a critical role in various mental health conditions, such as depression, anxiety disorders, and post-traumatic stress disorder. Despite its importance, the exact neuronal mechanisms underlying emotional processing are not yet fully understood. Here, using a unique opportunity to record single-neuron activity from patients suffering from intractable epilepsy, we gathered neuronal responses to emotional images and words. Stimuli were rated on six scales—valence, arousal, disgust, sadness, happiness, and fear. By choosing continuous emotional dimensions, we aimed to estimate neuronal response magnitude as a function of emotion intensity. Single-neuron activity was isolated using the OSort algorithm, and neuronal selectivity was assessed through generalized linear models and permutation testing. The analysis revealed a significant number of emotion-selective neurons in areas such as the amygdala and hippocampus. Furthermore, neurons responded according to the intensity of the presented emotions. This finding, for the first time, demonstrates a graded response to emotional stimuli at the neuronal level, indicating conjunctive coding of emotion category and intensity. These results advance our understanding of the neuronal coding of emotions and establish a foundation for future investigations into the decoding of emotional states from neuronal population activity.

PERSISTENT ACTIVITY IN MEDIAL TEMPORAL LOBE NEURONS ENCODES WORKING MEMORY CONTENT INSIDE AND OUTSIDE THE ATTENTIONAL FOCUS

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The role of persistent neural activity in working memory storage is well documented, particularly in tasks where memorized items are of equal importance. However, the encoding mechanism for unattended items, often considered as 'activity silent', remains poorly understood. Here, we recorded the activity of image-selective neurons in the medial temporal lobe (MTL) while subjects (n=12) shifted attention between concurrently stored memory items. Our results demonstrate that both attended and unattended mem-

ory items are encoded through persistent activity. Additionally, we observed a dynamic transformation in the neuronal subspace following cue presentation, reflecting a shift in how information was maintained. While information about the unattended item was decodable at the single-trial level from pre-selected image-selective cells, it was not decodable from the entire population of MTL cells. These findings support models of persistent activity and challenge the notion that unattended items are stored *via* 'activity silent' mechanisms.

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CHANGES IN BRAIN ACTIVITY AFTER CBT INTERVENTIONS FOR PROCRASTINATION: PRELIMINARY RESULTS FROM A RANDOMIZED CONTROLLED fMRI STUDY

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Procrastination, a self-regulatory failure involving voluntary but irrational delay, affects many individuals by impairing their psychological well-being. While cognitive-behavioral therapy (CBT) interventions are regarded as the most promising treatments for procrastination, their neural mechanisms remain unexplored. We aimed to investigate the therapy-induced changes in brain activity using longitudinal task-based fMRI. Data were collected from 70 (target n=120) help-seeking, high-procrastinating university students before and after a 5-week group CBT intervention (intervention group, IG) or a matched waiting period (wait-list control group, WL). The intervention was found to be effective in reducing procrastination with large effect sizes (d=-1.24). During the fMRI task, participants were instructed to read procrastination-relevant, emotionally negative, and neutral short scenarios, and to imagine the described situations. At baseline, processing of procrastination-relevant (*vs.* neutral) scenarios elicited increased activation in regions involved in self-referential processing within the default mode network (precuneus, posterior cingulate cortex, medial prefrontal cortex, angular gyri), salience and emotional processing (ventral anterior cingulate cortex), and language comprehension (middle temporal gyri). Negative (*vs.* neutral) scenarios engaged similar regions related to self-referential processing. From pre- to post-intervention, a group × time interaction was observed in the right dorsolateral prefrontal cortex (dlPFC), with increased activation during processing procrastination-relevant scenarios after the intervention in the IG, as compared to WL. No significant interaction was found for negative scenarios. These preliminary results suggest that CBT for procrastination may work through enhancing recruitment of the dlPFC, an area associated with cognitive control and executive functioning, when facing situations potentially leading to procrastination.

DEVELOPMENT OF A NEW RABIES VIRUS WITH CHANGED TROPISM

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Vision plays a critical role at every stage of our lives, and it's our "window to the world". The World Health Organization reports that over 2.2 billion individuals struggle with visual impairment or blindness. It is necessary to search for new, innovative, and effective treatment methods for retinal degenerative diseases. Gene and/or viral therapy stands out as a promising tool among these approaches. An unconventional solution may be Rabies virus (RV), a (-)RNA rhabdovirus that has the ability to infect neurons *via* retrograde transport (from postsynaptic to presynaptic cells) and can carry very large cargos. Delivery of therapeutic genes to the target cells is a key step in gene therapy, and RV infections are not cell-type unique, therefore gene delivery cannot be specifically controlled. In order to address this challenge, our project focuses on modifying the tropism of a G-deleted Rabies virus (RVΔG) by pseudotyping it with a chimeric protein designed to recognize and bind to a receptor selectively expressed on ON-bipolar cells in the retina. We hypothesize that this targeted pseudotyping strategy will enable specific infection of bipolar cells, avoiding off-target effects. As a first step we prepared lentivirus expressing the chimeric glycoprotein and used it to transduce BHK-21 and stable cell line gen-

eration. The next step involves producing pseudotyped Rabies virus, which was injected into the eyes of mice. The specificity of the virus was verified through the use of immunohistochemistry assays. The project seeks to advance our understanding of pseudotyping techniques and their potential applications in gene therapies, ultimately paving the way for innovative treatments for retinal neurodegenerative disorders.

