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# Abstract book

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# Neuroplasticity and Synaptic Dynamics

**MODULATION OF BULBAR ACTIVITY IN AN IMPAIRED MODEL OF INTRINSIC EXCITABILITY IN MITRAL AND TUFTED CELLS**

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Homeostatic intrinsic plasticity is a critical yet understudied aspect of neuronal excitability and is essential for maintaining stable neural activity in response to perturbations. However, uncovering the molecular regulators of intrinsic plasticity is challenging due to its dynamic and cell-specific nature as well as the interplay between synaptic and intrinsic mechanisms. The microphthalmia-associated transcription factor (*Mitf*) known for its role in melanocyte development, has cell-specific expression in the olfactory bulb. Primary neurons from a *Mitf* mutant model are hyperactive which is linked to *Mitf* regulation over target genes involved in ion channel expression and neuronal excitability.

Using whole-cell patch clamp electrophysiology in acute slices from wild type and *Mitf* knockout mice we investigated the role of *Mitf* in transcriptional control of intrinsic excitability in projection neurons of the olfactory bulb. Focusing on both passive and active properties we found that mutant mice have a hyperactive phenotype when the circuit is maintained. Moreover, analysis of action potential waveforms suggests that this hyperactivity is potassium based. Additionally activity induction reveals that MITF mutants fail to properly adjust their excitability that is correlated with changes in differential gene expression. Together these results offer a new perspective over the transcriptional control of homeostatic mechanisms that contribute to excitability.

**Keyword 1**: Olfaction
**Keyword 2**: Excitability
**Keyword 3**: Mitral Cells

**EXPERIENCE DRIVEN PLASTICITY IN VISUAL THALAMUS, A MULTI-OMIC APPROACH TO UNDERSTANDING THE NEURAL MECHANISMS OF AMBLYOPIA**

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Amblyopia affects around 3% of the general population and is the most common cause of vision loss in young children. Even though no ocular abnormalities are present, an unbalanced binocular experience in early childhood results in a loss of visual acuity, stereopsis, position acuity, and contrast sensitivity due to the suppression of neuronal responses from the weaker, amblyopic eye.

We investigated visual experience-driven plasticity in the dorsolateral geniculate nucleus (dLGN), the primary visual thalamic nucleus essential for image perception. This structure is not only composed of relay cells but also receives afferents from both eyes and undergoes plasticity of excitability.

Employing a proteomic approach, we identified activity-dependent changes in the protein composition of dLGN neurons from young rat subjected to monocular deprivation by eyelid closure for 4, 7, and 10 days, durations producing distinct changes of excitability in the dLGN.

By microdissection, we isolated deprived and spared regions of the dLGN. The protein content was analyzed by LC-MS/MS mass spectrometry. Isobaric labelling was used to enhance mass spectrometry sensitivity and enable relative quantification.

We observed significant changes in the levels of proteins essential for intrinsic and synaptic neuronal excitability, such as ion channels or proteins involved in synaptic transmission and identified novel activity-regulated proteins.

We are analyzing the corresponding transcripts using spatial transcriptomic which enables *in situ* single-cell mRNA quantification in specific cell population. This work will provide insight into the coordinated excitatory and inhibitory molecular changes, allowing a better comprehension of the mechanism underlying amblyopia.

**Keyword 1**: neuronal plasticity
**Keyword 2**: amblyopia
**Keyword 3**: proteomic
**Keyword 4**: spatial transcriptomic

**CAN SINGLE ACTION POTENTIALS IN THE AXONLESS RETINAL AII AMACRINE CELL DRIVE OUTPUT AT CHEMICAL SYNAPSES?**

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The narrow-field AII amacrine cell, an inhibitory interneuron, links the rod and cone pathways in the mammalian retina. AIIs integrate chemical (excitatory, inhibitory) and electrical synaptic inputs and generate outputs at chemical (glycinergic) and electrical synapses in different regions of the dendritic tree, corresponding to lobular and arboreal dendrites, respectively. Surprisingly, AIIs also generate $Na_v$-dependent action potentials at a single process (the axon initial segment-like process; AII-AIS), from which spikes backpropagate passively across the dendritic tree. This raises the question whether single spikes contribute to signal processing by triggering exocytosis of glycine at the lobular dendrites after passive propagation from the AII-AIS. Here, we have used whole-cell recording and computational modeling to investigate the ability of spikes to activate voltage-gated $Ca^{2+}$ channels in AIIs. With voltage-clamp recording in rat retinal slices, we observed robust $Ca^{2+}$ currents ($I_{Ca}$) with threshold of ~-55 mV, rise time of 2-3 ms, amplitude of ≲200 pA, and little inactivation. After blocking $Na_v$ channels (TTX), voltage clamping AIIs with physiologically recorded soma spike waveforms evoked transient, inward $I_{Ca}$ responses, potentially driving exocytosis. We also combined whole-cell recording and 2-photon imaging (followed by immunolabeling for ankyrin-G to identify the location of the AII-AIS), to developed multicompartmental AII models. Modeling suggested that single spikes evoked depolarizations at the lobular dendrites that may activate $I_{Ca}$, as observed in the physiological recordings. These results suggest that AII spiking might trigger exocytosis at the output synapses, potentially adding an element of spike coding to the otherwise graded control of synaptic output.

**Keyword 1**: Compartmental models
**Keyword 2**: 2-photon imaging
**Keyword 3**: Signal integration
**Keyword 4**: Voltage-gated calcium currents
**Keyword 5**: Back-propagating action potentials

**INVESTIGATING THE ROLE OF MENA IN THE FORMATION AND FUNCTION OF INHIBITORY SYNAPSES**

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A hallmark of cortical circuit organization is the highly precise pattern of connectivity between neurons. This specificity is established by common cellular and molecular mechanisms like local mRNA translation and actin cytoskeleton remodeling, that dictate the precise placement of neurons and their synaptic connections. However, our understanding of the coordination between such mechanisms and the specific molecular components that underlie synapse formation and function, remains elusive. Previous work from our lab has established the dual role of the protein Mena in actin cytoskeleton remodeling and the regulation of local translation of specific mRNAs in developing neurons. We hypothesize that this dual function of Mena is conserved in adult neurons, and may be crucial for synapse formation and plasticity. To this end, we report Mena to be preferentially localized in inhibitory synapses *(*of cortical PV interneurons in particular*),* and also its tendency to co-immunopercipitate with several key organizers of inhibitory synapses and regulators of local translation. In good agreement with these findings and our initial hypothesis, we observe significant synaptic defects in mice with genetic ablation of Mena (Mena KO), including hindered formation of inhibitory synapses, hastened maturation of dendritic spines and formation of perineuronal nets around PV interneurons. Based on those observations, we speculate that Mena, along with its network of interneuron-specific interactors, may be implicated in the molecular and structural organization of inhibitory synapses. Therefore, our data uncover novel mechanistic insights into synapse biology, and contribute to our understanding of inhibitory synapse establishment and potential function.

**Keyword 1**: Synapses
**Keyword 2**: Interneurons
**Keyword 3**: Local Translation

**SEROTONIN AND SEROTONERGIC INPUTS MODULATE CHOLINERGIC INTERNEURONS IN THE STRIATUM ON DIFFERENT TIMESCALES**

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The striatum, the predominant input structure of the basal ganglia, receives converging glutamatergic and GABAergic inputs from cortical and subcortical regions. It also receives modulatory input from midbrain dopamine neurons, critical for motor initiation, learning and reward. Additionally, the striatum also receives serotonergic input with one third of serotonergic neurons projecting from the dorsal raphe nucleus to the striatum. Here, we studied the impact of serotonin, and serotonergic inputs, on the cholinergic interneurons (ChINs) in the striatum. Through inhibitory control on medium spiny neurons, ChINs modulate striatal functions such as action selection and transmission of reinforcement-related information. They also form polysynaptic connections between each other, enabling them to elicit a concerted signal across large areas of the striatum. Using simultaneous whole-cell recordings of ChINs and pharmacological manipulation, we showed that serotonin altered their intrinsic properties as well as attenuate the polysynaptic inhibitory responses between them, both via the 5-HT2 receptor. Using optogenetics to stimulate serotonergic fibres in the striatum, we also uncovered a fast monosynaptic response in ChINs that we found was glutamatergic. These data present a novel way by which networks of ChINs are modulated by serotonin and serotonergic neurons, adding to the complexity of interactions between acetylcholine, dopamine, and serotonin in striatal circuit modulation. Due to the broad effects of acetylcholine release in the striatum, serotonin likely has far-reaching effects on striatal subtypes and inputs, which may provide insight into the mechanisms underlying non-motor symptoms of Parkinson's Disease.

**Keyword 1**: Striatum
**Keyword 2**: Cholinergic Interneurons
**Keyword 3**: Serotonin
**Keyword 4**: Basal Ganglia
**Keyword 5**: 5-HT

**TTX-INDUCED ULTRASTRUCTURAL REMODELING OF SYNAPSES AT APICAL DENDRITES IN HIPPOCAMPAL PRINCIPAL NEURONS**

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Synaptic plasticity within the hippocampal neuronal network modulates critical processes underlying learning and memory. In this study, we used transmission electron microscopy to examine both the formation and the recycling of distinct synaptic membranes during tetrodotoxin (TTX)-induced synaptic plasticity in murine entorhino-hippocampal tissue cultures. Additionally, using pharmacological microglia depletion, we assessed the microglial contribution to synaptic changes. Specifically, we aimed to (1) analyze the density and turnover of presynaptic vesicles and to (2) assess membrane dynamics at postsynaptic spines across different neuronal compartments of both dentate granule cells and CA1 pyramidal neurons. Our findings reveal significant alterations in the ultrastructure of excitatory synapses, particularly within cortical projections, indicating activity-dependent remodeling in neuronal networks of entorhino-hippocampal tissue cultures. These results underscore the complexity of neuronal membrane remodeling and highlight the importance of microglia in modulating synaptic function and plasticity.

**Keyword 1**: membrane recycling
**Keyword 2**: quantitative electron-microscopical analysis
**Keyword 3**: excitatory synapses

**ELECTRICAL COUPLING SYNCHRONIZES ACTION POTENTIALS IN THE RAT HYPOTHALAMIC TUBEROINFUNDIBULAR DOPAMINE (TIDA) CIRCUIT**

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Neuroendocrine tuberoinfundibular dopamine (TIDA) neurons in the hypothalamus provide tonic inhibition of the hormone, prolactin, from the pituitary gland. The efficiency of this inhibition is reflected in the low basal prolactin levels of male rats, where TIDA neurons exhibit highly rhythmic, slow oscillations between periods of discharge and quiescence, synchronized by electrical synapses within the network. However, whether gap junctions also synchronize the discharge of individual action potentials (APs) in the TIDA network, remains unknown. We addressed this issue by dual whole-cell patch-clamp recordings of TIDA neurons in slices from male THcre-tdTomato rats (P21-30) and analyzed spike-time cross-correlation for each TIDA pair. AP synchronicity correlated with the strength of electrical coupling: $r_P$=0.93, p<0.0001, n=19 pairs. In some electrically coupled pairs, the recipient neuron (voltage-clamp mode) responded by spikelet generation when receiving spontaneous APs from the donor neuron (current-clamp mode), a relationship that was bidirectional. Electrophysiological and dye coupling experiments revealed that a single TIDA neuron could be coupled to more than one neuron and generate discrete amplitudes of spikelets. The TIDA spikelets were independent of fast synaptic transmission, but were abolished by the blocker of voltage-gated $Na^+$channels, tetrodotoxin. In addition, silencing (no APs) the donor neuron by somatic hyperpolarization of the membrane potential eliminated spikelets in the coupled recipient neuron. Thus, gap junctions regulate TIDA spike synchrony and network excitability, suggesting that synchronization occurs within this network on a faster time scale than previously assumed and reinforcing the relevance of electrical coupling for hormonal homeostasis.

**Keyword 1**: oscillations
**Keyword 2**: spikelets
**Keyword 3**: firing synchrony
**Keyword 4**: prolactin

**SPECIALIZED AXON INITIAL SEGMENT ENABLES LOW FIRING THRESHOLD WITH RAPID ACTION POTENTIAL GENERATION IN FAST-SPIKING INTERNEURONS IN THE HUMAN NEOCORTEX**

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In the human brain, we can see differences in the microanatomical structure of neurons and the molecules they contain. This is best seen in the neocortical mantle, the part of the brain that controls the most complex functions. However, we still have a poor understanding of why these "human-specific" neuronal features exist. We studied biopsies of human brain tissue taken during surgery and compared the results with those from mouse brains. Whole-cell recordings from identified pvalb neurons showed that human neurons have a lower threshold for generating action potentials than mouse brain cells. In addition, the human neurons had an elongated axon initial segment (AIS), a specialized axonal structure that determines the action potential (AP) threshold. The human AIS was also low in voltage-gated inhibitory Kv1.1 and Kv1.2 potassium channels, whereas both Kv1 ion channels were prominent in the AIS of mouse neurons. Computational simulations of fast-spiking interneuron models showed that human-type AIS lowered the AP threshold and shortened the time lag for AP generation. This means that human AIS supports fast in-fast out electrical circuit function in human pvalb neurons, which suffer from electrically slow (compared to mouse) membrane potential kinetics in the cell body.

**CROSS-SPECIES VALIDATION OF PERINEURONAL NET DISTRIBUTION IN AFFECTIVE STATE RESEARCH: INSIGHTS FROM HUMAN AND RAT DATA**

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Perineuronal nets (PNNs) are extracellular matrix structures critical for synaptic plasticity and emotional regulation. PNNs primarily enwrap parvalbumin interneurons and contain chondroitin sulphate proteoglycans (CSPGs) like aggrecan. The aggrecan knockout literature further highlights its functional role in PNN formation. Understanding PNN distribution in affective states requires translationally relevant models. Here, we compare human postmortem data with rat brain tissue to validate PNN spatial patterns and explore implications for affective states and neuroimmune interactions.
Human gene expression data from the Allen Brain Atlas was analysed to determine regional ACAN (aggrecan) distribution. Rat brain slices from male animals housed in enriched (EE) or isolated conditions for 3 or 21 days were stained with Wisteria Floribunda Agglutinin to visualize PNNs. Mean intensity and total staining metrics were analysed.
ACAN expression in both human (mRNA) and rodent (WFA imaging) was highest in the reticular thalamic nucleus (RTN) and cingulate gyrus (CG) regions, with lower expression in hippocampal subfields (CA1, CA2, CA3, DG). These findings support conserved PNN distribution across species. In the rat experiments there is significant correlation in PNN intensity in hippocampal regions across both day 3 and day 21. EE rats showing stronger correlations between CG and RTN regions.
This cross-species alignment in the brain PNN signatures strengthens the rat model's validity for studying PNNs in affective states. Future analyses of additional CSPGs (brevican, neurocan, versican) could deepen our understanding of PNN dynamics in emotion and cognition. This work bridges molecular, neuroimmune, and behavioural insights, advancing therapeutic possibilities for affective disorders.

**Keyword 1**: Perineuronal nets
**Keyword 2**: Affective state
**Keyword 3**: parvalbumin GABAergic interneurons
**Keyword 4**: plasticity
**Keyword 5**: excitatory/inhibitory balance

**AGE-RELATED DECLINE IN ACTION POTENTIAL OF FAST-SPIKING INTERNEURONS IN THE HUMAN NEOCORTEX**

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Aging is associated with the slowdown of neuronal processing and cognitive performance in the brain, but now, thanks to cutting-edge research, we're finally starting to understand the exact cellular mechanisms behind this deterioration in humans. Excitingly, recordings in human acute brain slices prepared from tissue resected during brain surgery are enabling the investigation of neuronal changes with age. Neocortical fast-spiking cells are widely associated with neuronal network activities underlying cognitive processes, and their high metabolic demand makes them vulnerable to neurodegeneration. In this study, we analyzed the electrical properties of 166 fast-spiking cells in neocortex samples from individuals aged 11–84 years. And by studying the electrophysiological features of action potentials and intrinsic excitability, we report that action potential overshoot significantly decreases and spike half-width increases with age. We also found that the maximum rise speed of action potentials (but not the repolarization speed or the afterhyperpolarization amplitude) changes significantly with age, suggesting a particular weakening of the sodium channel current generated in the soma. Excitingly, cell intrinsic excitability, measured as input resistance, membrane time constant and cell capacitance, remained unaffected by senescence. This suggests that the action potential in fast-spiking interneurons may weaken significantly with age in the human neocortex, which could contribute to the deterioration of cortical functions with age.

**Keyword 1**: aging
**Keyword 2**: action potential
**Keyword 3**: neocortical interneurons
**Keyword 4**: electrophysiology

**BRIDGING SYNAPTIC MOLECULAR ARCHITECTURE TO FUNCTION: A MULTIMODAL APPROACH TO STUDY SYNAPSE HETEROGENEITY**

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Connectomics studies have revealed the staggering complexity of mammalian neural circuits, arising from highly organized diversity. Synapses play a pivotal role here, constantly remodeled by activity-dependent mechanisms, enabling adaptive behaviors such as learning and memory. They are the locus of numerous psychiatric and neurodegenerative diseases, including autism spectrum disorders(ASDs) and Alzheimer's disease, where maladaptation precedes symptoms. Classical approaches typically consider synapses at the population level, i.e. average synapses. However, synapses exhibit significant structural, molecular and functional heterogeneity. Variability in synapse size, receptor composition, and plasticity has been further dissected by super-resolution imaging, revealing nanoscale sub-synaptic domains and highlighting the need for a deeper understanding of individual synapses. Depending on these parameters, individual synapses may exhibit different plastic properties and respond differently to activity-dependent, genetic or pharmacological perturbations, whether physiological, pathological, or therapeutic. Thus, investigating synaptic heterogeneity is crucial.

To comprehend rules shaping synaptic herteogenity, experimental approaches integrating multiple modalities at single-synapse resolution are necessary. Previous methods, while allowing this, often lack integration of spatial, functional, structural, or molecular correlates. To overcome these limitations, we developed a strategy combining patch-clamp recordings, single-cell transfection, high-resolution live imaging, and expansion microscopy. This enables: 1/genetic manipulation and imaging of pre- and postsynaptic elements at individual synapses embedded in the local connectome; 2/mapping and monitoring at structural, molecular, and functional levels to investigate their contribution to neuronal output; 3/longitudinal investigation of synaptic plasticity and disease-related mutations. Here, we present an example, overexpression of Neuroligin-1, an ASD-implicated transynaptic adhesion molecule, to understand synapse heterogeneity through pertubation.

**Keyword 1**: Synapse heterogenity
**Keyword 2**: Hippocampus

**SYNAPTIC PLASTICITY MANIFOLDS SUPPORTING MEMORY ABILITIES IN SPIKING NETWORKS**

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Synaptic plasticity is thought to underlie learning and memory. Plasticity rules can be active in different synaptic connection types, such as excitatory-excitatory (EE) and inhibitory-excitatory (IE) connections, with experimental evidence suggesting distinct mechanisms for each type. However, it remains unclear how these co-active rules subserve memory, as previous studies have primarily focused on synapse-type specific plasticity rules in isolation. Here, we leverage recent work that inferred a manifold of spike-timing-dependent plasticity (STDP) rules acting co-actively across four synapse types (EE, EI, IE, and II). These quadruplets of plasticity rules, selected solely for their ability to stabilize network dynamics, also support memory when embedded in recurrent spiking networks. First, we study the properties of this manifold by testing plasticity rules interpolated between different rule quadruplets. Network statistics, such as population firing rate, vary smoothly between quadruplets on the manifold, while a subspace of rules that stabilize network dynamics emerges in their neighborhood. Second, we find that only a few plasticity rules match those reported in previous studies. Third, most rules within a quadruplet lead to instability when simulated in isolation. Such fragility suggests that the flexibility conferred by the rule co-activity reduces the need for fine-tuning of individual rules. As a result, the manifold of stable rule quadruplets appears continuous and well-behaved, despite being non-linear. Overall, we show that co-activity gives rise to a subspace of stable plasticity rules with memory abilities, highlighting the importance of studying plasticity rules in combination to understand how networks achieve stability and memory.

**Keyword 1**: Synaptic Plasticity
**Keyword 2**: Spiking Networks

**VOLTAGE IMAGING OF COLLATERAL VMH NEURONS REVEALS DEFENSE BEHAVIOR PLASTICITY MODULATION**

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The ventromedial hypothalamic nucleus (VMH) plays a critical role in defensive behaviors, projecting to key brain regions such as the anterior hypothalamic nucleus (AHN) and the periaqueductal gray (PAG). Depending on the integration of threat-related network activity, mice exhibit distinct behavioral responses, including escape (jumping/running) or freezing. While the VMH's involvement in these responses is well established, how its collateral projections influence the AHN and PAG at the synaptic level remains poorly understood.
To address this gap, we investigated the post-synaptic responses in the AHN and PAG following stimulation of VMH axons that collateralize to both regions. We employed all-optical voltage imaging, enabling precise stimulation of VMH axons while simultaneously recording voltage dynamics in post-synaptic cells. Our custom-made software, **VoltView**, extracted diverse electrophysiological features, allowing us to classify and cluster distinct response types in both AHN and PAG. Furthermore, high-frequency stimulation revealed plasticity-related changes in post-synaptic responses, suggesting dynamic modulation of defensive circuits.
These findings provide new insights into how VMH collateral projections shape neural activity in AHN and PAG, advancing our understanding of the synaptic mechanisms underlying defensive behaviors.

***PLASTICITY OF LAYER 2/3 VIP-IN EXCITATORY SYNAPSES IN THE MOUSE SOMATOSENSORY CORTEX IS INFLUENCED BY MGLUR-1 ACTIVITY***

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Long-term potentiation (LTP) is a form of synaptic plasticity, where specific stimulation results in long-lasting strengthening of the synapse efficacy through pre- or/and postsynaptic mechanisms. However exact mechanisms of LTP in specific synapses require further investigations. Here, we study vasoactive intestinal polypeptide-expressing interneurons (VIP-INs), which are a subset of one of three major cortical GABAergic neurons. The main function of VIP-INs is to disinhibit excitatory cell from inhibition mediated through other interneurons. In present study we aim to electrophysiologically characterize excitatory synapses onto layer 2/3 VIP-INs and receptor/signaling pathways necessary for LTP induction.

Using in vitro whole-cell patch clamp technique, we patched fluorescently-labeled VIP-INs in transgenic mice. We evoked LTP by pairing extracellular stimulation with postsynaptic membrane depolarization to 0 mV, and then compared amplitudes of EPSCs (excitatory postsynaptic currents) before and post-LTP induction. Here, pharmacological blockage of mGluR (metabotropic glutamate receptors) abolished LTP, revealing that plasticity on excitatory synapses on VIP-INs is dependent on those receptors, specifically on mGluR-1 but not mGluR-5. mGluR-1 act through several signaling pathways. Here we show that upon application of Src kinase inhibitor the LTP is blocked, meaning that those tyrosine kinases are crucial for LTP induction through mGluR-1. Moreover, our results show that synaptic plasticity onto VIP-IN inputs is NMDAR-independent. Collectively, our study contributes to characterizing LTP induction research, revealing molecular mechanism of excitatory synaptic plasticity on L2/3 VIP-INs.

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**Keyword 1**: long-term potentiation
**Keyword 2**: VIP-interneurons
**Keyword 3**: excitatory synapses
**Keyword 4**: mGluRs
**Keyword 5**: barrel cortex

**O-GLCNACASE REGULATES DENDRITIC SPINE MORPHOGENESIS AND SYNAPTIC FUNCTION**

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Dendritic spines are essential for synaptic transmission, neural circuit organization, and cognitive function. Their morphology and density influence synaptic plasticity, learning, and memory. O-GlcNAc modifications regulate neuronal function and are implicated in neurodegenerative disorders, yet the role of O-GlcNAcase (OGA) in dendritic spine regulation remains unclear. This study examines OGA's role in spine morphogenesis and synaptic function using cultured hippocampal neurons. Immunohistochemical and biochemical analyses reveal OGA enrichment in dendritic spines. Functional assays show that OGA overexpression promotes spine maturation, increases spine density, and alters synapse size. Additionally, OGA modulates AMPA receptor composition, selectively regulating GluA2 and GluA3 expression in developing and mature neurons. These findings highlight OGA as a key regulator of excitatory synaptic remodeling, with implications for neurological disorders such as Alzheimer's disease and autism. By elucidating OGA's role in synaptic plasticity, this study underscores its potential as a therapeutic target for synapse-related pathologies.

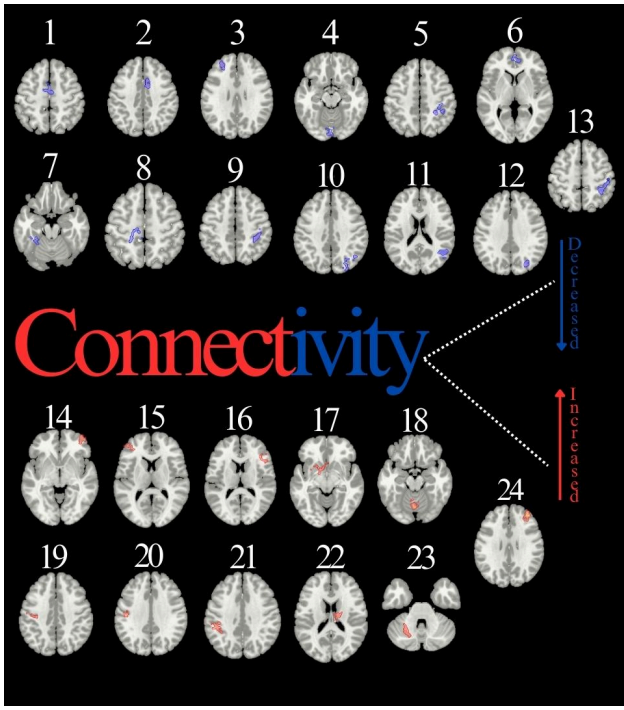
**Keyword 1**: Dendritic spines
**Keyword 2**: O-GlcNAcase (OGA)
**Keyword 3**: spine morphogenesis

**NEURAL INFLUENCES BETWEEN DISTANT BODY REGIONS: A PRELIMINARY FMRI STUDY ON THE INFLUENCE OF IMPROVED SUBTALAR JOINT MOBILITY ON CHRONIC CERVICAL PAIN**

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Emerging data suggests neural interconnectivity between anatomically distant body regions. This study is based on our clinical observation of varying degree of ankle joint stiffness in chronic neck pain patients. We investigated whether improving subtalar joint function influences cervical muscle pain and brain neural connectivity in neck pain patients.
Three participants (2 females and 1 male) with chronic neck pain underwent fMRI scans and pain assessments using the Visual Analog Scale (VAS) manual palpation of the sternocleidomastoid and suboccipital muscles. The intervention involved improving subtalar abduction and adduction by relaxing the lateral and medial ankle muscles through appropriate stretch and Golgi tendon organ reflex activations. fMRI was acquired on a 3T scanner with 3D T1-MPRAGE and EPI resting state fMRI scans at pre and post-intervention. VAS scores were compared to assess changes.
After intervention, all three participants showed a reduction in pain VAS scores for the studied muscles. fMRI findings revealed altered neural connectivity in different brain networks such as an increase in the Right and Left lateral sensorimotor, and a decrease in the default mode network prefrontal cortex and regions associated with pain modulation, including the insula and thalamus.
This study provides preliminary evidence of neural influence between the subtalar joint and cervical region. This finding points to the importance of considering global neural connectivity in physiotherapy and pain management techniques, offering new possibilities for integrative treatment approaches. Further research is warranted to elucidate the underlying mechanisms and long-term efficacy of such interventions

**Keyword 1**: fMRI
**Keyword 2**: Physiotherapy
**Keyword 3**: Pain




| Connection Number | Connection | Coordinates (x,y,z) | Cluster size | peak p-FWE |
|---|---|---|---|---|
| 1 | Default Mode Network Left Lateral Parietal - Juxtapositional Lobule Cortex Right | +08 -12 +62 | 173 | 0.002524 |
| 2 | Default Mode Network Left Parietal Cortex - Paracingulate Gyrus Right | +04 +12 +48 | 131 | 0.0031432 |
| 3 | Default Mode Network Right Parietal Cortex - Frontal Pole Left | -24 +44 +28 | 132 | 0.002715 |
| 4 | Default Mode Network Right Parietal Cortex - Intracalcarine Cortex Right | +12 -86 +02 | 245 | 0.002161 |
| 5 | Left Insular Cortex - Postcentral Gyrus Right | +18 -44 +54 | 196 | 0.002721 |
| 6 | Superior SensoriMotor - Middle Paracingulate Gyrus | -08 +48 +10 | 220 | 0.002052 |
| 7 | Superior SensoriMotor - Temporal Fusiform Cortex Posterior division Left | -32 -50 -34 | 132 | 0.002028 |
| 8 | Left Thalamus - Precentral Gyrus Left | -06 -36 +66 | 166 | 0.001753 |
| 9 | Left Thalamus - Superior Parietal Lobule Right | +36 -44 +54 | 205 | 0.002551 |
| 10 | Left Thalamus - Lateral occipital cortex superior division Right | +40 -72 +46 | 166 | 0.002724 |
| 11 | Right Thalamus - Angular Gyrus Right | +58 -66 +16 | 308 | 0.001859 |
| 12 | Right Thalamus - Lateral occipital cortex, superior division Right | +28 -74 +34 | 177 | 0.00222 |
| 13 | Right Thalamus - Postcentral Gyrus Right | +26 -40 +74 | 298 | 0.001939 |
| 14 | Default Mode Network Right Parietal Cortex - Frontal Pole Right | +36 +56 -02 | 143 | 0.002696 |
| 15 | Default Mode Network Medial Pre Frontal Cortex - Left Frontal Pole | -40 +50 +06 | 100 | 0.002377 |
| 16 | Left Insular Cortex - Inferior Frontal Gyrus Right | +46 +32 +08 | 166 | 0.002151 |
| 17 | Left Insular Cortex - Left insular cortex | -40 +10 -04 | 186 | 0.00195 |
| 18 | Left Thalamus - Vermis 6 | -04 -60 -08 | 149 | 0.002255 |
| 19 | Left Lateral SensoriMotor - Postcentral Gyrus Left | -50 -14 +32 | 184 | 0.00256 |
| 20 | Right Lateral SensoriMotor - Postcentral Gyrus Left | -52 -14 +40 | 116 | 0.002918 |
| 21 | Superior SensoriMotor - Supramarginal Gyrus, anterior division Left | -46 -22 +44 | 225 | 0.002414 |
| 22 | Left Thalamus - Right Thalamus | +14 -18 +22 | 169 | 0.002448 |
| 23 | Left Thalamus - Cerebelum 6 Left | -24 -54 -30 | 162 | 0.001965 |
| 24 | Right Thalamus - Frontal pole Right | +36 +42 +26 | 114 | 0.002949 |

**LINKING CIRCUITS AND NEUROPLASTICITY: THE PFC > REUNIENS > HIPPOCAMPUS NETWORK**

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Major depressive disorder (MDD) is a prevalent psychiatric condition with significant individual and socioeconomic impacts. Existing therapeutic interventions exhibit limited efficacy and tolerability, underscoring the need for innovative treatment strategies. While recent research has independently explored neuroplasticity, neural circuits, and antidepressant mechanisms, an integrative understanding remains elusive. Our study aims to bridge this gap by investigating the interplay between neural circuits and neuroplasticity, focusing on the medial Prefrontal Cortex (mPFC) and its downstream effects on the hippocampus. Using the Chronic Despair Model, we induced depressive-like behavior in mice and manipulated mPFC activity with DREADDs chemogenetics. We employed immunohistochemistry, Patch Clamp, fiber photometry, and various behavioral assays to assess the impact of mPFC stimulation on depressive-like phenotype and studied the downstream effector regions, particularly the hippocampus. Our findings demonstrate that chemogenetic stimulation of the infralimbic mPFC produces antidepressant effects in a wide range of behavioural tests. Conversely, prelimbic mPFC stimulation showed no antidepressant effects. We observed overexpression of immediate early genes (cFos, NPAS4) in the ventral hippocampus and restoration of hippocampal structural and associative plasticity, evidenced by LTP recovery at Schaffer collateral synapses. We identified the Reuniens Nucleus (nRe) as a critical relay, as its inhibition abolished both antidepressant effects and hippocampal LTP restoration. Our study highlights the mPFC > Reuniens > hippocampus > PFC loop as a potential circuit modulating neuroplasticity in mood disorders, suggesting the Reuniens nucleus as a novel therapeutic target. This integrative perspective offers insights into depression mechanisms and may inform more targeted therapeutic approaches.

**Keyword 1**: depression
**Keyword 2**: LTP
**Keyword 3**: Reuniens

**ARC/ARG3.1 BINDS THE NUCLEAR POLYADENYLATE-BINDING PROTEIN RRM AND REGULATES NEURONAL ACTIVITY-DEPENDENT FORMATION OF NUCLEAR SPECKLES**

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ARC is a neuronal activity-induced protein interaction hub with critical roles in synaptic plasticity and memory. ARC localizes to synapses and the nucleus, but its nuclear functions are little known. Following in vivo LTP induction in the dentate gyrus, we show that ARC accumulates in the nucleosol fraction and interchromatin space of granule cells. Proteomic analysis of immunoprecipitated ARC complexes identifies proteins involved in pre-mRNA processing. We demonstrate endogenous ARC protein-protein interaction with polyadenylate-binding nuclear protein 1 (PABPN1) and the paraspeckles protein PTB-associated splicing factor (PSF). In vitro peptide binding arrays show direct binding of purified ARC to the PABPN1 polyA-RNA recognition motif. 3D morphometric imaging reveals structural changes in PABPN1 foci corresponding to classical nuclear speckles following in vivo and in vitro LTP. Depletion of ARC disrupts the maintenance and activity-dependent formation of PABPN1 speckles, thus implicating ARC in regulation of nuclear speckle dynamics and pre-mRNA processing.

**Keyword 1**: synaptic plasticity
**Keyword 2**: activity-regulated cytoskeleton-associated protein (ARC)
**Keyword 3**: mRNA processing
**Keyword 4**: gene expression
**Keyword 5**: nuclear speckles

**TARPS IN THE REGULATION OF M-CHANNEL FUNCTION AND NEURONAL EXCITABILITY**

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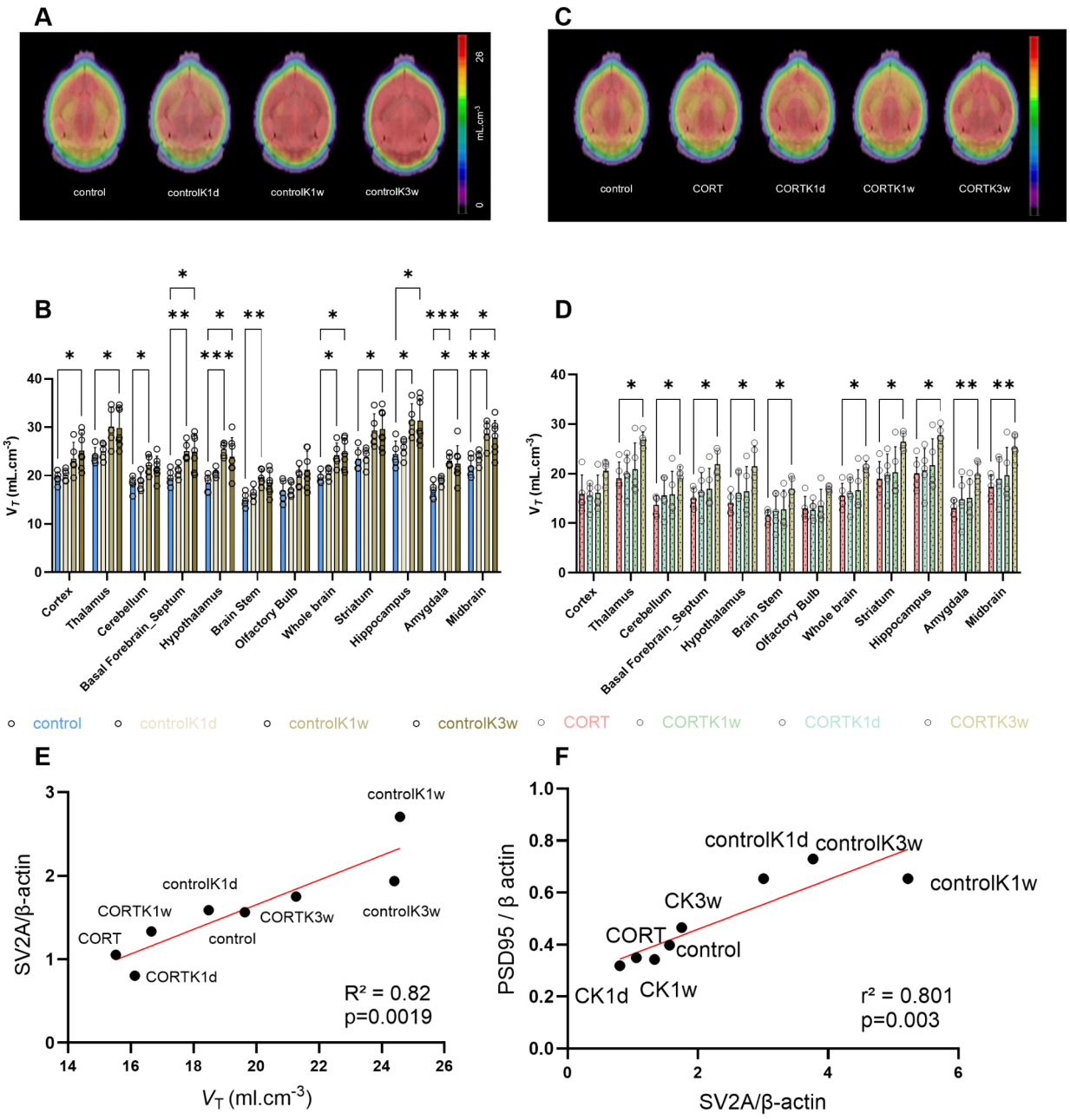
Neuronal excitability dysfunction is a key feature of neurodevelopmental disorders like epilepsy. Understanding the underlying mechanisms associated with this dysfunction is crucial for developing therapeutic strategies. Excitability is regulated by ion channels, including M-type (Kv7) channels, which are low-threshold, slowly activating potassium channels that modulate neuronal activity and network function. Due to their role in seizure suppression, M-channels are an important drug target; however, available M-channel activators have very adverse side effects and have been withdrawn from clinical use. An alternative approach is to target channel interactors to modulate their activity indirectly and bypass the side effects.
M-channels are tetrameric and assemble in the brain as Kv7.2 homomers or Kv7.2/Kv7.3 heteromers. We identified a novel Kv7.2 interactor, Stargazin, a TARP (Transmembrane AMPA receptor regulatory protein) family member essential for AMPA receptor regulation and fast excitatory neurotransmission. Our previous studies demonstrated that Stargazin affects M-currents. Here, we developed new tools to investigate this interaction further and assess whether other TARPs regulate Kv7.2 channels.
Using SplitFAST, a fluorescence complementation assay that allows the visualization of protein-protein interactions in live cells, we confirmed the Stargazin-Kv7.2 interaction and showed its regulation upon cellular depolarization and PKC activity. Our findings also revealed that additional TARP family members interact with and differentially regulate Kv7.2.
Overall, this study provides strong evidence of the Stargazin-Kv7.2 interaction and identifies TARPs as bona-fide auxiliary subunits of Kv7.2. The SplitFAST assay enabled real-time analysis, offering spatial-temporal insights. These findings lay the foundation for exploring modulators targeting newly characterized Kv7.2 regulatory mechanisms.