THE NASAL EPITHELIUM'S ROLE IN ELECTROPHYSIOLOGICAL RHYTHMS IN THE RAT OLFACTORY BULB

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Changes in the sense of smell are emerging as early indicators of several major neurological conditions, including Alzheimer's disease. These disorders are also linked to abnormal brain activity patterns. Olfactory sensory neurons (OSNs) transmit signals from the nasal epithelium (NE) to the olfactory bulb (OB). In this study, we investigated how sensory input from the NE contributes to the generation of electrical activity patterns in the OB. Adult male Wistar rats were implanted with electrodes in the OB, prefrontal cortex (PFC), and ventral striatum (VS), as well as with EEG electrodes placed on the frontal and parietal regions of the skull. The NE was damaged in one group of rats by administering gadolinium to both nares, while a control group received saline. Olfactory performance was measured using the hidden cookie test, combined with recordings of local field potentials at the end of each session. Additionally, sleep-related brain activity was recorded every four days. Integrity of the NE was assessed using olfactory marker protein (OMP) at 5, 15, and 22 days after treatment, and hematoxylin and eosin to evaluate NE thickness. Rats receiving gadolinium took longer to find the hidden cookie than controls, an effect that persisted for roughly 10 days. Analysis of OB local field potentials during wakefulness showed that these rats had lower amplitudes in both the respiration rhythm (1–10 Hz) and gamma frequency range (30–90 Hz), compared to controls. Across all animals, better performance on the hidden cookie test was positively associated with stronger nasal respiration rhythms. Although wake-related OB oscillatory activity was disrupted by gadolinium, classical slow-waves activity during deep sleep remained intact. Immunohistochemical results showed that NE of rats receiving gadolinium had reduced OMP expression, indicating damage to OSN. The findings show gadolinium to the nares can be used in rats to model anosmia, and that functional OSNs are critical for maintaining normal OB rhythms.

REGULATION OF NR2B TRAFFICKING AND GOLGI LOCALIZATION THROUGH 5-HT7 RECEPTOR SIGNALING AND AMPK ACTIVATION: IMPLICATIONS FOR SYNAPTIC PLASTICITY AND STRESS-INDUCED SYNAPTOPATHY

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The serotonin 5-HT7 receptor (5-HT7R) plays a pivotal role in synaptic remodeling and resilience to stress-related neuropsychiatric disorders by modulating intracellular signaling pathways. Among these pathways, NMDA receptor (NMDAR) trafficking, particularly the NR2B subunit, is critical for synaptic plasticity and stress-adaptive synaptic function. This study investigates how 5-HT7R signaling and AMPK activation regulate NR2B trafficking and its intracellular localization. Treatment of hippocampal primary neurons with the 5-HT7R agonist 5-CT and AMPK activator A-769662 induced significant NR2B internalization, with prominent accumulation in the Golgi and some retention in the endoplasmic reticulum (ER), as confirmed by immunocytochemistry and confocal fluorescence microscopy imaging analyses. Ongoing studies focus on dissecting how AMPK-dependent regulation of palmitoyl-acyl-transferases (e.g., zDHHC13) modulates NR2B palmitoylation, trafficking, and localization, impacting NMDAR's synaptic functions. Accordingly, electrophysiological assessments will be conducted to correlate NR2B localization with NMDAR-dependent synaptic functions and plasticity. Future directions include validating these findings in hippocampal tissues from CUS-induced anhedonic and 5-HT7R agonist-treated mice to establish their physiological relevance. This work provides novel insights into 5-HT7R-mediated synaptic plasticity and its role in stress-induced synaptopathies, identifying potential therapeutic targets for neuropsychiatric disorders.

KETAMINE-INDUCED OSCILLATORY DYNAMICS IN FREELY MOVING RATS REVEAL DISTINCT NEURAL CIRCUIT MECHANISMS

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Ketamine has long been used as a pharmacological model of schizophrenia and has more recently shown promise in treating depression. However, the neural networks affected by ketamine remain only partially understood. In this study, we investigated the effects of a subanesthetic dose of ketamine on gamma (30–80 Hz) and high-frequency oscillations (HFO, 130–180 Hz) across multiple brain regions in freely moving rats. Ketamine produced region-specific changes in oscillatory power: gamma power significantly increased in frontal and parietal cortical areas, while HFO power was most prominently elevated in the olfactory bulb and ventral striatum, with smaller changes in cortical regions. Infusion of AMPA/kainate receptor antagonists (NBQX and CNQX) into the olfactory bulb suppressed both spontaneous and ketamine-enhanced HFO in the bulb and ventral striatum, but had no effect on cortical gamma power. These findings highlight a crucial role for AMPA/kainate receptor signaling in the olfactory bulb in generating ketamine-induced HFO locally and in modulating HFO activity in associated brain regions.