**Keyword 1**: Kv7.2
**Keyword 2**: TARPs
**Keyword 3**: Excitability
**Keyword 4**: PPIs
**Keyword 5**: SplitFAST

**DYNAMICS OF THE EFFECTS OF KETAMINE ON SYNAPTIC DENSITY: AN SV2A PET STUDY IN A MOUSE MODEL OF DEPRESSION**

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R,S-ketamine is now prescribe to relief depressive symptoms in patients with major depressive disorders. Although, its mechanism of action remains poorly describe, it seems to modulate synaptic density. In healthy (control) and corticosterone-treated (CORT, 35mg/mL) male mice, ketamine (10mg/kg) was injected (i.p) either 24h (K1d; CORTK1d), one (K1w; CORTK1w) or three (K3w; CORTK3w) weeks before a PET imaging Brain [$^{11}$C]UCB-J microPET acquisition was performed under anesthesia. Volumes of distribution ($V_T$, Logan method) were determined to estimate the brain distribution of [$^{11}$C]UCB-J using the time activity curve extracted from brain region and left heart ventricle. Western blots were performed to measure brain protein expression of SV2A and PSD95 as markers of synaptic density.
$V_T$ was decreased (whole brain; $p<0.05$) in the CORT group compared with the control group. $V_T$ did not change in CORTK1d or K1d group. $V_T$ increased in the cortex, thalamus, striatum, and cerebellum ($p<0.05$) of the K1w group, but not in the CORTK1w group. $V_T$ increased in both the K3w and CORTK3w groups. Interestingly, 3 weeks of ketamine significantly increased [$^{11}$C]UCB-J $V_T$ by 26.5±3.7% in control mice ($p<0.001$) and 41.6±7.5% in CORT mice ($p<0.05$) . Western blot results were very well correlated with $V_T$ obtained in PET. No differences were found in PSD95 but it was strongly correlated with SV2A protein level.
CORT-induced deficiency in SV2A expression alters the dynamics and intensity of the effect of ketamine in the brain. These are the first results showing a correlation between in vivo [$^{11}$C]UCB-J $V_T$ and ex vivo SV2A.

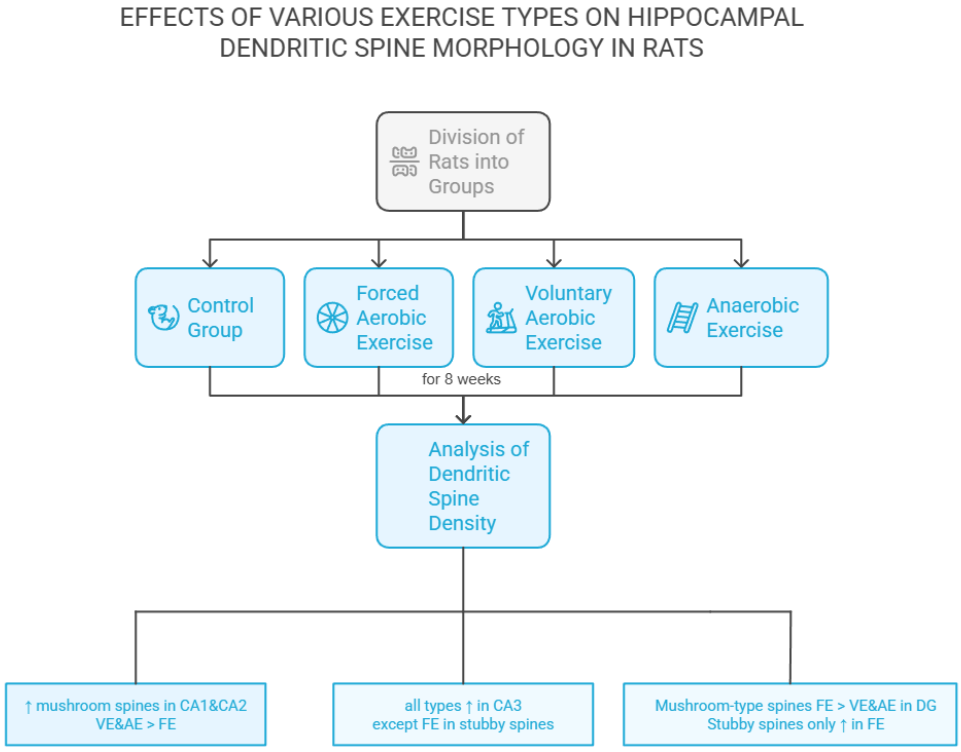



**Keyword 1**: PET imaging
**Keyword 2**: psychiatry
**Keyword 3**: major depressive disorders
**Keyword 4**: SV2A
**Keyword 5**: synaptic density

**EFFECTS OF VARIOUS EXERCISE TYPES ON HIPPOCAMPAL DENDRITIC SPINE MORPHOLOGY IN RATS**

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While it is well-established that physical exercise (PE) positively impacts neuronal proliferation, growth, and synaptic plasticity, the specific effects of different exercise modalities on hippocampal dendritic morphology remain incompletely understood. In the present study, we divided 32 adult rats into four groups: (1) control group (C) housed in a standard environment; (2) forced aerobic exercise group (FE), performed forced aerobic exercise on a rotarod machine; (3) voluntary aerobic exercise group (VE), voluntarily performed aerobic exercise on a running wheel in their home cage; (4) anaerobic exercise group (AE), performed weighted ladder climbing. The exercise protocols were 8 weeks long. Following Golgi-Cox histochemistry, we analyzed dendritic spine density and morphology in the hippocampus. While each PE paradigm had an overall positive impact on spine density, significant differences were present for subfields and spine types. In CA1 and CA2, only mushroom-type spines were increased in PE groups, though this increase was more prominent in VE and AE ($p<0.001$) compared to FE ($p<0.05$). In CA3, except FE in stubby spines, all paradigms had increased density for mushroom, thin, and stubby spines ($p<0.001$). Interestingly, in the dentate gyrus, FE had the most significant increase in the mushroom spine and the only increase in stubby spines, contrasting other subfields ($p<0.001$). Overall, our data suggests that while physical exercise positively impacts hippocampal spine density, this effect varies remarkably between the subfields and spine types.




**Keyword 1**: Physical Exercise
**Keyword 2**: Hippocampus

**PHOSPHORYLATION OF PLPPR3 MEMBRANE PROTEINS AS SIGNALING INTEGRATOR AT CNS SYNAPSES**

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The growth of neuronal processes play an essential role in establishing neuronal circuits. Phospholipid-phosphatase related protein 3 (PLPPR3, previously known as Plasticity Related Gene 2 or PRG2) belongs to a family of transmembrane proteins is highly expressed during neuronal development, it regulates critical axonal growth processes during guidance, filopodia formation and branching. Prior work established crucial functions of PLPPR3 in axon guidance, filopodia formation and axon branching. Through the inhibition of PTEN PLPPR3 directs neuronal growth to branches and accelerates actin based filopodia formation even in the absence well known nucleators such as ARP2/3 or ENA/VASP. However, little is known regarding the signaling events regulating PLPPR3 function. We identify here 26 high-confidence phosphorylation sites in the intracellular domain of PLPPR3 using mass spectrometry. Biochemical characterization established one of these – S351 – as a bona fide phosphorylation site of PKA. Experiments in neuronal cell lines suggest that phosphorylation of S351 does not regulate filopodia formation. Instead, it regulates binding to BASP1, a signaling molecule previously implicated in axonal growth and regeneration. Using neuronal imaging and cutting-edge analysis methods we show that both PLPPR3 intracellular domain and BASP1 enrich in presynapses in primary neurons. Additionally, live neuron activity imaging experiments suggest that PKA-dependent S351 phosphorylation of PLPPR3 controls neurotransmitter release. Based on these findings we propose that a PLPPR3-BASP1 complex under the control of PKA activity is a novel signaling integrator of presynaptic activity in neuronal synapses.

**Keyword 1**: Presynapse
**Keyword 2**: Membrane protein
**Keyword 3**: Phosphorylation
**Keyword 4**: Neuronal development
**Keyword 5**: Filopodia formation

**COPPER TRANSPORTER 1 CONTRIBUTES TO OLFACTORY PATHWAY IN MICE**

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*[3]University of Maryland, Department of Animal and Avian Sciences, Maryland, United States*

Transition metal ions, including Cu, Zn, and Fe, are essential in various biological processes, including neurotransmitter coordination, protein conformational and structural changes, and the generation of reactive oxygen species. Despite extensive research on transition metal transporters, the potential role of copper transporters in brain function remain poorly understood. This study aimed to analyze behavioral, histological, and molecular in the olfactory bulb (OB) of changes in copper-deficient diet and copper transporter 1 (CTR1) conditional knockout (cKO) mice. Both models exhibited olfactory impairments in odor detection and discrimination. Golgi staining indicated morphological changes in OB granule cells of CTR1 cKO mice, correlated with a decrease in the expression of synaptic plasticity-related proteins, including pERK, c-fos, Arc, synapsin, and PSD-95. Interestingly, RNA sequencing identified significant alterations in multiple genes associated with mitochondrial complexes in the OB of CTR1 cKO mice. Furthermore, transmission electron microscopy revealed the existence of abnormal mitochondria. The deletion of CTR1 resulted in alterations in mitochondrial function in the OB, characterized by decreased expression of oxidative phosphorylation proteins, particularly mitochondrial complex IV, and a reduction in the oxygen consumption rate. Our findings suggest that reduced neuroplasticity and abnormal mitochondria resulting from CTR1 deficiency contribute to olfactory dysfunction.

**Keyword 1**: copper
**Keyword 2**: mitochondria
**Keyword 3**: neuroplasticity
**Keyword 4**: olfactory function

**MICE EXPERIENCING MILD CHRONIC STRESS EXHIBIT A REDUCED LEVEL OF CYTOPLASMIC HDA4 IN THE NUCLEUS ACCUMBENS: IMPLICATIONS FOR GLUTAMATERGIC SYNAPTIC TRANSMISSION**

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The histone deacetylase 4 (HDAC4), able to target histone and non-histone proteins, has gained substantial interest in translational neuroscience because its alteration has been observed in several neuropsychiatric diseases. However, the molecular and cellular mechanisms by which HDAC4 contributes to neuroadaptations associated with depression-like behaviors are unknown. Here, we aimed at investigating whether HDAC4 dysfunction in the nucleus accumbens (NAc) impacts synaptic neuroadaptation in the chronic unpredictable mild stress (CUMS) mouse model of depression.
We found decreased levels of HDAC4 in the NAc of CUMS mice in total lysates and at the level of synaptosomes. This reduction did not depend on HDAC4 delocalization to the nucleus but was induced by GSK3β-dependent phosphorylation of HDAC4 and its targeting to degradation. Indeed, GSK3β inhibition (CT-99021) or treatment with the proteosome inhibitor MG132 reversed HDAC4 degradation. Decreased HDAC4 in NAc of CUMS mice was paralleled by reduced levels of synapsin I at the presynaptic compartment and by altered AMPA phosphorylation at the postsynaptic density regions.
To correlate these data directly to HDAC4 function, we inhibited its activity with the selective inhibitor LMK235 and evaluated synaptic transmission within the NAc by whole-cell patch-clamp recordings. We found that the peak amplitudes and frequencies of miniature excitatory postsynaptic currents (mEPSCs) were significantly decreased compared to the control, while there was no significant difference in mEPSC kinetics. These results suggest that CUMS-dependent downregulation of HDAC4 impairs glutamatergic synaptic transmission in the NAc.

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**Keyword 1**: HDAC4
**Keyword 2**: Synaptic transmission
**Keyword 3**: Nucleus accumbens
**Keyword 4**: Chronic stress

**UNEXPECTED ROLE OF REST/NRSF IN SUPPORTING NMDA RECEPTORS AND SYNAPTIC STRENGTH**

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Molecular studies of gene expression during long-lasting plasticity initially focused on the identification of positive gene regulators, but increasing evidence revealed that the fine tuning of synaptic plasticity also requires activity-dependent activation of silencing transcription factors. Recent studies suggest that the neuron-restrictive silencer factor (NRSF), also known as REST (Repressor Element-1 Silencing Transcription Factor), plays a key role in maintaining memory function and synaptic plasticity during aging (Lu et al., 2014; Zullo et al., 2019). REST suppresses several neuronal genes, including grin2b (Rodenas-Ruano et al., 2012), which encodes a subunit of NMDA receptor.
To explore REST's impact on NMDAR function, we used electrophysiology and $Ca^{2+}$ imaging in hippocampal neurons. Surprisingly, REST inhibition for 48 hours via a decoy oligodeoxynucleotide (ODN) — which sequesters REST from its genomic targets — resulted in a decrease in NMDA-dependent current density and $Ca^{2+}$ waves following NMDA perfusion, suggesting an unexpected role for REST in supporting both synaptic and extra-synaptic NMDARs function or expression. Additionally, a reduction in electrically evoked NMDA-dependent excitatory post-synaptic currents and synaptic $Ca^{2+}$ waves indicated a specific positive effect of REST on synaptic NMDA Rs. These findings suggest that REST may contribute to hippocampal long-term potentiation, a hypothesis we intend explore soon. Given its role in memory maintenance and synaptic plasticity during aging (Aron et al., 2023), as well as cognitive protection in Alzheimer's disease models (Yang et al., *2023)*, REST may represent a novel target for neuroprotective strategies.

**Keyword 1**: REST/NRSF
**Keyword 2**: NMDA Receptor
**Keyword 3**: electrophisiology

**EXPRESSION, SUBCELLULAR LOCALIZATION, AND FUNCTIONAL ANALYSIS OF INTELLECTUAL DISABILITY SYNDROME PROTEIN ABBA**

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A missense mutation in *MTSS2*, a human gene encoding ABBA, was found to cause an intellectual disability syndrome. Experimental evidence suggests that the mutated protein is more harmful when expressed than when absent. Thus, to better understand the harm of *MTSS2* mutation, it is important to know which cells express ABBA and what are ABBA´s functions in these cells to get insights into which cells and processes might be dysfunctional in mutation-carrying patients. We investigated age-, brain region-, and cell type-specific expression of ABBA in the mouse brain. We found that ABBA was expressed in GABAergic inhibitory neurons, such as parvalbumin-positive interneurons in the hippocampus or Purkinje cells in the cerebellum. ABBA was also expressed in radial glial cells, as shown before. At the subcellular level, ABBA localizes to the edges of membrane protrusions, suggesting a role in cell migration and spinogenesis. ABBA colocalizes with PIP2 and both active and inactive Rac1. We showed that ABBA enhances actin dynamics, particularly by enhancing actin depolymerization. Overexpression of ABBA in pyramidal excitatory and inhibitory neurons increased dendritic spine density. In hippocampal neurons, this effect required Arp2/3 complex activity. Against our expectations, the expression of inactive or active Rac1 further increased spine density in ABBA-expressing pyramidal neurons. Our findings provide insights into ABBA's expression, localization, and functional mechanisms, advancing our understanding of its role in neurodevelopmental processes and disorders. Based on our results, we would expect that disease mutation in *MTSS2* would mostly affect inhibitory neurons and radial glial cells.

**EFFECTS OF PSYCHEDELICS AND CLASSICAL ANTIDEPRESSANTS ON TRKB DIMERIZATION, SPINOGENESIS AND NEUROTROPHIC SIGNALING**

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Accumulating evidence indicates that the plasticity-inducing and therapeutic effects of conventional antidepressant drugs are mediated by their direct binding to TrkB. It has additionally been reported that LSD and other psychedelic compounds also bind to TrkB with higher affinity.
In this study, we investigated whether psychedelics and classical antidepressants compounds promote TrkB dimerization and neurotrophic signaling in a differential manner. The effects of psychedelics on the TrkB dimerization dynamics, spinogenesis, and neurotrophic signaling associated with plasticity were studied treating N2a cells and primary cortical neuronal cultures with LSD and fluoxetine. Dimerization of the TrkB receptor in the presence of compounds is assayed by protein-fragment complementation assay (PCA). N2a cells, transiently transfected with TrkB constructs C-terminally tagged with either half of Gaussian luciferase, were treated for 40 minutes. Primary cortical cultures (DIV21) were treated with fluoxetine (10μM) or LSD (100nM) overnight or for 1 hour. Spine formation in hippocampal neurons after treatment was determined. The phosphorylation state of the neuronal TrkB receptor in different tyrosines was checked as marker of its activation by Western blot. These experiments showed a significant increase on spine count and phosphorylation in TrkB Y816 after the treatments except for fluoxetine treatment.
These results support that LSD binds to TrkB and act as a potent TrkB signaling promoting agent. The study provides further evidence that TrkB is a critical mediator of the effects of psychedelics and conventional antidepressants on neurotrophic signaling and neuroplasticity. These assays could enable testing other potential antidepressants for their effects on TrkB signaling.

**Keyword 1**: TrkB
**Keyword 2**: Psychedelics
**Keyword 3**: Antidepressants
**Keyword 4**: Plasticity

**ENVIRONMENTAL ENRICHMENT AND NEUROPLASTICITY-RELATED BEHAVIOURS**

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Environmental enrichment (EE) is the external stimulation by physical and social surroundings. Like antidepressants, EE promotes neuronal plasticity in the brain, although the exact mechanisms of action are unknown. The aim of the study was to further elucidate the role of BDNF-TrkB in the behavioural outcomes of EE exposure, as well as to assess the effect of EE in conjunction with the antidepressant fluoxetine. The effect of long term environmental enrichment on neuroplasticity was investigated in female wildtype and BDNF-het mutant mice, with chronic fluoxetine treatment in drinking water. The behavioural tests, open field, elevated plus maze, novel object recognition and fear conditioning, were conducted to assess exploratory behaviour as well as memory and learning. EE had a BDNF-dependent effect on exploratory behaviour in the open field and BDNF-hets demonstrated reduced extinction in fear conditioning, which was further emphasised by EE. These results indicate that the BDNF-TrkB pathway plays a role in generating behavioural effects of EE, and further elucidate the molecular mechanisms behind EE effect on plasticity. Studies understanding the role of different components of EE as well as the different paradigms are needed to further understand the mechanisms behind the EE effect.

**Keyword 1**: Neuroplasticity
**Keyword 2**: Mouse behaviour
**Keyword 3**: Environmental enrichment
**Keyword 4**: Anxiety-like behaviour
**Keyword 5**: Memory and learning

**IMPACT OF DIVERSE ANTIDEPRESSANTS AND VOLUNTARY EXERCISE THROUGH TRKB ON VISUAL PLASTICITY IN ADULT MICE**

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Depression is a severe mood disorder that significantly disrupts neural plasticity. Both pharmacological treatments and environmental interventions have been shown to promote brain plasticity, with the brain-derived neurotrophic factor (BDNF) and its receptor TRKB serving as key elements in this process. The ocular dominance plasticity (ODP) model is a well-established tool for investigating plasticity, wherein the forced closure of one eye induces a shift in ocular dominance during a specific early post-natal period, the critical period. Notably, both pharmacological and environmental strategies can reactivate ODP in adult mice. Recent studies have reported a cholesterol-recognition motif (CARC) within the transmembrane region of TRKB, clarifying its interactions with cholesterol and antidepressant drugs. We assessed ODP by performing optical imaging of intrinsic signals on female wild-type (wt) mice and heterozygous mice possessing the Y433F mutation within the TRKB.CARC motif (TRKB.het). Our findings show that subanaesthetic doses of ketamine and its active derivative, 2R,6R-hydroxynorketamine (HNK), facilitate ODP in adult mice, thus aligning with previous research. In TRKB.het mice, the Y433F mutation disrupts the ODP induced by fluoxetine and HNK treatment, while the effects of voluntary exercise on ODP are preserved. Overall, our results highlight the significant influence of the Y433F mutation in TRKB.CARC on visual plasticity specifically modulated by antidepressants, but not voluntary exercise.

**Keyword 1**: TRKB
**Keyword 2**: Visual plasticity
**Keyword 3**: Antidepressants
**Keyword 4**: Voluntary exercise

**NEURAL PLASTICITY IN SOMATOSTATIN-EXPRESSING INTERNEURONS TO SUPPRESS COCAINESEEKING BEHAVIOUR**

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Addiction is a highly debilitating condition consisting of compulsive self-administration and seek for the substance of abuse, and its most challenging feature is the high rate of relapse. Addiction and relapse share similarities with a brain process called neural plasticity which acts through the Brain-Derived Neurotrophic Factor and its receptor TrkB. Moreover, Somatostatin (SST) expressing interneurons are involved in neuronal plasticity and are important in modulating cocaine-seeking
behaviour in mice. We tested the role of TrkB in Somatostatin (SST)-expressing neurons in the formation and extinction of cocaine-seeking behaviour, using mice in which TrkB has been knocked out specifically in SST neurons. Using a conditioned-place preference paradigm, we observed that in the transgenic mice, cocaine conditioning is slightly reduced and its extinction through behavioural training is impaired, showing how the acquisition of a cocaine-seeking behaviour and its extinction rely on neural plasticity in SST neurons. We then activated plasticity using a light-activable TrkB in SST neurons in the prefrontal cortex (area involved in the cocaine-seeking suppression) of cocaine-conditioned mice, during extinction training, and we managed to promote local neural plasticity and prevent relapse of cocaine-seeking in the mouse model. Identifying the network behind the extinction of reward-seeking behaviour and the role of synaptic plasticity will shed light on targets for new treatments to prevent relapse and improve the outcomes of addiction treatments.

**Keyword 1**: Plasticity
**Keyword 2**: TrkB
**Keyword 3**: Somatostatin
**Keyword 4**: Addiction
**Keyword 5**: Cocaine

**INTERACTION OF BDNF AND GLUCOCORTICOIDS SIGNALING IN MODULATING NEUROPLASTICITY**

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The synergistic effects on neuronal plasticity of acute glucocorticoids levels increase and the BDNF-TRKB signaling indicate a deep interconnection between the two pathways. Moreover, chronic stress with elevated glucocorticoids levels and downregulation of TRKB signaling associated with reduced BDNF are both involved in the pathophysiology of different psychiatric disorders. In this study we investigated in vitro the molecular mechanisms underlying the interplay of glucocorticoids and TRKB signaling. While glucocorticoids can enhance TRKB activity under certain physiological conditions, chronic stress or prolonged glucocorticoid exposure suppresses BDNF-TRKB signaling, leading to detrimental effects on neuroplasticity, mood regulation, and cognitive functions. However, the mechanism by which TRKB and glucocorticoid receptors are recruited together in the modulation of neuronal plasticity is not currently known.
In this study we investigated in vitro the molecular mechanisms underlying the interplay of glucocorticoids and TRKB signaling. We found that the glucocorticoid receptor physically interacts with TRKB, modulating its dimerization and activity both in presence and in absence of glucocorticoids. Additionally, the transmembrane domain of TRKB is important for the interaction and for an effective signaling of the receptor, suggesting an overlap between the two signaling pathway.
These results shed light on the interconnected effects of glucocorticoids and TRKB signaling both in physiological and in pathological condition, highlighting the need for a more comprehensive understanding of the role and the dysfunction of different cellular players contributing to synaptic plasticity.

**Keyword 1**: TRKB
**Keyword 2**: glucocorticoid receptor
**Keyword 3**: neuropsychiatric disorders
**Keyword 4**: BDNF

**IMAGING OF BRAIN PLASTICITY USING FUNCTIONAL ULTRASOUND**

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Functional ultrasound imaging (fUS) is an emerging neuroimaging modality with high sensitivity and spatiotemporal resolution, making it a promising tool for studying brain activity. This study aimed to validate fUS for investigating plasticity in the visual cortex of adult mice, a model commonly used to understand neural network reorganization in response to sensory changes. Additionally, the effects of two anesthetics, medetomidine and isoflurane, on plasticity were evaluated. Monocular deprivation (MD), achieved by closing one eye for four days, was used to assess visual cortex plasticity. In adult mice, MD alone is insufficient to trigger plasticity due to the closure of the critical period. fUS imaging was performed on control and MD groups under medetomidine (1.0 mg/kg) or isoflurane (1.8% 15 minutes for induction and 1.2 % for maintenance) anesthesia, using flickering light stimulation. Immunohistochemical analysis of parvalbumin (PV) interneurons and perineuronal nets (PNNs) was conducted to examine potential cellular changes accompanying functional plasticity.
The results indicate that medetomidine reactivates juvenile-like plasticity in the adult visual cortex, as reflected by a shift in the ocular dominance index (ODI), while isoflurane did not induce plasticity. No significant changes were observed in PV interneuron or PNN density for either anesthetic. These findings suggest that medetomidine-induced plasticity is independent of PV or PNN alterations. This study highlights fUS as a valuable tool for non-invasive monitoring of neural plasticity and provides new insights into how anesthetics influence brain function at the network level.

**Keyword 1**: Functional Ultrasound
**Keyword 2**: Neuronal plasticity
**Keyword 3**: Induced plasticity

**THE SERINE-THREONINE KINASE NDR2 IMPAIRS SPATIAL MEMORY THROUGH AN INTERPLAY OF AUTOPHAGY AND PROTEOSTASIS ALTERATIONS IN THE AGEING HIPPOCAMPUS**

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Aging evolves through the natural selection of a few genes with antagonistic pleiotropic features; these genes benefit early life but contribute to decline in later stages by affecting a wide range of processes. In the brain, these include gradual cognitive decline, altered neuronal connectivity, changes in synaptic activity, disruptions in proteostasis and autophagy, and increased neuroinflammation, among others. However, only a few genes that affect such a broad range of features in the brain have been identified, making them valuable targets for therapeutic interventions aimed at alleviating cognitive aging.

Ndr2 is a serine-threonine kinase involved in numerous neuronal functions. Previous work from our group demonstrated that Ndr2 participates in spatial memory, long-term potentiation, and spine formation. Moreover, Ndr2 also regulates autophagy, cell cycle regulation, inflammation, and intercellular communication. This suggests that Ndr2 may be an important factor in aging, particularly in the central nervous system.

In this study, we demonstrate that knocking down Ndr2 in aged mice impairs hippocampal autophagic flux, prevents age-related memory decline, and induces changes in the hippocampal proteome. We show that Ndr2 drives neuronal dysfunction through two main processes by increasing neuroinflammation and disrupting synaptic maintenance. Mechanistically, we show that Ndr2 increases autophagosome build-up at synapses and alters autophagic-lysosomal integrity. A meta-analysis revealed that Ndr2 favours the upregulation of a protein network associated with age-related and pathological cognitive decline.

These findings show that Ndr2 is an important modulator of brain aging, positioning it as a promising target for interventions aimed at slowing age-related neurodegeneration.

**Keyword 1**: Ageing
**Keyword 2**: Autophagy
**Keyword 3**: Neuroinflammation
**Keyword 4**: Proteostasis
**Keyword 5**: Cognition

**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.

**Keyword 1**: 5-HT7 receptor signaling
**Keyword 2**: AMPK activation
**Keyword 3**: NMDAR trafficking
**Keyword 4**: Synaptic plasticity
**Keyword 5**: Palmitoylation

**PROTEIN TRANSPORT FROM SYNAPSE-TO-NUCLEUS IS ESSENTIAL FOR LONG-LASTING ACTIVATION OF CREB FOLLOWING INDUCTION OF SYNAPTIC PLASTICITY**

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Neuronal plasticity is a tightly regulated cellular mechanism that is essential for the formation of memory. A complex interplay of signaling pathways and molecular players orchestrate the activity of the transcription factor CREB, a central driver of neuronal plasticity, during distinct phases and on different timescales. While the induction of long-term potentiation causes an initial upregulation of CREB activity through electrochemical signaling, we show that the maintenance of its activity critically depends on another type of signaling that is mediated by the messenger protein Jacob, which translocates from dendritic spines to the nucleus via the molecular motor dynein.
As hub for protein interaction, Jacob forms a signalosome with other proteins. We show that a trimeric complex of Jacob with importin-α and –β constitutes the core of the signalosome. It couples the complex to dynein and grants a high degree of stability which is critical to protect it from disintegration during the translocation process. Importantly, the signalosome also contains the kinase (p)ERK which, once the complex reaches the nucleus, maintains the phosphorylation and activity of CREB to drive neuronal plasticity. These processes are critical for the formation of memory and the underlying neuronal connectivity and engrams.
Intriguingly, only those spines that contain the whole molecular machinery for the formation of the signalosome may participate in this form of signaling. Therefore, we predict the existence of a distinct set of spines that play an instrumental role in synapse-to-nucleus signaling and subsequent gene expression.

**Keyword 1**: Plasticity
**Keyword 2**: Synapse
**Keyword 3**: CREB
**Keyword 4**: Transport
**Keyword 5**: Jacob/NSMF

**INOSITOL 1,4,5-TRISPHOSPHATE RECEPTOR TYPE 1 (IP 3 R1) AND CALCIUM IN SYNAPTIC VESICLES**

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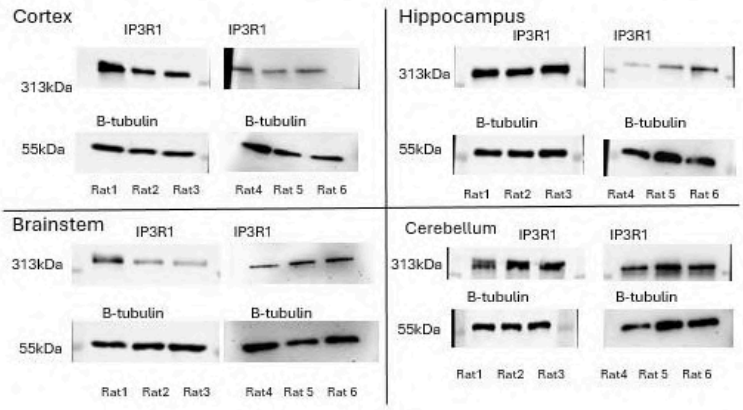
Neurotransmitter release depends on the fusion of synaptic vesicles with the plasma membrane, a process tightly regulated by intracellular calcium dynamics. Synaptic vesicles are a heterogeneous population of organelles, categorized into functionally distinct pools based on their release probability and response to stimulation. While voltage-gated calcium channels (VGCCs) are the primary mediators of calcium influx, we suggest that synaptic vesicles themselves may act as calcium-sequestering organelles, analogous to the smooth endoplasmic reticulum (sER). However, the physiological relevance of intravesicular calcium and its potential release mechanisms remain unclear.
We hypothesize that inositol 1,4,5-triphosphate receptor type 1 (IP3R1), a well-characterized calcium-release channel in the smooth endoplasmic reticulum (sER), is expressed on a subset of synaptic vesicles, where it regulates neurotransmitter release by modulating intravesicular calcium efflux. To investigate this, we employed a combination of biochemical assays, imaging modalities, and electrophysiological recordings. Synaptic vesicle fractions were isolated via subcellular fractionation, with their purity verified through electron microscopy. The presence of IP3R1 was examined using immunoprecipitation and western blotting. The highest concentrations were found in the cerebellum. Additionally, calcium imaging using confocal microscopy and field potential recordings (fEPSP) were performed in wild-type and heterozygous IP3R1 knockout mice to evaluate its functional role in synaptic plasticity.

Preliminary findings indicate that IP3R1 is present in synaptic vesicle fractions and co-localizes with vesicular markers. Functional analyses suggest that IP3R1-mediated calcium release influences synaptic efficacy. These findings provide novel insights into an alternative calcium source within presynaptic terminals, with potential implications for synaptic plasticity and neurological disorders.

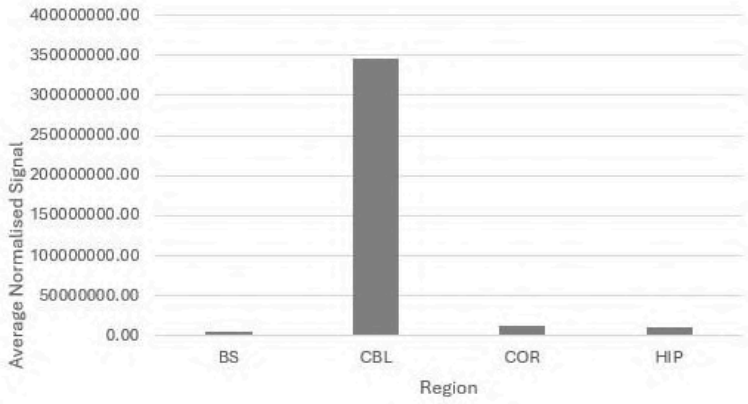
**Keyword 1**: Synaptic vesicles
**Keyword 2**: IP3R1
**Keyword 3**: Calcium signaling
**Keyword 4**: Synaptic plasticity
**Keyword 5**: Neurotransmittor release

Regional distribution of IP3R1: A quantitative, comparative investigation of the distribution of IP3R1 within the hippocampus, cerebellum, cortex and brainstem.

**A**




**B**



**TIPPING THE SCALES: HOW DIFFERENTIAL SYNAPTIC VULNERABILITY TO AMYLOID-BETA OLIGOMERS MAY UNDERLIE NETWORK DISRUPTIONS DURING EARLY ALZHEIMER'S DISEASE**

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Early Alzheimer's disease (AD) is marked by a gradual accumulation of amyloid-β oligomers (AβOs) that disrupt synaptic function and subsequently disturb the excitation/inhibition (E/I) balance in neuronal networks. This occurs at preclinical stages of AD, prior to the manifestation of cognitive impairment, yet the early molecular and cellular mechanisms leading to this disruption remain unknown.
Our previous work demonstrated that 24-hour exposure to AβOs in hippocampal slices impair synaptic inputs at GABAergic synapses, prominently at dendritic inhibitory synapses. To investigate the differential vulnerability of synapses to AβOs, we now employ advanced imaging and live synaptotagmin-1 antibody uptake assays in primary hippocampal neurons. We found that AβO exposure triggers overall hyperexcitability and specifically disrupts synaptic vesicle (SV) turnover at inhibitory boutons. To expand these findings, we are currently using live-cell imaging and genetically encoded sensors for glutamate (iGluSnFR3) and GABA (iGABASnFR2). Thus far, we observe that AβO-induced synaptic dysfunction leads to hyperactivity, hypersynchronicity of networks, and increased spatial correlation of hyperactive and synchronous synapses implying that AβO-induced synaptic defects may contribute to the overall loss of network E/I balance.
To assess whether selective AβO binding could explain the observed vulnerability of GABAergic synapses, we performed immunostainings for AβOs. Surprisingly, AβOs do not bind to GABAergic neurons; instead, they cluster at synaptic and extra-synaptic locations on the membranes of glutamatergic neurons. Our results suggest that in early AD, AβOs accumulate on excitatory neurons, thereby triggering signaling events that lead to the impairment of GABA release at inhibitory synapses.

**Keyword 1**: Live cell imaging
**Keyword 2**: Glutamate sensors
**Keyword 3**: Inhibitory synapses
**Keyword 4**: Excitatory synapses
**Keyword 5**: GABA sensors

**PIANP CONTROLS GABA$_B$ RECEPTOR LOCALIZATION VIA ITS SUSHI-DOMAIN IN CEREBELLAR PURKINJE CELLS TO REGULATE DENDRITOGENESIS, POSTNATAL SYNAPTIC DEVELOPMENT AND AUTISM-LIKE BEHAVIOUR**

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The Autism-spectrum disorder (ASD) is a multicausal medical condition that manifests with a broad phenotypic and symptomatic spectrum. Cerebellar dysfunction is strongly implicated with ASD-development and an imbalance of glutamatergic/GABAergic neurotransmission has been proposed as common cellular phenotype. Previously, we studied the PILRα associated neural protein (*PIANP*) gene, the chromosomal region of which has been highlighted in a meta-analysis from a cohort of patients with developmental delay and ASD. We showed that global loss-of *Pianp* in mice resulted in a salient ASD-like behavioral phenotype. Here, we focused on the molecular and cellular mechanisms by which PIANP in Purkinje cells (PCs) controls neuronal morphogenesis and mouse behavior. Using PC-specific knockout (KO) models and viral vectors for the overexpression (OE) of PIANP in murine PCs, we show that both, decreased and increased PIANP expression, reduced dendritic morphogenesis *in vivo*. In contrast, PC distal spine density was increased in global and PC-specific KO animals but decreased after PIANP-OE. Our data suggest that PIANP regulates spine stability through trans-interaction and recruitment of GBR-subunit GB1a to PC dendrites, through PIANPs sushi-binding domain (SBD). On this end, OE of PIANP, but not a SBD-deficient variant, in PCs led to ASD-like behavioral changes, too. In summary, we identified PIANP-mediated interactions such as with GB1a in PCs as modulator of dendritic and synaptic functionality, the dysregulation of which promote ASD-like behavioral changes. The molecular mechanisms revealed by our findings may open new avenues for therapeutic approaches to target glutamatergic/GABAergic neurotransmission in the context of ASD.

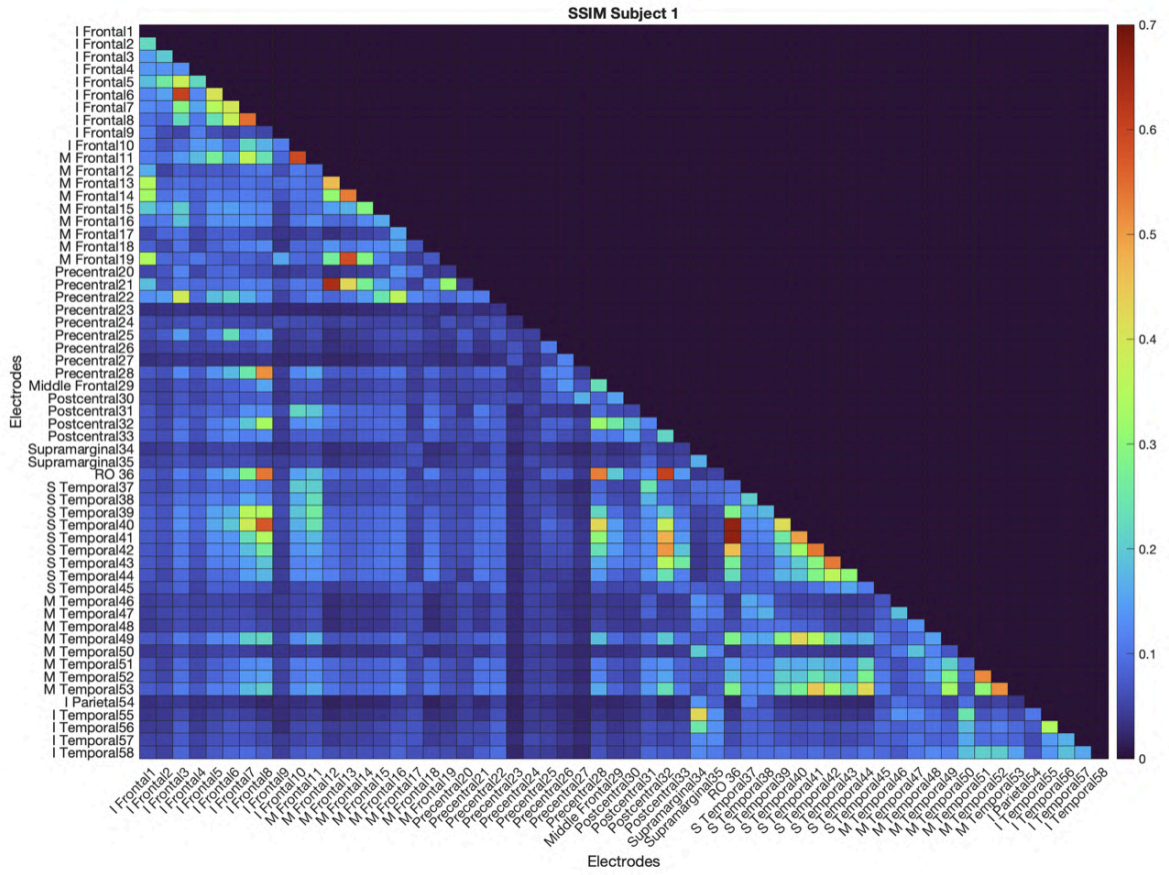
**Keyword 1**: Purkinje cell
**Keyword 2**: cerebellar development
**Keyword 3**: GABAB receptor
**Keyword 4**: synaptogenesis

**HOMEOSTASIS OF SLEEP: EXPLORING HUMAN BRAIN OSCILLATIONS THROUGH ELECTROCORTICOGRAPHY**

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The regional differences in oscillatory activity during human sleep remain largely unexplored. Sleep is commonly perceived as a universal process that uniformly affects the entire brain. However, emerging evidence suggests that sleep regulation may occur at a more localized level. This study aims to investigate the dynamics of brain oscillations throughout the night using electrocorticography (ECoG) in frontal, temporal, and parietal regions.
Intracranial recordings were obtained from eight patients with intractable epilepsy who underwent pre-surgical monitoring with implanted subdural ECoG electrodes. Patients were recruited from UC Christus Hospital in Santiago, Chile. Electrode placement was determined solely by clinical considerations, and all procedures were approved by the local ethics committee.
Results show that slow-wave activity (SWA, 0.5–4 Hz) exhibits higher power during the first part of the night, after wakefulness, and progressively decreases across the brain as the night advances, reflecting sleep homeostasis. A detailed electrode-level analysis revealed differences in sleep homeostasis and spectral patterns, assessed using structural similarity analysis (SSIM). The similarity index between electrodes was low in ECoG subjects, with an average of 0.0606, indicating significant spectral power variability among electrodes, even in anatomically adjacent regions. This highlights the heterogeneity of brain activity during sleep.
These findings provide new insights into the regional specificity of sleep homeostasis in the human brain, emphasizing the complex and heterogeneous nature of brain oscillations during sleep.