AGING BRAIN: FROM THE BEHAVIOR TO BRAIN CONNECTIVITY

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Understanding age-dependent differences in behavior and neuronal function is crucial for elucidating the mechanisms underlying cognitive decline and age-related neurologic disorders. This study investigates the behavioral patterns and functional neuronal networks in mice across different age groups using IntelliCage system and whole brain iDISCO clearing coupled with c-Fos immunostaining. IntelliCage, an automated system, allows for continuous and comprehensive monitoring of mouse behavior in close-to-ecologic conditions, providing insights into cognitive and social interactions, activity levels, and response to environmental changes. We observed significant variations in behavior between young (3-5 months old) and old (>18 months old) mice, particularly in exploratory activity, social behaviors, learning and memory tasks. To correlate these behavioral findings with neuronal activity, we employed the iDISCO followed by whole brain c-Fos immunostaining. Our analysis revealed age-related differences in brain volume and the activation patterns of specific brain regions associated with cognitive functions and behavioral responses. In particular we observed that brains are smaller and brain networks less complex in old mice. Our study contributes to a deeper understanding of the neurobiological basis of age-dependent behavioral changes and may help with finding new therapeutic strategies for age-related cognitive decline and neurological diseases.

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SPATIAL LEARNING IS FOLLOWED BY RAPID, TIME-DEPENDENT, AND PROTEIN-SPECIFIC MODULATION OF PROTEIN S-PALMITOYLATION

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Synaptic plasticity, manifested through the reorganization of synapses and changes in synaptic efficacy, plays a central role in learning and memory formation. S-palmitoylation (S-PALM) is a reversible lipid-based posttranslational modification of proteins. Similar to phosphorylation, S-PALM is thought to dynamically regulate the localization, stability, and function of synaptic proteins, thereby facilitating the synapse's ability to rapidly adapt to fluctuations in neuronal network activity. We have previously demonstrated a role for

S-PALM in supporting long-term synaptic potentiation; however, its direct involvement in learning processes remain unclear. In this study, we tested the hypothesis that spatial learning and memory formation affect the S-PALM status of synaptic proteins in the hippocampus. To this end, we combined behavioral training with advanced proteomic analyses to examine how learning-related processes modulate the synaptic S-palmitoylome. Specifically, we aimed to capture temporal dynamics by comparing early-phase memory formation (minutes to hours) with long-term memory consolidation (days). Rats were subjected to spatial learning in the Morris water maze, and hippocampal proteins were analyzed by mass spectrometry at two time points: 1 hour and 5 days after the onset of training. Our results revealed that 8–24% of S-palmitoylated proteins exhibited significant, protein-specific alterations in S-PALM following learning. Notably, the 1-hour time point was characterized predominantly by hyper-palmitoylation, whereas the 5-day time point showed a more balanced pattern of both palmitoylation and depalmitoylation. These findings suggest that synaptic plasticity and learning are supported by rapid, time-dependent, and protein-specific modulation of S-PALM.

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STUDYING THE EVOLUTIONARY ASPECTS OF SYNAPSE MATURATION AND DYNAMICS USING iPSCs-DERIVED iNeurons

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Dissecting the molecular and cellular basis that underlie human unique cognitive abilities and set us apart from the great apes, is one of the main questions of humankind. The differences between human and non-human primates are thought to depend on the increased size and connectivity of the human cerebral cortex. In a previous study we found that human neurons show a slower maturation process when compared to non-human primates, this is evident both at the transcriptional level and at the functional level. Genes involved in dendrite and synapse development are expressed earlier in chimpanzee iNeurons compared to human iNs. Discrepancies at the transcript level, also result in striking differences in the timing and dynamics of functional maturation. The prolonged process of human neurons maturation, neoteny, is one of the most relevant characteristics of the human brain. We now aim to get further insight on the intrinsic cellular mechanisms of human neoteny focusing on synapse maturation and dynamics. To test the cellular basis of evolutionary differences, we generated induced excitatory neurons (iNeurons, iNs) from chimpanzee, and human induced pluripotent stem cells (iPSCs). We followed the structural maturation of the synapses, with immunofluorescence and electron microscopy, and the functional maturation of neuronal networks with calcium imaging. Calcium imaging detected spontaneous activity at the network level in both species. However, the spontaneous activity was visibly more synchronous in chimpanzee iNs, whereas human iNs showed a sparser and less correlated activity. The functional data were supported by the co-localization analysis of synaptic markers that shows a higher number of synapses formed in chimpanzee iNs. Overall, our results indicate that human neurons present a delayed maturation and a late synaptic formation. We are now using a biochemical approach to identify differences between human and non-human primates in the synapse's molecular makeup.

THE ROLE OF MYOCARDIN-RELATED TRANSCRIPTION FACTORS A AND B IN SOCIAL BEHAVIOR AND NEURONAL DEVELOPMENT

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Myocardin-related transcription factors A and B (MRTF A and MRTF B) are coactivators of serum response factor, a major transcription factor in the brain. Both MRTFs are found in dendritic spines, where they may interact with G-actin. Activity-dependent actin polymerization facilitates their shuttling to the nucleus, where they regulate transcription, making them novel synaptic-nuclear messengers that directly link synaptic changes with gene expression. The identification of single nucleotide polymorphisms in human *MRTF A* and *MRTF B* genes has associated them with a spectrum of neurodevelopmental disorders, including autism spectrum disorder and schizophrenia.

THE SWITCHBOARD TEST: A FLEXIBLE, LOW-COST SETUP FOR ASSESSING SPATIAL MEMORY IN RATS DURING SEQUENTIAL INSTRUMENTAL TASKS

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Appetitive instrumental conditioning is widely used to shape animals' associations between actions and outcomes under controlled conditions. When implemented in automated environments with more complex tasks, it enables the collection of high-resolution data across hundreds of trials, supporting fine-grained behavioral analysis. Our work introduces an automated, interactive environment for studying spatial memory in rats. The system enables training and testing of location- and sequence-specific responses, with adjustable parameters including spatial layout, sequence length, cues, timing, and reward size. It is an open-field environment (64 × 64 cm) featuring a single reward dispensing area and nine equally spaced floor buttons (3 × 3 arrangement) that rats can press using a fraction of their body weight. The system operates using an affordable Arduino controller and a PC to manage floor switches, a reward dispenser, an amplifier with a speaker for playback of feedback sounds, and a 64-LED array that signals reward delivery. It is designed for compatibility with electrophysiology and deep brain stimulation (DBS), providing a flexible tool for behavioral studies. Rats (n=16) were gradually trained to memorize a target sequence (7→4→2), selected as a moderate-difficulty option based on a free-choice control protocol. Each trial was self-initiated and, if successfully executed, concluded with a distinct sound and a light cue signaling reward delivery and marking the end of the trial. Errors resulted in a negative feedback sound and termination of the trial. Upon activation, each switch triggered a unique sound from a set of complex natural sounds, such as those of crickets, small frogs, and dolphins, which are intended for use in future experiments to test the transfer of spatial memory to the abstract auditory domain. In expert rats, removing all feedback sounds had a minimal effect. Based on experimental data, we proposed a memory score calculation method for generating learning curves, which can serve as a sensitive measure of spatial memory performance. We will apply this method to assess the effects of DBS on spatial memory, as well as the impact of different modes of social cooperation on individual performance. This study proposes a new, sensitive approach for evaluating spatial memory performance within a flexible operant conditioning framework.

SOCIABILITY AND ALCOHOL ADDICTION-LIKE TRAITS IN MALE AND FEMALE MICE

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Alcohol consumption poses a significant social challenge, leading to negative consequences for both individuals and society. Even occasional drinking can escalate into serious alcohol-related issues. While some individuals consume alcohol without developing dependence, others are more vulnerable to addiction. Social influences play a crucial role in shaping drinking behaviors, affecting the likelihood of progression to alcohol use disorders. Understanding these factors is essential and animal models provide valuable insights into the mechanisms underlying alcohol addiction. The aim of this study was to examine if social factors – such as the interactions between pairs of animals within the group and their social status – influence excessive alcohol consumption and development of addiction-like traits in mice. To investigate this we used the IntelliCage training system. Female (n=14) and male (n=13) C57BL6 mice were used. First, animals were allowed to freely explore the cage, next for three weeks they were given access to 20% ethanol for two hours each day. Motivation was assessed by providing access to 20% ethanol under progressive ratio schedule. Withdrawal lasted for one week where no access to ethanol was granted. Social interactions and addiction-like traits were analyzed. Females and males exhibit slightly different activity with females being more active in terms

of overall cage exploration. We observed individual differences in alcohol consumption within one sex. Similarly individual mice differed in the number of interactions between pairs of mice. While some mice actively followed others, some did not. We found no correlation between overall sociability (per whole experiment) and alcohol consumption.