**Keyword 1**: Sleep
**Keyword 2**: Sleep homeostasis
**Keyword 3**: epilepsy
**Keyword 4**: slow waves
**Keyword 5**: spindles


SSIM Subject 1
Electrodes
Electrodes
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0

**ALTERED SYNAPTIC ACTIVITY IN PRIMARY HIPPOCAMPAL NEURONS OF *SHANK3*-DEFICIENT AND VALPROATE-INDUCED AUTISM MODELS**

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Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterised by synaptic dysfunction leading to impairments of neuronal communication and network plasticity. Current ASD research utilises both genetic and environmental animal models to investigate its pathophysiology. The monogenic *Shank3-deficient* mouse model with disrupted synaptic scaffolding protein mimics Phelan-McDermid syndrome showing autistic phenotype, while the environmental valproate (VPA)-induced model based on prenatal exposure to valproic acid, leads to neurodevelopmental abnormalities associated with autistic symptomatology. Despite different aetiology, both models exhibit distinct but overlapping synaptic alterations such as impaired excitatory-inhibitory balance, postsynaptic density organization, impaired glutamatergic signalling and altered synaptic plasticity. We aimed to compare the synaptic activity changes in both models, focusing on inhibitory GABAergic activity, by examining the postsynaptic currents of hippocampal neurons in wild-type and *Shank3-deficient* mice, as well as in VPA-induced and control rats. The spontaneous postsynaptic currents were recorded in the whole-cell configuration of the patch-clamp method. Our functional analysis of hippocampal synaptic activity revealed a decrease in the frequency of spontaneous inhibitory currents but an increase in the median event amplitude in *Shank3-deficient* mice compared to their wild-type counterparts. Similarly, hippocampal neurons derived from VPA-induced animals exhibited a significant decrease in the frequency of inhibitory GABAergic postsynaptic currents while no change in the median amplitude of the events was observed. These findings suggest an excitatory-inhibitory imbalance in hippocampal neurons due to reduced inhibitory synaptic function, potentially affecting memory and social interaction, and contributing to autism symptoms like impaired social behaviour. Supported by VEGA 2/0073/22 and APVV-21-0189.

**Keyword 1**: autism
**Keyword 2**: synaptic activity
**Keyword 3**: hippocampal neurons

**DECODING MEMORY FORMATION: HOW DOES EARLY ALZHEIMER'S DISEASE-RELATED PATHOLOGY ALTER SYNAPTIC PLASTICITY?**

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Alzheimer's disease (AD) is pathologically characterized by accumulation of beta-amyloid (Aβ) deposits and tau inclusions. Animal studies suggest that this leads to memory impairment by disrupting synaptic plasticity. Although often studied in hippocampi, long-term memory also resides in the six-layered neocortex. Here we investigated how AD-related pathology alters synaptic plasticity in human brain by leveraging cortical biopsies obtained during shunt surgery of idiopathic normal pressure hydrocephalus (iNPH) patients. Since many of these patients exhibit AD-related pathology, they offer a unique window to investigate cellular responses occurring during pathological progression of AD.
Pairing Training Protocol (PTP) was used to induce synaptic strengthening in the biopsies in whole cell patch clamp recordings. These experiments were complemented by inducing plasticity in the biopsies using Theta Burst Stimulation (TBS) protocol on 60-electrode Microelectrode Arrays (MEAs). We established a novel computational pipeline to extract temporal, spatial and potentiation features from the evoked field post synaptic potential waveforms. These were used to train a supervised machine-learning algorithm to classify the samples for the presence of Aβ and tau pathologies. In addition, Xenium spatial transcriptomic technology was used to discover differentially regulated transcripts induced by the synaptic strengthening protocols.
Our data show that iNPH cortical biopsies elicit synaptic plasticity upon both PTP and TBS protocols and led to induction of pathways related to synaptic plasticity. TBS-induced waveforms were capable of predicting the presence of AD-related pathology in the biopsies. These unique samples provide physiologically relevant model to study AD-induced alterations in synaptic function in human cortex.

**Keyword 1**: Long term potentiation
**Keyword 2**: Multielectrode Array
**Keyword 3**: Alzheimer's disease
**Keyword 4**: iNPH

**TEMPORAL PROFILE OF NEUROARCHITECTURE IN THE HIPPOCAMPUS OF 5×FAD ALZHEIMER'S DISEASE MOUSE MODEL**

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Structural plasticity is critical for regulating neuronal activity and brain function in the hippocampus. Alzheimer's disease (AD) is characterized by progressive cognitive decline, neuroinflammation, and synaptic dysfunction. The 5×FAD mouse model is the most commonly used model for AD, successfully mimicking the key pathological features including amyloid-β (Aβ) deposition, tau hyperphosphorylation, and glial activation. However, the temporal progression of neuroplasticity-related impairments in the hippocampus of 5×FAD mice remains unclear. The present study investigated the features of behavioral alteration, AD histopathology, and hippocampal structural plasticity in 5×FAD mice at 3, 6, and 12 months of age. We demonstrated progressive fine motor dysfunction and hippocampus-related behavioral dysfunction, with deficits in cognitive performance in the T-maze test, in 5×FAD mice. Significant increases in Aβ and p-Tau accumulation, along with elevated levels of microglia, astrocytes, and inflammatory cytokines, were observed in the hippocampus. In Golgi staining, dendritic complexity was significantly reduced in the cornu ammonis 1 (CA1) and dentate gyrus (DG) subregions of the hippocampus, with age-dependent decreases in number of crossing dendrites, total dendritic length, and branch points. The dendritic spine density and morphology were significantly altered in the CA1 apical/basal and DG subregions following age-dependent. Furthermore, the expression of activity-regulated cytoskeleton-associated protein and postsynaptic density protein 95 was significantly reduced in 12-month-old 5×FAD mice. Collectively, these results suggest that the interplay between neuroinflammation, structural plasticity deficits, and synaptic dysfunction in the hippocampus of 5×FAD mice, could contribute to Alzheimer's disease pathology.

**Keyword 1**: Alzheimer's disease
**Keyword 2**: Golgi staining
**Keyword 3**: hippocampus
**Keyword 4**: neuroinflammation
**Keyword 5**: structural plasticity

**SYNAPTIC LOCALIZATION OF A-SYNUCLEIN IN CHOLINERGIC TERMINALS OF THE HEALTHY BRAIN**

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Alpha-synuclein (α-synuclein) is a presynaptic protein that regulates synaptic vesicle trafficking, playing a crucial role in presynaptic homeostasis. While being widely associated with various neurotransmitter systems its distribution in cholinergic brain regions is unclear. Given recent interest in the role of cholinergic systems in Parkinson's disease, we mapped the synaptic localization of α-synuclein in cholinergic systems across healthy mouse brains, as a reference for future pathological studies. Fixed brain tissue from five adult α-synuclein knockout (SNCA KO) mice and six adult wild type mice were coronally sectioned and immunolabeled for α-synuclein, vesicular acetylcholine transporter (VAChT), choline acetyltransferase (ChAT), and cellular markers. Digital multi-channel fluorescence microscopy images of whole serial sections were acquired by slide scanning and co-registered to the Allen mouse brain Common Coordinate Framework (CCFv3, 2017) using the EBRAINS QuickNII tool (RRID:SCR 016854). Preliminary qualitative assessment of these images indicates α-synuclein presence in cholinergic terminals in several brain regions, including the substantia nigra, known to receive input from cholinergic projection neurons in the pedunculopontine nucleus (PPN) and neocortex, and the cingulate cortex, known to receive cholinergic projections from the basal forebrain. Additional STED microscopy confirmed localization of α-synuclein in cholinergic terminals from PPN and basal forebrain. Absence of α-synuclein staining in SNCA KO mice confirmed specificity. Our findings support the hypothesis that α-synuclein is present in cholinergic synapses in the healthy brain, suggesting a physiological role for α-synuclein in cholinergic neurotransmission. Our histological image collection will be openly shared via the EBRAINS Knowledge Graph (https://search.kg.ebrains.eu/).

**A NEW CYCLE IN THE BRAIN: NEURON-GLIA-ECM SHUTTLING VIA EXOSOMES**

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The brain extracellular matrix (ECM) is a lattice-like structure that occupies the intercellular space in the central nervous system (CNS). The ECM constituents are extremely long-lived and are widely believed to stabilize neurons and synapses in the brain. They are thought to be renewed only rarely, dependent on neuronal activity, by the activation of matrix metalloproteinases (MMPs) that cleave the existing ECM components, followed by de novo ECM protein synthesis. However, various studies have demonstrated that synapses change their structural organization on minutes to hours time scale, calling for additional mechanisms for ECM remodeling. Using advanced microscopy techniques, molecular biology tools and biochemical assays, we report here novel insights on the ECM dynamics. The proteoglycan neurocan (Ncan) frequently shifts between the neuronal surface ECM and astrocytic intracellular compartments. Ncan is secreted initially by glia cells and predominantly resides on their surfaces in young cultures. It is later transferred to neurons, and continues to shuttle between these cells throughout later developmental stages, a process dependent on extracellular vesicles (EVs). Inhibiting this process with specific drugs or mutations, as well as, suppressing neuronal activity, results in Ncan accumulation in glia. Our findings challenge the prevailing view of ECM remodelling, highlighting a previously unknown mechanism of dynamic transcellular molecular exchanges within the CNS.

**Keyword 1**: extracellular matrix
**Keyword 2**: exosomes
**Keyword 3**: neurocan
**Keyword 4**: astrocytes
**Keyword 5**: neurons

**EARLY SYNAPTIC DYSFUNCTION CONTRIBUTES TO NETWORK-WIDE STRUCTURAL AND FUNCTIONAL DISRUPTIONS IN MOTOR NEURONS DERIVED FROM AN ALS PATIENT**

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Accumulating evidence suggests that neurodegenerative diseases lead to widespread alterations in neuronal communication before substantial cell death and symptom manifestation are observed. However, it is less clear how altered network dynamics contribute to the increased vulnerability of affected neuronal populations. Here, we aimed to identify how ALS pathology affects the structural and functional dynamics of motor neuron networks, and the molecular underpinnings contributing to cause such dysfunction. Multielectrode arrays (MEAs) enable longitudinal assessment of electrophysiological activity from engineered neural networks, thus facilitating the study of network-wide disruptions associated with neurodegenerative processes. We examined over a period of six weeks the structural and functional development of ALS patient-derived motor neuron networks and healthy control networks. Systematic recordings revealed specific time points for the emergence of potentially maladaptive neural network behavior in the ALS networks. Specifically, the ALS networks exhibited decreased firing rate, lower spike amplitude and reduced bursting compared to controls, but a higher overall synchronization of network activity. These changes coincided with structural alterations within the networks. Additionally, mRNA sequencing revealed dysregulated expression of genes involved in synapse development and maintenance, cell adhesion and neuroplasticity in patient-derived motor neurons. Overall, our findings suggest that ALS-predisposing mutations can lead to inherent vulnerabilities in motor neurons in terms of synaptic impairment, resulting in compensatory mechanisms at the network level. Such compensation could be highly energy demanding and render the network particularly vulnerable to insults, ultimately leading to network degeneration.

**Keyword 1**: electrophysiology
**Keyword 2**: transcriptomics
**Keyword 3**: connectivity
**Keyword 4**: neuroplasticity
**Keyword 5**: human induced pluripotent stem cells

**MECHANICAL STRESS MODULATES THE EXPRESSION OF IMMEDIATE EARLY GENES IN CULTURED NEURONS**

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A variety of cells are sensitive to mechanical stress, eliciting intracellular signals. Meanwhile, it has not been well understood whether neurons in the central nervous system could sense mechanical stress. To understand the possible sensitivity of neurons to mechanical stress and its effect on neuronal activity, we analyzed the effects of mechanical stress on gene expressions of immediate early genes (IEGs) in cultured neurons.
Neural progenitor cells were harvested from the cerebellum of mice and incubated in Neurobasal Medium with 1% P/S and 2% B-27. Subsequently, the cells were cultured in the presence of Arac and differentiated to neurons with neuronal network on the culture plate. Mechanical stress was applied to neurons by fluid force induced by shaking the culture plate 30 min a day for five days. In the control cells, the culture plate was placed on the shaker without shaking. After the intervention, mRNA was purified from collected cells for quantitative PCR to measure the expression of IEGs including c-fos and BDNF.
Shaking for 5 days significantly decreased the expression of both c-fos and BDNF in neurons, indicating that mechanical stress inhibited the expression of the IEGs.
This study showed that mechanical stress induced by fluid force inhibited the expression of c-fos and BDNF, markers of neuronal activity. Thus, this study suggests that neurons could sense mechanical stress, and the mechanical stress could inhibit neuronal activity. Based on the findings, further studies are expected to explore the mechanism underlying the sensitivity of neurons to mechanical stress.

**Keyword 1**: mechanical stress
**Keyword 2**: neuron
**Keyword 3**: immediate early gene

**KETOGENIC DIET IN A MOUSE MODEL OF GRIN2A SYNDROME: EFFECTS ON BEHAVIOUR, NEUROPLASTICITY AND NEUROINFLAMMATION**

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**Background** Developmental and epileptic encephalopathies (DEE) are early-life onset syndromes characterized by refractory epilepsy and cognitive impairment. GluN2A(N615S)-mutated mice carry a mutation in the *Grin2a* gene coding for the GluN2A subunit of the NMDA glutamate receptor and display symptoms similar to those described in DEE patients carrying analogous mutations, thus representing a valuable murine model for *GRIN*-related DEEs. **Methods** We investigated the effects of a ketogenic diet (KD) in this model on the epileptic phenotype and behavior and, after behavioral and seizure testing, mice were sacrificed and several tissues were collected. Brains slices were stained for different markers in order to investigate neuroplasticity by evaluating perineuronal nets and parvalbumin-positive interneurons, and neuroinflammation by studying microglia. **Results** We demonstrated for the first time in this model that KD reduces the susceptibility of mutant mice to audiogenic seizures (AGS), and the use of both sexes allowed us to evaluate differences in behavioral performances, AGS susceptibility, neuroplasticity, and neuroinflammation in response to KD based on sex. **Conclusion** KD is a proven treatment for drug-resistant epilepsy, but there are conflicting data regarding potential cognitive improvements and metabolic and physiological effects beyond epilepsy: results of our behavioral testing, together with *ex-vivo* analysis of the brains, could lead us to identify potential molecular and physiological mechanisms underlying the effects of KD.

**Keyword 1**: Ketogenic diet
**Keyword 2**: GRIN-related disorders
**Keyword 3**: Epilepsy
**Keyword 4**: Perineuronal nets

**ROLE OF CEREBELLAR NETWORK EXCITABILITY AND PLASTICITY IN THE PATHOPHYSIOLOGY OF DYSTONIA**

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DYT1 is an autosomal dominant form of dystonia, linked to a deletion (ΔGAG) in the TOR1A gene. The basal ganglia have been found to play a crucial role in the development of dystonia in the TOR1A$^{\Delta GAG}$ murine model. Experimental evidence has also indicated that the cerebellar cortex is likely to play a pivotal role in the pathophysiology of this disorder. High-density multielectrode array (HD-MEA) and whole-cell patch-clamp recordings were performed in acute parasagittal cerebellar slices to investigate cerebellar cortex activity and synaptic plasticity in WT and TOR1A$^{\Delta GAG}$ mice. HD-MEA allowed the simultaneous recording of the spontaneous activity of several neurons distributed in the whole network and the recording of local field potentials in the granular layer, elicited by mossy fiber stimulation. Patch-clamp recordings were used to analyze passive membrane properties, intrinsic excitability, and excitatory postsynaptic currents in cerebellar granule cells following mossy fiber stimulation. The analysis of Purkinje cells' spontaneous activity did not show significant differences in firing frequency between WT and dystonic mice. TOR1A$^{\Delta GAG}$ mice granule cells showed increased excitability and a larger input resistance than WT counterparts upon inhibition blockade. Moreover, in TOR1A$^{\Delta GAG}$ granule cells, the induction of long-term plasticity resulted in the depression of synaptic transmission, while WT granule cells showed both potentiation and depression. These data will be crucial to understand the role of different neuronal types in determining the alterations in signal integration and processing in the cerebellar cortex of the DYT1 pathological model.

**Keyword 1**: Cerebellum
**Keyword 2**: Dystonia
**Keyword 3**: Mouse model
**Keyword 4**: Neurophysiology
**Keyword 5**: Electrophysiology

**ALTERED CEREBELLAR PURKINJE CELL EXCITABILITY AND SYNAPTIC TRANSMISSION IN THE IB2 KO**

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Autism spectrum disorder (ASD) is a neurodevelopmental condition characterized by social interaction deficits, restricted interests, and repetitive behaviors. Growing evidence suggests a crucial role of the cerebellum in ASD. Previous studies in the IB2 KO mouse model of ASD showed disrupted synaptic plasticity in the cerebellar granular layer, together with alterations of Purkinje cell (PC) morphology. To investigate how IB2 loss affects PC activity, we performed whole-cell patch-clamp recordings in acute cerebellar slices. While IB2 WT PCs displayed typical electrophysiological properties, IB2 KO PCs segregated into two groups: IB2 KO-1 showed reduced firing frequency and excitability, whereas IB2 KO-2 exhibited burst-like spontaneous activity, increased excitability, and complex bursts in response to depolarizing currents. Blocking inhibition during depolarizing current steps reduced both the firing rate and complex bursts in IB2 KO-2, aligning its activity with that of the IB2 WT group. This suggests that inhibitory mechanisms contribute to the observed electrophysiological differences. Moreover, in response to five-pulse parallel fiber stimulation at varying frequencies (4–200 Hz), IB2 KO-2 PCs exhibited an abnormal response compared to IB2 WT PCs, with a burst followed by a protracted pause. To further investigate these alterations, we are developing a computational PC model replicating the properties of IB2 WT and IB2 KO-1/KO-2 PCs. The presence of two distinct IB2 KO PC subtypes indicates a complex physio-pathogenetic process that may include compensatory mechanisms, highlighting the need for further investigation and a thorough assessment of the inhibition role in this ASD model.

**Keyword 1**: cerebellum
**Keyword 2**: autism spectrum disorder
**Keyword 3**: mouse model
**Keyword 4**: neurophysiology
**Keyword 5**: patch-clamp

**COPPER OXIDE NANOPARTICLES REPROGRAM NEUROINFLAMMATION AND ENHANCE NEUROPLASTICITY IN TRAUMATIC CNS INJURIES**

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Traumatic central nervous system (CNS) injuries, including spinal cord injury (SCI) and traumatic brain injury (TBI), trigger complex neuroinflammatory cascades that exacerbate neuronal loss and glial scarring, ultimately impairing functional recovery. This study explores copper oxide nanoparticles (CuO-NPs) as a novel therapeutic intervention targeting secondary injury mechanisms in rat models of SCI and TBI. CuO-NPs, known for their redox activity and immunomodulatory properties, were locally administered to the lesion sites immediately after injury. In the SCI model, CuO-NP treatment significantly reduced lesion cavity size, suppressed ED1-positive microglial and macrophage infiltration, and promoted serotonergic axon sprouting at 8 weeks post injury. Behavioral assessments revealed improved hindlimb motor function as indicated by superior performance in the BBB and ladder tests. In the TBI model, CuO-NPs reduced cortical lesion volume and neuroinflammation while improving neurological function (mNSS) and motor coordination (rotarod test). Mechanistically, CuO-NPs shifted macrophage polarization toward the reparative M2 phenotype, preserved axonal integrity via NF200 expression, and suppressed pro-inflammatory cytokines such as IL-1β and TNF-α. In addition, enhanced VEGF signaling suggested pro-regenerative revascularization. These findings demonstrate the potential of CuO-NPs to simultaneously modulate neuroinflammation and enhance neuroplasticity, providing a dual-action approach to promote neuroprotection and repair in acute CNS trauma. This work highlights the translational promise of nanoparticle-based immunoengineering in neurotrauma as a novel strategy to mitigate secondary injury cascades and restore functional outcomes after SCI and TBI.

**Keyword 1**: Neuroinflammation
**Keyword 2**: copper oxide nanoparticles
**Keyword 3**: spinal cord injury
**Keyword 4**: traumatic brain injury
**Keyword 5**: macrophage polarization

**FREEDIVING AS A NATURAL HUMAN MODEL TO STUDY HIPPOCAMPAL FUNCTIONAL CONNECTIVITY UNDER HYPOXIA**

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Accidental hypoxia has detrimental effects on the brain, particularly on the hippocampus, which is highly sensitive to oxygen deprivation. Most resting-state functional MRI (rs-fMRI) studies on accidental hypoxia have reported altered brain connectivity, including hippocampal rs-functional connectivity (rsFC). However, it remains unclear whether voluntary hypoxia from freediving training, which involves prolonged breath-holding, leads to functional brain changes. We aimed to clarify the impact of voluntary hypoxia experienced during freediving training on hippocampal rsFC. rs-fMRI were acquired on 17 freedivers before and after 7-months of regular training, alongside 20 non-freedivers control athletes. Data were acquired at 3 Tesla using a T2* weighted EPI sequence (TR = 2500 ms, TE = 26 ms, FOV = 192 mm, voxel size = 1.7 × 1.7 × 1.7 mm, 68 slices; 4 rs-fMRI runs of 3'30'': 2' apnea + 1'30'' resting state + 1' rest interval between runs). Preprocessing and analysis were performed using the CONN toolbox. A permutation test was conducted on hippocampal rsFC values in each hemisphere (i) during apnea and rest and (ii) before and after training. Significant differences in rsFC values were observed in both the right and left hippocampus (Figure 1). As expected, hippocampal rsFC increased during apnea. This original finding suggests compensatory mechanisms aimed at preserving hippocampal functions under reduced oxygen availability, in agreement with our previous study showing no damage of episodic memory in this population. Such an original model in healthy individuals could provide insights into potential therapeutic strategies for neurological conditions involving hippocampal damage.

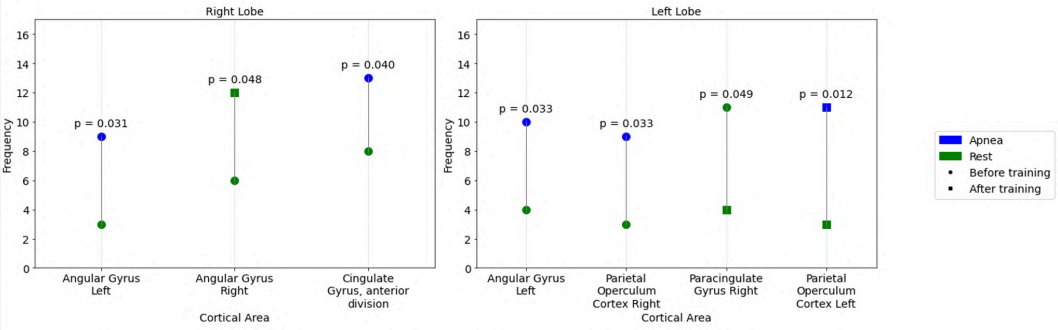


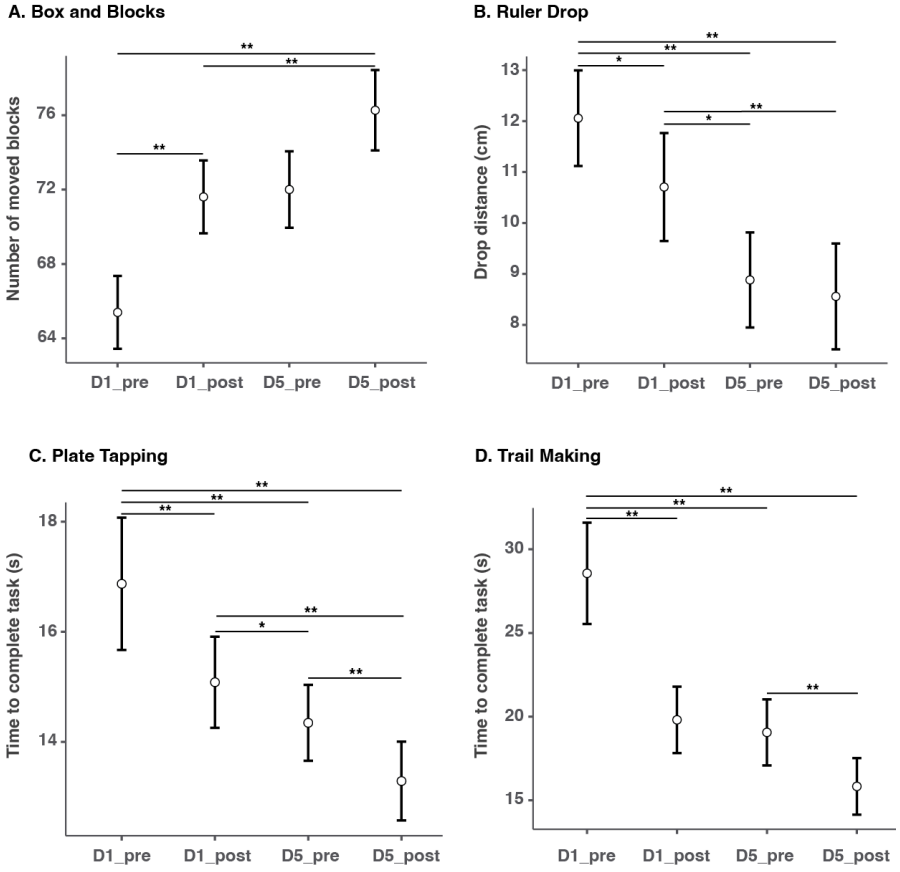

**Fig. 1**: Subjects (frequency) exhibiting significant differences in FC between specific brain regions and the left or right hippocampus during apnea or rest, before and after training (including only regions showing connectivity changes). *P-values* are permutation test with 10,000 permutations.

**Keyword 1**: Hippocampus
**Keyword 2**: Freediving
**Keyword 3**: Hypoxia
**Keyword 4**: rsfMRI

**STUDY OF GRIN DISORDERS - MOUSE MODELS CARRYING MISSENSE OR FRAMESHIFT *GRIN2B* GENE VARIANTS ASSOCIATED WITH NEURODEVELOPMENTAL DISORDERS**

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NMDA receptors, whose subunits are encoded by GRIN genes, are ionotropic glutamate receptors that are crucial for synaptic transmission and neuronal plasticity underlying the processes of learning and memory formation. Pathogenic variants of GRIN genes are associated with intellectual disability, developmental delays and epileptic seizures. Most frequent are missense variants resulting in a single amino acid substitution, while nonsense or frameshift variants introduce a premature stop codon. Interestingly, protein-truncating variants (PTVs) tend to be associated with less severe symptoms than missense variants. We have previously reported the effects of human GRIN2B transmembrane and CTD variants on NMDAR functions. However, the molecular mechanisms associated with presence of these variants are not well understood. Here, we present, at the molecular level, the analysis of this clinically important difference between the variants by comparing mouse lines carrying Grin2b variants localized in the TMD: a missense variant (L825V) and a frameshift variant (L825FfsTer15). First, analysis of NMDAR localization in *Grin2b*$^{+/L825V}$ and *Grin2b*$^{+/L825fs}$ neurons revealed a significant difference in which GluN2B$^{L825fs}$ variant decreases NMDAR surface expression. In addition, mRNA expression of the other functionally important subunits GluN1 and GluN2B was impaired in the brains of *Grin2b*$^{+/L825fs}$ mice compared to WT and *Grin2b*$^{+/L825V}$ mice. Finally, MS analysis revealed a greater proportion of proteins with altered expression, including GluN1 and 2B in *Grin2b*$^{+/L825fs}$ mice. In summary, our mouse models suggest that less pathogenic PTV has stronger effects on overall gene expression. These data have also improved our understanding of the etiology of certain cases of GRIN disorders.




**Keyword 1**: GRIN disorders
**Keyword 2**: NMDAR
**Keyword 3**: mRNA, protein expression
**Keyword 4**: mouse model

**TEMPERATURE-DEPENDENT NEURONAL PLASTICITY IN A MOUSE MODEL OF INDUCED HYPOTHERMIA**

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In small hibernating mammals, synaptic plasticity occurs in the form of rapid and reversible retraction and remodeling of dendrites and spines that is followed by an overshoot of synapses in early arousal. Whether this also happens in non-hibernating species subjected to hypothermia is not clear, but in mice, hypothermia induces expression of certain “cold-shock” proteins that is directly related to synapse formation and neuronal plasticity. Consequently, higher amplitude LTP is found upon early arousal from torpor/hypothermia in mice and several hibernator species. This implies that hypothermia can induce a state of hyperplasticity, but the caveat of these experiments is that hypothermia is induced indirectly and secondarily to a hypometabolic state that might promote adaptive mechanisms responsible for observed plasticity. In this project, we actively induce hypothermia or normothermia in mice under general anaesthesia with isoflurane and can therefore assess more directly the contribution of hypothermia on proposed neuroplasticity in mice with similar metabolic conditions.
To test if induced hypothermia under isoflurane anaesthesia alters neuronal connections, we evaluate structural changes in neuronal morphology from in cooled and normothermia anesthetized mice, as well as awake mice using systemic injections of AAV’s to sparsely label neurons with fluorescent proteins. To test if induced hypothermia changes neuronal connections and transiently alters synaptic plasticity, we evaluate the strength of fear memories conditioned directly after anesthesia, anesthesia with hypothermia or from naive awake animals.

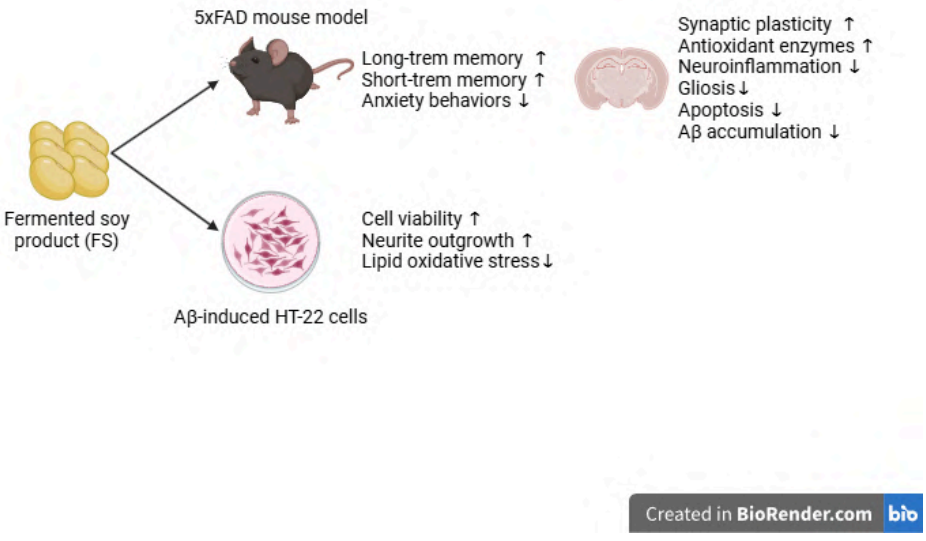
**Keyword 1**: hypothermia
**Keyword 2**: neuronal plasticity
**Keyword 3**: hibernation
**Keyword 4**: cold-shock proteins
**Keyword 5**: viral vectors

# Neurodegenerative Disorders and Therapeutic Approaches

**NEUROPROTECTIVE EFFECTS OF FERMENTED SOY IN ALZHEIMER'S DISEASE: EVIDENCE FROM 5XFAD MOUSE AND IN VITRO MODELS**

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Alzheimer's disease (AD) is the most common neurodegenerative disorder of the central nervous system, characterized by memory loss and changes in cognitive behavior. The two main hypotheses regarding Alzheimer's disease involve the accumulation of amyloid-β (Aβ) forming amyloid plaques and the intracellular hyperphosphorylation of tau protein, leading to neurofibrillary tangles (NFT). These processes are accompanied by neuroinflammation and oxidative stress, ultimately resulting in neuronal death. In this study, we found that oral administration of fermented soy powder (FS), derived from microbial fermentation of soybeans, improved cognitive deficits in 5xFAD mouse models. These improvements include enhanced short-term and long-term memory and reduced anxiety symptoms. FS activated the Nrf2 pathway and inhibited NF-κB signaling via regulation of the AKT/GSK3β pathway in the hippocampus, which led to upregulation of antioxidant enzymes and inhibition of neuroinflammatory responses. Additionally, FS increased the expression levels of brain-derived neurotrophic factor (BDNF), PSD95, and Synaptophysin, inhibiting hippocampal cell apoptosis. Immunohistochemical analysis revealed that the FS-treated group exhibited a significant reduction in hippocampal amyloid plaque accumulation and gliosis compared to the control group which fed saline only. In vitro experiments showed that FS, at concentrations of 0.001 μg/ml and 0.01 μg/ml, significantly enhanced the cell viability of Aβ-induced HT-22 cells cytotoxicity, reduced lipid oxidative stress, and promoted neurite outgrowth. Overall, FS shows potential as a therapeutic agent for alleviating cognitive behavioral impairments in Alzheimer's disease.




**Keyword 1**: Alzheimer's disease
**Keyword 2**: Fermented soy
**Keyword 3**: AKT/GSK3β/NRF2
**Keyword 4**: BDNF

**PROTECTIVE EFFECTS OF DEHYDROEPIANDROSTERONE ON THE CHOLINERGIC SYSTEM AND GLIAL CELLS IN A NEUROTOXIC ALZHEIMER'S DISEASE MOUSE MODEL**

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Alzheimer's disease (AD) is the most common neurodegenerative disorder. Accumulation of pathological proteins like Aβ and pTau affect not only neurons but also glial cells, altering their morphology and increasing their activity. Cholinergic system dysfunction plays a key role in AD pathology. Dehydroepiandrosterone (DHEA), an endogenous steroid hormone, is believed to have neuroprotective effects. Our aim is to investigate the protective effects of DHEA/S on microglia, astrocytes and the cholinergic system. We used a neurotoxic AD model induced by Aβ microinjection into the cholinergic nucleus basalis magnocellularis (NBM) region of C57BL6/J male mice. One hour after the stereotaxic surgery an intraperitoneal treatment with 10 mg/kg DHEA/S or vehicle (0,9% saline solution) was performed. The animals were transcardially perfused, and immunohistochemical stainings (Iba-1, GFAP, ChAT, AChE) were performed to investigate microglia, astrocyte and cholinergic cell morphology. The Aβ injection decreased the number of ChAT positive neurons in the NBM and the density of their AChE positive fiber projections in the somatosensory cortex (SSC). Furthermore, increased the activation of microglia and astrocytes in the injection site. DHEA/S had a protective effect on the cholinergic fibers, decreasing the Aβ induced neurotoxicity. The treatment also decreased the glial cells activation. Overall, DHEA/S or similar compounds may provide new insights into understanding the pathology of AD and could represent a new therapeutic target.

**Keyword 1**: dehydroepiandrosterone
**Keyword 2**: Alzheimer's disease
**Keyword 3**: cholinergic system
**Keyword 4**: glia cells
**Keyword 5**: neurotoxicity

**ENGINEERED NEURAL NETWORKS FOR ALZHEIMER'S DISEASE MODELING**

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Engineered biological neural networks are powerful models for investigating neural function and dysfunction at multiple scales. Advanced neuroengineering approaches are particularly valuable for replicating the topological and functional organization of brain networks, providing insights into disease progression. Here, we employed custom-designed two- or four nodal microfluidic platforms with Tesla valve-inspired microtunnels to reverse-engineer feedforward cortical and hippocampal neural networks derived from rats. These networks were interfaced with nanoporous microelectrode arrays (mMEAs) to monitor dynamic structure-function relationships under both healthy and pathological conditions. To model Alzheimer's disease pathology, we introduced either Aβ fragments or P301L mutated tau protein into specific nodes of the engineered networks, enabling precise manipulation and long-term monitoring of disease-related changes. Overall, our findings revealed progressive alterations in network dynamics, including axonal retraction, increased synchrony, and decreased signal propagation between pre- and post-synaptic nodes. These perturbations resulted in a decline in overall firing rate and bursting activity, mimicking electrophysiological shifts observed in neurodegenerative diseases. Our results highlight the potential of microfluidic-based engineered neural networks for studying the evolving interplay between structural and functional reconfigurations in neurodegenerative disease. By enabling precise control over network connectivity and pathology induction, these platforms provide a powerful tool for investigating Alzheimer's disease progression and evaluating potential therapeutic interventions.

**Keyword 1**: Alzheimer's disease
**Keyword 2**: Engineered neural networks
**Keyword 3**: Microfludic chips

**MICROSCALE DYSFUNCTION AND MESOSCALE COMPENSATION IN DEGENERATING NEURONAL NETWORKS**

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Changes in brain network structure and function are increasingly recognised as crucial in neurodegenerative disease. Despite differences in pathological aspects, such as type of misfolding proteins involved and neuronal subtypes vulnerable to such pathology, network-level changes appear to follow common patterns. Some changes precede clinical symptoms, indicating that functional reconfigurations can maintain information processing capacity despite structural damage. Increased connectivity within hub node communities is a common reconfiguration which supports network function. However, this can increase the hubs' metabolic demand, making them prone to failure, leading to loss of network function. The extent of this response, involving hyperconnectivity, centralisation and subsequent hub failure in neurodegenerative disease remains unclear.