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NEUROPIXELS OPTO: COMBINING HIGH-RESOLUTION ELECTROPHYSIOLOGY AND OPTOGENETICS

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High-resolution extracellular electrophysiology is the gold standard for recording spikes from distributed neural populations, and is especially powerful when combined with optogenetics for manipulation of specific cell types with high temporal resolution. We integrated these approaches into prototype Neuropixels Opto probes, which combine electronic and photonic circuits. These devices pack 960 electrical recording sites and two sets of 14 light emitters onto a 1 cm shank, allowing spatially addressable optogenetic stimulation with blue and red light. In mouse cortex, Neuropixels Opto probes delivered high-quality recordings together with spatially addressable optogenetics, differentially activating or silencing neurons at distinct cortical depths. In mouse striatum and other deep structures, Neuropixels Opto probes delivered efficient optotagging, facilitating the identification of two cell types in parallel. Neuropixels Opto probes represent an unprecedented tool for recording, identifying, and manipulating neuronal populations.

MITOCHONDRIA DYSFUNCTION IN Trap1 MUTANT MICE

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TRAP1 is a mitochondrial chaperone of the HSP90 family which was shown to be involved in protection from oxidative stress and cell death, implicated in metabolic regulation and mitochondrial dynamics. The postzygotic mutation p.Q639* in the *TRAP1* gene was found in ASD patient whose identical twin was unaffected. Additional screening of 176 ASD probands revealed an identical TRAP1 variant in the male patient who had inherited it from a healthy mother. We generated a new transgenic knock-in mouse line with identical mutation as the one identified in ASD patients. The truncation of Trap1 (p.Gln641*) results in significant downregulation of Trap1 expression. We found that Trap1 mutant mice (Trap1 MUT) exhibit sex-specific changes in social behavior and neuronal synaptic plasticity compared to wild-type mice. Since synapses are the place where about 20% of whole body energy is used and this energy is produced by mitochondria , in the current study we aimed at investigating the effects of Trap1 mutation on mitochondria functioning in the brain. We studied the CA1 region of the hippocampus of Trap1 MUT and WT mice using 3D electron microscopy to analyze the morphological signs of mitochondrial stress, namely mitophagy and endoplasmic reticulum–mitochondria contact sites. Next, we analyzed level of the proteins involved in cellular stress response in hippocampi of Trap1 mice. We found a significant increase in the level of phospho-p70 S6 kinase and phospho-eukaryotic initiation factor-2α in Trap1 MUT mice compared to WT mice suggesting impaired response to

mitochondrial stress. In addition we used mitochondria isolated from hippocampus and cortex of Trap1 mice for measure respiration using Seahorse. We found significant changes in the activity of respiratory complexes in Trap1 MUT mitochondria compared to WT. We also analyzed the level of reactive oxygen species and ATP in isolated synaptoneurosomes.

WHERE DID I LEAVE MY CHEESE: DYNAMICS OF SPATIAL DECISIONS IN FEMALE MICE

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It is widely recognized that hippocampal plasticity, particularly long-term potentiation, is necessary for formation of spatial memory. However, recent studies using genetically altered mice have challenged these claims showcasing the potential role of the plasticity in shaping spatial decisions, that require spatial information, rather than in spatial memory *per se*. In order to understand the molecular basis of spatial choices within the hippocampus we have designed a new system for monitoring mice activity and navigation. Our apparatus is built of fully automated and integrated modules including camera system, cue display system, liquid reward dispensers and door control system. Animals are tested within 3 connected corridors, parted with automatic doors, where they can roam undisturbed and collect a reward at the reward sites. Here, we present the results of our experiments on C57BL/6 female mice within the system. We have tested the effect of food motivation, cue complexity, reward proximity and dorsal hippocampal (dCA1) plasticity on efficiency of spatial choice. To manipulate the dCA1 plasticity, we injected the lentiviral vectors encoding shRNA for PSD-95 or luciferase as a control into dCA1. Our results show that hunger affects motivation to perform both working-memory related task and associative-memory task, as well as the dynamics of decision making. Complex cues render better performance than the simple ones; one-modality cues are necessary for non-random choices. Closer proximity of the reward promotes faster learning and more efficient performance. Additionally, PSD-95-dependent activity of CA1 synapses regulates decision-making and attention. Finally, after analyzing the choice patterns on an individual level, we have discovered a division for better, average and poorly performing animals within groups. Together, our results demonstrate how spatial decision-making in mice is shaped, highlighting the prominent role of dCA1 hippocampal synaptic plasticity in the process.

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A NOVEL METHOD FOR PARAMETERIZATION OF EEG TRAVELLING WAVES

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Part of the oscillatory activity in the brain can be described as "traveling waves" – patterns of neural activity that extend across space, where the peaks in amplitude appear to move progressively over the cortical surface. This apparent motion is driven by a gradient in phase, which provides a direction for the wave to propagate. Traveling waves are mostly observed below 30 Hz, including beta, alpha and theta bands and are related to human perception and actions. This study proposes a novel method to detect and analyze this phenomenon in multichannel EEG recordings. Multivariate Matching Pursuit decomposition allows for a selective tracing of selected oscillations in all the recorded EEG derivations simultaneously, allowing for inter-electrode variations in phase. We fit a linear model to these phases across the spatial dimension spanned by the positions of the EEG electrodes. This method assesses the significance of the phase correlation (i.e., wave being directed along a given axis) and provides an estimate of the wave speed. Proposed method is validated on the dataset from, where the propagation of alpha waves (8-12 Hz) in the sagittal plane was shown. Our results showed alpha waves propagation along the sagittal axis, with dominant directions dependent on visual processing, consistent with the original study. Compared to traditional methods, our approach provides an automated, sensitive and selective parameterization of phase changes, eliminating the need for extensive manual selection of epochs, channels, or time windows.

MACHINE LEARNING CLASSIFICATION OF MENTAL DISORDERS FROM RESTING STATE EEG – A PRELIMINARY STUDY

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Presently, diagnosis of mental disorders is based on subjective methods, like self-reported behavioral symptoms. In the search of objective, biophysical indicators for psychological disorders, machine learning (ML) classification based on electroencephalography (EEG) data was suggested in the current literature. This approach is very promising, reported accuracies fall in the range of 75-95%. However, current studies focus mainly on comparing people suffering from single disorder with healthy controls, while in reality, psychiatric diagnosis is a multicategorical choice. Moreover, these results were obtained on small sample sizes, which poses a danger of overfitting. From the psychiatric hospital's archival database, we obtained about 14000 resting-state EEG signals of psychiatric patients diagnosed with a wide range of disorders, which enables training of multicategorical choice algorithms on sufficient sample. In this poster, I present results of a preliminary experiment on this data. As the EEG signal is complex and irregular, one of the most important steps in its analysis is feature extraction and selection. We tested the importance of different EEG features in distinguishing between different groups of disorders and tested performance of different state-of-art classification algorithms, using selected feature set. We plan to develop a multicategorical classifier, and train it on this data. Results from this preliminary experiment could serve as a baseline for evaluation of multicategorical classification algorithms, as the literature about ML classification between different disorders is scarce.

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μHOLLO: LENSLESS COMPUTATIONAL MICROSCOPY DEMONSTRATOR FOR LARGE FIELD-OF-VIEW LABEL-FREE BIOMEDICAL *IN VITRO* IMAGING

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Lensless digital holographic imaging represents a modern approach to label-free optical microscopy, enabling non-destructive, and large-area computational imaging with a field of view equal to the sensor matrix size. Biologically relevant structures, e.g., cell cultures and tissue slices, can be quantitatively investigated with very easy preparation as no staining is required. The absence of lenses eliminates common issues related to aberrations, narrow field of view, and limited depth of field, while simultaneously simplifying the system architecture and reducing overall costs. Holographic reconstruction – numerically propagated complex field, possibly done *via* deep learning – yields both amplitude (absorption features) and phase (refraction features) information of light transmitted through the sample. In case of translucent samples, with very low contrast in classical brightfield microscopy, lensless holographic microscope allows for the extraction of label-free high-contrast spatial distribution of specimen optical thickness (refractive index combined with physical thickness). These features are particularly desirable in biomedical *in vitro* imaging applications such as histological section analysis (e.g., mouse brain tissue slices) and cell phenotyping/tracking even under extremely low power of illumination (no photodamage nor photo-stimulation with possibility of long time lapse imaging). We have developed a complete stand-alone demonstrator of a lensless holographic microscope that integrates a light sources, sample holder and board-level camera, and custom-made holographic autofocusing and reconstruction algorithms capable of accounting for detrimental noise effects. Camera is mounted on a linear stage enabling its axial movement and acquisition of multiple defocused holograms utilized to enhance the sample's signal-to-noise ratio through iterative holographic reconstruction. The system is equipped with a specialized application that not only enables efficient data

acquisition and reconstruction but also supports advanced quantitative analysis (e.g., morphological and statistical operations). The functionality of the system has been successfully validated under real-world conditions on tissue sections and cell culture samples – both live and fixed – highlighting the potential of this technology for high-throughput, label-free, and preparation-light digital biomedical microscopy and quantitative diagnostics.