Here, we investigated ALS and healthy human motor neuron networks in terms of TDP-43 proteinopathy, structure, and functional network connectivity properties based on graph theory, using both conventional and high-density multielectrode arrays.
We found clear signs of proteinopathy in ALS networks, along with significant structural changes, including increased neurite density, branching and junctions, but reduced average neurite length. Functionally, we demonstrate consistently elevated firing rate alongside reduced spike amplitude in ALS networks. Network analysis revealed signs of compensation, including network centralisation and reliance on a rich-club, as well as increased network density.

In conclusion, we provide a novel, clear demonstration of functional network reconfiguration in neurodegenerative disease, indicating compensatory changes to pathological processes. Our results support the hypothesis of increasing network connectivity and centralisation to maintain network function, which results in mounting demands on a rich-club and more vulnerable networks.

**Keyword 1**: Neurodegeneration
**Keyword 2**: Microelectrode array
**Keyword 3**: Graph theory
**Keyword 4**: Rich-club
**Keyword 5**: Hyperactivity

**INVESTIGATING THERMOREGULATORY IMPAIRMENTS IN ALZHEIMER'S DISEASE: THE EFFECTS OF SENKTIDE AND ROLIPRAM IN MALE 3XTG-AD MICE**

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Emerging evidence highlights a critical link between thermoregulation and metabolism, both of which are often disrupted in Alzheimer's Disease (AD). Disturbed thermoregulation, influenced by metabolic dysfunction, may exacerbate AD progression. However, the mechanism is not fully understood but can be studied in the popular triple transgenic AD mice (3xTg-AD). We investigated core body temperature (Tc) changes in 6-month-old male 3xTg-AD mice after hypothermic provocation by senktide (NK3 receptor) substance used for modelling menopausal hot flushes. To test whether the changes are due to vasodilatation or some more specific NK3 effect, another vasodilatory agent, the rolipram (PDE4 inhibitor) was also used. Tc was continuously monitored using a telemetry system. Mice were subjected to treatments (intraperitoneally) with senktide (0.5 mg/kg), rolipram (1mg/kg), or a vehicle (2% DMSO in saline 0.9% - 10mL/kg) control. Senktide significantly decreased Tc in WT, but not in KO animals, consistent with its role as an NK3 receptor agonist that modulates central thermoregulatory pathways and vasodilation. It seems to have a specific effect, as rolipram had no effect on Tc and there was no genotype difference after its administration, either. Our results suggest that neuroinflammation, amyloid plaque deposition, and tau pathology in the thermoregulatory center (probably in the medial preoptic area) may disrupt the NK3 signaling and its downstream effects. Next, we will examine the molecular details of this disrupted thermoregulation using immunohistochemistry and PCR both in the brain and in the brown adipose tissue.

**Keyword 1**: Alzheimer's Disease
**Keyword 2**: Thermoregulation
**Keyword 3**: NK3 receptor
**Keyword 4**: 3xTg-AD mouse
**Keyword 5**: Vasodilation

**CNEURO-201 AT LOWER DOSES IMPROVES SPATIAL LEARNING AND MEMORY IN A TRIPLE-TRANSGENIC MOUSE MODEL (3XTG-AD) OF ALZHEIMER'S DISEASE**

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Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by cognitive decline and β-amyloid (βA) plaque accumulation, with no effective treatments currently available. CNEURO-201 (CN) is a novel compound with dual activity, inhibiting βA aggregation and acting as a σ1 receptor agonist, making it a potential AD treatment candidate. Previous studies showed that CN at 1.0 mg/kg reduced Aβ deposits in the hippocampal subiculum of 3xTg-AD mice, with slight memory improvements. Recent findings revealed that lower doses (0.03–0.10 mg/kg) improved memory in a dizocilpine-induced amnesia model. This study evaluated the effects of 0.1 mg/kg CN on spatial learning and memory in 8-month-old female 3xTg-AD mice, using non-transgenic (NoTg) mice as controls. Mice were divided into three groups (n=10): I) 3xTg-CN (CN-treated 3xTg-AD), II) 3xTg-VEH (vehicle-treated 3xTg-AD), and III) NoTg-VEH (vehicle-treated NoTg). All received daily intragastric administration of CN or vehicle for eight weeks. Spatial learning and memory were assessed using the Morris water maze. Results showed that 0.1 mg/kg CN significantly improved cognitive performance in 3xTg-AD mice, with a ~45% reduction in escape latencies compared to 3xTg-VEH. CN treatment also shifted search strategies, increasing direct over indirect strategies, indicating enhanced spatial memory. These findings highlight CNEURO-201's therapeutic potential at lower doses, supporting its development as a promising AD treatment.

**Keyword 1**: Alzheimer's disease
**Keyword 2**: Spatial memory
**Keyword 3**: CNEURO-201

**COGNITIVE IMPAIRMENT AND MOLECULAR ALTERATIONS IN A PSEN1 THR119ILE MUTATION MOUSE MODEL OF EARLY-ONSET ALZHEIMER'S DISEASE**

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Alzheimer's Disease (AD) is a neurodegenerative disorder characterized by cognitive decline, with early-onset forms often linked to genetic mutations. The PSEN1 Thr119Ile mutation has been reported in Korean patients with early-onset AD, prompting further investigation into its role in disease pathogenesis. In this study, we generated and characterized a PSEN1 Thr119Ile mutation mouse model using CRISPR/Cas9 technology to elucidate the mechanisms underlying early-onset AD. The PSEN1 Thr119Ile mutation was introduced into C57BL/6N mice, resulting in wild type (WT), homozygous, and heterozygous groups. Behavioral assessments, including Y-maze, Novel object recognition, Morris water maze, and Contextual fear conditioning, were conducted at 3, 6, 9, and 12 months to evaluate cognitive function. Homozygous mice exhibited significant cognitive dysfunction compared to WT mice, with deficits observed across all behavioral tests starting at 3 months and progressively worsening over time. Heterozygous mice showed a similar pattern of cognitive decline. mRNA sequencing analyzed molecular changes in the prefrontal cortex (PFC) and hippocampus. The analyses revealed a downregulation of genes associated with synaptic plasticity, neuronal activity, inflammation, and cognitive function in the homozygous mice. The PSEN1 Thr119Ile mutation mouse model demonstrates cognitive deficits and molecular alterations consistent with early-onset AD. These findings provide valuable insights into the pathogenic mechanisms of this mutation and establish a foundation for future studies. Further functional analyses, including immunohistochemistry and Western blotting, are warranted to elucidate the molecular mechanisms associated with this mutation in early-onset AD.

**Keyword 1**: Alzheimer’s disease
**Keyword 2**: neurodegenerative disorder
**Keyword 3**: Thr119Ile mutation
**Keyword 4**: presenilin-1
**Keyword 5**: cognitive function

**THERAPEUTIC MECHANISMS OF TRANSCRANIAL ALTERNATING CURRENT STIMULATION AND TREADMILL EXERCISE IN A MOUSE STROKE MODEL**

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Non-invasive transcranial alternating current stimulation (tACS) is a promising therapy for brain disorders. To improve the efficiency of cortical stimulation, we designed a novel electrode that attached the needle to the conventional flat ring based high-definition (HD) electrode. Male C57BL/6N mice with cerebral ischemic stroke were treated with HD-tACS (50 μA/mm$^2$, 60 kC/m, 20 min) using conventional flat type electrode (C-HD-tACS) and a novel needle type (CN-HD-tACS), and compared to treadmill exercise therapy (15 m/min, 30 min). To demonstrate the therapeutic effect, behavioral tests for motor function were performed, and the mechanisms were identified using proteomic and other biomedical analyses. All treatments examined improved motor function, and the volume of the brain infarction was significantly reduced. In particular, CN-HD-tACS shown greater therapeutic efficacy in alleviating motor deficits. Based on proteomic analysis, we focused on mitochondrial activation, particularly oxidative phosphorylation and ATP synthase, and then selected 8 of the most potent candidates (mitochondria complex, Ndufa2, Ndufa13, Atp5e, Atp5o, Atp5c1, Atp11b, Ak4) with cell death factors, Bax, Bcl2 and cleaved caspase3. Our findings show that all treatments promote neuronal survival by regulating mitochondria and other beneficial factors (Gabra4, CD47, SIRPα). In conclusion, tACS and treadmill exercise enhance motor functional recovery via regulation of mitochondrial processes. We suggest that tACS may be a useful therapy for patients with stroke to alleviate motor dysfunction.

**Keyword 1**: Transcranial alternative current stimulation
**Keyword 2**: Stroke
**Keyword 3**: High-definition electrode
**Keyword 4**: Exercise
**Keyword 5**: Mitochondria

**SELECTIVE VULNERABILITY TO TAU CHALLENGE IN SPECIFIC SUBSETS OF NEURONS IN THE LATERAL ENTORHINAL CORTEX IN A RAT MODEL OF AD**

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Alzheimer's Disease (AD) is distinguished by two pathological hallmarks known as plaques and tangles, consisting of aggregations of beta-amyloid and microtubule- associated protein tau respectively. Tau pathology correlates far more strongly with the cognitive disease progression than plaques. Pathological staging of AD patients shows that the tau pathology begins in particular neurons of Layer II of the lateral parts of the entorhinal cortex (EC, Braak stage I), then spreads throughout the EC (stage II), and then to synaptically connected regions like the hippocampus and subiculum (stage III) and then throughout the rest of the cortex (stages IV-V). This and other evidence has led to the idea of “prion-like” transsynaptic spread of tau pathology from these ECLII neurons, and raises the question of why the neurons of this particular brain region are so susceptible to AD-related tauopathy. To explore these questions, we stereotactically injected AAV virus with a human-Tau payload into the LEC of WT rats and performed a comprehensive immunohistochemical staging at 1, 2, and 5 months post-injection using a variety of antibodies probing various aspects of tau biology. We demonstrate that not only is the EC particularly susceptible to Tau pathology as compared to adjoining cortical regions, different neuronal subtypes within the EC have markedly distinct responses to tau overexpression. To explore the molecular bases for these celltype-specific differences in the development of distinct pathological tau species, we will present data exploring transcriptomic response of EC celltypes to human tau overexpression in the rodent EC.

**Keyword 1**: Alzheimer's disease
**Keyword 2**: Tau
**Keyword 3**: Transynaptic
**Keyword 4**: Cell vulnerability
**Keyword 5**: Lateral entorhinal cortex

**EFFECTS OF VISUAL AND ACOUSTIC SENSORY STIMULATION ON MICROGLIA ACTIVATION AND AMYLOID B PLAQUE FORMATION IN THE APP $^{NL\text{-}G\text{-}F/NL\text{-}G\text{-}F}$ MOUSE MODEL OF ALZHEIMER'S DISEASE**

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Alzheimer's disease is a major cause of illness and death, with current treatments like Lecanemab (Leqembi) carrying risks such as cerebral oedema and haemorrhage. This study explores a non-invasive approach with minimal side effects to clear amyloid-beta plaques. I hypothesise that 12 days of sensory stimulation will alter microglial number and size while reducing amyloid plaques. Cognitive improvements will be assessed using the fear conditioning test. Twelve-month-old wild-type and APP $^{NL\text{-}G\text{-}F/NL\text{-}G\text{-}F}$ mice underwent one hour of 40Hz light (LED) and sound stimulation daily for 12 days. A one-hour dark period served as control. This was followed by contextual fear conditioning and post-mortem histological analysis of microglia and amyloid-beta plaques. Sensory stimulation significantly increased freezing rates in wild-type and APP mice, suggesting cognitive effects. Microglial analysis revealed a slight increase in inactive microglia (10–1000μm$^2$) in the retrosplenial cortex (RSC) but no change in the CA1 region. In contrast, active microglia (>1000μm$^2$) decreased in number and percentage area in the CA1, with no effect in the RSC. Amyloid plaque area significantly decreased in CA1 but remained unchanged in RSC, possibly reducing the need for microglial activation. Sensory stimulation induces region-specific microglial and amyloid changes in CA1 and RSC, supporting its therapeutic potential for Alzheimer's disease. Further investigation is required to confirm these effects.

**Keyword 1**: Alzheimer's disease
**Keyword 2**: microglia
**Keyword 3**: Amyloid plaque

**A SYSTEMATIC REVIEW OF HUMAN STEM CELL-DERIVED DOPAMINERGIC PROGENITOR SURVIVAL AND DOPAMINERGIC MATURATION IN PARKINSONIAN MODELS**

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A promising emergent therapy for Parkinson's disease is stem cell-derived brain repair. The clinical success of this approach is based on the ability of the transplanted cells to survive and differentiate to mature dopaminergic neurons *in situ*. However, the pre-clinical literature regarding human stem cell-derived dopaminergic progenitor transplantation in parkinsonian models suggests high variability in terms of survival and differentiation between studies. Therefore, the aim of this systematic review was to assess these two parameters in studies where human ESC or iPSC-derived dopaminergic progenitors were transplanted in animal models of Parkinson's disease. To do so, we performed a systematic search of the database, PubMed, to identify relevant studies published up to March 2024. From the selected manuscripts (n= 76), we could assess graft survival in 52 studies and dopaminergic differentiation in 129 studies. Although graft survival was variable, between <0% and 500% of transplanted cells, the overall median of 51% was relatively high. Dopaminergic differentiation ranged from 0% to 46% of transplanted cells, with a median of 3%. Differentiation was negatively correlated with the number of cells transplanted and was lowest in the largest hosts (mice > rats > primates) with a median of just 1% in primate models. Interestingly, differentiation was also significantly lower in immunosuppressed compared to immunodeficient hosts. Ultimately, this systematic review suggests that there is considerable room for improvement in the differentiation of stem cell-derived dopaminergic progenitors to maximise the therapeutic benefit of this approach.

**Keyword 1**: Parkinson's disease
**Keyword 2**: Human iPSCs
**Keyword 3**: Human ESCs
**Keyword 4**: Dopaminergic differentiation

**DOPAMINE BUT NOT GLUTAMATE RELEASE IS DISRUPTED IN PARKINSON'S PATIENT-DERIVED DOPAMINERGIC NEURONS DUE TO IMPAIRED SYNAPTIC VESICLE LOADING**

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Dopamine release defects are an early and consistently-observed pathological feature of Parkinson's disease, however the underlying molecular mechanisms have not yet been elucidated. Here we use HPLC to demonstrate KCl-evoked dopamine release is impaired in patient-derived human dopamine neurons with *SNCA* triplication despite reaching equivalent maturations as assessed by whole-cell patch clamp electrophysiology. We find that this synaptic dysfunction is due to impaired vesicle loading as assessed by VMAT2 substrate, fluorescent false neurotransmitter (FFN) 206, which leads to reduction in synaptic vesicle pool size as determined using cypher and synaptopHluorin-based live imaging assays. This disruption in dopamine loading coincides with reduced VMAT2 levels, and results in reduced dopamine content and increased dopamine degradation while dopamine synthesis remains intact. Importantly, supplementation with 100 µm L-DOPA temporarily allows for the rescue of these observed dopamine content and release defects. In contrast, neither glutamate release nor VGLUT2 levels are impaired in Parkinson's patient's cells, revealing a specificity of dysfunction towards dopaminergic neurons. Combined, these data reveal dopamine synaptic vesicle loading and specifically, VMAT2, as a critical target in Parkinson's disease therapies.

**Keyword 1**: neurodegeneration
**Keyword 2**: dopamine release
**Keyword 3**: ipsc
**Keyword 4**: parkinson's disease
**Keyword 5**: dopamine

**DEPRESSIVE DISORDERS IN PARKINSON'S DISEASE: INVOLVEMENT OF MONOAMINERGIC SYSTEMS**

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Parkinson's Disease (PD) is a common neurodegenerative disorder characterized by depletion of dopamine in the nigrostriatal system, leading to the onset of motor and non-motor symptoms, including psychiatric disorders. Depression represents a major clinical challenge as it is generally unresponsive to dopamine replacement therapy and its neurological substrates are still elusive. PD typically leads to alterations in the noradrenergic and serotonergic systems, which are implicated in non-motor affective comorbidities.
To investigate the involvement of monoaminergic transmission in PD-associated depression, we examined noradrenergic activity and noradrenaline release in a 6-hydroxydopamine (6-OHDA) mouse model of PD exhibiting depression-like behavior. Using fiber photometry with calcium and noradrenaline sensors, we monitored neuronal activity and neurotransmitter release, respectively, during the tail suspension test, a despair-based paradigm for depression.
We found that struggling, reflecting the reaction of the animals to the inescapable stress of being suspended, increased the activity of noradrenergic neurons in the locus coeruleus (LC, the main noradrenergic nucleus in the brain) and enhanced noradrenaline release in the dorsal raphe nucleus (DRN, the primary source of central serotonin). Notably, when compared to control mice, 6-OHDA-lesioned mice displayed reduced activity of noradrenergic neurons in the LC and impaired noradrenaline release in the DRN during the tail suspension test.
These results indicate that the LC-to-DRN noradrenergic pathway plays a key role in the response to inescapable stress, but is impaired in the PD model, thereby representing a potential neuronal target to counteract depressive symptoms in PD.

**Keyword 1**: Parkinson's Disease
**Keyword 2**: Depression
**Keyword 3**: Noradrenaline

**A2A RECEPTOR-MEDIATED DARPP-32 SIGNALING REGULATES EDS IN PARKINSON'S DISEASE**

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Excessive daytime sleepiness (EDS) and sleep fragmentation are common in Parkinson's disease (PD) and significantly reduce patients' quality of life. Effective treatments against these comorbidities remain elusive, likely due to an incomplete understanding of their etiology. Emerging evidence suggests that dopamine- and adenosine-mediated signaling in the striatum are involved in sleep regulation. A key intracellular effector of these neuromodulators is DARPP-32 (dopamine- and cAMP-regulated phosphoprotein of 32 kDa), but its role in sleep remains unknown.
Here, we investigated the contribution of DARPP-32 in D1 and A2A receptor (D1R and A2AR)-expressing neurons. Using targeted genetic depletion combined with EEG/EMG recording, we show that DARPP-32 plays a key role in the control of NREM sleep quantity and quality in distinct neuronal subpopulations. We found that disruption of DARPP-32 signaling in A2AR neurons reduces NREM sleep during the active phase of the sleep-wake cycle, whereas the same intervention in D1R neurons enhances NREM stability in the inactive phase. Notably, we also found that, in a 6-hydroxydopamine mouse model of PD, deletion of DARPP-32 in A2A neurons counteracts somnolence during the active period, thereby protecting against EDS.
In line with these results, we found that the selective A2AR antagonist istradefylline, a drug recently found to improve sleep disturbances in PD patients, reduces EDS in the mouse PD model. Our study provides a mechanistic explanation for this effect, opening the way for the development of interventions acting specifically on DARPP-32-mediated signaling in A2AR neurons to restore normal sleep patterns and enhance patient well-being.

**Keyword 1**: DARPP-32
**Keyword 2**: NREM Sleep
**Keyword 3**: Parkinson's disease
**Keyword 4**: A2A neurons
**Keyword 5**: Excessive daytime sleepiness

**DISTINCT CONTRIBUTIONS OF DOPAMINE AND NORADRENALINE DYSFUNCTION TO SLEEP DISTURBANCES IN A PARKINSON'S DISEASE MODEL**

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A broad spectrum of sleep disturbances is associated with Parkinson's disease (PD), significantly impairing patients' quality of life and often persisting under pharmacological treatments. This study examined sleep architecture in a PD mouse model generated by unilateral administration of catecholamine neurotoxin 6-hydroxydopamine (6-OHDA) into the medial forebrain bundle.
Electroencephalogram and electromyogram electrodes were implanted over the left parietal cortex and neck muscles of both sham-lesioned and 6-OHDA-lesioned mice. Continuous 24-hour recordings were performed, and wakefulness, REM sleep, and non-REM (NREM) sleep states were classified and analyzed using a MATLAB-based algorithm.
Mice with 6-OHDA lesions exhibited reduced wakefulness and increased NREM sleep during the active phase of the sleep-wake cycle, consistent with excessive daytime sleepiness (EDS). Additionally, these animals showed a higher number of NREM episodes with shortened duration during the inactive phase, indicative of sleep fragmentation. Notably, pre-administration of desipramine, which preserves noradrenergic function, prevented sleep fragmentation but did not mitigate EDS. Treatment with reboxetine, a selective norepinephrine reuptake inhibitor, reduced the number of NREM episodes but failed to prolong their duration during the inactive phase. These findings demonstrate that the PD mouse model employed in this study recapitulates sleep abnormalities observed in PD patients, including EDS and sleep fragmentation. Importantly, our results indicate that while nigrostriatal dopamine loss is sufficient to induce EDS, sleep fragmentation is primarily driven by noradrenergic dysfunction. In conclusion, these findings suggest that while targeting noradrenergic transmission can improve disrupted sleep architecture in PD, further studies are necessary to identify optimal pharmacological interventions.

**Keyword 1**: Parkinson's disease
**Keyword 2**: Sleep
**Keyword 3**: Sleep fragmentation
**Keyword 4**: Excessive daytime sleepiness
**Keyword 5**: Mouse

**EFFECT OF GENERAL ANESTHESIA ON POSTOPERATIVE CHANGES IN INFLAMMATORY CYTOKINES IN PATIENTS WITH PARKINSON'S DISEASE**

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**Introduction:**
While the exact mechanisms underlying neuronal loss in Parkinson's disease (PD) remain unclear, growing evidence suggests that inflammation and the peripheral immune system play a critical role. The immune response may influence both the central and peripheral nervous systems. General anesthesia and surgical stress can trigger perioperative immune and inflammatory responses, leading to the activation of immunosuppressive mechanisms. Despite these clinical implications, no studies have yet investigated the effects of general anesthesia on serum cytokine levels in PD patients. Therefore, this study aims to examine the impact of general anesthesia on IL-6, IL-17, IL-10, IL-8, IFN-γ, and TNF-α levels in patients with Parkinson's disease.
**Methods:**
Patients aged 40–80 years with Parkinson's disease undergoing orthopedic surgery under general anesthesia were included. Peripheral blood samples were collected before the induction of general anesthesia (T0). Anesthesia was induced using propofol (1–2 mg/kg) and rocuronium (0.6–1 mg/kg), followed by endotracheal intubation after 1–2 minutes. Anesthesia was maintained with sevoflurane (1–2 vol.%), remifentanil (0.01–0.2 μg/kg/min), and an oxygen/air mixture ($FiO_2$ 0.4). Blood samples were collected again 24 hours postoperatively (T1).
**Results:**
Only IFN-γ demonstrated a significant decrease, dropping from 41.9 pg/mL preoperatively to pg/mL postoperatively. Other cytokines did not show consistent changes.
*Note:* Participant recruitment is still ongoing, and a control group of healthy individuals is currently being enrolled. By the time of the conference, recruitment is expected to be complete, and cytokine analysis finalized.

**Keyword 1**: Parkinson's disease
**Keyword 2**: general anesthesia
**Keyword 3**: IFN-γ

**CARRIES OF *LRRK2* PATHOGENIC VARIANTS SHOW A MILDER, ANATOMICALLY DISTINCT BRAIN SIGNATURE OF PARKINSON'S DISEASE**

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*LRRK2* gene variants are a major genetic risk factor for both familial and sporadic Parkinson's disease (PD), opening an unattended window on the disease's mechanisms and potential therapies. Investigating the influence of pathogenic variants in *LRRK2* gene on brain structure is a crucial step toward enabling early diagnosis and personalized treatment. Yet, despite its significance, the ways in which *LRRK2* genotype affects brain structure remain largely unexplored. Work in this domain is plagued by small sample sizes and differences in cohort composition, which can obscure genuine distinctions among clinical subgroups. In this study, we overcome such important limitations by combining explicit modeling of population background variation and pattern matching. Specifically, we leveraged a large cohort of 641 participants (including 364 with a PD diagnosis) to examine MRI-detectable cortical atrophy patterns associated with the *LRRK2* pathogenic variants in people with PD and non-manifesting individuals. LRRK2 PD patients exhibited milder cortical thinning compared to sporadic PD, with notable preservation in temporal and occipital regions, suggesting a distinct pattern of neurodegeneration. Non-manifesting LRRK2 carriers showed no significant cortical atrophy, indicating no structural signs of subclinical PD. We further analyzed the relationship between aggregated alpha-synuclein in cerebrospinal fluid and atrophy. We found that those with evidence of aggregated alpha-synuclein experienced pronounced neurodegeneration and increased cortical thinning, possibly defining another aggressive PD subtype. Our findings highlight avenues for distinguishing PD subtypes, which could lead to more targeted treatment approaches and a more complete understanding of Parkinson's disease progression.

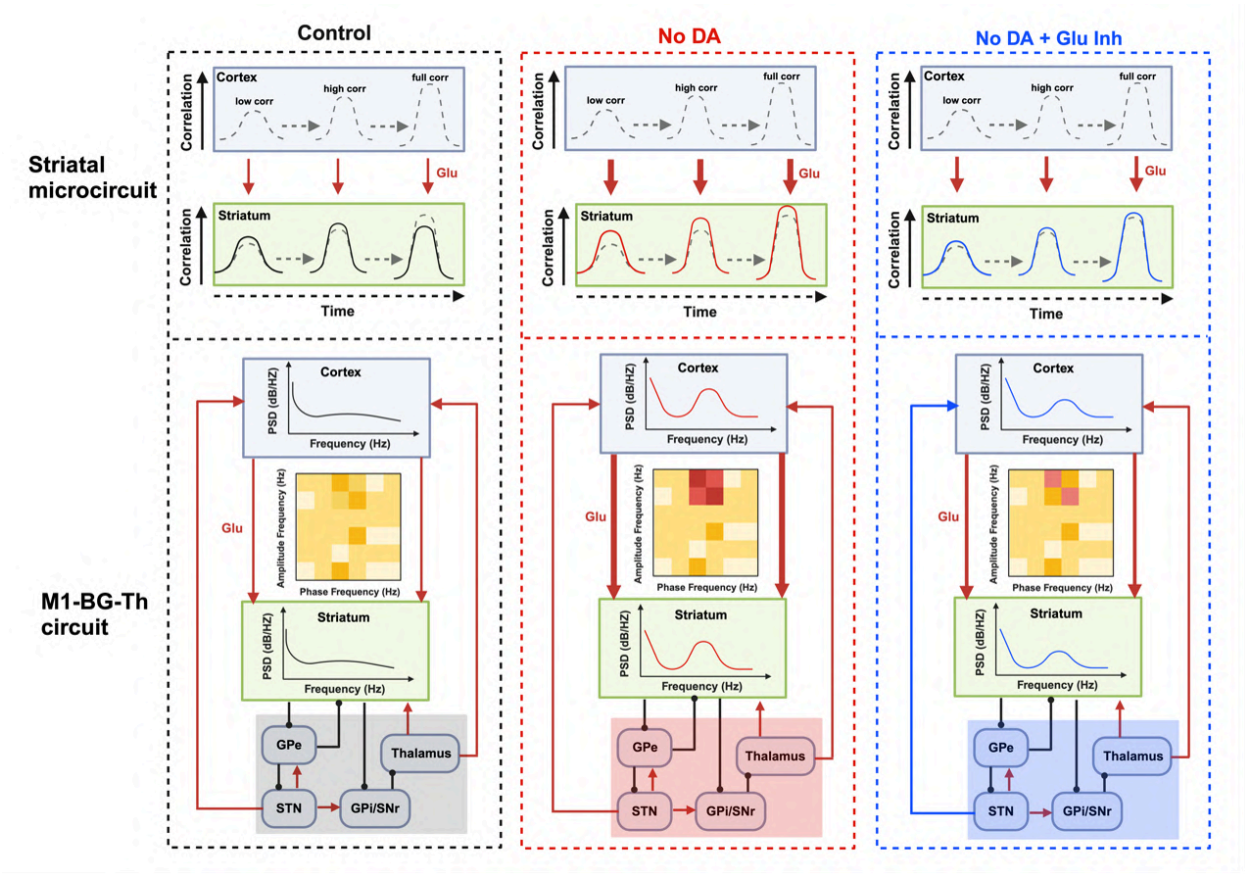
**Keyword 1**: Parkinson's disease
**Keyword 2**: LRRK2
**Keyword 3**: neuroimaging
**Keyword 4**: structural MRI
**Keyword 5**: Lewy body

**CORTICO-STRIATAL GLUTAMATE HYPERACTIVITY MEDIATED BETA OSCILLATION DYNAMICS IN COMPUTATIONAL PARKINSONIAN MODELS**

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Parkinson's disease (PD) is a debilitating neurodegenerative disorder characterized by severe motor deficits. Previous studies have linked hypokinetic symptoms to aberrant beta oscillations and synchronization in the cortex-basal ganglia circuit. The degeneration of dopamine (DA) neurons also leads to hyperactive glutamatergic transmission in the cortico-striatal pathway, yet the relationship between this dysregulated glutamate (Glu) signaling and altered striatal beta oscillation dynamics remains unclear. In the current study, we develop computational models of the striatal microcircuit and the cortico-basal ganglia-thalamic circuit to investigate the interaction between glutamatergic hyperactivity and beta oscillation dynamics in PD. Our findings reveal that DA depletion-induced cortico-striatal glutamatergic hyperactivity significantly disrupts striatal responsivity, reduces resistance to increased cortical input correlation, and is associated with increased beta power and cortico-striatal phase-amplitude coupling (PAC). Notably, inhibition of excessive Glu transmission, specifically targeting postsynaptic N-Methyl-D-Aspartate (NMDA) receptors, partially restores normal striatal decorrelation, reduces beta power and decreases PAC within the cortico-striatal circuit. Furthermore, this modulation normalizes neuronal oscillations and synchronizations across the cortico-basal ganglia circuit and sustains cortical function via the subthalamic nucleus-cortex feedback loop. Our results demonstrate that the overactive glutamatergic transmission, particularly through the NMDA receptor, is a crucial factor driving pathological beta oscillation patterns in the cortico-basal ganglia circuit in PD. Targeting this dysregulated Glu signaling may offer a novel therapeutic approach to normalize beta oscillation dynamics and alleviate motor dysfunction in PD.

**Keyword 1**: Cortico-striatal glutamate hyperactivity
**Keyword 2**: Beta oscillations dynamics
**Keyword 3**: Cortico-basal ganglia-thalamocortical loop
**Keyword 4**: Computational models
**Keyword 5**: Parkinson’s disease


Control
No DA
No DA + Glu Inh
Striatal microcircuit
M1-BG-Th circuit
Cortex
Striatum
Correlation
low corr
high corr
full corr
Glu
Time
PSD (dB/HZ)
Frequency (Hz)
Amplitude Frequency (Hz)
Phase Frequency (Hz)
GPe
STN
GPi/SNr
Thalamus

**MODELING PARKINSON'S DISEASE IN 3D: INTEGRATING SH-SY5Y ENCAPSULATION AND OPTOGENETIC CONTROL FOR ENHANCED NEURODEGENERATIVE RESEARCH**

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Parkinson's disease (PD) is a neurodegenerative condition characterized by progressive loss of dopaminergic neurons. Traditional 2D cell culture models often fail to mimic the intricate cellular architecture and interactions in the human brain, limiting their ability to accurately represent neurodegenerative processes or effectively test potential therapies. To overcome these limitations, we have developed an innovative 3D multiplex model using SH-SY5Y cells, a human neuroblastoma line encapsulated in a Gelatin methacrylate, combined with optogenetic tools to control neuronal activity precisely. This model provides a more biologically relevant environment, enabling the study of key disease features such as dopaminergic differentiation, neurite growth, and cellular responses to neurotoxic damage. The integration of optogenetic control allows for real-time modulation of neuronal activity, providing a unique opportunity to explore the effects of stimulation or inhibition on the progression of PD. The fusion of 3D cellular encapsulation with optogenetic presents a robust platform for investigating Parkinson's disease pathophysiology, uncovering novel therapeutic targets, and assessing the effectiveness of potential treatments. This advanced model significantly enhances neurodegenerative disease research, offering valuable insights that bridge the gap between experimental and clinical studies.

**MAY THE OFFSPRING OF SLEEP DEPRIVED MOTHERS PREDISPOSE THEM TO PARKINSON'S DISEASE?**

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Parkinson's Disease (PD) depends on environmental and genetic factors. Maternal sleep deprivation can lead to metabolic disorders and increased neuroinflammation in the offspring. Maternal inflammation reduces the amount of mitochondrial DNA, increases the rate of mitochondrial fragmentation, and consequently affects glial and neural cells in brain development. In our study, we aimed to examine the response to Parkinson's disease in offspring of mothers exposed to sleep deprivation. Rats were subjected to an 18-hour REM sleep deprivation procedure for 21 days after the detection of pregnancy. The REM sleep deprivation model utilizes the use of a multi-platform technique. The control group is formed by offspring of normal female rats. When the puppies were 2 months old, the PD model was induced by 6-OHDA. After 21 days, behavioral and rotation tests were performed, and total antioxidant capacity was measured from blood. When we compare rats that are offspring of sleep deprived mothers (OSDM) and offspring of control mothers (OCM), behavioral tests (anxiety, locomotor activity, and memory) are non-significant between the two groups. Rotation numbers increased in the OSDM group ($p=0.0106$). Total antioxidant capacity highly decreased in the OSDM group ($p=0.0006$). There is a potential link between sleep deprivation in the mother rat and an increased neuroinflammation in the offspring. It may predispose to PD.

**Keyword 1**: maternal sleep deprivation
**Keyword 2**: parkinson's disease

**IDENTIFICATION OF ALTERED PATHWAYS IN PARKINSON'S DISEASE PATIENTS-DERIVED MESODIENCEPHALIC DOPAMINERGIC (MDDA) NEURONS CARRYING NEW GENETIC RARE VARIANTS**

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Parkinson’s disease (PD) pathophysiology is still an aim of study; PD is characterized by an interplay of several altered cellular mechanisms. In the last years, new genes emerged associated to PD insurgence. Recently, we demonstrated the existence of a polygenic model of inheritance associated to both familial and sporadic PD forms. In this study we generated 10 induced Pluripotent Stem Cells (iPSC) from 6 PD patients carrying rare mutations in new genes such as AIMP2, HMOX2, SLC6A3, KIF21B, LRRK2, RHOT2, TMEM175, TOMM22, TVP23A, ZSCAN21 and 4 matched healthy subjects. We used these iPSCs-derived mesodiencephalic dopaminergic neurons (mdDA) to disclose deregulated pathways and monitor their electrophysiological properties. Patch-clamp experiments disclosed a defect in current recording and cell capacitance in patient-derived neurons when compared to healthy subjects. Particularly, patients carrying heterozygous mutations in *SLC6A3* and in *HMOX2* genes displayed a more pronounced electrophysiological immature profile compared to those of other patients and controls. Furthermore, we also integrated data from untargeted proteomic and lipidomic approach with transcriptomic analysis of mdDA neurons and we revealing a common signature of altered transcript, proteins and lipids involved in the most relevant step of cellular energy production and related organelles like mitochondria and peroxisomes, affecting the most of PD patients-derived mdDA neurons. These findings were also supported by expression studies performed with droplet digital PCR. In conclusion, we highlight that multilayer integrative analysis could corroborate new potential cellular mechanisms and target in support of personalized medicine application for PD diagnosis and prognosis.

**Keyword 1**: Neurodegeneration
**Keyword 2**: Parkinson's disease
**Keyword 3**: Dopaminergic neurons
**Keyword 4**: rare genetic variants

**UNRAVELING SENESCENCE INDUCTION IN ALPHA-SYNUCLEIN-OVEREXPRESSING NEURONS: A NOVEL CELLULAR MODEL SYSTEM AND ADVANCED SENESCENCE MEASUREMENT APPROACH**

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While alpha-synuclein (aSyn) aggregation is a well-established hallmark of synucleinopathies, its potential interplay with cellular senescence remains unexplored. Recent evidence suggests that neuronal senescence, traditionally associated with proliferative cells, may be a critical yet overlooked mechanism in neurodegeneration. However, the lack of suitable models and reliable detection methods has hindered progress in this field. We established a novel neural model system with aSyn overexpression and developed an innovative, specific, and transient senescence-reporting system to dissect this relationship.
We generated neural cell lines with stable aSyn overexpression (wild-type and A53T mutant variants) and implemented a newly designed, versatile senescence-detection system based on transient reporter constructs controlled by senescence-associated promoters (p16INK4a and p21Cip1/Waf1). This approach allows precise quantification of senescence induction via fluorescence-based imaging and flow cytometry. We applied diverse senescence-inducing agents, including oxidative stress to assess senescence susceptibility in aSyn-overexpressing neurons. Our findings indicate that aSyn overexpression sensitizes neural cells to senescence-inducing stimuli, leading to reporter activation and distinct senescence-related featured including increased β-galactosidase activity and senescence-associated secretory phenotype signature. This supports the hypothesis that aSyn-related pathology may involve or exacerbate cellular senescence.
By introducing an entirely new experimental framework, our study provides the first robust platform to investigate neuronal senescence in the context of aSyn overexpression. Our transient detection system enables highly specific and reproducible assessment of senescence with potential intervention strategies for neurodegenerative disorders.

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**Keyword 1**: alpha-synuclein
**Keyword 2**: senescence
**Keyword 3**: neurodegeneration
**Keyword 4**: reporters

**THE SYNERGY OF DOPAMINERGIC SYSTEM AND ADULT HIPPOCAMPAL NEUROGENESIS IN A PRE-CLINICAL MODEL OF PARKINSON'S DISEASE**

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Depressive disturbances are prevalent in 40% to 50% of clinical cases of Parkinson's Disease (PD), alongside a common reduction in adult hippocampal neurogenesis observed in both PD and its related conditions. This neurogenesis deficit may affect the clinical course of the disease. With this in mind, we set an experiment using the glial fibrillary acidic protein-thymidine kinase (GFAP-TK) transgenic rat model to assess the impact of impaired adult cytogenesis induced by the antiviral Ganciclovir on PD. The experiment involved a combination of the GFAP-TK model and a 6-hydroxydopamine (6-OHDA) model of PD, while behavioral analyses focused on anxiety, depression, and motor skills. From the results, histological examinations revealed decreased proliferative cells and reduced dopaminergic innervation. Additionally, analysis of newborn and immature neurons occurred in the hippocampus, subventricular zone, and olfactory bulbs, while dopaminergic loss was assessed in regions like the substantia nigra and striatum. Findings indicated that the model exhibited anxiety/depressive-like behaviors and motor impairments, linked to the notable loss of dopaminergic neurons, which appeared to correlate with reduced doublecortin-positive cells in the hippocampus. Moreover, results suggested subtle differences between ipsilateral and contralateral sides, highlighting the dopaminergic system's role in hippocampal adaptation. Therefore, these findings suggest a connection between reduced neurogenesis and dopaminergic neuron loss, hinting that these phenomena might be interrelated. Therefore, investigating this potential regional interconnection may augment our understanding of non-motor dimensions in PD pathophysiology related to motor functions, thereby facilitating the development of enhanced therapeutic strategies for individuals in the early stages of PD.

**Keyword 1**: Depression
**Keyword 2**: Parkinson's Disease
**Keyword 3**: Neurogenesis
**Keyword 4**: Hippocampus

**METABOLIC SPIKE LOSS AS THE CRITICAL DRIVER OF PARKINSON'S DISEASE**

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A key mystery in Parkinson's disease (PD) is the absence of dopamine in the striatum, and the cell loss of the dopamine-releasing substantia nigra pars compacta (SNcDA) neurons. Paradoxically, a dysregulation cascade from excess cytosolic dopamine in SNcDA causes the loss of SNcDA. Why is the readily available cytosolic dopamine not being released into the striatum and instead causing cell death? Here, we apply our recent theory wherein neurons self-initiate action potentials to maintain metabolic homeostasis. Our work demonstrates that the loss of such self-initiated metabolic spiking is the critical driver of PD, establishing a comprehensive causal pathway from initial dysfunction to eventual cell death. In our *in silico*, metabolism-coupled SNcDA neuron model that senses ROS and autonomously initiates spikes, we identify four categories of dysfunction that diminish metabolic spikes and deplete striatal dopamine. These categories align with several reported environmental (e.g., rotenone), lifestyle (e.g., stress, sleep), and genetic factors implicated in PD etiology.
To connect these dysfunctions to accelerated SNcDA cell death in PD, we developed a spike-dependent dopamine release model, which integrates key dopamine processes: release, reuptake, *de novo* dopamine synthesis, and its spontaneous degradation into disruptive aldehyde DOPAL, specifically at axonal terminals. Further, the clearance of these DOPAL disruptions relies on retrograde endosomal transport, predicting increased cellular maintenance costs in SNcDA that accumulate over days or weeks. To investigate the nearly decade-long disease progression, our computational model can incorporate key aspects of aging at temporal resolutions previously inaccessible, allowing us to model the slow progression of PD.

**Keyword 1**: Metabolism
**Keyword 2**: Sleep
**Keyword 3**: Dopamine
**Keyword 4**: Parkinson's disease
**Keyword 5**: Reactive Oxygen Species

**SENSORY PROCESSING AND MEMBRANE PROPERTIES OF EXTERNAL GLOBUS PALLIDUS NEURONS IN DOPAMINE DEPLETED MICE**

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Parkinson's disease (PD) is characterized by the loss of dopamine in the basal ganglia (BG), leading to aberrant activity patterns in BG neuronal circuits. The external globus pallidus (GPe) is a central nucleus in the BG playing a pivotal role in the function of the indirect pathway. Two populations of GPe cells were described, the prototypic and arkypallidal neurons, which are distinct developmentally, anatomically and electrophysiologically. GPe cells have been shown to process tactile information, mediated by different input pathways. How dopamine depletion affects such processing is unknown. We use *in vivo* whole cell patch clamp recordings to study the membrane properties of GPe neurons and examine their responses to whisker stimulation in anesthetized healthy and dopamine depleted mice. Following dopamine depletion, prototypic neurons exhibited a decrease in spike frequency, spiking regularity, and input resistance compared to prototypic neurons recorded in control animals, while no such differences were observed in arkypallidal neurons. Prototypic and arkypallidal neurons recorded in dopamine depleted mice exhibited a triphasic response to bilateral whisker stimulation. The response of prototypic neurons to whisker stimulation remained almost unchanged after dopamine depletion, whereas in arkypallidal neurons, all three phases decreased in the amplitude. Thus, out findings show alteration in the membrane properties of prototypic neurons and a reduced amplitude of the response of arkypallidal neurons to sensory stimulation following dopamine depletion. Understanding how the GPe circuitry and function are affected by dopamine depletion is essential for our understanding of PD and its potential treatment.

**Keyword 1**: GPe
**Keyword 2**: Parkinson
**Keyword 3**: in vivo recordings

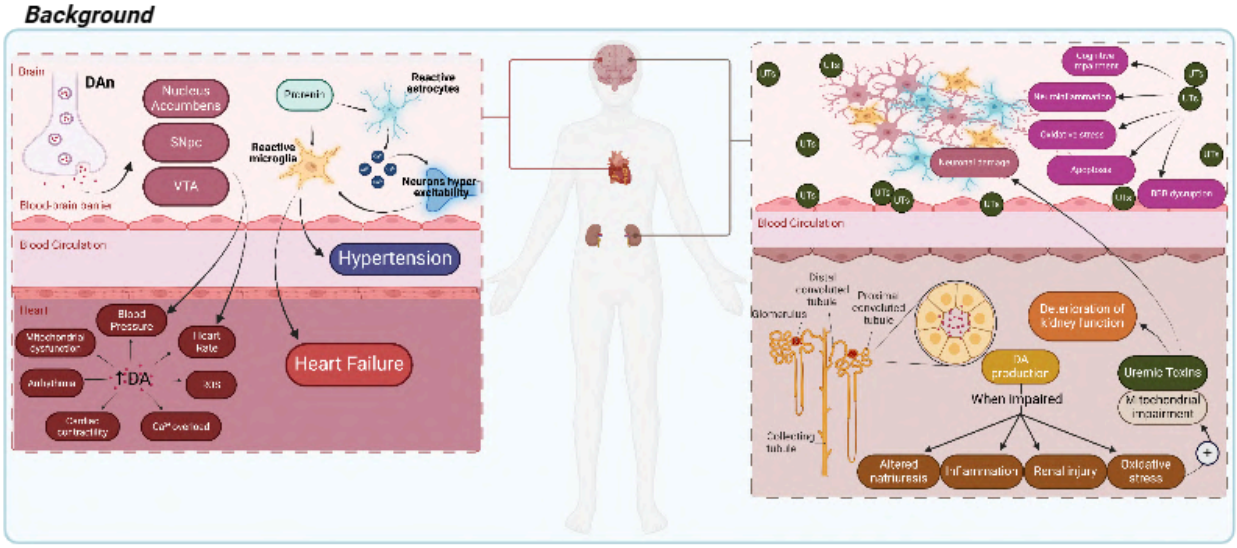
**BEYOND THE BRAIN: THE HIDDEN ROLE OF CARDIORENAL DYSFUNCTION IN PARKINSON'S DISEASE**

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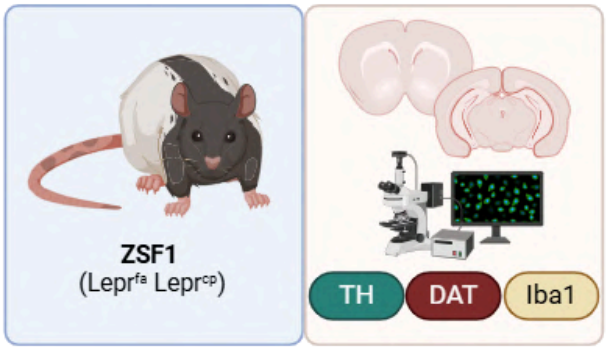
Parkinson's disease (PD) is the second most common neurodegenerative disorder, marked by the progressive loss of dopaminergic neurons in critical areas of the brain, particularly the striatum and substantia nigra. PD's complex nature suggests its interactions with various systemic health issues, particularly those affecting organs outside the central nervous system (CNS), which may increase the risk of developing PD and affect treatment outcomes. Research indicates that individuals with cardiovascular disease (CVD) and chronic kidney disease (CKD) face significantly higher risks of PD, even when controlling for shared risk factors. Notably, alpha-synuclein aggregations, a hallmark of PD, have also been found in the renal and cardiac tissues of patients with PD, CKD, and CVD, highlighting the interconnectedness of these systems. The Zucker fatty and spontaneously hypertensive (ZSF1) rats model metabolic syndrome, which includes kidney issues and heart failure. This study aimed to explore how the ZSF1 phenotype impacts the integrity of dopaminergic neurons and neuroinflammatory processes. Brain tissues from ZSF1 rats were analyzed through immunostaining with markers specific to dopaminergic and glial cells. The results showed a significant decrease in dopaminergic markers in the striatum and substantia nigra, indicating a potential link between cardiorenal dysfunction and neurodegenerative pathways. These findings suggest that systemic health conditions can directly influence PD pathology, emphasizing the complex interactions between the brain, heart, and kidneys, and presenting new opportunities for targeted PD therapies.

**Keyword 1**: Parkinson's disease
**Keyword 2**: Brain-heart-kidney axis
**Keyword 3**: Chronic kidney disease
**Keyword 4**: Cardiovascular diseases

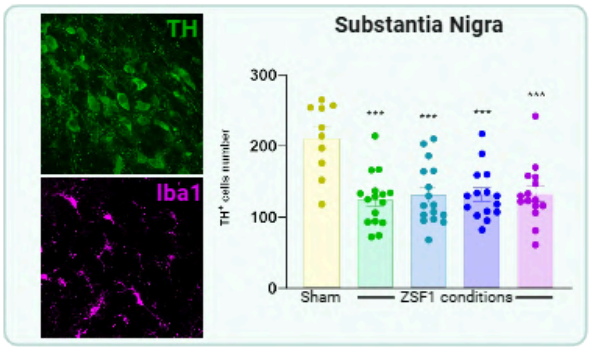
***Background***




***Methodology***




***Results***



**EVALUATION AND TREATMENT OF IMBALANCE / DIZZINESS IN ELDERLY PACIENTS WISH PARKINSON'S DISEASE**

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**OBJECTIVES**
The prevalence of balance problems in people aged more than 70 years, with Parkinson's Disease (PD) is rapidly increasing. The terms Imbalance cover a variety of symptoms, which affect a stable gaze and normal gait. The aim of this study was to recognize main signs of this problem and determine rapid support recommendations.
**METHODS**
19 patient wish PD, with acute dizziness, admitted to Neurological department, were investigated. The main symptoms was acute vertigo with fall (n=4) and acute vertigo with unsteadiness (n=15). After exclusion of acute vascular pathologies, investigation of balance system was provided by vestibular Romberg test, Fukuda step test, vestibular ocular reflex (VOR) and Dix–Hallpike maneuver (DHM). In some cases (n=5), after the fast stabilization, video head impulse testing (VHIT) was done.
**RESULTS**
In 7 cases, reason of dizziness was bilateral vestibulopathy, in 2 cases, - benign paroxysmal positional vertigo, in 3 cases, - vestibular neuritis and in 7 cases, somatosensory loss. In all cases, VOR and DHM were effective for balance recovery as well as first medical support. After the acute period (24hr) vestibular rehabilitation (VR) exercises were more effective than DHM.
**CONCLUSIONS**
Imbalance / Dizziness in PD patients remains a difficult subject, given the underlying factor of vestibular impairment due to aging in the form of presbystasis. The diagnostic and therapeutic approach must be oriented to the visual, proprioceptive, and vestibular systems.
Imbalance / Dizziness in patients with PD, can be treated both with physical and pharmacologic therapy to help improve symptoms.

**SCHIZOPHRENIA RISK ENRICHED GENE-PROGRAM CO-DYSREGULATES PROTEIN MODIFICATION, ENERGY SUPPLY AND ION TRANSPORT IN THE PREFRONTAL CORTEX**

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Schizophrenia is a devastating, genetically complex disorder characterized by persistent and idiopathic cognitive deficits, delusions and hallucinations. Schizophrenia has been associated with impaired nervous system development and an excitation/inhibition imbalance in the prefrontal cortex. On a molecular level, hundreds of risk genes have been identified, spanning a heterogeneous landscape dominated by loci that confer relatively small risk. Bioinformatic analyses of genetic associations point to excitatory cortical neurons, but other analyses suggest the importance of astrocytes and microglia. To understand different cell type roles in schizophrenia and reveal novel aetiologically relevant perturbations, our study integrated genetic analysis with single nucleus RNA-seq of 536,618 nuclei from postmortem samples of dorsal prefrontal cortex (Brodmann Area 8/9) of 43 cases with schizophrenia and 42 neurotypical controls. Gene expression in excitatory layer 2-3 intra-telencephalic neurons had the most differentially expressed transcripts and, together with excitatory deep layer intra-telencephalic neurons, conferred most of the genetic risk for schizophrenia. Most differential expression of genes was dominated by down-regulated transcripts, which were enriched in transmembrane transport, mitochondrial function, protein folding, and cell-cell signaling pathways. Up-regulated transcripts were enriched in gene sets related to RNA processing, including RNA splicing in neurons. Co-regulation network analysis identified 40 schizophrenia-relevant programs across 13 cell types. A gene program largely shared between neuronal subtypes, astrocytes, and oligodendrocytes was significantly enriched for schizophrenia risk, supporting an aetiological role for perturbed protein modification, ion transport, and mitochondrial function. Our study integrates genetic analysis with transcriptomics to reveal novel cell-type specific aetiologically relevant perturbations in schizophrenia.

**Keyword 1**: schizophrenia
**Keyword 2**: single nuclei RNA-sequencing
**Keyword 3**: human prefrontal cortex
**Keyword 4**: co-expression network analysis
**Keyword 5**: integration of genetic risk

**BIOLOGY OF SUICIDE: (EPI)GENETIC AND PROTEOMIC APPROACH**

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Aims: Suicidality is a serious public health problem that affects individuals, their immediate environment and the wider society. Slovenia is a country with one of the highest suicide rates worldwide. The risk factors are numerous, with biology becoming increasingly more acknowledged. With our work we want to investigate the biological background of suicide in a Slovenian sample. Methods: The subjects are men, aged 18 to 65 who died by suicide by hanging or by sudden cardiac death (control group). The samples were various brain regions and blood taken posthumously during a routine autopsy. The epigenetic state was analysed using the genome-wide RRBS method and targeted 5mC sequencing. Gene expression was analysed using hydrolysis assays and qPCR. Investigations at the protein level are also underway. Results: Genome-wide methylation analysis revealed changes most notable in genes involved in cell structural integrity and nervous system regulation. These changes were confirmed by targeted sequencing and gene expression analyses. We are currently analysing the gene expression of genes identified by RRBS that may be involved in protein aggregation (*CRMP1, DISC1, TSNAX-DISC1, MAPT, SOD1, PARK2, GABARAPL1, GRIN2A* and *GRIN2B*). Conclusions: The occurrence of suicidal behaviour is influenced by many factors. Although it is not the only factor, the biological background is very important, which is reflected in the results of our study in Slovenian subjects. By continuing the study and shifting the focus to proteins and their possible aggregation in suicidal behaviour, we would like to contribute to the understanding and more appropriate treatment of suicidality.

**Keyword 1**: suicide
**Keyword 2**: psychiatry
**Keyword 3**: epigenetics
**Keyword 4**: protein aggregation
**Keyword 5**: mental health

**INVESTIGATING THE EFFECTS OF STRESS ON THE TRANSCRIPTOME AND PROTEOME *IN VITRO***

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Elevated glucocorticoid levels or chronic stress have been linked with cognitive deficits and an increased risk of neurological conditions such as Alzheimer's disease. Chronic stress partly exerts its negative effects through prolonged activation of the hypothalamic-pituitary-adrenal axis and subsequent excess glucocorticoid production. Glucocorticoids are liposoluble, enabling them to cross the blood-brain barrier and directly influence neural function. Evidence from animal models shows deleterious effects of long-term exposure to glucocorticoids including suppression of neurogenesis, decreased dendritic branching and inhibition of synaptogenesis, ultimately contributing to neuronal atrophy. However, research using human neural tissue is limited, which is crucial for understanding the mechanisms by which stress affects the human brain. Moreover, most studies have focused on transcriptomic changes in neural tissue, with proteomic responses remaining largely unexplored. Thus, our study aims to investigate the transcriptomic and proteomic changes elicited by the stress response in iPSC-derived neurons. To achieve this, wildtype NGN2-neurons are treated with the glucocorticoid receptor agonist, hydrocortisone. This work is currently being undertaken. RNA sequencing and mass spectrometry will be used to identify differentially expressed genes and proteins between stressed and non-stressed neurons. This research will provide insights into how neurons respond to stress at multiple molecular levels. Future work could look at whether different genotypes respond differently to stress in order to identify individuals who are most susceptible to the negative effects of stress.

**SMALL COMPOUNDS: A NEW PROMISING STRATEGY TO SAFEGUARD BDNF RECEPTOR IN ALZHEIMER'S DISEASE**

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Brain-Derived Neurotrophic Factor (BDNF) promotes neuronal protection through the activation of its full-length receptor, TrkB-FL. In Alzheimer's Disease (AD), this BDNF/TrkB-FL system is compromised due to an amyloid-beta (Aβ)-mediated calpain TrkB-FL cleavage originating two fragments: a truncated receptor (TrkB-T') and an intracellular fragment (TrkB-ICD) leading to neuroprotection loss. Focusing on the BDNF/TrkB-FL signaling reestablishment as a potential therapeutic strategy for AD, the aim of this work was to search for small compounds able to prevent TrkB-FL cleavage. A computer drug design protocol based on docking prediction, was applied to select the most promising compounds that would bind TrkB-FL at its cleavage site. The top eight compounds (A-H) were added to primary cortical neuronal rat cultures, with and without Aβ, to assess their effect on preventing TrkB- FL cleavage. To evaluate the effect of the compounds upon hippocampal synaptic transmission, electrophysiological experiments were performed. In basal conditions, compound B reduced by 70% TrkB-ICD/TrkB- FL levels in all studied concentrations compared to control condition ($p<0.05$,$n=5$). Compound C, at the 100 nM concentration, decreased TrkB-ICD/TrkB-FL levels in 70% when compared to its control ($p<0.05$,$n=4$). Remarkably, compound H showed potential in decreasing TrkB-ICD/TrkB-FL levels (30%) in the presence of Aβ ($p<0.05$,$n=9$). Importantly, compound H did not affect BDNF-mediated phosphorylation of TrkB-FL receptor nor hippocampal synaptic transmission ($p>0.05$,$n=7$). Altogether, compounds B and C prevented TrkB-FL basal cleavage being now important the test them with Aβ. Compound H co-incubated with Aβ, prevented TrkB-FL cleavage without interfering with basal synaptic transmission nor TrkB-FL phosphorylation.

**Keyword 1**: Alzheimer's disease
**Keyword 2**: BDNF
**Keyword 3**: TrkB-FL cleavage
**Keyword 4**: Small compounds

**TRKB-ICD AND TAT-TRKB IN MICROGLIA: UNRAVELLING THEIR ROLE IN NEURON-MICROGLIA INTERACTIONS IN ALZHEIMER'S DISEASE CONTEXT**

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In Alzheimer's disease (AD), BDNF/TrkB-FL signalling is disrupted due to amyloid-beta (Aβ)-induced cleavage of TrkB-FL receptor, generating TrkB-ICD. Initially identified in neurons, TrkB-ICD is a stable fragment that phosphorylates nuclear and axonal proteins, altering transcriptomics and proteomics. Notably, it is present in secretome, suggesting its release from neurons and potential effects on surrounding cells. Different cell types, like microglia, also express TrkB-FL. To prevent TrkB-FL cleavage, we developed TAT-TrkB, a novel peptide. This study aims to: 1) evaluate TAT-TrkB efficacy *in vivo* and 2) explore whether microglia produce TrkB-ICD and/or internalize neuron-derived TrkB-ICD, alongside the effects of TrkB-ICD and TAT-TrkB on microglial function.
The effect of TAT-TrkB (25 mg/Kg), intraperitoneally administered 5x/week for two months, was evaluated in 5xFAD mice (AD model). Animals were submitted to behavioural tests, afterwards, electrophysiological recording, immunohistochemistry and western-blot analysis were performed. Primary microglia were isolated and incubated with Aβ and/or TAT-TrkB or neuronal secretome to assess TrkB-FL and TrkB-ICD levels.
Results show that: 1) TAT-TrkB prevented hippocampal memory deficits, long-term potentiation impairments, dendritic spine loss, TrkB-FL cleavage, Aβ aggregation, and tau hyperphosphorylation; 2) Microglia expressed TrkB-FL, NMDA receptors, and calpains, indicating the potential for TrkB-FL cleavage. Preliminary results suggest that Aβ did not alter TrkB-FL or TrkB-ICD levels in microglia, nor did TAT-TrkB affect TrkB-FL levels. However, when exposed to neuronal secretome, data suggests that microglia engulfs neuronal-TrkB-ICD. TAT-TrkB effectively prevented AD-related deficits in 5xFAD mice, while findings suggest that microglia may play a role in TrkB-ICD processing.

**Keyword 1**: Microglia
**Keyword 2**: TAT-TrkB
**Keyword 3**: TrkB-FL
**Keyword 4**: TrkB-ICD

**BDNF RECEPTOR FRAGMENT DRIVES SYNAPTIC ALTERATIONS AND IS MIRRORED IN PLASMA-DERIVED EXTRACELLULAR VESICLES OF PATIENTS**

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The neuroprotective BDNF/TrkB-FL system is compromised in Alzheimer's disease (AD). Amyloid-beta triggers calpain-mediated TrkB-FL receptor cleavage, leading to TrkB-ICD formation, a novel intracellular fragment. Biological fluids are used to pinpoint potential disease biomarkers and extracellular vesicles (EVs) are cell-specific carriers of promising pathological biomarkers. Thus, this work aimed to 1) investigate TrkB-ICD effects and 2) its presence in AD human samples and EVs. To that endeavour, neurons were transduced with either LV-CamKII-GFP or LV-CamKII-TrkB-ICD-IRES-ZsGreen and used for patch clamp, dendritic spine and transcriptomic analysis. For plasma samples, patients fulfilled the criteria for Mild Cognitive Impairment due to AD ($MCI_{AD}$), whereas controls ($MCI_{CONTROL}$) reported cognitive complaints but had no evidence of Aβ pathology or neuronal injury. $MCI_{AD}$ plasma-derived EVs (pdEVs) were isolated using the ExoQuick reagent and characterized. EVs from 48-hour conditioned medium of control, GFP- and TrkB-ICD-V5-transduced differentiated SH-SY5Y cells were also isolated through differential ultracentrifugation and characterized to understand TrkB-ICD EV incorporation. Neurons overexpressing TrkB-ICD were enriched in phosphotyrosine proteins (p=7.62x10-3, n=3) and possessed fewer dendritic spines (p=1.15x10-10, n=13-15 cells/3 cultures). TrkB-ICD neurons are also more hyperexcitable given by mEPSC increase (p=2.46x10-2, n=7-8 cells/3cultures) and concomitant decrease in resting membrane potential (p=1.26x10-2, n=7-8 cells/3 cultures). Transcriptomic data revealed that TrkB-ICD upregulates 23 GO terms, encompassing genes implicated in for synaptic structure/function. PDEVs of $MCI_{AD}$ patients contained higher levels of TrkB-ICD (p=0.010, n=17-18). TrkB-ICD and TrkB-ICD-V5 were detected in both SH-SY5Y EV subpopulations (p>0.05, n=3). Altogether, these data demonstrate TrkB-ICD extracellular secretion, alluding for its potential toxicity dissemination.

**Keyword 1**: BDNF signaling
**Keyword 2**: TrkB-ICD
**Keyword 3**: Extracellular Vesicles
**Keyword 4**: Synapse
**Keyword 5**: Plasma

**INVESTIGATING THE EFFECT OF CAFFEINE ON -AMYLOID () INDUCED NEUROTOXICITY AND ITS LINK WITH THE GABAERGIC SYSTEM IN MOUSE PRIMARY HIPPOCAMPAL CULTURES**

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Alzheimer's disease (AD) is a neurodegenerative disorder characterized by synaptic loss and neurotransmitter remodeling, leading to cognitive deficits. A hallmark of AD is the formation of toxic β-amyloid (Aβ) plaques, and current pharmacological treatments remain limited due to its complex pathophysiology. As a result, research is shifting toward dietary interventions and nutraceuticals. Caffeine, a widely consumed psychoactive substance, has shown conflicting effects in AD models. Gamma-aminobutyric acid (GABA), the primary inhibitory neurotransmitter, plays a key role in AD, though its precise molecular involvement remains unclear. This study investigated caffeine's effects on Aβ-induced neuronal loss, adenosine receptor 1 (A1R), and GABA transporter 1 (GAT1) expression using an **in vitro** AD model. We found that caffeine significantly reduced neuronal death in primary hippocampal cultures across all concentrations tested (0.1, 1, 25, and 50 μM) five days post-Aβ treatment, with 25 μM also effective in acute (6h) conditions. A1R expression, which was downregulated by Aβ, was restored with caffeine treatment. However, neither Aβ nor caffeine significantly affected GAT1 expression, as assessed by immunocytochemistry and western blot analysis. These findings suggest that Aβ-induced neurotoxicity may involve disruptions in the adenosinergic system, and caffeine could mitigate these effects. Further research is needed to clarify caffeine's potential role in the GABAergic system and its broader therapeutic implications in AD.

**Keyword 1**: Alzheimer's disease
**Keyword 2**: amyloid-beta
**Keyword 3**: caffeine
**Keyword 4**: GABAergic
**Keyword 5**: adenosine receptor

**B-AMYLOID-INDUCED INCREASED TONIC CONDUCTANCE, IMPAIRED LTP AND COGNITIVE DEFICITS CHARACTERISTIC OF ALZHEIMER'S DISEASE ARE REVERSED BY AN A5 GABA TYPE A RECEPTOR INVERSE AGONIST**

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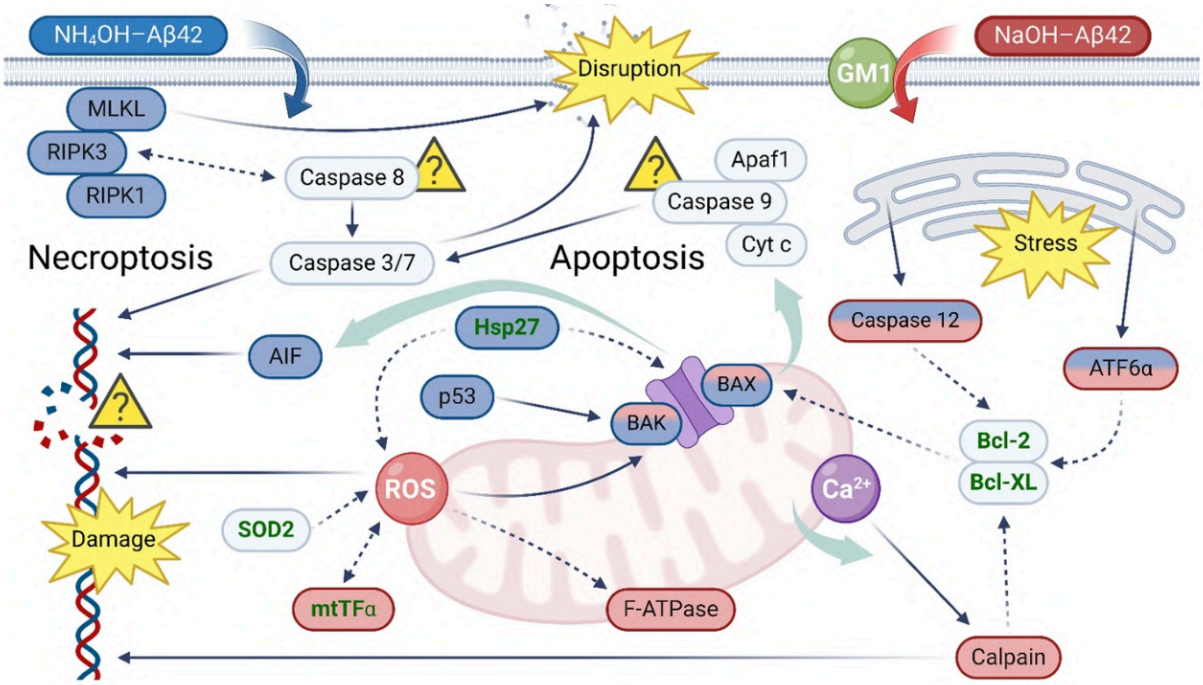
Alzheimer’s disease (AD) is a chronic progressive neurodegenerative disorder characterized by cognitive impairment, which may arise from disruptions in the excitatory/inhibitory balance within the brain. Gamma-aminobutyric acid (GABA), the principal inhibitory neurotransmitter in the central nervous system, plays a crucial role in maintaining the excitatory/inhibitory balance and regulating neuronal activity involved in memory. In AD, changes in α5-GABA type A receptors (α5GABAAR) expression and activity increase tonic inhibition, disturbing the neuronal excitatory/inhibitory balance and ultimately impairing cognitive processes. Therefore, targeting α5GABAAR offers a promising therapeutic strategy to mitigate the impairment of these processes. This study examined the potential of an α5GABAAR-selective inverse agonist, α5IA, for treating β-amyloid-induced cognitive deficits, and the underlying mechanism of action, using *ex vivo* microelectrode array and patch clamp electrophysiology. The inverse agonist, α5IA, significantly reduced β-amyloid-induced long-term spatial memory deficits and long-term potentiation. It reversed Aβ-induced increases in neuronal excitability, as indicated by input-output curves, and mitigated elevated tonic conductance. These findings highlight α5IA’s ability to restore excitatory/inhibitory balance and thereby cognitive function. The selective targeting of GABAARs by enhancing the efficacy of α5-GABAAR inverse agonists, such as α5IA, represents a promising direction for developing novel AD therapies.

**Keyword 1**: Alzheimer’s disease
**Keyword 2**: Neurodegenerative disease
**Keyword 3**: Microelectrode array

**DIFFERENT AMYLOID B42 PREPARATIONS INDUCE DIFFERENT CELL DEATH PATHWAYS IN THE MODEL OF DIFFERENTIATED SH-SY5Y NEUROBLASTOMA CELLS**

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Alzheimer's disease (AD) is characterized by the accumulation of amyloid-β42 (Aβ42) protein, forming toxic aggregates that damage neuronal cells. Various cell death pathways, including apoptosis, necroptosis, pyroptosis, oxidative stress, ferroptosis, and disturbances in mitophagy, autophagy, and endo/lysosomal functions, are linked to amyloid-β42 activity. This study investigated the cellular responses triggered by two structurally similar but functionally distinct Aβ42 preparations (NaOH-Aβ42 and $NH_4OH$-Aβ42 solubilization) in differentiated SH-SY5Y neuroblastoma cells. Despite the similar appearance in both preparations in dynamic light scattering and atomic force microscopy analysis, they triggered different cellular responses. NaOH-Aβ42 mainly causes mitochondrial dysfunction and impaired energy metabolism by increasing the production of reactive oxygen, which can cause a decline in ATP production. However, $NH_4OH$-Aβ42 induced early apoptosis and the formation of necrosomes, leading to necroptosis. Notably, $NH_4OH$-Aβ42 also activated the heat shock protein (Hsp)27 expression, which helps mitigate cellular stress. Both Aβ preparations elevated levels of general stress markers like Hsp70 and Hsp90, indicating a broad stress response. Interestingly, the toxicity of NaOH-Aβ42 was linked to the presence of ganglioside GM1, while $NH_4OH$-Aβ42's toxic effects occurred independently. These findings suggest that the specific structures of Aβ42 species are crucial for their toxic features. The presented differences between NaOH-Aβ42 and $NH_4OH$-Aβ42 preparations show the importance of evaluating varieties of Aβ42 in AD research. In conclusion, these variations in Aβ42 oligomers significantly influence the toxicity and trigger very specific cellular stress responses, emphasizing the importance of aggregate specificity in understanding AD.



**Keyword 1**: Alzheimer's disease
**Keyword 2**: Amyloid β42 oligomers
**Keyword 3**: Neurodegeneration

**PROBING THE MOLECULAR IMPACT OF TAU PATHOLOGY ON NEURONAL INTEGRITY USING ADVANCED CRYO-ELECTRON TOMOGRAPHY**

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In many neurodegenerative diseases, including Tauopathies, the relationship between the structures formed by protein aggregates, their impact on the neuronal environment, and the subsequent development of dementia remains poorly understood. This is largely due to the complexity of the interactions between these structures and their progressive accumulation, which are difficult to model. This research introduces a novel workflow that recapitulates Tau pathology in three-dimensional hippocampal slice cultures, which are subsequently imaged at high resolution using correlative light and cryo-electron tomography. This approach enables the targeting and identification of pathological Tau aggregates, specifically neurofibrillary tangles, and provides detailed insights into their structure and spatial arrangement in their native environment. Furthermore, evidence suggesting autophagic responses to Tau tangle formation has been observed, characterised by large, sheet-like intracellular aggregates and disruption of normal organelle structures. Notably, enlarged, diffuse calcium deposits were observed within sequestered mitochondria in Tau-accumulating tissues. The framework developed in this study offers the opportunity for high-resolution analysis of local interactions, shedding light on their impact on organelles and ultrastructural features within the neuronal landscape. This work contributes to a deeper mechanistic understanding of Tauopathies and establishes a versatile platform for investigating various neurodegenerative diseases *in situ*.

**Keyword 1**: Cryo-Electron Tomography
**Keyword 2**: Tau
**Keyword 3**: Dementia
**Keyword 4**: Hippocampus

**INVOLVEMENT OF THE *DROSOPHILA* GENE *YATA* THAT IS RELATED TO ALZHEIMER'S DISEASE IN THE METABOLISM, GROWTH AND LONGEVITY SIGNAL**

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We have identified YATA as a molecule that is required for the intracellular trafficking of APPL, the *Drosophila* homolog of the Alzheimer's disease-causing molecule APP. YATA is involved in the formation of COPI-coated transport vesicles that travel from the Golgi to the endoplasmic reticulum. *yata* null mutants show phenotypes including aberrant accumulation of APPL in the endoplasmic reticulum, progressive brain shrinkage, a shortened lifespan and progressive tissue vacuolization. Some phenotypes of *yata* null mutants are exacerbated in double null mutants with *Appl* and are rescued by the overexpression of APPL. Insulin/IGF-1 signal (IIS) and mechanistic target of Rapamycin (mTOR) pathway are known to regulate metabolism, growth, and lifespan. Suppression of these signals results in a reduction in cell size, the increased stress tolerance and longer lifespans. Since Ppk32, a fission yeast homolog of *yata*, has been reported to interact with mTOR, we investigated the involvement of *yata* in the IIS/mTOR pathway. Compared with wild-type flies, *yata* mutants presented smaller cell sizes. Furthermore, *yata* mutants showed increased resistance to heat stress. These phenotypes of *yata* mutants are similar to those of loss-of-function mutants of IIS/mTOR pathway components, which have longer lifespans However, surprisingly, analysis of the genetic interaction between *yata* and *chico*, a component of the IIS pathway, revealed that *yata* and the IIS signal act antagonistically. Our results suggest that the *yata* gene is involved in the regulation of the IIS/mTOR pathway.

**Keyword 1**: Drosophila
**Keyword 2**: APP
**Keyword 3**: Alzheimer's disease
**Keyword 4**: Amyloid precursor protein

**A FRUITS AND VEGETABLES-DERIVED COMPOUND AA INDUCES MITOPHAGY AND ALLEVIATES TAU PATHOLOGY IN ALZHEIMER'S DISEASE LIKE MODELS**

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Alzheimer's disease (AD) is the most case of dementia, which is the global health issues due to accelerated ageing population worldwide. Hyper-phosphorylated microtubule-associated protein Tau (p-Tau) forms abundant insoluble aggregates known as neurofibrillary tangles, is the hallmarks of AD. Imbalances in production of soluble Tau and clearance of aggregated hyper-phosphorylated Tau (p-Tau) with additional inactive autophagy (especially its subtype mitophagy) speed up AD progression. Our previous studies suggest turning up mitophagy may be an effective therapeutic strategy. In the present study, a network proximity approach and in-house-generated protein-protein interaction analysis are employed for predication of the fruit-derived bioactive compounds with anti-AD potential. We find a fruit-derived compound, αA can enhance cellular mitochondrial health, and induce autophagy/mitophagy in neurons. Using the cross-species models of AD, we confirm that αA attenuates aggregation of hTau P301S assemblies and suppresses expression of p-Tau at sites Thr181, Ser199, and Thr217. αA alleviates cognitive deficits in hTau[P301L] nematodes and hTau[P301S] mice. In addition, αA exhibits a favorable pharmacokinetic profile, with a half-life (t1/2) =10.1 hours in plasma, and crosses the blood-brain barrier in mice. Altogether, our study supports therapeutic strategies that target autophagic/mitophagic clearance of intracellular pathological Tau, with further clinical studies of αA on AD indicated.

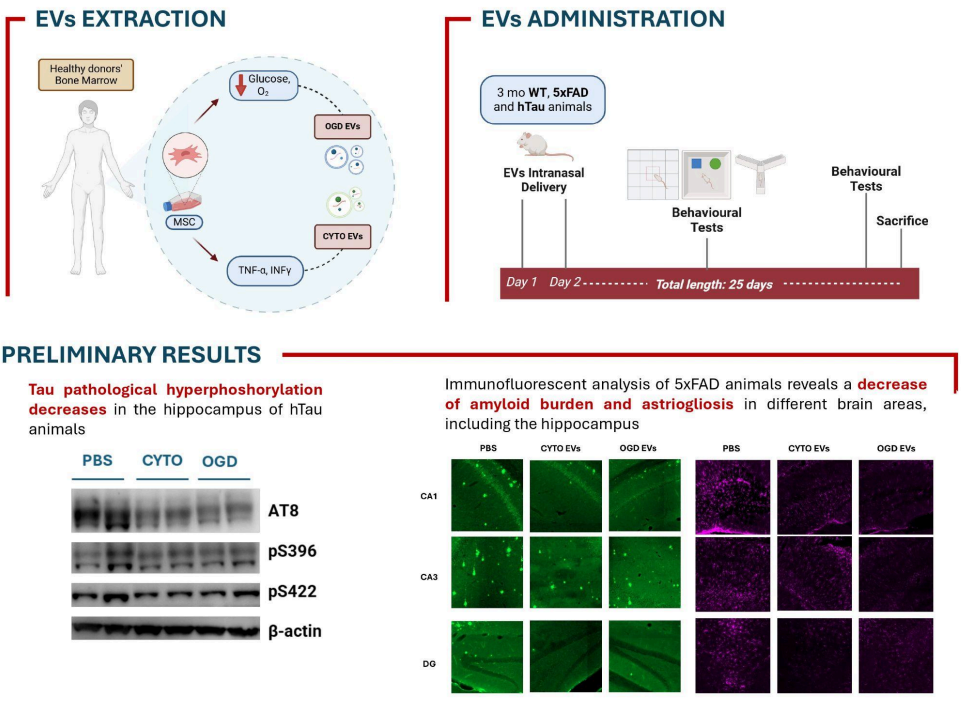
**Keyword 1**: mitophagy
**Keyword 2**: drug discovery
**Keyword 3**: Tau

**EXTRACELLULAR VESICLES (EVS) DERIVED FROM PRECONDITIONED MESENCHYMAL STEM CELLS AS A NOVEL THERAPY AGAINST ALZHEIMER'S DISEASE**

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**Alzheimer's disease (AD)** is the most common form of age-related dementia. While no single causative factor has been identified, the role of the **immune system** in AD pathogenesis is becoming increasingly evident, thus highlighting the need to identify novel strategies to target glial cells in the brain, namely **astrocytes** and **microglia**. In this perspective, **mesenchymal stem cells (MSCs)** could represent an important ally, as they can secrete **extracellular vesicles (EVs)** with relevant paracrine anti-inflammatory effects. In our study, we tested the role of *pro-inflammatory cytokine-preconditioned EVs* and *oxygen-glucose deprivation-preconditioned EVs*, which were **intranasally injected** in wild-type animals as well as two distinct murine models of AD: the **5xFAD model**, which exhibits amyloid pathology, and the **hTau model**, which mimics AD tauopathy. Our findings suggest improved performance during behavioural tests in the WT group, suggesting an intrinsic **trophic effect** of MSC-EVs, however they seem unable to revert cognitive decline in the transgenic animals. Despite this, molecular data reveal a **reduction in astrogliosis** and **amyloid plaque burden** in 5xFAD, as well as a significant decrease in the accumulation of levels of **tau phosphorylation** in the hippocampus of hTau mice. A deeper investigation is required to understand how EVs modulate glial activity and which downstream processes are activated to improve AD pathology. Nevertheless, these preliminary data suggest that MSC-derived EVs play a crucial role in modulating AD-related pathology, potentially offering a novel therapeutic approach for neuroinflammation and disease progression.