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NEURAL MECHANISMS OF SPONTANEOUS AND REQUESTED VISUAL IMAGERY

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Visual mental imagery activates brain regions also involved in visual perception, as shown in fMRI and EEG studies with simple stimuli. However, less is known about brain responses to more complex, real-world imagery. This study examined whether visual areas are engaged when imagining real-world scenes, both intentionally and spontaneously. Spontaneous imagery is particularly interesting as it reflects natural processes like daydreaming, creativity, and memory recall. Sixty healthy adults completed an EEG study; 40 individuals with the highest scores on the Vividness of Visual Imagery Questionnaire were selected for the analysis. Participants completed three conditions: Induced imagery-imagining an activity and deciding on engagement; Spontaneous imagery-deciding without being prompted to visualize; Perception-watching videos of the activities and deciding. EEG was recorded throughout. We applied beamformer source analysis to assess activation in the visual cortex. Functional connectivity at the source level was examined using Phase Locking Value (PLV), while directionality of information flow was analyzed at the electrode level using Directed Transfer Function (DTF). PLV showed increased alpha (8-12 Hz) and theta (4-7 Hz) connectivity in both imagery conditions , indicating visual area involvement. DTF revealed directed flow from visual to widespread regions during induced imagery, across alpha, beta (12-20 Hz), and theta bands. Spontaneous imagery exhibited similar but weaker patterns and additional sources were emerging in central regions. In the perception condition, central and parietal electrodes emerged as primary sources of directed connectivity, with visual regions playing a less dominant role. Spontaneous and induced imagery may share a common functional patterns, shown by similar source-level PLV connectivity in alpha and theta bands, both engaging visual areas. DTF results revealed a broader pattern of connectivity, showing not only the involvement of the visual cortex but also engagement of other brain regions. DTF patterns were more consistent in induced imagery, while spontaneous imagery showed weaker and more variable flow. Perception was characterized by bottom-up flow from central regions. These findings highlight shared neural mechanisms between imagery types and their connection to visual perception.

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RUSH, REGRET, REPEAT: DELAY DISCOUNTING, ANXIETY AND ALCOHOL USE DISORDER IN MICE

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Alcohol use disorder (AUD) involves persistent alcohol-seeking behavior and impaired decision-making, often rooted in underlying traits such as impulsivity and anxiety. To investigate these behavioral predictors, we developed a longitudinal mouse model integrating delay discounting and anxiety assessment. Using a custom-designed, fully automated E-maze, we implemented a decision-making task in which mice chose between two reward sides: one delivering a smaller, immediate reward, and the other offering a larger reward at varying delay intervals. Mice were first conditioned to develop a strong baseline preference (≥80%) for the larger reward. Once established, they underwent testing across five randomized delay conditions—0, 1, 3, 5, and 7 seconds—each presented in blocks of 10 trials. We found that the preference of the big reward site was affected by delay time in a cohort of mice, indicating elevated impulsivity. Following the delay discounting phase, the same cohort underwent anxiety testing using the elevated plus maze, which assessed open-arm

avoidance behavior as a proxy for anxiety levels. This dual-paradigm approach enabled us to explore the interaction between impulsive choice behavior and trait anxiety, both of which are hypothesized to contribute to addiction vulnerability. In the final phase of the study, mice will be transferred to IntelliCage systems for automated, long-term monitoring of alcohol-seeking and consumption behaviors, allowing us to correlate early behavioral phenotypes with subsequent AUD-like traits. This multi-stage experimental design offers a robust framework for identifying behavioral predictors of alcohol addiction and may inform early intervention strategies.

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THE EFFECT OF NR2B KNOCK-OUT ON APPETITIVE LEARNING IN MICE

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The brain is a highly dynamic structure capable of adapting to environmental changes and encoding experiences as memories. Learning involves activity-dependent modifications of synaptic connectivity, including the formation of new synapses through long-term potentiation (LTP) and the elimination of existing ones *via* long-term depression (LTD). Both processes can transiently produce glutamatergic synapses that are functionally silent, characterized by the presence of NMDA receptors and the absence of AMPA receptors. Our previous work demonstrated the formation of silent synapses in the central nucleus of the amygdala (CeA) during appetitive learning in mice. However, the underlying mechanisms—specifically whether these synapses result from LTP or LTD—remain unclear. This study aimed to evaluate the role of LTP in appetitive learning by selectively knocking out the NR2B subunit of the NMDA receptor in the CeA, thereby impairing LTP. Behavioral assay and electrophysiological patch-clamp recordings were performed on NR2B knockout mice. Our results indicate a significant alteration in neuronal functional activity.

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LIPOCALIN-2 INFLUENCE ON ELECTROPHYSIOLOGICAL PROPERTIES OF NEURONS IN MATERNAL IMMUNE ACTIVATION MODEL

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Maternal infections during pregnancy have been shown to affect the brain development of the offspring. The infection process triggers the production of inflammatory proteins, including lipocalin-2 (Lcn2). Lcn2 is a small secreted glycoprotein involved in the innate immune response. It has been reported to influence neuronal plasticity, and its deficiency impairs neuronal excitability, long-term potentiation, and spine density. Exogenous Lcn2 also shifts spine morphology toward more immature forms. However, its role in offspring neurodevelopment remains unclear. The aim of this study was to investigate how maternal immune activation (MIA) affects the electrophysiological properties of CA1 pyramidal neurons in offspring, and to determine the involvement of Lcn2 deficiency in this process. Pregnant Lcn2HET mice were intraperitoneally injected with either saline or lipopolysaccharide (LPS, 40 µg/kg) on gestational days 16–18 to mimic the infection. Adult offspring (8-12 weeks) of both sexes and genotypes (WT/KO) were used for whole-cell patch clamp recordings of miniature excitatory postsynaptic currents (mEPSCs) and dendritic spine density analysis. The results revealed sex-dependent differences in the inter-event interval (IEI) of mEPSCs. Notably, Lcn2 deficiency led to a significant decrease in IEI in control KO females, indicating an increased frequency of synaptic events. In WT males, MIA caused a significant increase in IEI. To assess whether these IEI changes were associated with alterations in excitatory synapse number, we analyzed dendritic spine density. No significant differences were found, suggesting that the observed changes in IEI likely result from altered presynaptic release rather than synapse number. These findings indicate that both MIA and Lcn2 deficiency influence neuronal synaptic properties in a sex-dependent manner.

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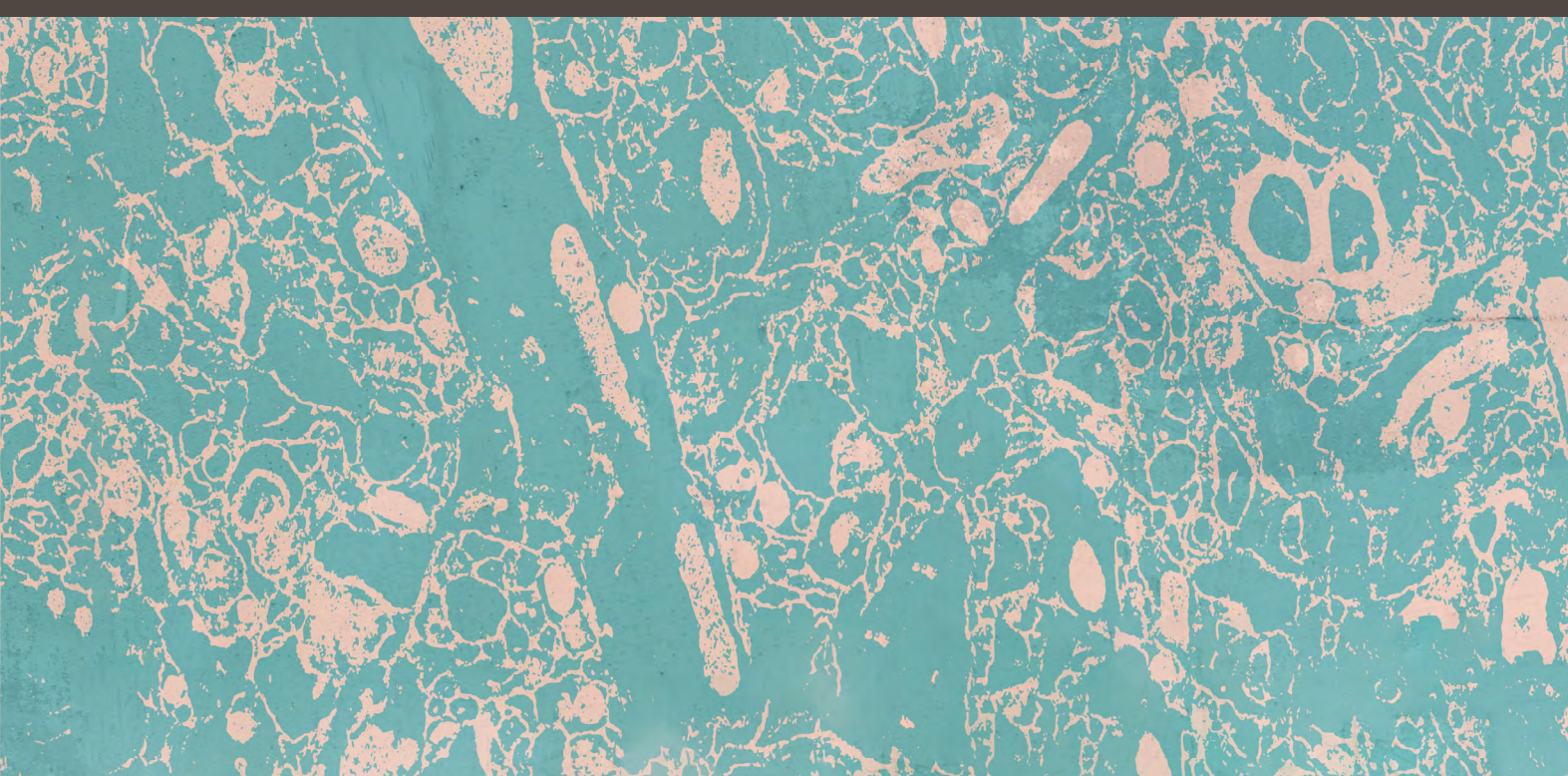

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