**Keyword 1**: Alzheimer's disease
**Keyword 2**: Mesenchymal Stem Cells
**Keyword 3**: Extracellular Vesiscles
**Keyword 4**: Tauopathy
**Keyword 5**: Glial cells

**NORADRENALINE ENHANCES SURVIVAL AND REDUCES AB AGGREGATION AND LPS-INDUCED NEUROINFLAMMATION IN HUMAN MICROGLIAL CELLS (HMC3)**

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The study aimed to investigate the protective effects of noradrenaline (NA) on human microglial cells (HMC3) exposed to lipopolysaccharides (LPS) and Aβ aggregates –Alzheimer's Disease (AD) *in vitro* model. The cytotoxicity of the LPS (5-1000 μg/ml) and Aβ (1-200μM) and the evaluation of the effect of NA (1-200μM) on HMC3 cells exposed to IC50 LPS or IC50 Aβ, were measured using XTT and LDH Assays. Then, immunocytochemical staining for Aβ, an alkaline version of comet assay, caspase-3 assay, qRT-PCR and Western blot (DDIT3, BAX, BCL2, HIF-1α expression assessment) were performed on HMC3 cells exposed to IC50 LPS or IC50 Aβ and treated with 25 μM and 50 μM NA. NA treatment at 25 μM and 50 μM concentrations in HMC3 cells exposed to IC50 LPS or IC50 Aβ resulted in an increased proliferation of HMC3 cells, their return to normal morphology, decreased levels of DNA damage, reduced caspase-3 activity, decreased expression of pro-apoptotic DDIT3 and BAX, and increased expression of anti-apoptotic BCL-2 genes and proteins, leading to enhanced cell survival, when compared to that of the HMC3 cells treated only with IC50 LPS or IC50 Aβ. Furthermore, NA induced the degradation of both extracellular and intracellular Aβ deposits and downregulated HIF-1α. These findings indicate that NA holds promise as a therapeutic target to address microglial dysfunction and potentially slow the progression of AD. The project was funded by grant no. SKN\SP\602622\2024 from the state budget allocated by the Minister of Science within the programme 'Student Scientific Circles Create Innovations'.

**Keyword 1**: Alzheimer's disease
**Keyword 2**: Noradrenaline
**Keyword 3**: Human Microglia
**Keyword 4**: Apoptosis
**Keyword 5**: Cytotoxicity

**EZH2 DOWNREGULATION MODULATES NEUROINFLAMMATION AND ALLEVIATES NEURONAL APOPTOSIS IN AN *IN VITRO* MODEL OF ALZHEIMER'S DISEASE**

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The study aimed to investigate the impact of EZH2 inhibition/knockdown on microglial activation and microglia-mediated neurodegeneration in the context of Alzheimer's Disease (AD) *in vitro*.
Experiments on HMC3 microglia and SH-SY5Y neurons examined AD-related phenotypes. HMC3 cells were treated with LPS (100ng) and Aβ1-42 (2.5μM), followed by treatment with EZH2 inhibitor EPZ6438 (5μM) or degrader MS1943 (4μM). Groups included control, LPS+Aβ, LPS+Aβ+EPZ (EPZ) and LPS+Aβ+MS (MS). The Aβ aggregation was evaluated by Thioflavin T (ThT) assay. The relative expression of EZH2, cytokines (TNFα, IL-1β, IL-6, TGFβ, IL-10) and ER stress markers (ATF4, Nrf2, DDIT3) was measured by qRT-PCR. SH-SY5Y neurons were treated with the conditioned media from HMC3 groups and cell death was analysed by annexin-V/PI/Hoechst staining.
ThT assay confirmed Aβ aggregation in a concentration-dependent manner. AD microglia demonstrated markedly increased expression of EZH2 and proinflammatory cytokines (TNFα, IL-1β, IL-6). After 24h, MS induced severe ER stress in AD microglia, elevating ATF4, Nrf2, and DDIT3, and triggering massive increase in IL-1β and IL-6 expression. However, after 48h, ER stress was alleviated, and both EPZ and MS downregulated EZH2 and mitigated pro-inflammatory cytokines expression, with EPZ increasing anti-inflammatory TGFβ and IL-10 expression. SH-SY5Y-derived neurons exposed to LPS+Aβ-conditioned media showed enhanced Annexin-V/PI signal, while EPZ- and MS-conditioned media alleviated neuronal death.
EZH2 inhibition/knockdown modulated microglial proinflammatory activation and attenuated microglia-mediated neurodegeneration in AD model *in vitro*.
The project was funded by grant SKN\SP\602622\2024 from the state budget allocated by the Minister of Science within the programme 'Student Scientific Circles Create Innovations'.

**Keyword 1**: Alzheimer's Disease
**Keyword 2**: Microglia-mediated neuroinflammation
**Keyword 3**: Neurodegeneration
**Keyword 4**: Histone methyltransferase EZH2

**INVESTIGATING THE IMPACT OF KETOGENIC DIET ON CEREBELLAR GENE EXPRESSION IN ALZHEIMER'S DISEASE**

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Alzheimer's disease (AD) is a neurodegenerative disorder characterized by β-amyloid accumulation, neurofibrillary tangles, and chronic neuroinflammation. Genetic factors contribute to AD pathogenesis, but their roles remain unclear. Ketogenic diets (KDs), high-fat, low-carbohydrate regimens inducing ketosis, may offer neuroprotection by enhancing mitochondrial function and reducing neuroinflammation. Clinical studies suggest KD improves cognitive function in AD patients. The cerebellum, traditionally considered less affected in AD, may benefit from KD's metabolic effects, potentially contributing to its resilience. We analyzed cerebellar expression of *Bdnf, Foxo3, Il-6, Sirt1, Gabra1*, and *Sst* in an AD rat model induced by streptozotocin (STZ) injection. RT-qPCR was performed on cerebellar tissue from three groups: STZ-AD (n=6), therapeutic intervention (n=7), and sham controls (n=5). *Bdnf* expression was significantly higher in the therapeutic group than in STZ-AD and controls ($p < 0.0001$). Sirt1 was also elevated in the therapeutic group compared to STZ-AD ($p = 0.048$). *Il-6* levels increased in STZ-AD and therapeutic groups but were not statistically significant. *Foxo3* was significantly reduced in the therapeutic group ($p = 0.0047$), while *Gabra1* was lower in STZ-AD compared to controls ($p = 0.0392$). Sst expression was reduced in both STZ-AD and therapeutic groups. Our findings suggest KD may modulate cerebellar gene expression in AD, potentially contributing to neuroprotection. Upregulated Bdnf and Sirt1 align with KD's neuroprotective effects, while *Foxo3, Gabra1*, and *Sst* reductions suggest regulatory effects on neurodegeneration. These findings highlight the cerebellum's role in AD and warrant further investigation.

**Keyword 1**: Alzheimer's disease
**Keyword 2**: cerebellum
**Keyword 3**: gene expression
**Keyword 4**: ketogenic diet

**BIOSAFETY CHARACTERIZATION OF BIOCOMPATIBLE POLYMERIC NANOGELS AS POTENTIAL NEUROPROTECTIVE DRUG CARRIERS IN ALZHEIMER'S DISEASE**

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Alzheimer's disease (AD) progresses through distinct stages, from preclinical stage to mild cognitive impairment and ultimately dementia. Despite extensive research, precise understanding of AD pathophysiology lacks, and no curative therapies are currently available. The midbrain and hippocampus are among the earliest affected brain regions, making their preservation a potential strategy for slowing disease progression. However, conventional drug delivery systems are ineffective due to their inability to penetrate the blood-brain barrier (BBB), limiting pharmacological intervention. Natural polyphenols like hydroxytyrosol are promising AD anti-inflammatory/antioxidant therapeutic agents, playing neuroprotective roles. Nevertheless, their therapeutic potential is hindered by poor bioavailability and rapid systemic clearance, resulting in minimal BBB penetration. To enhance targeted delivery and controlled release of polyphenols, nanovector-based delivery systems offer a novel approach to preserve neuronal integrity, mitigating neuroinflammation, and potentially improving AD-associated symptoms. Here, we synthesized and characterized nanogels (NGs) as potential AD drug delivery systems, with the long-term objective of enhancing drug transport across the BBB for effective prevention strategies. A comprehensive evaluation of NG safety and biocompatibility was conducted both *in vitro* and in C57BL/6N mice using a multidisciplinary approach, integrating molecular biology, confocal microscopy, electrophysiology and behavioral assays. We assessed NG uptake in SH-SY5Y cells, translocation across the BBB to distinct brain regions, biodistribution, and the absence of neurotoxicity and neuroinflammatory responses. Overall, our findings demonstrate the successful synthesis and validation of biocompatible NGs as smart drug carriers, facilitating targeted delivery to brain areas implicated in early AD and providing a foundation for future preventive strategies.

**Keyword 1**: Alzheimer's disease
**Keyword 2**: Neuroinflammation
**Keyword 3**: Blood-brain barrier
**Keyword 4**: Nanogels
**Keyword 5**: Neuroprotection

**RICE BRAN EXTRACT AMELIORATES METAL MIXTURE- INDUCED CEREBRAL CORTEX TOXICITY. POSSIBLE ROLE OF NRF-2/HMOX-1/BDNF SIGNALLING PATHWAYS**

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Heavy metal mixtures (MM) pose a significant public health concern due to their adverse health risks. Rice bran extract (RBE) exhibits antioxidant, anti-inflammatory, and anti-apoptotic properties. This study aimed to investigate the neuroprotective effects of RBE on MM-induced cerebral toxicity and its underlying cellular and molecular mechanisms. Rats were exposed to MM alone, with doses of lead acetate (Pb, 20 mg/kg), aluminum (Al, 35 mg/kg), and manganese (Mn, 0.564 mg/kg) body weight, or co-exposed with RBE at doses of 125, 250, and 500 mg/kg body weight for 60 consecutive days. Thereafter, levels of Pb, Al, Mn, antioxidant enzymes and non-enzymatic antioxidants, lipid peroxidation, inflammation (cyclooxygenase-2), and protein levels of occludin, amyloid precursor proteins (Aβ40 and Aβ42), apoptotic marker (caspase-3), HMOX-1, BDNF, and transcription factor Nrf-2 in the cerebral cortex were investigated.RBE treatment reduced the bioaccumulation of Pb, Al, and Mn, and reversed the MM-induced decrease in antioxidant enzymes and GSH, as well as the increase in lipid peroxidation, cyclooxygenase-2, and caspase-3. Furthermore, our results show that MM significantly increased levels of Aβ40, Aβ42, and Nrf-2, while significantly decreasing BDNF, occludin, and HMOX-1 compared to the control group. Taken together, RBE mitigates MM-induced oxidative stress, neuronal inflammation, and hippocampal apoptosis via the regulation of amyloid precursor proteins, BDNF, and HMOX-1 through Nrf-2-dependent pathways, reversing MM-induced cerebral toxicity

**Keyword 1**: Nrf-2
**Keyword 2**: amyloid precursor proteins
**Keyword 3**: occludin
**Keyword 4**: rice bran extract

**HIGH-THROUGHPUT KNOCKDOWN SCREENING FOR MODIFIERS OF NEURONAL MORPHOLOGY IN PATIENT-DERIVED NEURONS**

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Leigh syndrome (LS) is a rare, incurable mitochondrial disease causing intellectual and motor disabilities in children. It is caused by mutations in mitochondrial respiratory chain genes, including mitochondrial complex IV assembly factor *SURF1*.

SURF1-deficient animals failed to recapitulate neurodegeneration in the midbrain and basal ganglia seen in human LS, hampering the discovery of treatments. We therefore turned to human models, specifically induced pluripotent stem cells (iPSCs) derived from LS patients. Differentiation of these cells into neurons and brain organoids revealed an impaired capacity for neuronal outgrowth.

Based on these results, we developed a high-content analysis approach to automatically quantify neuronal outgrowth and branching. Next, we incorporated a genetic knockdown (KD) step by developing a novel transfection protocol that allows efficient short interfering RNA (siRNA)-based knockdown in human neurons. Control iPSC lines and an isogenic line carrying mutant *SURF1* were engineered to express *Neurogenin 2*, allowing rapid generation of pure inducible neurons (iNs) upon doxycycline exposure. A proof-of-concept siRNA KD screen in control iNs confirmed the ability of this approach to identify known modulators of neuronal morphology.

Next, we will conduct a comprehensive siRNA KD screen in LS iNs, targeting the ‘druggable genome’ (~800 genes associated with FDA-approved drugs). Candidate genes enhancing neuronal outgrowth upon knockdown will be further tested with corresponding FDA-approved drugs in LS brain organoids.

Our innovative KD screening strategy has the potential to identify novel modulators of neuronal outgrowth and drugs for repurposing for the treatment of LS and related disorders.

**Keyword 1**: iPSC
**Keyword 2**: mitochondria
**Keyword 3**: screening
**Keyword 4**: branching

**TISSUE DISTRIBUTION OF KYNURENIC ANALOGUE SZR104 IN THE BRAIN, CSF, AND PLASMA: *IN VIVO* ANALYSIS OF DOSAGE, CONCENTRATIONS, AND CLEARANCE TIMES**

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In recent years, our research group has focused on the *in vitro* electrophysiological examination of the SZR104 kynurenic acid analogue in the context of cerebral ischaemia. Given the positive results from our studies and the confirmation of the molecule's beneficial effects by other research groups at the University, we are encouraged to continue our investigations.
We aimed to determine whether the SZR104 analogue can cross the blood-brain barrier within the expected therapeutic range under *in vivo* conditions.
The analogue was administered intravenously (♂W 250-270 grams). The concentration was 0.015 mM/kg and had four termination time points (30 minutes, 60 minutes, 120 minutes, and 24 hours). Three types of tissue samples were collected (plasma, blood-perfused brain tissue, and cerebrospinal fluid).
We obtained sufficient samples for a total of 1167 analytical measurements. The analogue reaches its peak concentration in brain tissue approximately after 60 minutes and begins to clear from both cortex and hippocampus by the 120th minute. After 24 hours, it is almost completely cleared. Compared to the literature, the analogue may not alter the kynurenic acid nor quinolinic acid concentration in brain tissue. This is crucial because quinolinic acid is neurotoxic, and if the administered substance were to increase its concentration, the risk-benefit balance could shift toward harm despite therapeutic intentions. In plasma samples, kynurenine concentration changes dramatically following analogue administration.
The present measurements lay the groundwork for future studies of the analogue in an *in vivo* ischaemic model (MCAo).

**Keyword 1**: Kynurenic acid analogue
**Keyword 2**: Tissue distribution
**Keyword 3**: Tryptophan metabolism
**Keyword 4**: Excitotoxicity

**BALANCING NEUROINFLAMMATION: TNFR1 AND TNFR2 AS THERAPEUTIC TARGETS IN ALZHEIMER'S DISEASE**

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Tumor necrosis factor alpha (TNF-α), a pro-inflammatory cytokine, has been implicated in the pathogenesis of AD. However, anti-TNF therapies failed in treating Alzheimer's disease due to the opposing functions of TNF receptors: TNFR1 drives neurodegeneration, while TNFR2 promotes neuroprotection. Thus, targeting TNFR could represent a promising strategy for AD treatment.

This study evaluates the efficacy of a TNFR1 antagonist and a novel TNFR2 agonist in two AD mouse models. Mice are treated with the TNFR1 antagonist or the novel TNFR2 agonist via intraperitoneal injections while controls will receive phosphate-buffered saline (PBS) injections. After the treatment, behavioral tests will be conducted to assess spatial memory and working memory. FACS analysis from the blood samples will be performed to determine the peripheral effects of the treatment. The brain sections will be immunohistochemically stained for different markers, including Aβ and tau pathology, microglial activation and phagocytic microglia.

Our group has demonstrated that a TNFR2 agonist ameliorated neuropathology and improved cognition in an AD mouse model, while a TNFR1 antagonist alleviated memory deficits in an acute mouse neurodegeneration model. Thus, in this study, we expect improved cognition, reduced Aβ plaques and tau pathology, and increased phagocytic microglia around the plaques. We also expect to see changes in T-cell response and pro-inflammatory response.
Our study aims to evaluate the efficacy of the TNFR1 antagonist and a novel TNFR2 agonist in mitigating AD neuropathology and improving cognition as well as finding peripheral changes to support the hypothesis that AD is more than a brain disease.

**Keyword 1**: Alzheimer`s Disease
**Keyword 2**: Neuroinflammation
**Keyword 3**: TNF alpha
**Keyword 4**: Neurodegeneration

**SELECTIVE TNF RECEPTOR 2 ACTIVATION ALLEVIATES INFLAMMATION AND NEUROLOGICAL DEFICITS IN MULTIPLE SCLEROSIS**

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Multiple sclerosis (MS) is an autoimmune disease of the central nervous system (CNS) characterized by demyelination, inflammation, and axonal damage in both white and gray matter. Current therapeutic strategies focus on modulating certain immune responses but provide only partial and temporary relief.

Numerous studies have implicated the pro-inflammatory cytokine tumor necrosis factor-α (TNF-α) in MS, as elevated levels are found in active lesions and correlate with disease severity. While TNF- inhibitors are effective in treating other autoimmune diseases like rheumatoid arthritis, their use in MS patients exacerbated symptoms in clinical trials. This may be due to the opposing roles of TNF-α receptors: TNF receptor 1 (TNFR1) promotes inflammation and apoptosis, whereas TNF receptor 2 (TNFR2) supports neuroprotection and tissue regeneration. Thus, selective activation of TNFR2 emerges as a promising therapeutic strategy.

In this study, we used the experimental autoimmune encephalomyelitis (EAE) mouse model of MS, which replicates key disease features, including progressive hind-limb paralysis, axonal demyelination, and immune cell infiltration. Mice were treated with a novel TNFR2 agonist to assess its therapeutic potential in ameliorating MS-related pathology. Our results demonstrate that systemic administration of the TNFR2 agonist significantly improved motor function and alleviated mechanical allodynia. Moreover, inflammation and demyelination were significantly reduced in the CNS of treated animals.

Altogether, these findings suggest that TNFR2 activation may represent a viable therapeutic approach for MS.

**Keyword 1**: Multiple Sclerosis
**Keyword 2**: TNF
**Keyword 3**: Neuroinflammation
**Keyword 4**: Therapy
**Keyword 5**: EAE

**TAU DEPENDENT MICROTUBULE ORGANIZATION IN DORSAL ROOT GANGLION (DRG) NEURONS AND ITS IMPACT ON AXON REGENERATION AND FUNCTION**

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In axons from peripheral neurons, microtubules form a dynamic and spatially regulated network critical for structural integrity and regeneration. However, how Tau, a microtubule-associated protein, contributes to maintain this organization and support axonal regrowth remains unclear. We used DNA-PAINT, a super-resolution microscopy technique, followed by 2-D algorithmic image analysis, to investigate microtubule organization in cultured DRG neurons from control and Tau Knock-out (TauKO) mice. We found that in TauKO neurons, mean microtubule length was reduced, while microtubule density was increased. In addition, we observed that microtubule straightness was decreased in TauKO neurons compared with controls. To determine changes in microtubule spacing, we developed 3D spatial reconstruction.
To determine the functional consequences of these structural alterations, we assessed axonal regeneration in TauKO and control DRG cultures. Surprisingly, despite the more fragmented microtubule network, TauKO neurons exhibited significantly longer axonal processes**,** indicating increased axonal sprouting. These findings suggest that tau restricts regeneration in peripheral neurons by modulating axonal microtubule organization and stiffness.
Our study demonstrates that DNA-PAINT combined with 2D and 3D algorithmic image analysis is a powerful approach to quantitatively assess microtubule organization and its role in axonal function. We identify Tau as a key regulator of microtubule architecture and axonal regeneration, providing novel insights into Tau’s role in neuronal repair.

**INVESTIGATING THE NEUROPROTECTIVE POTENTIAL OF EZETIMIBE AND SERICIN AGAINST LEAD-INDUCED NEUROTOXICITY IN RAT MODEL**

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Environmental pollution caused by heavy metals is becoming a major global health concern worldwide. Lead (Pb) is a pervasive environmental neurotoxin that poses significant risks to brain function and development. Oxidative stress, mitochondrial dysfunction, apoptosis, and cognitive impairment are implicated in pathophysiology in lead-induced neurotoxicity. The chelating agents are available in the management of lead-induced neurotoxicity with various side effects. Therefore, this study proposed to investigate the neuroprotective effect of ezetimibe and sericin in lead induced neurotoxicity in rat models.
Adult Wistar rats were divided into seven groups: control, lead acetate (50 mg/kg), lead with ezetimibe (10 and 20 mg/kg), sericin (200 and 400 mg/kg), and a combination of ezetimibe (10 mg/kg) and sericin (200 mg/kg). Lead acetate was administered for 55 days, with treatments of Ezetimibe and Sericin initiated on day 29 and continued until day 55. Behavioral assessments, including motor coordination, anxiety, and cognitive function, were performed every 14 days. On day 56, animals were sacrificed, and hippocampal tissues were analyzed for biochemical, neurochemical, and histopathological changes.
The results demonstrated that ezetimibe and sericin significantly mitigated lead acetate-induced behavioral impairments, including motor dysfunction, anxiety, and cognitive deficits. Both compounds ameliorated hippocampal morphological alterations and restored neurotransmitter levels, including glutamate, GABA, and acetylcholine.
These findings suggest that ezetimibe and sericin may alleviate the neurotoxic effects of lead acetate.

**Keyword 1**: Neurotoxicity
**Keyword 2**: Lead acetate
**Keyword 3**: Ezetimibe
**Keyword 4**: Sericin
**Keyword 5**: Environmental pollution

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**COMBINED USE OF OLFACTORY MUCOSAL MESENCHYMAL STEM CELLS SECRETOME AND NEURAL GUIDE CONDUITS PROMOTES NERVE REGENERATION IN AN OVINE MODEL**

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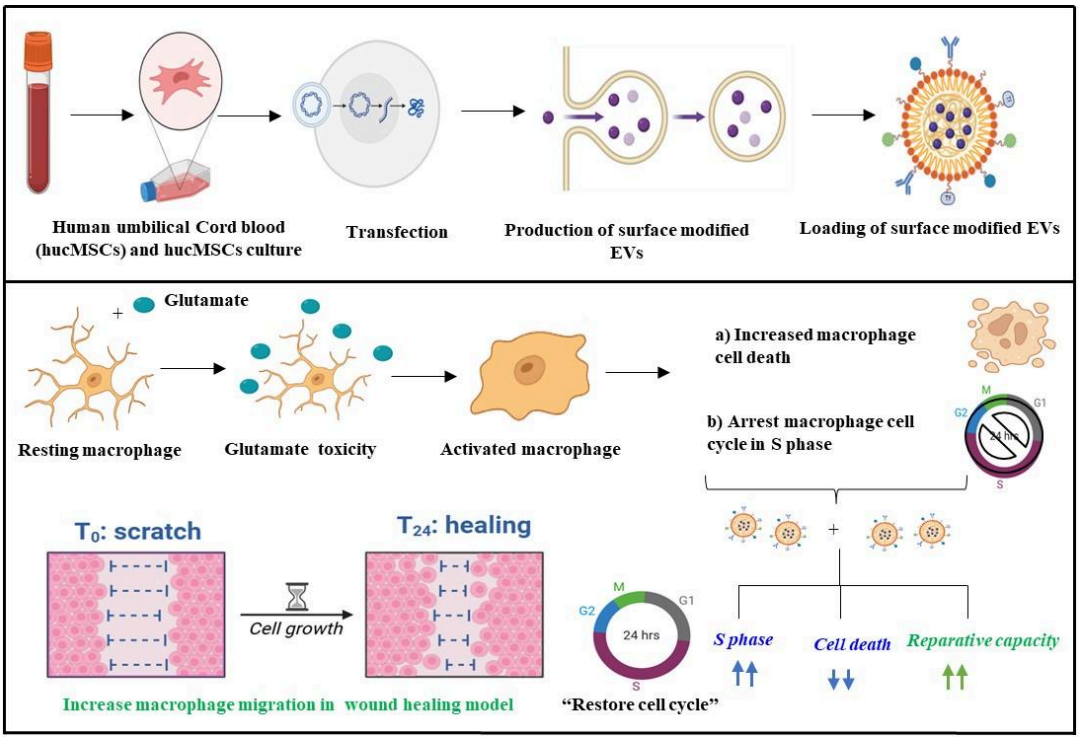
Peripheral nerve injuries remain a significant clinical challenge, particularly in severe neurotmesis injuries requiring complex therapeutic interventions to restore functionality. This study evaluated the pro-regenerative potential of combining neural guide conduits with secretome from olfactory mucosa mesenchymal stem cells, compared to gold-standard surgical techniques, in a validated ovine model of common peroneal nerve injury. Recovery was assessed over 24 weeks using functional, kinematic, ultrasonographic, and electrophysiological evaluations, complemented by *post-mortem* nerve stereology and muscle histomorphometry. All therapeutic approaches promoted nerve and muscle regeneration, resulting in notable functional and structural improvements. However, irregularities were observed, as neural guide conduits and secretome did not consistently outperform standard techniques. Additionally, recovery often fell short of normal values in the control group, highlighting the complexity of peripheral nerve regeneration in challenging surgical scenarios. These findings underscore the translational potential of biomaterials and cell secretome-based therapies while emphasizing the need for further research in complex animal models before its application in real clinical cases. Such studies are essential to refine therapeutic strategies, address observed irregularities and establish cell secretome as a viable tool in peripheral nerve regeneration and repair.

**Keyword 1**: peripheral nerve injury
**Keyword 2**: peripheral nerve regeneration
**Keyword 3**: common peroneal nerve
**Keyword 4**: mesenchymal stem cells secretome
**Keyword 5**: regenerative medicine

**GENETICALLY ENGINEERED EXTRACELLULAR VESICLES DERIVED FROM HUMAN UMBILICAL CORD MESENCHYMAL STEM CELLS TARGET MACROPHAGES AND MITIGATE GLUTAMATE-INDUCED CELL TOXICITY**

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Precise targeting of macrophages presents a promising therapeutic approach for modulating neuroinflammatory pathways. Extracellular vesicles (EVs) are emerging as a revolutionary drug delivery system for neuroinflammatory diseases, though their limited targeting ability often restricts therapeutic efficacy. To overcome this, we genetically engineered EVs derived from human umbilical cord mesenchymal stem cells (hucMSCs) using a glycosylated pcDNA3.1-GNSTM-RGV-Lamp2b-HA plasmid, enabling them to target acetylcholine receptors on macrophages. Post-transfection, the modified EVs retained inherent vesicle properties, such as size, membrane protein expression (TSG101, Flotillin), and morphology. Additionally, by incorporating CHPG [(RS)-2-Chloro-5-hydroxyphenylglycine], an selective agonist of drug molecule of metabotropic glutamate receptors (mGlu5), into these surface-modified EVs, we noted a sustained drug release profile lasting up to 36 hours. These surface-modified drug loaded EVs exhibited enhanced uptake by naïve macrophages, resulting in significantly increased proliferation at 24 hours compared to control cells. In in vitro experiments, these EVs reduced annexin/PI-positive macrophages under glutamate toxicity and facilitated their transition into the S phase, boosting their reparative capacity. Additionally, the engineered EVs improved macrophage migration in a wound healing model, underscoring their therapeutic potential. This novel targeting strategy not only optimizes EV uptake by macrophages but also establishes a foundation for advanced regenerative treatments.




**Keyword 1**:

Extracellular vesicles
**Keyword 2**: hucMSCs
**Keyword 3**: Glutamate toxicity
**Keyword 4**: Macrophage
**Keyword 5**: Drug delivery

**PSYCHOLOGICAL PROFILE AND QUALITY OF LIFE IN PATIENTS WITH OSA**

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OSA is the most common sleep-related breathing disorder with a large number of comorbidities. The impairment of neuro-psychological state takes the leading place. The aim of the present cross-sectional study was to analyze the personality characteristics and quality of life (QoL) of patients with OSA and their relationship with the severity of the disease.
A total of 276 individuals were included – 224 patients with OSA and 52 healthy controls. The diagnosis was confirmed by polysomnography. Cognitive function, affective disorders, personality changes and QoL were investigated. Their relationship with the main clinical and PSG data in patients with OSA was analyzed.
In patients with OSA, we found impaired general cognitive functioning, reduced concentration and attention, disorders in short- and long-term memory. There was a significantly higher frequency of affective disorders – symptoms of depression in 64.7% and anxiety in 41.97%. Results showed a significant worsening in QoL in the OSA patients compared to the controls – $F=14.57$, $p<0.001$, measured by EQ-VAS. We found significantly worsening in Mobility, Self-care, and Usual activities. We observed the following pathological manifestations in the personality characteristics of the studied patients: somatic distress, hypochondria, symptoms of obsessive-compulsive disorder, impaired interpersonal sensitivity and primary psychotic deviations. Changes in the psychological state and QoL of OSA patients significantly correlated with the affective disorders, impaired cognitive function and disease severity.
In the course of the progressive development of OSA, psychological deviations, personality changes and worsened QoL occur, determined by the severity of the disease. Their early diagnosis is important for clinical practice.

**Keyword 1**: obstructive sleep apnea (OSA)
**Keyword 2**: cognitive function
**Keyword 3**: affective disorders
**Keyword 4**: psychotic deviations
**Keyword 5**: quality of life

**INVESTIGATING THE FUNCTIONAL IMPACT OF RBM12 MUTATION IN PATIENT-DERIVED NEURONS**

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Mutations in the *RBM12* gene have been associated with neuropsychiatric and neurodevelopmental disorders such as familial schizophrenia and neurodevelopmental deficits. However, the functional consequences of these mutations on neuronal activity remain poorly understood. This study aims to investigate the cellular and electrophysiological phenotypes associated with *RBM12* mutation using patient-derived neuronal models. Peripheral blood mononuclear cells (PBMCs) were isolated from a family male affected proband carrying an RBM12 mutation and both healthy parents. The PBMCs were immortalized using Epstein-Barr virus (EBV) and subsequently reprogrammed into induced pluripotent stem cells (iPSCs) via episomal reprogramming. iPSCs were differentiated into functional cortical neurons, and comprehensive phenotypic analyses were performed. Electrophysiological properties were assessed using whole-cell patch-clamp recordings, while network activity and calcium dynamics were examined through calcium imaging assays. Preliminary findings reveal distinct electrophysiological alterations in patient-derived neurons compared to parental controls. These functional deficits may underlie the neurological manifestations observed in individuals with *RBM12* mutations. This study provides novel insights into the impact of *RBM12* mutations on neuronal function. The patient-derived iPSC model offers a robust platform for elucidating the pathogenic mechanisms of *RBM12*-related disorders and may guide future therapeutic strategies targeting neurodevelopmental dysfunctions associated with this gene.

**Keyword 1**: RBM12
**Keyword 2**: induced pluripotent stem cells (iPSCs)
**Keyword 3**: whole-cell patch clamp
**Keyword 4**: neurodevelopmental disorders

**OLIGODENDROCYTE PRECURSOR CELL DYNAMICS DICTATE REMYELINATION EFFICIENCY IN AGING**

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Cortical myelin is organized in a patchy and discontinuous pattern, with oligodendrocytes (OLs) contributing to both developmental myelination and homeostasis. While cortical myelin is thought to be linked to higher brain function, the extent of lifelong myelin remodeling and its response to local disruptions remain largely unclear. Although the decline of remyelination in very old rodents has been shown, little is known about OL replacement in mid-aged animals or whether deficits stem from changes in oligodendrocyte precursor cell (OPC) dynamics. To address this, we used longitudinal in vivo two-photon imaging in transgenic mice to study myelin turnover across aging. Building on previous work demonstrating that OL loss in the adolescent cortex triggers a localized response, with newly generated OLs precisely restoring myelin sheaths, we investigated how this process changes with age. Already starting from mid age, we found that remyelination capacity is reduced in both, the number of replaced OLs and restoration of myelin patterns. By tracking OPC behavior over weeks, we observed a decline in OPC dynamics with age. Furthermore, by experimentally modulating OPC motility, we demonstrated a correlation between OPC dynamics and remyelination efficiency. These findings establish OPC dynamics as a key determinant of successful myelin repair. We also showed that OPC retain their intrinsic differentiation capability despite their reduced motility when a sufficient number of axonal targets are in close proximity. By identifying impaired OPC dynamics as central mechanism underlying age-related remyelination failure, our study highlights a potential therapeutic target for enhancing myelin repair in aging.

**Keyword 1**: Remyelination
**Keyword 2**: Aging
**Keyword 3**: Oligodendrocyte precursor cells

**MICROPRINTED 3D SCAFFOLDS FOR REPAIR OF PERIPHERAL NERVE INJURY**

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Peripheral nerve injury (PNI) is a common clinical problem that often results in irreversible motor and sensory deficits due to insufficient or misdirected axonal regeneration. Here we propose a novel artificial 3D-printed hollow microscaffolds with a biomimetic architecture, constructed using 2-photon photopolymerization (2PP). The scaffolds were placed into a silicone conduit (length: 10 mm, outer diameter: 2 mm, inner diameter: 1 mm) and implanted in a 6 mm gap in the sciatic nerve of seven-week-old Wistar rats to guide axons and vessels after PNI. Structural guidance was provided by the tubular scaffold design, with multiple densely packed parallel micro-tunnels (221 hexagonal-shaped tunnels per scaffold, inner size: 50 μm, wall thickness: 5 μm) running through their whole length.
We found that the scaffolds integrated well into the excision site with minimal fibrotic response, indicating good biocompatibility. Immunohistochemical analysis showed axon growth in 90-95% of the tunnels, mainly aligned with the rostrocaudal axis. The regenerated tissue within the scaffold was properly vascularized. Additionally, implantation improved the motor and sensory functions in the ipsilateral limb by the 12th week, and the recovery was maintained until the end of the fifth month post-operation compared to a nerve graft.
Thus, 3D-oriented hollow scaffolds with a large internal surface area create conditions for enhancing sciatic nerve regeneration and recovering motor and sensory function in the limb.
Supported by NRFU grant # 2021.01/0328

**Keyword 1**: Nerve repair
**Keyword 2**: Neural tissue engineering
**Keyword 3**: 3D printing
**Keyword 4**: 2-photon photopolymerization (2PP)
**Keyword 5**: Structural support

**THE EFFECT OF HYBRID CARBON COMPOSITES IN MESENCEPHALIC CELL CULTURES**

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Pharmacotherapy of neurodegenerative diseases (ND) including Parkinson's disease so far is only symptomatic. Among alternative methods of treatment is electric brain stimulation via implantable metal electrodes. In recent years has been proposed carbon-based materials for electrodes like carbon fibers (CFs), carbon nanomaterials and pyrolytic carbon (PyC) which are expected not to evoke inflammatory response and to prevent glial scar formation. The aim of this work was testing various hybrid carbon composites modified with carbon nanotubes (CNTs) before and after functionalization with hydroxyl/carboxylic and amine groups (CF1-CF5) in the contact with dopaminergic neuronal cells. We demonstrated that the tested nanomaterials are not cytotoxic for 2 and 7 days in vitro (DIV) mouse mesencephalic neuronal cell cultures as confirmed by LDH release assay. By morphological analysis of tyrosine hydroxylase (TH)-immunostained cells at 7 DIV, we showed that the tested nanomaterials did not evoke any changes in the cell and neurites number of TH-positive neurons. Interestingly w observed a significant increase in the number of NeuN-positive cell in the distance to 1000 mm from the rod for all tested nanomaterials (CF1-CF5), whereas in the distance 1000-2000 mm such effect was maintained only by CF2 and CF3. The obtained results point not only to biosafe profile of tested nanomaterials, especially to dopaminergic cells but also to their pro-survival effect on non-dopaminergic neurons. The above findings justify further research on development of new electrodes based on hybrid carbon composites for brain stimulation for various ND.
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**EPIGENETIC TRIGGERS IN THE MAMMALIAN RETINAL RESPONSE TO DAMAGE AND AGING**

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Retinal regeneration—and consequently, the response of retinal cells to injury and aging—depends on the neuronal regenerative capacity of Müller glial cells and their epigenomic plasticity. Specific antibodies targeting epigenetic marks associated with permissive (5-hydroxymethylcytosine, H3K4me3) or restrictive (5-methylcytosine, MeCP2) states revealed that, in an experimental paradigm of retinal damage, the epigenetic landscape of murine Müller cells undergoes a transition from a restrictive to a permissive state. We have proposed that dysfunction in epigenetic mechanisms linked to DNA methylation and demethylation may contribute to a diminished regenerative response in aged or injured mammalian retinal cells. To sustain this proposal we evaluated the role of specific DNA methyltransferases (DNMTs) and DNA demethylases (TET) enzymatic activities in the response of rodent Müller cells to retinal damage in primary cultures. Gene expression alterations using CRISPR/dCas9 and siRNA technology provided evidence that, specifically, DNMT3a and TET3 are critical for the damage-induced change in the epigenetic landscape. Furthermore, both enzymatic activities are required for the induction of expression of pluripotency genes (Ascl-1, Lin28), the acquisition of a stem cell-like phenotype of Müller cells and the further development of the neurogenic capacity. We conclude that the processes of DNA methylation and demethylation are crucial to control the silenced neurogenic capacity of mammalian Müller cells. The development of DNA methylation-based tools could emerge as a potential therapeutic approach in the context of mammalian retinal degeneration.
This work was supported by VELUX STIFTUNG project 1852.

**AUTOPHAGY DYNAMICS IN AXONAL GROWTH, INJURY AND REGENERATION**

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Autophagy, a crucial cellular process for degrading intracellular components, plays a key role in maintaining cellular homeostasis and has been increasingly recognized for its involvement in neuronal regeneration. However, its exact role remains debated due to conflicting findings. This study investigates autophagy's impact on axonal growth, injury response, and regeneration by analyzing autophagic vesicle quantity, transport, and maturation in adult dorsal root ganglion (DRG) neurons from RFP-EGFP-LC3 transgenic mice using live-imaging microscopy. Our findings reveal a strong correlation between autophagic vesicle dynamics and axonal growth potential. In uninjured neurons, axons with limited growth exhibited impaired vesicle maturation, leading to the accumulation of stationary autophagosomes. Following axotomy, a similar maturation blockage occurred, accompanied by disrupted vesicle transport. Regenerating axons restored autophagosome maturation, whereas static axons retained flux impairment, and retracting axons showed increased autophagic activity. Notably, regenerating axons accumulated stationary vesicles at the axon tip, in stark contrast to the dynamic vesicle movement seen in uninjured conditions. Additionally, pharmacological modulation of autophagy further clarified its role. Inhibiting autophagosome formation or blocking their maturation impaired axonal growth and regeneration, whereas enhancing autophagy promoted both processes. A comparative analysis of treatments before and after injury highlighted distinct roles for autophagy in healthy versus injured axons. These findings provide new insights into the complex interplay between autophagy, axonal injury, and regeneration, offering potential therapeutic strategies to enhance neuronal repair. This project was supported by EXREGMED project CZ.02.01.01./00/22_008/0004562.

**Keyword 1**: autophagy
**Keyword 2**: live-imaging
**Keyword 3**: axon growth
**Keyword 4**: axon regeneration

**NEUROPROTECTIVE EFFECTS OF *CANNABIS SATIVA* ON ALUMINIUM CHLORIDE-INDUCED NEUROTOXICITY IN LONG EVANS RATS**

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**Objective:** This study aims to evaluate the neuroprotective effects of *Cannabis sativa,* known for its antioxidant and anti-inflammatory properties, against aluminium chloride ($AlCl_3$)-induced neurotoxicity in Long Evans rats.

**Methods:** Twenty adult Long Evans rats were randomly divided into four groups (N=5). Group 1 (control) received distilled water for 28 days. Group 2 was administered 1000 mg/kg of $AlCl_3$ for 21 days. Group 3 received 166.5 mg/kg ethanolic extract of *Cannabis sativa* for 7 days. Group 4 received 1000 mg/kg of $AlCl_3$ for 21 days, followed by 166.5 mg/kg ethanolic extract of *Cannabis sativa* for 7 days. Neurobehavioral studies, including the Elevated Plus Maze and Morris Water Maze, along with haematological and acetylcholine assays, were performed.

**Results:** Ethanolic extract of *Cannabis sativa* treatment significantly improved neurobehavioral performance by enhancing cognitive function and reducing anxiety-like behaviour in tests impaired by $AlCl_3$. The extract also had a positive impact on haematological parameters and increased acetylcholine levels in the brain, which had been reduced by $AlCl_3$ exposure.

**Conclusion:** *Cannabis sativa* demonstrates neuroprotective effects against $AlCl_3$-induced neurotoxicity by improving cognitive and behavioural functions and restoring neurotransmitter and haematological imbalances. These findings suggest its potential as a therapeutic agent for aluminium-related neurodegenerative conditions.

**Keyword 1**: Acetylcholine
**Keyword 2**: Neurobehavioral
**Keyword 3**: Aluminium chloride
**Keyword 4**: Cannabis sativa
**Keyword 5**: Neurotoxicity

**GENE THERAPY FOR EFFICIENT SUPPRESSION OF T-TYPE CHANNELS IN TREATING DIABETIC NEUROPATHY**

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Painful diabetic neuropathy (PDN), a chronic and often incurable syndrome, is one of the most common and unpleasant complications of diabetes. Effective clinical interventions for PDN are very limited and already developed approaches are characterized by lack of molecular or cellular target specificity and a short duration of therapeutic effects. Numerous investigations causally link upregulation of the $Ca_v3.2$ T-type $Ca^{2+}$ channels in peripheral nociceptive neurons to painful symptoms of PDN. Here we suggest an approach to alleviate these symptoms based on implementation of virus-mediated cell-specific delivery of vectors expressing small hairpin RNAs (shRNAs). Processed by Dicer into specific small interfering RNA (siRNA), they would suppress the translation of T-type $Ca^{2+}$ channels. In order to experimentally validate this approach we have initially confirmed the ability of designed Dicer-substrate small interfering RNAs (DsiRNAs) to suppress expression of Cav3.2 channels in neurons of primary hippocampal and DRG cultures. Target sequences of the effectively interfering DsiRNA were then used to design shRNAs and the coding sequences of shRNAs were cloned into the vector pAAV under U6 promotor. Suppression of the T-type $Ca^{2+}$ current was revealed in cultured nociceptive DRG neurons transfected with the plasmid pAAV-shRNA::Cav3.2. The expression cassette of these plasmids will be packed into AAV6 particles with tropism to unmyelinated fibers to suppress T-type channel expression in nociceptive DRG neurons and to alleviate painful symptoms of PDN.

**Keyword 1**: diabetes
**Keyword 2**: neuropathy
**Keyword 3**: T-type channels
**Keyword 4**: siRNA
**Keyword 5**: adeno-associated virus

**MULTIOMICS ANALYSIS REVEALS GLYCOSYLATION DEFECTS AND NEUROPATHOLOGICAL MECHANISMS IN ALG13-CDG HUMAN CORTICAL ORGANOIDS**

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Asparagine-linked glycosylation 13 (ALG13) is an enzyme critical for adding GlcNAc sugar to glycan chains. ALG13-congenital disorder of glycosylation (CDG), an X-linked condition caused by ALG13 missense variants, is the second most common CDG. Despite the absence of glycosylation defects in patient blood and fibroblasts, ALG13-CDG primarily affects the brain, leading to seizures, intellectual disability, and developmental delays.
To investigate its neuropathology, we generated human cortical organoids (hCOs) from patient-derived iPSCs. At 77–92 days of differentiation, we performed X-inactivation studies and multiomics profiling, including single-cell RNA-seq, proteomics, glycoproteomics, lipidomics, and metabolomics.
Our findings revealed reduced glycosylation of proteins essential for extracellular matrix (ECM) integrity, neuronal migration, lipid metabolism, and excitability. Dysregulation of these same pathways was evident in scRNA-seq and proteomics data, along with aberrant developmental trajectories and intercellular communication in ALG13-deficient hCOs. Metabolomics showed increased GlcNAc levels, while lipidomics uncovered disturbances in neurologically relevant lipids. Additionally, both iPSCs and hCOs exhibited X-inactivation skewing, a previously unreported feature in ALG13-CDG.
These results demonstrate, for the first time, that ALG13-CDG causes glycosylation defects specifically in the human brain. Our multiomics analysis suggests that disruptions in lipid metabolism, ECM function, neuronal migration, and excitability contribute to disease pathology. This novel insight paves the way for targeted therapeutic strategies for ALG13-CDG.

**DAPAGLIFLOZIN-MEDIATED NEUROPROTECTION IN *IN VITRO* AND *IN VIVO* TOXICITY MODELS**

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Dapagliflozin, a sodium-glucose cotransporter-2 (SGLT2) inhibitor widely used in diabetes treatment, has demonstrated neuroprotective effects in several experimental models. This study investigated dapagliflozin's impact on neuronal viability, dendritic morphology, and cognitive function using both in vitro and in vivo neurotoxicity models. In our in vitro experiments, primary hippocampal neurons and NIH/3T3 fibroblasts exposed to $H_2O_2$-induced oxidative stress showed significant protection when pretreated with subtoxic concentrations of dapagliflozin. Cell viability assays and flow cytometry confirmed the absence of apoptotic or necrotic cell death, while morphological analyses revealed preservation of cellular and dendritic architecture. Notably, dapagliflozin treatment significantly enhanced dendritic field complexity and increased spine density in hippocampal neurons following oxidative stress. In our in vivo studies, Wistar rats exposed to aluminum chloride ($AlCl_3$) demonstrated cognitive impairments in Morris water maze and Y-maze behavioral tests. Dapagliflozin co-administration significantly ameliorated these deficits, with treated rats showing decreased latency in locating the hidden platform, increased quadrant crossings, and enhanced novel arm exploration. Complementary immunohistochemical and morphological analyses of brain tissue confirmed dapagliflozin's neuroprotective effects. These findings suggest that dapagliflozin protects against neurodegeneration by preserving dendritic architecture, mitigating oxidative stress-induced damage, and improving cognitive function, highlighting its potential as a therapeutic agent for neurodegenerative conditions beyond its established antidiabetic role.

**Keyword 1**: Dapagliflozin
**Keyword 2**: Neuroprotection
**Keyword 3**: Oxidative stress
**Keyword 4**: Cognitive function

**LONG NONCODING RNA (LNCRNA) FRAGMENT PROMOTING PERIVASCULAR ASTROGLIOSIS IN THE CEREBRAL CORTEX**

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Perivascular lesions with accumulation of abnormally structured proteins (Onoda et al. *Front. Cell. Neurosci.* 11:92, 2017) are associated with neurodegenerative diseases such as dementia. Along with upregulation of the genes (Gfap, Aqp4) (Umezawa et al. *Part. Fibre Toxicol.*, 15:36, 2018) and endoplasmic reticular stress (Onoda et al. *Sci. Rep.*, 10:10028, 2020) involved in the pathogenesis, a number of long noncoding RNAs (lncRNAs) are differentially expressed in the lesion; however, their roles and functions remained unclarified. This study aimed to reveal their functions potentially involved in the pathogenesis of the perivascular astrogliosis. RNA expression profiles in the cerebral cortex of mice with perivascular lesion model (GSE250286) were analyzed using principle component analysis to extract lncRNAs highly correlated to the lesion level. Common sequences of the lncRNA groups and mRNAs having their complementary sequences were extracted; then, the mRNAs were subjected to functional analysis using DAVID. We found a common sequence in the lncRNAs positively correlated with the lesion level. The functions of mRNAs with the complementary sequences were enriched in “cytoskeleton”, “cell projection”, “cell cortex”, and “postsynaptic density”, which play roles in the elongation of astrocytic endfoot and in neural functions in the brain. When liposome encapsulating RNA fragment with the common sequence was injected to the ventricle of the newborn rats, the number of GFAP-positive astrocytes, an astrogliosis marker, was significantly increased in the cerebral cortex. The lncRNAs may play a key role in the perivascular astrogliosis that can be linked to neurodegenerative and neurodevelopmental disorders.

**Keyword 1**: long noncoding RNA
**Keyword 2**: astrogliosis
**Keyword 3**: dementia
**Keyword 4**: developmental disorder
**Keyword 5**: transcriptomics

**ABERRANT INTERHEMISPHERIC RESTING-STATE BOLD FUNCTIONAL CONNECTIVITY AT ~0.1 HZ IN PATIENTS WITH ONE-SIDED INTERNAL CAROTID ARTERY STENOSIS**

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Although without clinical symptoms, individuals with one-sided internal carotid artery stenosis (ICAS) have mostly one-sided, diverse vascular-hemodynamic impairments. However, it is unclear whether interhemispheric fMRI-based functional connectivity (FC) at rest, which strongly depends on vasomotion at ~0.1 Hz, is also impaired in ICAS (**Fig.1A**). Resting-state blood oxygenation level dependent (BOLD) fMRI and multiparametric MRI were measured in 26 unilateral asymptomatic ICAS (>70% NASCET, mean-age 70.3y, stenoses aligned to the right side) and 25 age-matched (mean-age 70.4y) healthy controls (HC) at 3T (**Fig.1B&C**). An atlas of intrinsic connectivity of homotopic areas (AICHA) parcellation (**Fig.1D**) was applied, and interhemispheric differences of multiple parameters were calculated (**Fig.1E**). For homotopic and heterotopic BOLD-FC analysis, power spectral densities (PSDs) and coherence were calculated within 0.01-0.1 Hz. Two-sample t-tests were applied for statistical significance (*p-value<0.05 and **p-value<0.01). Single subject averaged PSDs demonstrated no peak at 0.08-0.1 Hz in ICAS compared to HC (**Fig.1F**). On group level, interhemispheric PSDs difference at ~0.1 Hz in ICAS was significantly higher than in HC (vs. no significance at <0.05 Hz, **Fig.1G**). Homotopic as well as heterotopic coherence (**Fig.1J-L**) was significantly reduced at 0.08-0.1 Hz in ICAS. Across brain networks, coherence was strikingly reduced at 0.08-0.1 Hz in ICAS (**Fig.1Hvs.I**). Our work demonstrates diminished homotopic FC specifically at ~0.1 Hz in ICAS. Besides CBF, significant difference in CBV between HC and ICAS supports our hypothesis that the reduction of homotopic FC at ~0.1 Hz may arise from impaired vasomotor activity while comparable CMRO2 across groups suggests preserved neuronal activity (**Fig.1C&E).**

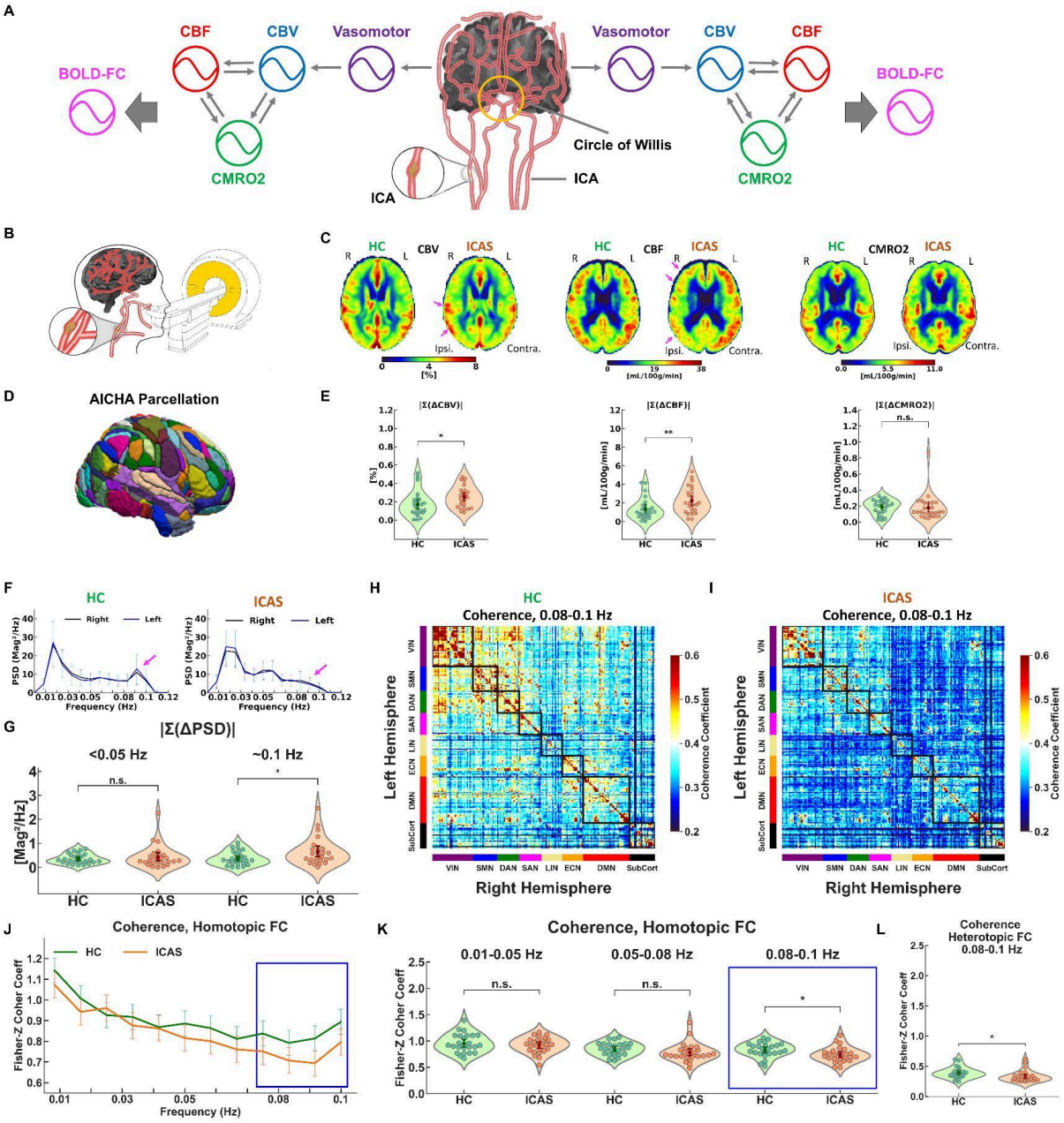



**Fig. 1. A.** Central hypothesis model in this work. **B.** MRI experiment. **C.** Multiparametric MRI maps. **D.** AICHA Parcellation. **E.** Group-level comparison of multiple parameters (Left and right hemisphere difference of CBF, CBV, and CMRO2). Wilcoxon rank sum tests were applied for statistical significance (n.s.=no significance, *p-value<0.05, and **p-value<0.01). **F.** Averaged PSDs from a representative subject in HC and ICAS. **G.** On group level, inter-hemispheric PSDs difference at less than 0.05 Hz vs. ~0.1 Hz. **H-I.** Coherence matrices sorted by seven networks at 0.08-0.1 Hz in HC (**H**) and ICAS (**I**). **J.** Homotopic coherence at 0.01-0.1 Hz. **K.** Statistical tests for homotopic coherence at 0.01-0.1 Hz. **L.** Statistical test for heterotopic coherence at 0.08-0.1 Hz. For PSD and coherence, two-sample t-tests were applied for statistical significance (n.s.=no significance and *p-value<0.05).

**Keyword 1**: Internal Carotid Artery Stenosis
**Keyword 2**: Vasomotion
**Keyword 3**: Functional Connectivity
**Keyword 4**: Resting-state fMRI
**Keyword 5**: Multiparametric MRI

**ASSESSMENT OF CORNEAL SENSITIVITY IN AN OCULAR ROSACEA MOUSE MODEL EXPOSED TO ULTRAVIOLET B**

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Ocular rosacea is a chronic disease of the ocular surface affecting the eyes and eyelids. It is a common cause of redness and painful sensations such as burning, stinging and itching. It is characterised by persistent inflammation of the cornea, eyelid margins and dysfunction of the Meibomian glands. Clinical signs include telangiectasias around the eyelids, blepharitis, conjunctivitis and keratitis, often marked by the appearance of corneal vascularization. This pathology is multifactorial and can be triggered by various factors such as sunlight exposure or emotional stress. We aimed to develop a new model by exposing mouse eyes to ultraviolet B (UVB) radiation. Mice were exposed to UVB at 3,94mW/cm² for 3 minutes per day over one, three or five days. To assess the validity of the model, we evaluated ocular surface sensitivity using eye-wiping and ocular Von Frey tests. Additionally, corneal inflammation was measured by ELISA using lipocalin-2, MPO and IL-8 markers. We found that one day of exposure did not alter corneal sensitivity in mice. After three days of exposure, corneal sensitivity only increased with one compartmental test. Finally, in the five-day UVB exposure protocol, corneal sensibility increased with the eye-wiping test but decreased with the ocular Von Frey test. The concentration of corneal inflammatory markers was significantly elevated, indicating substantial ocular inflammation in mice exposed to UVB for five days. These results demonstrate that repeated exposures for 5 days are required to increase ocular surface sensitivity and inflammation.

**MEMORY DEFICITS IN THE POST-COVID CONDITION – RELATION TO STRUCTURE AND FUNCTION OF THE MEDIAL TEMPORAL LOBE**

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Post-COVID condition (PCC; “Long Covid”) is a multi-systemic condition with diverse clinical presentations. Some PCC patients report neurocognitive symptoms, including subjective memory deficit and anosmia. These specific deficits may be related to alterations in the medial temporal lobe (MTL), a region associated with both memory and olfactory function. It is of clinical relevance to contextualize subjective memory deficit in PCC using objective memory assessments and to advance the understanding of the underlying neuropathology. We address, in PCC cases relative to controls, the relationship between anosmia, memory symptoms, and objective memory performance. Next, we assess grey matter (GM) volumes and functional activity in the MTL. Participants were characterized in terms of symptom burden and memory performance and underwent structural and functional 3T MRI and performed an in-scanner spatial memory paradigm. The PCC group (n=68) scored lower than the control group (n=33) in objective memory assessments. In cases, increased subjective memory deficit correlated to self-reported fatigue and brain fog, but not to objective memory performance or objective olfactory function. Structurally, PCC cases showed significantly lower anterior hippocampal grey matter volume relative to controls, and there were no other significant MTL GM differences between groups. Our next steps will be to assess memory-related MTL functional activity, and how it relates MTL GM volume, and to objective and subjective memory deficit and anosmia.

**Keyword 1**: Long Covid
**Keyword 2**: Medial Temporal Lobe
**Keyword 3**: Hippocampus
**Keyword 4**: Entorhinal Cortex
**Keyword 5**: Memory Pathology

**EPIGENETIC EDITING ALLEVIATES ANGELMAN SYNDROME PHENOTYPE IN MICE BY UNSILENCING PATERNAL UBE3A**

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Angelman syndrome (AS) is characterized by severe neurodevelopmental disorders caused by abnormalities in the maternally inherited UBE3A gene, leading to a complete loss of UBE3A specifically in neurons, where the intact paternal UBE3A is silenced by an endogenous antisense long-noncoding RNA known as UBE3A-ATS. The expression of UBE3A-ATS is regulated by the imprinting center Snrpn (Snrpn-IC)1, which is highly methylated in the maternal but not paternal allele. Recent studies using small- molecule inhibitior2, RNA targeting3–5, artificial transcription factor6,7, and CRISPR/Cas9-mediated AAV vector integration8 have shown that silencing of UBE3A-ATS was effective in allowing paternal UBE3A expression and alleviating AS phenotypes in mice. Another potential approach to silencing UBE3A-ATS is to introduce DNA methylation through epigenetic editing at the Snrpn-IC locus. Here we report that efficient DNA methylation on Snrpn-IC can be achieved by catalytically inactive Cas9 (dCas9) fused with peptide epitopes that recruit catalytic domains of two DNA methyltrans- ferases, leading to the unsilencing of paternal Ube3a via inhibition of Ube3a-ATS expression in mouse neurons. By generating AS transgenic mice carrying this epigenetic editing system, the expression of paternal Ube3a was markedly increased and AS phenotypes in mice were alleviated. Taken together, these results demonstrate that epigenetic editing can unsilence paternally inherited Ube3a without causing DNA sequence break, suggesting a potential therapeutic approach for AS treatment.

**LEVERAGING 3RS PRINCIPLES FOR GLIAL STUDIES: FUNCTIONAL RECORDINGS FROM EX VIVO HUMAN ASTROCYTES**

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Studying human brain tissue obtained through surgical biopsy poses significant challenges due to the rapid deterioration of tissue post-resection, allowing only a narrow window of several hours for functional cellular recordings. This research presents a novel and optimized approach for using viable brain slices from temporal lobe resection samples taken during epilepsy surgery. Preliminary findings from ex vivo human cortical astrocytes analyzed via whole-cell patch clamp, two-photon microscopy, and fluorescence lifetime imaging microscopy (FLIM) techniques demonstrate that human astrocytes, although morphologically analogous to rodent astroglia, exhibit larger cell bodies and more structurally complex processes. Electrophysiological studies reveal linear current-voltage relationships across all recorded astrocytes. Notably, a distinctive subtype of astroglia with varicose processes was identified in cortical layer V. Contrary to rodent models, where astrocytes are effectively labeled by glial syncytium loading with cell-permeant dyes, human astrocytes labeling is complicated due to strong neuronal uptake of the astroglial marker SR-101. These findings provide valuable insights into the properties of human astroglia and emphasize the imperative need for direct studies on human neural cells to gain a complete understanding of their functions in both health and disease.

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**CHARACTERIZATION OF MRNA-LOADED NANOPARTICLES TO NEURONAL CELLS: A STEP TOWARD CNS TREATMENT**

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Acute central nervous system (CNS) damage presents a major treatment challenge due to limited neuroregenerative capacity. Brain-derived neurotrophic factor (BDNF) is a promising candidate for promoting neuronal survival and growth. mRNA-based therapies have recently gained attention for their potential in targeted treatments. This study aimed to develop a stable, non-toxic polymeric carrier for mRNA delivery to neuronal cells.
Poly(2-oxazolines)-based nanoparticles were prepared via a standard polyplex formation method. mRNA encoding eGFP and BDNF was generated using the mMESSAGE mMACHINE T7 Ultra Kit. Primary hippocampal neurons from neonatal Wistar rats were used as an in vitro model, while adult male Wistar rats were employed for in vivo experiments. Cytotoxicity was assessed using the MTS assay. Polyplex (polymer + mRNA) nanoparticles were nasally administered to adult rats, and mRNA delivery (eGFP and BDNF expression) was analyzed via confocal microscopy (Leica TCS SP8 STED 3X).
We confirmed the internalization of polymer and polyplex nanoparticles into hippocampal neurons within 4 hours. Cytotoxicity results indicated that nanoparticles at 1 mg/ml were non-toxic. In vivo, nanoparticles were detected in the olfactory bulb. Expressions of eGFP and BDNF were observed in primary hippocampal neurons after nanoparticle delivery.
In conclusion, our polymer nanoparticles are non-toxic, effectively deliver mRNA to specific cell types, and enable protein expression of selected genes, highlighting their potential for CNS treatment.
Work was supported by PostdokGrant APD0092.

**STUDYING THE CONTRIBUTION OF VOLTAGE-GATED CALCIUM CHANNELS FOR TRKB-FL CLEAVAGE**

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In Alzheimer's disease (AD), amyloid-beta accumulation triggers excessive calcium influx through N-methyl-D-aspartate (NMDA) receptors, leading to calpain-mediated cleavage of the brain-derived neurotrophic factor (BDNF) receptor (TrkB-FL). This process generates TrkB-ICD, an intracellular fragment with known detrimental effects. Voltage-gated calcium channels (VGCC), particularly L-type, regulate calcium entry in excitable cells during membrane depolarization and are commonly targeted in hypertension treatment. Whether these channels contribute to BDNF receptor cleavage is unclear. This study aimed to explore alternative calcium influx sources as potential triggers of TrkB-FL cleavage.
Neocortical neurons (DIV7) were incubated with KCl to induce depolarization and/or EGTA, a calcium chelator, to assess extracellular calcium's role in TrkB-FL cleavage. VGCC involvement was evaluated by co-incubating KCl with inhibitors targeting L-type (Nifedipine), P/Q-type (ω-Agatoxin), N-type (ω-Conotoxin), R-type (SNX-482), or T-type (Ethosuximide) calcium channels. Western blotting measured TrkB-FL, TrkB-ICD, and their ratios 24h post-treatment. A time chase assay with KCl ± Nifedipine assessed TrkB-FL and TrkB-ICD at 8, 16, and 24h.
KCl-depolarized neurons increased TrkB-ICD levels ($p<0.05$, n=4-8) without altering TrkB-FL, an effect reversed by EGTA ($p<0.05$). Among VGCC inhibitors, only Nifedipine reduced TrkB-ICD formation ($p<0.05$, n=3-5) and TrkB-ICD/TrkB-FL ratios ($p<0.05$, n=3-5) at 24h. Time chase results confirmed this trend, showing reduced TrkB-ICD levels at 16h ($p<0.05$, n=4) and 24h ($p<0.05$, n=4).These findings suggest VGCCs may have an important contribution for TrkB-FL cleavage, highlighting the therapeutic potential of VGCC blockers in AD.

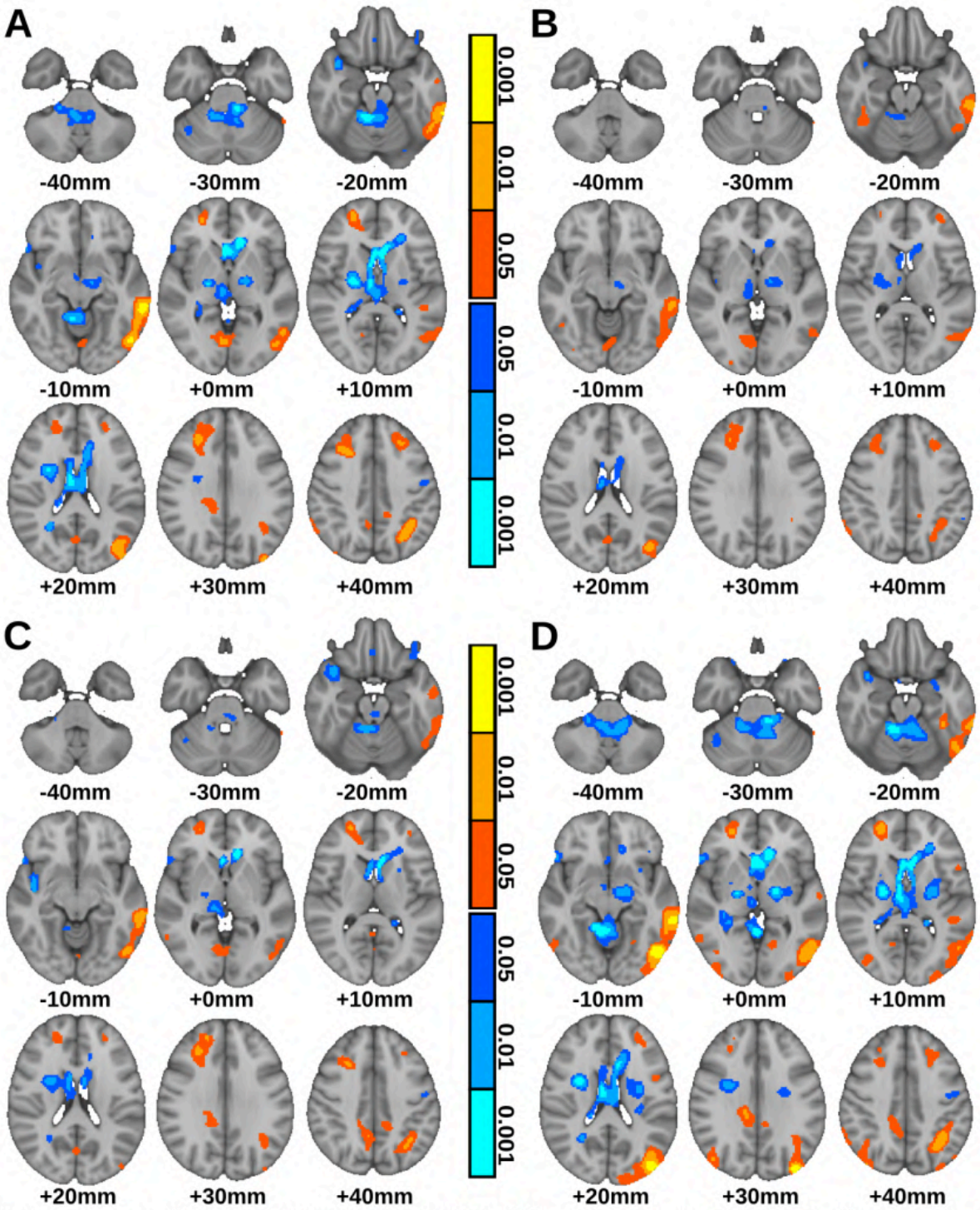
**Keyword 1**: Alzheimer's Disease
**Keyword 2**: TrkB-ICD
**Keyword 3**: BDNF
**Keyword 4**: VGCC
**Keyword 5**: NMDA

**ALTERED CEREBROSPINAL FLUID DYNAMICS IN ALZHEIMER'S DISEASE: REGIONAL HOMOGENEITY ANALYSIS OF PHYSIOLOGICAL BRAIN PULSATIONS**

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Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by cognitive impairment, amyloid-beta (Aβ) plaque deposition, and the formation of tau neurofibrillary tangles. AD also involves significant cerebrovascular alterations, such as arterial stiffening and decreased vascular compliance. Recent research has focused on the role of the glymphatic system in AD, a clearance pathway responsible for removing soluble metabolites, including Aβ and tau proteins, from the brain. The glymphatic system relies on cerebrospinal fluid (CSF) flow dynamics, which are influenced by physiological pulsations originating from cardiac and respiratory cycles. These pulsations drive CSF through perivascular spaces, facilitating metabolite clearance from neural tissue. In this study, we employed fast magnetic resonance encephalography (MREG), an advanced fMRI sequence with high temporal resolution, to investigate the regional homogeneity (ReHo) of physiological brain pulsations in patients with AD (n=43) compared to healthy controls (n=72), with both groups comparable in age. ReHo analysis quantifies local coherence by evaluating the similarity of fMRI signals among neighboring voxels. With MREG, we can separate the signal into cardiac, respiratory, and very low-frequency bands, assessing coherence across the full-frequency range and within each frequency band. Our primary objective was to characterize potential alterations in physiological pulsation coherence associated with AD pathology. We hypothesized that AD patients would exhibit reduced coherence, particularly within the cardiac frequency band, reflecting disease-related changes in arterial structure and function. Such findings could offer novel insights into impaired CSF dynamics and compromised glymphatic clearance mechanisms in Alzheimer's disease.

**Keyword 1**: Alzheimer's Disease
**Keyword 2**: Cerebrospinal fluid dynamics
**Keyword 3**: Functional magnetic resonance imaging
**Keyword 4**: Resting state
**Keyword 5**: Regional Homogeneity




**Statistical maps of Regional Homogeneity (ReHo) differences between Alzheimer's disease patients and healthy controls in different frequency bands.** Panels A-D display statistical maps overlaid on axial slices of the brain in MNI space, showing the results of ReHo analysis. **(A)** Full-band (0.008–0.5 Hz), **(B)** Very Low Frequency (VLF; 0.008–0.1 Hz), **(C)** Respiration (0.10–0.65 Hz), and **(D)** Cardiac (0.65–5 Hz) frequency bands. Blue regions indicate areas where ReHo is significantly higher in controls compared to Alzheimer's patients, whereas red regions indicate higher ReHo in Alzheimer's patients compared to controls. The color bar represents the level of statistical significance, with darker shades indicating p = 0.05, middle shades indicating p = 0.01, and lighter shades indicating p = 0.001. The images are displayed from z-coordinates ranging from -40 mm to +40 mm in the MNI space.

**UPREGULATION OF THE NAD+/SIRT1-REST AXIS INHIBITS ALZHEIMER'S DISEASE**

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Downregulation of the Repressor Element 1-Silencing Transcription factor (REST) is a significant marker of and likely contributes to Alzheimer's disease (AD), but the exact molecular mechanism linking REST reduction to AD remains unclear. We examined the changes in REST expression in the entorhinal cortex and hippocampus across different stages of AD in postmortem human brain samples. Using a cross-species approach, we show that alterations in REST expression and cellular localization are partially responsible for AD pathology. REST overexpression improves cognitive function, reduces amyloid beta (Aβ) deposition and accumulation of misfolded and phosphorylated tau, and restores mitochondrial and synaptic homeostasis. Research indicates that mitochondrial dysfunction plays a crucial role in the development of AD, we and others propose that enhancing mitophagy through the nicotinamide adenine dinucleotide ($NAD^+$)-mitophagy pathway could be a promising target for drug development. Effects of $NAD^+$ are mainly regulated by $NAD^+$-dependent enzymes, such as SIRT-1. Recent studies point to the $NAD^+$/SIRT1-mitophagy axis as a contributor to neuroprotection and memory preservation. Moreover, REST regulates mitochondrial biogenesis and mitochondrial autophagy (mitophagy). Therefore, we propose a $NAD^+$/SIRT1-REST axis that regulates mitochondrial quality control in AD. Mechanistically, the $NAD^+$/SIRT1 axis modulates REST expression through chromatin remodeling in the promoter region of REST, leading to changes in the expression of REST target genes involved in mitophagy and synaptic function. These findings reveal a new mechanism of action for $NAD^+$ and highlight REST as a promising therapeutic target for AD.

**Keyword 1**: REST
**Keyword 2**: SIRT1
**Keyword 3**: NAD+
**Keyword 4**: Alzheimer's disease
**Keyword 5**: Dementia

**NEUROPSYCHIATRIC AND NEUROCOGNITIVE SYMPTOMS AS PREDICTORS OF SLEEP PROBLEMS IN MILD COGNITIVE IMPAIRMENT: A LATENT VARIABLE APPROACH**

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Sleep problems constitute an intrinsic characteristic of mild cognitive impairment (MCI), whereby mounting evidence suggests that they may worsen neurocognitive decline and possibly contribute to the underlying neural pathophysiology. In this direction, Partial Least Squares Structural Equation Modeling (PLS-SEM) was applied to investigate the latent relationships between "psychological burden", "cognitive status", and "sleep problems" amongst 131 individuals with MCI. The psychological burden-including depression, anxiety, and stress was identified as the most salient predictor of sleep problems ($\beta$ = 0.296, $p$ < 0.001), thus indicating a close linkage between neuropsychiatric processes and disturbed sleep. Declining cognitive status ($\beta$ = -0.183, $p$ = 0.011) and younger age at MCI onset ($\beta$ = -0.152, $p$ = 0.012) were also implicated in sleep problems, thereby further entrenching their interrelatedness. These findings underscore the critical role of psychological burden and cognitive decline in shaping sleep patterns in MCI. Possible neural mechanisms - hippocampal atrophy and HPA axis dysregulation- are marked as important factors linking these variables with sleep-cognition interactions. The results may also support the importance of early clinical interventions targeting psychological health and sleep quality to delay the progression of MCI. By integrating latent variable modeling into the neurocognitive and neuropsychiatric framework, this work points to the importance of sleep in the neuropathophysiological progression of MCI while drawing attention to the need to investigate sleep-related mechanisms in both neuroscience and clinical care.

**Keyword 1**: Mild Cognitive Impairment
**Keyword 2**: Latent-variable approach
**Keyword 3**: Cognitive functioning
**Keyword 4**: Psychological symptoms
**Keyword 5**: Sleep problems

**REAL-TIME FMRI NEUROFEEDBACK OR MENTAL STRATEGIES IMPROVE PATTERN SEPARATION PERFORMANCE IN INDIVIDUALS AT RISK OF ALZHEIMER'S DISEASE**

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Alzheimer's disease (AD) pathogenesis begins at least a decade before any clinical symptoms. The degree of hippocampal activation during memory tasks is a promising neuroimaging biomarker for improved AD risk prediction. In manifest AD, it is typically *lower* than in healthy volunteers. In patients with Mild Cognitive Impairment (MCI) it is markedly *higher* and associated with memory deficits and faster clinical progression. Pattern separation, a process that critically depends on the hippocampus, is particularly prone to elicit hyperactivity in patients with MCI. In a previous study, we examined whether real-time fMRI neurofeedback can reduce hippocampal hyperactivity and improve pattern separation. With this method, participants train to control region specific brain activity by using mental strategies. The training is accomplished by continuously measuring brain activity and providing feedback to the participant about the ongoing activity. We found that 2 sessions of real-time fMRI neurofeedback significantly reduced hippocampal activity and improved pattern separation performance. While these results are promising, neurofeedback with real-time fMRI is not practical when treating many people. We therefore extracted the most successful downregulation strategies and examined in a follow-up study, whether these strategies alone lead to similar results. So far, 15 participants (70±6.1years old, 8 female) that took part in both studies were analysed. Their pattern separation performance significantly improved after neurofeedback ($p<.05$, Fig. 1) as well as after 4 or 6 strategy trainings ($p<.05$, Fig. 1) indicating both interventions improve episodic memory performance.

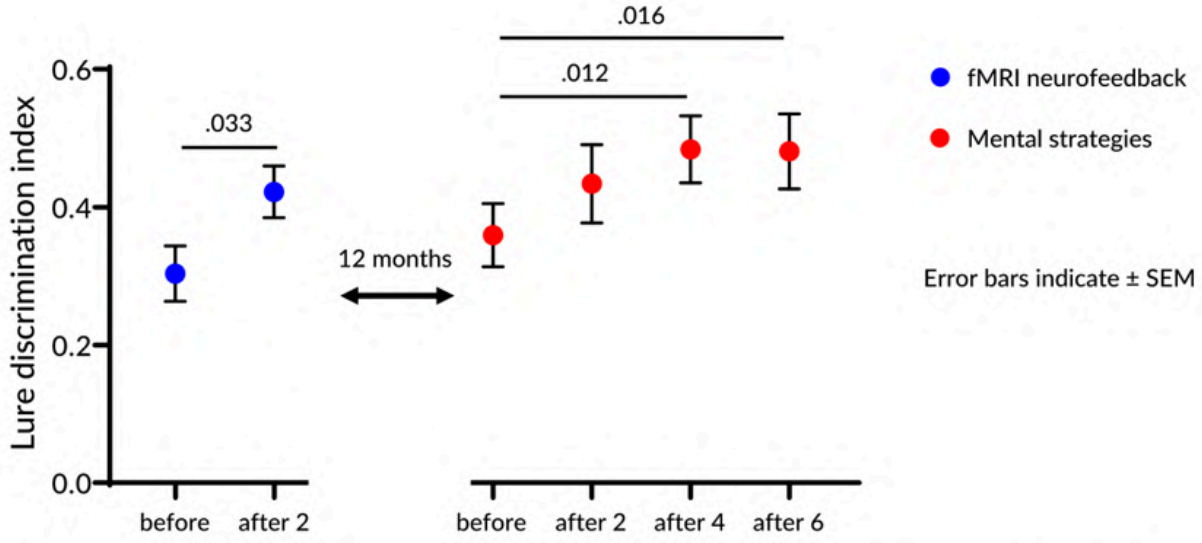



**Fig. 1.** Mean lure discrimination index before or after 2 sessions of neurofeedback (blue) or 2, 4 or 6 sessions of mental strategy training (red).

**PREDXCLEARANCE, EXPLORING NEW BIOMARKERS TO PREDICT ARIA/CAA AND PERSONALIZED THERAPY IN AD**

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New Aβ-specific antibody immunotherapies show promise in the treatment of Alzheimer's disease (AD). However, up to 40% of patients experience Amyloid-Related Imaging Abnormalities (ARIA), a potentially life-threatening complication linked to Cerebral Amyloid Angiopathy (CAA). Research suggests compromised Abeta clearance systems may predict immunotherapy complications, with monocyte/macrophage clearance capacity playing a key role in AD and ARIA/CAA risk.
Based on the information presented above, we are developing an in vitro tool capable of measuring monocytic Abeta clearance capacity using a proprietary monocytic culture system derived from AI-Mind Biobank samples. The prepared cell culture is spiked with Abeta1-40 and incubated for several hours. Intracellular Abeta1-40 and Abetax-34 are measured using immunoassay with proprietary antibodies at different time points to evaluate the Abeta proteolytic capacity of the monocytes. In addition, pTau217 was measured in plasma with the MSD S-PLEX Human Tau(pT217) Kit.
Our results showed that pTau217 concentration is linked to APOE4+ genotypes in a cohort with mild cognitive impairment (MCI). We found that pTau217 alone cannot distinguish different subsets of AD/MCI patients that could indicate a higher risk of ARIA. By measuring monocytic clearance capacity with our cell culture system, AD patients with higher pTau217 concentration and APOE4+ genotypes can be further subgrouped into those with high and low Aβ clearance capacity, with the latter being more likely to develop ARIA.
The findings of this study will lead to more effective, accessible, and safer treatments for AD patients.

**Keyword 1**: Alzheimer's Disease
**Keyword 2**: immunotherapy
**Keyword 3**: ARIA/CAA
**Keyword 4**: monocytes
**Keyword 5**: Abeta clearance

**BRIDGING THE GAP BETWEEN CONNECTOME AND METHYLOME: DIFFERENTIAL DNA METHYLATION WITHIN CA1 AS A FRAMEWORK FOR ALZHEIMER'S DISEASE PROGRESSION?**

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Alzheimer's disease (AD) is the most common form of dementia and a progressive neurodegenerative disease of the central nervous system. Ever since the first description of the disease, the classical biomarkers A-beta and Tau have been used to characterize AD neuropathologically. In recent years, differential DNA methylation across brain regions and across the disease continuum has emerged as a new hallmark of AD neuropathology at a molecular level. DNA methylation appears to be driven by changes in neuronal activity. Within the hippocampal formation, proximal and distal CA1 receive differential input from different subregions of the entorhinal cortex, leading to e.g. different place field dynamics in both areas. It is known that neuronal activity is disturbed early on within the entorhinal cortex in AD, largely driven by A-beta pathology. Here, we investigate if both the methylome and transcriptome of the proximal versus distal CA1 subregion of the hippocampus are differentially altered in an APP/PS1 mouse model. We dissect both areas separately using a laser-capture microdissection approach and isolate nucleic acids from the dissectates. Methylation specific DNA sequencing combined with classical whole-transcriptome sequencing will tell us if the differential input of both areas within the CA1 subregion leads to an altered methylome and transcriptome in proximal versus distal CA1. We think that disturbed neuronal activity in one brain region leads to disturbed methylation and, effectively, transcription in a different brain region connected to the first. This can be a novel framework for understanding disease progression in AD and neurodegenerative diseases in general.

**Keyword 1**: Alzheimer's Disease
**Keyword 2**: DNA methylation
**Keyword 3**: Hippocampal-entorhinal connectivity
**Keyword 4**: Laser capture microdissection
**Keyword 5**: A-beta pathology

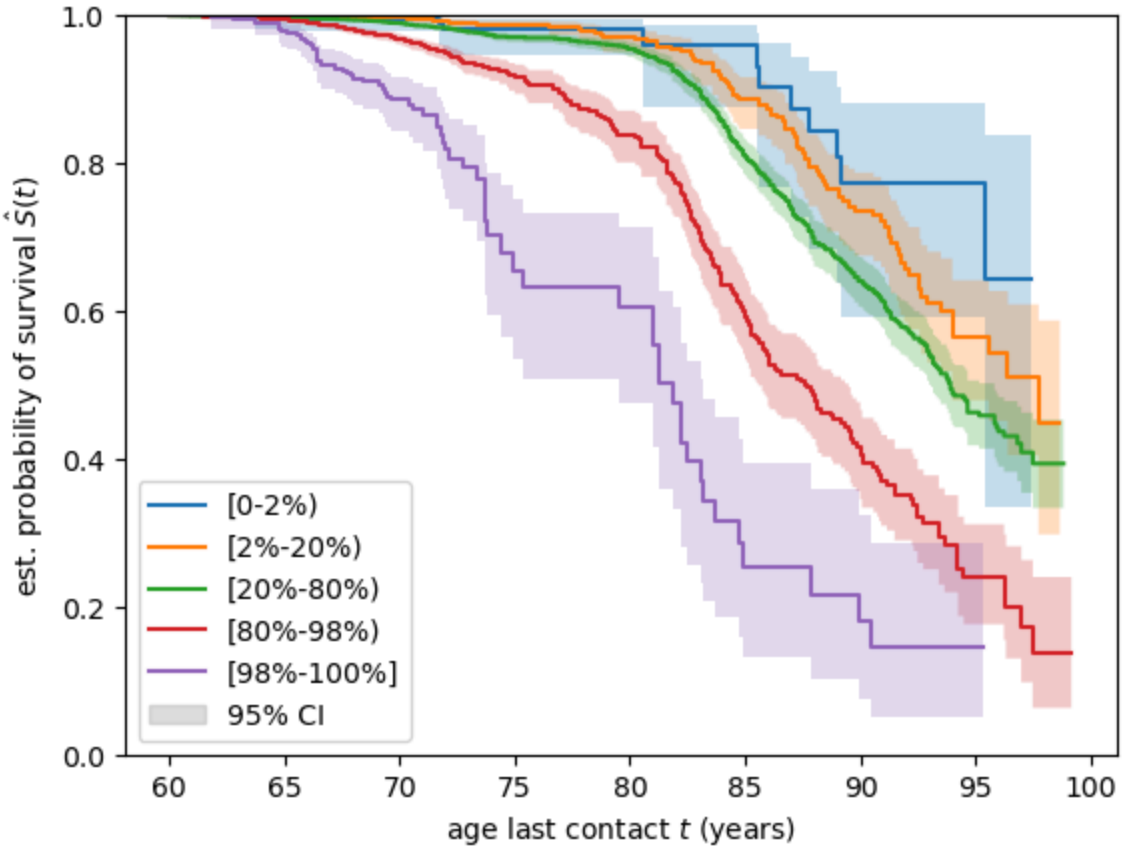
**PILOT STUDY FOR DEMENTIA AGE OF ONSET PREDICTION IN THE NORWEGIAN HUSK COHORT**

E. Hagen[1], V. Fominykh[1], B.C. Akdeniz[1,2], O. Frei[1,2], S. Bahrami[1,3], A. Shadrin[1], L. Rødevand[1], E. Moreno[4], B.M. Brumpton[4], I. Martinaityte[5], G. Selbæk[6,7,8], J. Haavik[9,10], O. Andreassen[1]
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Dementia accounts for the highest spending by GDP in the Norwegian healthcare system, increasing significantly by 2050 as the population ages [1]. The 2024 Lancet Standing Commission report on dementia [2] reviewed modifiable lifestyle- and environmental risk factors associated with dementia (e.g., education level, hypertension, cholesterol level, smoking, obesity, depression, physical activity, diabetes, alcohol use, social isolation). Individuals may be genetically predisposed, as Apolipoprotein E4 and other variants increase dementia risk [2]. Norwegian population cohorts and biobanks (Tromsø Study, https://uit.no/research/tromsostudy; HUNT Study, https://www.ntnu.edu/hunt; HUSK Study https://husk-en.w.uib.no) provide unique opportunities to study interactions between lifestyle- and environmental risk factors and genetics through biological samples and phenotypic information from questionnaire data, clinical assessments, health records data (Norwegian Patient Registry, Cause of Death Registry). Here, we employ polygenic scores and polygenic hazard score predictions [3] with lifestyle risk scores using Cox proportional hazards models [4] to investigate survival probabilities as a function of age for Alzheimer's disease and related dementias (ADRD) in the HUSK cohort (n=34.4K; born 1925-1956), accounting for risk factors listed above (Figure 1). The model improves predictive performance over established approaches [3]. We aim to extend the investigation to the HUNT and Tromsø studies.

1. Kinge, JM. et al. (2025). *BMC Med* 23:116
2. Livingston, G. et al. (2024). *The Lancet* 404(10452):572 – 628
3. Desikan RS. et al. (2017). *PLoS Med* 14(3):e1002258
4. Cox, DR. (1972). *J R Stat* 34(2):187–220

Figure 1. Kaplan-Meyer survival curves for ADRD cases and controls in the HUSK Study, accounting for PHS and risk factors.




**Keyword 1**: Dementia
**Keyword 2**: Alzheimer's disease
**Keyword 3**: Cox Proportional Hazards
**Keyword 4**: Survival analysis
**Keyword 5**: Genetics

**MECHANISM OF FERROPTOSIS IN ALZHEIMER'S DISEASE**

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As the most common form of dementia, Alzheimer's disease (AD) is a devastating neurodegenerative disorder without a cure. One possible reason is that the complex aetiology of AD is still not fully understood. Here we investigate the role of cell death mechanisms - in particular ferroptosis – and their linkages to age-dependent mitochondrial dysfunction in AD. Ferroptosis is form of cell death characterized by rampant iron-dependent lipid peroxidation. We analysed post-mortem human brain tissue covering a wide age range (20-100 years) and all stages of AD (Braak stages I-VI). Mass spectrometry based multi-omics approaches were used to analyse the lipidome, proteome and elemental distribution in post-mortem human brain tissue. These results were further validated by Western blotting, immunofluorescence imaging of brain slices and assessing iron distribution. Our analysis of three different brain regions shows that certain lipid species, such as bis(monoacylglycerol)phosphates, accumulate with aging without correlating with AD progression, while others, such as cardiolipin, show a decrease in chain length and unsaturation with both aging and AD progression, highlighting the central role of mitochondrial dysfunction in AD and as a modulator of ferroptosis.

**Keyword 1**: Alzheimer's Disease
**Keyword 2**: ferroptosis
**Keyword 3**: ageing

**ESTABLISHMENT OF A LARGE SPATIOTEMPORAL MULTI-OMICS DATABASE OF POSTMORTEM HUMAN BRAIN SAMPLES UNVEILS SOPHISTICATED MECHANISMS OF BRAIN AGEING AND ALZHEIMER´S DISEASE**

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Insights into the changes occurring during ageing of the human brain remain a mystery due to the difficulties in obtaining high quality human brain tissue and additional technical challenges. Recently, studies on human blood samples suggested that the trajectories of human ageing might be less linear than previously anticipated with two major peaks around 40 and 60 years old. However, knowledge about the changes occurring during ageing in the human brain remain elusive. We used carefully collected and pathologically well-characterized postmortem human brain tissues (n = 89 individuals, with a 3 brain regions per individual and a total of 267 samples) from individuals ranging from 20 to 100 years old from both cognitively normal and Alzheimer’s disease (AD) patients to investigate changes of human brain ageing. We performed multiple omics analyses, including single-nucleus RNA sequencing (snRNA-seq), proteomics, phosphoproteomics, metabolomics, and lipidomics. Our data show gender and age-driven, genetic dominant (e.g., *APOE4*), and brain-region (hippocampus, entorhinal cortex, and prefrontal cortex) specific profiles of the human brain during ageing and in AD. Specific pathways that are affected during ageing and especially in AD, include different cell death and survival pathways regulating mitochondrial function, mitophagy, and cellular metabolism, supporting an essential role of compromised mitochondrial homeostasis in brain ageing and AD.

**Keyword 1**: Alzheimer's disease
**Keyword 2**: multi-omics
**Keyword 3**: mitophagy
**Keyword 4**: brain ageing

**SLEEP DEPRIVATION EXACERBATES COGNITIVE DECLINE IN ALZHEIMER'S DISEASE RATS VIA ULTRASTRUCTURAL ALTERATIONS IN THE HIPPOCAMPUS**

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Alzheimer's disease (AD) is a prevalent neurobiological disorder. The bidirectional relationship between Alzheimer's and sleep architecture, suggests a potential role of sleep disturbances in disease progression. Understanding this relationship is crucial for comprehensive disease management and future therapeutic strategies. This study examined the effects of intracerebroventricular Streptozotocin (ICV-STZ) and Sleep Deprivation(SD) on sleep architecture in Wistar rats. Polysomnography and hippocampal ultrastructure changes were seen in control, AD, and SD+AD rats. SD+AD showed significant % increase in NREM (Mean diff: -7.62) and active wake (Mean diff: -5.14) stages in comparison to AD rats. Interestingly, REM sleep was significantly reduced in SD+AD rats in comparison to Controls (Mean diff: 6.51).On quantifying the probability % of sleep stage transitions, we observed that wake to NREM, NREM to REM, REM to NREM transition stages were significantly increased in AD and SD+AD rats in comparison to Controls ($F_{(3, 76)} = 17.5$; $p<0.001$; n=5). Two-way ANOVA results depicted treatment groups accounted for 4.35 of the variances (F = 0.17; DFd=76; $p<0.05$). Interestingly, REM to wake stage transition was significantly increased in AD rats whereas no significant change was observed in SD+AD rats in comparison to controls. Electron microscopy revealed swollen and vesicular types (activated) of mitochondria in AD and SD rats compared to controls. In conclusion, AD induction in SD animals alters sleep architecture, increasing various sleep stages, while SD exacerbates these alterations, particularly affecting REM sleep. Hippocampal ultrastructure analyses revealed that AD affects the mitochondrial topology and SD aggravates these alterations.

**Keyword 1**: Alzheimer's Disease
**Keyword 2**: Sleep Deprivation
**Keyword 3**: Hippocampal ultrastructure
**Keyword 4**: NREM – REM
**Keyword 5**: Sleep stage Transitions

**IDENTIFYING ENVIRONMENTAL RISK FACTORS FOR NEURODEGENERATIVE DISEASES IN NIGERIA USING A MULTI-FUNCTIONAL SENSOR EMBEDDED IN A BCI (EEG) DEVICE**

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Understanding the interplay between environmental factors and brain health is crucial for preventing neurodegenerative diseases like Alzheimer's, Parkinson's, and stress disorders. Nigeria, with over 50 million citizens facing neurological concerns, is particularly vulnerable. This study proposes a novel approach utilizing a multifunctional sensor integrated into a Brain-Computer Interface (BCI) to identify individuals at risk. By analyzing bioenvironmental data alongside EEG brain activity, we aim to uncover the complex factors contributing to neurodegenerative disease development. Environmental factors, such as pollutions, significantly impact brain health. The WHO estimates 12.6 million deaths annually due to unhealthy environments. This study addresses this critical issue by developing a sensor capable of real-time monitoring in selected Nigerian locations. Our approach involves: Neural Network Analysis: Advanced neural networks will analyze bioenvironmental data to identify patterns and correlations with brain health. A web application with information about environmental factors, their impact on brain health, and resources for well-being. EEG data will be collected simultaneously with environmental data. Key EEG parameters include brainwave frequencies, amplitude, and coherence. Environmental data will encompass pollution levels, noise exposure, natural disasters, and social indicators. Deep learning models will be trained on this combined dataset to identify patterns and predict the risk of neurodegenerative diseases. This study seeks to contribute to a deeper understanding of the environmental factors influencing brain health and develop innovative tools for early detection and prevention of neurodegenerative diseases. We aim to improve the quality of life for individuals at risk and pave the way for more effective interventions.

**Keyword 1**: Environment
**Keyword 2**: Brain Computer Interface (BCI)
**Keyword 3**: Neurodegenerative Diseases
**Keyword 4**: Sensor
**Keyword 5**: Neural Network

**C-FOS IN NEURODEGENERATION AND NEUROPROTECTION: MECHANISMS AND IMPLICATIONS IN NEURODEGENERATIVE DISORDERS**

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c-Fos, an immediate early gene and integral member of the AP-1 transcription factor family, plays a critical role in immune regulation, inflammation, and cancer. First identified as a viral oncogene in osteosarcoma, c-Fos serves as a link between transient cellular signals and sustained changes in gene expression. This review examines c-Fos's regulatory mechanisms and its implications in neurodegenerative disorders, particularly Alzheimer's and Parkinson's diseases. Notably, c-Fos exhibits a dual role: it can act as a neuroprotective factor or contribute to neurodegeneration, depending on the activation context. Key signalling pathways, such as MAP kinases (ERK and p38), govern its transcriptional activity and protein stability. c-Fos is rapidly induced by diverse stimuli, making it a reliable marker of neuronal activation. However, chronic or excessive c-Fos expression has been linked to neurodegeneration, particularly in Alzheimer's disease, where it disrupts amyloid precursor protein expression and exacerbates neuroinflammation. This inflammatory response may accelerate disease progression. By exploring the balance between c-Fos's protective and detrimental roles, this review highlights its potential as a therapeutic target. A deeper understanding of its involvement in neuroinflammatory pathways could inform strategies to mitigate neurodegeneration and enhance cognitive resilience. Targeting c-Fos offers a promising direction for innovative therapies to manage neurodegenerative diseases and preserve brain function.

**Keyword 1**: c-fos
**Keyword 2**: Alzheimer's Disease
**Keyword 3**: Parkinson's Disease
**Keyword 4**: Neuroinflammation
**Keyword 5**: Neurodegeneration

**SYSTEM $X_c^-$ DELETION ALTERS THE METABOLIC PHENOTYPE OF MURINE ASTROCYTES IN HEALTHY AND PATHOLOGICAL AGING**

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The cystine/glutamate antiporter system $x_c^-$ (specific subunit xCT) is in the central nervous system mainly expressed on astrocytes. Genetic deletion of xCT results in lifespan extension, memory preservation in aged mice and alterations in the hippocampal metabolome. To further understand the metabolic implications of xCT deletion, we performed a metabolic characterization of primary astrocytes isolated from young and aged xCT knockout ($xCT^{-/-}$) and wildtype ($xCT^{+/+}$) mice. A glucose metabolism-specific qPCR array showed that $xCT^{-/-}$ and $xCT^{+/+}$ astrocytes are metabolic distinct at the transcriptome level, with transcripts related to the TCA highly expressed in $xCT^{+/+}$ astrocytes, and glycolytic transcripts highly expressed in $xCT^{-/-}$ astrocytes, regardless of age. Accordingly, $xCT^{-/-}$ astrocytes tend to be better at maintaining glycolytic function than age-matched $xCT^{+/+}$ astrocytes, when being deprived of glucose. While we see an overall decrease in mitochondrial content and health with age, mitochondria from aged $xCT^{-/-}$ astrocytes seem to be more flexible. To investigate whether our metabolic flux findings can be extrapolated to pathological conditions, similar experiments were performed on astrocytes isolated from 5XFAD mice (model for Alzheimer's disease, AD), with and without xCT deletion. 5XFAD astrocytes show a stronger metabolic decline with a significant decrease in basal and maximal respiration, spare respiratory capacity, and ATP-coupled respiration. In the absence of xCT, 5XFAD astrocytes seem to maintain their flexibility and health. Altogether, our (preliminary) findings suggest that xCT deletion alters the metabolic phenotype of astrocytes, rendering them more resilient and flexible to the energetic stress that occurs during aging and AD.

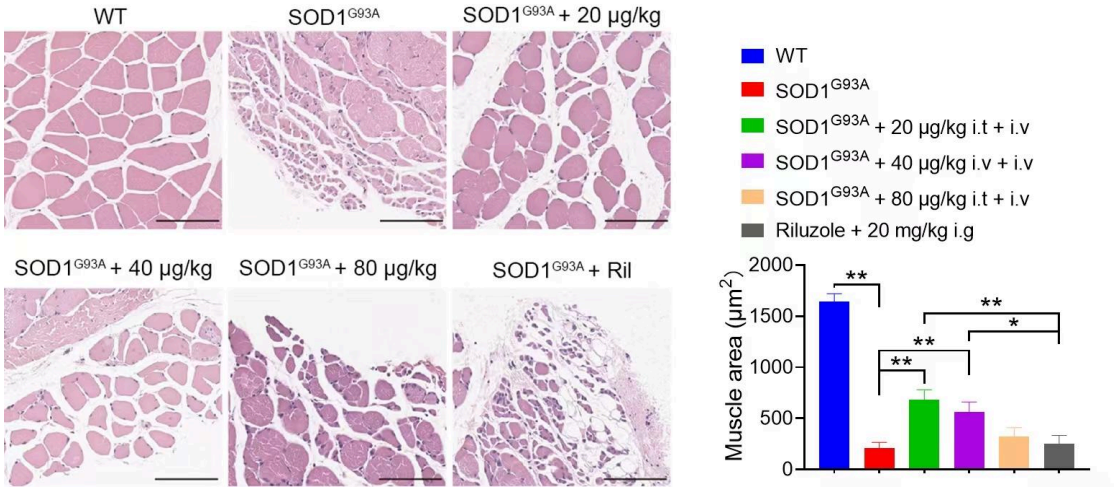
**Keyword 1**: xCT
**Keyword 2**: astrocytes
**Keyword 3**: aging
**Keyword 4**: energy metabolism

**NEURAL REPAIR REAGENT ALEETO™ CAN EFFICIENTLY TREAT AMYOTROPHIC LATERAL SCLEROSIS (ALS), FROM BENCH TO CLINIC**

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Amyotrophic lateral sclerosis (ALS) is a devastating neurodegenerative disease that affects the motoneurons. The pathogenic mechanisms remain largely elusive, and very few effective therapeutics for this disease are available. More efficient novel therapeutical methods are urgently needed.
Here we present a novel MSC-based drug, Aleeto™, which demonstrates promising clinical benefits. Both in vitro studies and IIT studies on more than 50 ALS patients are conducted.
In vitro experiments showed that cells treated with Aleeto™ restored cell viability after oxidative damage. Progressive loss of anterior horn motoneurons and muscle atrophy due to the lack of neuronal innervation are hallmarks of ALS. We found that Aleeto™ protected motoneurons against neurotoxicity and ameliorated muscle atrophy in SOD1G93A mice. In agreement with these results, the mutant mice treated with Aleeto™ exhibited improved motor functions and extended lifespan in comparison to mice treated with vehicle or Riluzole. We then carried out multicentered open-label IIT research on the efficacy of Aleeto™ on ALS patients. After 2 weeks of administration of Aleeto™, most patients reported positive outcomes, as demonstrated by elevated performance, ALSFRS-R, and Norris scores.

Such unprecedented therapeutic effects of Aleeto™ brought light to the treatment of this currently incurable disease




**Keyword 1**: Amyotrophic lateral sclerosis
**Keyword 2**: neurodegenerative disease
**Keyword 3**: ALS
**Keyword 4**: stem cells
**Keyword 5**: protein

**SMALL MOLECULE ACTIVATION OF M6A MRNA METHYLATION AS A NOVEL APPROACH FOR NEUROPROTECTION**

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N6-Methyladenosine (m6A) is the most common mRNA modification in eukaryotes. Methylation of adenosine contributes to regulation of splicing, transport, stability, and translation of mRNA. m6A formation is catalysed by methyltransferase-like 3 (METTL3) and METTL14. Recent data indicate that m6A levels are downregulated in several neurodegenerative disorders like Parkinson's disease (PD) and Alzheimer's disease. The mechanisms and role of m6A in neuronal survival, neuroprotection and neuroregeneration, however, remain poorly understood. We have previously identified four unique small molecule ligands that bind and activate the METTL3/14 complex thereby increasing m6A mRNA methylation. Now, we report that these compounds at nanomolar concentration support the viability of mouse dopamine (DA) neurons in culture in 6-hydroxydopamine (6-OHDA) neurotoxin model. In contrast, the METTL3/14 inhibitor STM2457 dose-dependently kills DA neurons emphasising the importance of m6A in these cells. For clinical translation, we tested the most effective compound C4 on human induced pluripotent stem cell-derived DA neurons and in rat model of PD. C4 protected human DA neurons in 6-OHDA model as good as GDNF and increased neurite outgrowth and the number of processes. In rat *in vivo* PD model, C4 improved motor behaviour and protected DA neurons and their fibres more efficiently than GDNF. Lastly, we pinpoint mitochondria as one of the targets for protection by increased m6A. We demonstrate that 6-OHDA causes fragmentation of mitochondria, whereas C4 restores their morphology. In conclusion, we discovered a completely new pathway of neuroprotection, provide proof-of-concept for novel therapeutic avenues and shed light into the mechanisms involved.

**Keyword 1**: Parkinson's disease
**Keyword 2**: Neurodegeneration
**Keyword 3**: m6A

**MITOCHONDRIAL DYNAMICS AND THERAPEUTIC INSIGHTS IN SPINAL MUSCULAR ATROPHY: PRELIMINARY RESULTS OF A MFN2 EXPRESSION ACTIVATOR DRUG IN HUMAN FIBROBLASTS AND MOTONEURONS**

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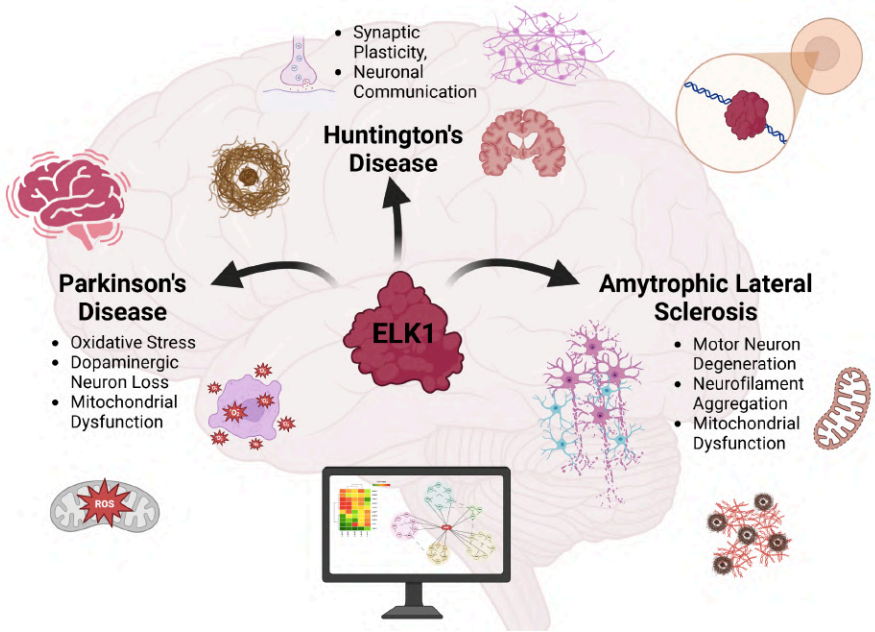
Mitochondria form a dynamic network where their spatial organization is regulated by the balance between fusion and fission, known as mitochondrial dynamics. These processes play a crucial role in mitochondrial turnover: fusion, mediated by Mfn2 and OPA1, promotes mitochondrial biogenesis, while fission, driven by Drp1, commits damaged mitochondria to mitophagy. Spinal Muscular Atrophy (SMA) is a neuromuscular disorder, where SMN (Survival Motor Neuron) protein deficiency leads to motor neuron (MN) degeneration. Since SMN protein is critical for mitochondrial homeostasis, several SMA models have shown mitochondrial dysfunctions. This study explored mitochondrial dynamics in SMA, first analyzing the mitochondrial network's morphology and function in a peripheral model, *i.e.* human fibroblasts derived from SMA patients. Here, we assessed the effects of a compound acting as a Mfn2 expression activator to promote mitochondrial fusion. Next, we characterized the network state in soma and neurites of motor neuron (MN) differentiated from SMA patients-derived iPSCs and primary MNs from the SMNΔ7 mouse model. In both models, western blot analysis of mitochondrial content markers highlighted a significant reduction in mitochondrial mass, and immunofluorescence analyses revealed a decreased mitochondrial distribution in the neurites of SMA MNs. Notably, western blot analysis of dynamics regulators indicated an increased fission state, localized in the neurites by performing the study of mitochondrial network morphology. The Mfn2 expression activator drug demonstrated its ability to enhance the mitochondrial mass and increase SMN protein levels. Our findings suggest that targeting mitochondrial dynamics could represent a promising strategy for the combinatorial treatment of SMA.

**Keyword 1**: Mitochondrial biology
**Keyword 2**: Spinal Muscular Atrophy
**Keyword 3**: Mitochondrial dynamics

**DISEASE-SPECIFIC GENE REGULATORY TARGETS OF ELK-1 IN NEURODEGENERATION**

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Neurodegenerative diseases are characterized by progressive neuronal degeneration, leading to severe motor and cognitive impairments. These conditions are associated with complex pathophysiological mechanisms, including oxidative stress, neuroinflammation, and dysregulation of gene expression. The ELK-1 transcription factor plays role as a critical regulator in these processes by influencing various cellular pathways that contribute to neuronal health and survival. This study integrates findings from transcriptomic analyses and gene regulatory network analysis to investigate the role of ELK-1 in neurodegeneration by focusing on amyotrophic lateral sclerosis (ALS), Parkinson's disease (PD) and Huntington's disease (HD). We have identified gene regulatory networks of ELK-1 specific to each disease using bulk RNA-seq data. In ALS dataset (GSE192755), ELK-1 modulates neurofilament deposition and mitochondrial function suggesting its involvement in maintaining neuronal structural integrity and motor neuron degeneration. In PD, ELK-1 regulates oxidative stress responses and mitochondrial pathways, highlighting its involvement in cellular energy metabolism and neuroprotection. In HD (GSE140118), ELK-1 influences genes related to neuronal signaling, synaptic plasticity which can contribute to the cognitive and motor symptoms associated with the disease. Collectively, these findings suggest that ELK-1 serves as a key transcriptional regulator in multiple neurodegenerative diseases, with distinct mechanisms adapted to each condition. Its role in modulating oxidative stress, mitochondrial function, and synaptic plasticity underscores its broad impact on neuronal health. Targeting ELK-1 may provide novel therapeutic strategies for neurodegenerative diseases, requires further research to uncover the precise molecular pathways affected by ELK-1 and explore its potential as a therapeutic target.

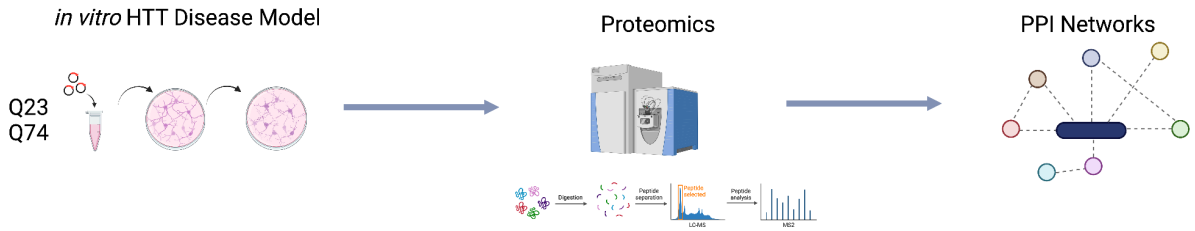



**Keyword 1**: Amyotrophic Lateral Sclerosis
**Keyword 2**: Huntington's Disease
**Keyword 3**: Parkinson's Disease
**Keyword 4**: transcriptome

**PREDICTED PROTEIN- PROTEIN INTERACTION NETWORKS FOR IN VITRO HTT DISEASE MODEL WITH MULTI-OMICS**

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Huntington's disease (HD) is a progressive neurodegenerative disorder caused by a CAG trinucleotide repeat expansion in the *HTT* gene, leading to an abnormally extended polyglutamine tract in the huntingtin protein. This mutation primarily affects neurons in the striatum, resulting in motor dysfunction and cognitive impairment. The aim of this study was to identify and characterize potential protein-protein interaction networks in an *in vitro* model of Huntington's disease using proteomics data to reveal therapeutic targets associated with disease progression. Mutant and wild-type *HTT* constructs were introduced into HEK293T cells, and aggregate formation was monitored over time. Cell death was observed during the process, with whole-proteome analysis conducted at the 72-hour time point. Proteome data analysis identified significantly altered proteins, which were categorized as mitochondrial proteins involved in energy metabolism. A potential *HTT*-specific interaction map was constructed using the STRING database. This study demonstrates that the Huntington's disease model exhibits unique proteomic signatures and protein interaction networks. The identified proteins represent promising therapeutic targets for Huntington's disease. Future work will focus on validating these findings through advanced proteomic and functional analyses.




**Keyword 1**: HTT
**Keyword 2**: Proteomics
**Keyword 3**: PPI
**Keyword 4**: AI
**Keyword 5**: mitochondria

**MODULATING MTOR-DEPENDENT ASTROCYTE SUBSTATE TRANSITIONS TO ALLEVIATE NEURODEGENERATION**

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Traditional approaches to studying astrocyte heterogeneity have mostly focused on analyzing static properties, failing to identify whether subtypes represent intermediate or final states of reactive astrocytes. Here we show that previously proposed neuroprotective and neurotoxic astrocytes are transitional states rather than distinct subtypes, as revealed through time-series multiomic sequencing. Neuroprotective astrocytes are an intermediate state of the transition from a nonreactive to a neurotoxic state in response to neuroinfammation, a process regulated by the mTOR signaling pathway. In Alzheimer's disease (AD) and aging, we observed an imbalance in neurotoxic and neuroprotective astrocytes in animal models and human patients. Moreover, targeting mTOR in astrocytes with rapamycin or shRNA mitigated astrocyte neurotoxic efects in neurodegenerative mouse models. Overall, our study uncovers a mechanism through which astrocytes exhibit neuroprotective functions before becoming neurotoxic under neuroinflammatory conditions and highlights mTOR modulation specifically in astrocytes as a potential therapeutic strategy for neurodegenerative diseases.

**Keyword 1**: astrocyte
**Keyword 2**: neurotoxic
**Keyword 3**: neuroprotective
**Keyword 4**: mTOR

**ENHANCED PROPERTY OF PROTEIN AGGREGATE DETECTION BY A NOVEL AGGREGATION MARKER IN NEURODEGENERATION MODELS**

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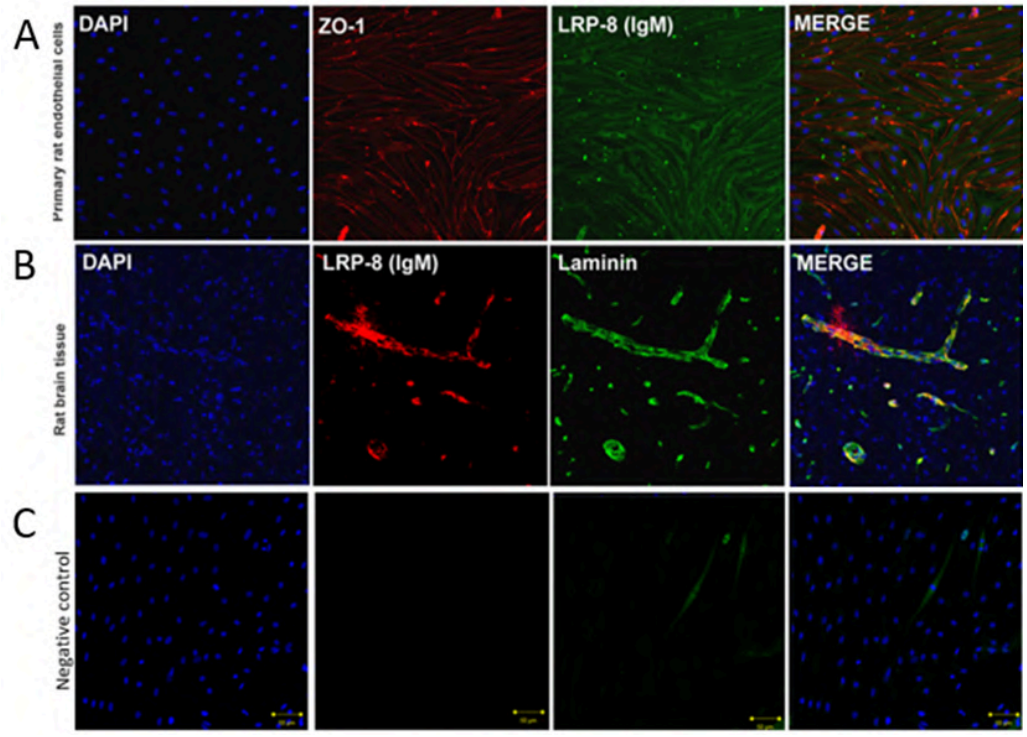
Thioflavin T (ThT) and Thioflavin S (ThS) are fluorescent dyes widely used in the study of protein aggregation, particularly in the context of neurodegenerative diseases such as Alzheimer's (AD) and Parkinson's Disease. These dyes are known for their high affinity for amyloid fibrils, which are aggregates of misfolded proteins that form beta-sheet-rich structures. The limitation of these flurophore molecule is its autofluorescence and low stoke shift properties. A nickel (II) complex (KRS) featuring a planar bis-benzothiazole ligand was designed and tested as novel flurophore to detect protein aggregation *in vitro* and *in vivo*. This novel molecule has stable photo-optic properties, broader excitation and emission spectra, no-autofluorescence, cost-effective, and is biocompatible compared to other available compounds available. For in-vitro system, SH-SY5Y cell line were incubated with amyloid-beta (Aβ) oligomers for 24 hours. Post-incubation the cells were fixed and stained with DAPI and KRS for image acquisition. In vivo analysis was carried out with a pharmacological model of AD by KRS administration intravenously and 48 hours post KRS injection, brain tissues were harvested for fluorescence imaging analysis.The study explores the efficacy of KRS in binding and highlighting protein aggregates in Alzheimer's Disease. Utilizing advanced imaging techniques such as Confocal and fluorescence microscopy, we observed that KRS selectively binds to these aggregates, enabling their visualization and quantification. Our findings also indicate that KRS successfully crosses the blood-brain barrier in pharmacologically induced AD mice and predominantly diffuses into the cortical areas of the brain.

**Keyword 1**: Dementia
**Keyword 2**: Alzheimer's Disease
**Keyword 3**: Fluorescent markers

**BLOOD-BRAIN BARRIER TRANSPORT SYSTEM FOR THERAPY AND DIAGNOSIS OF NEURODEGENERATIVE DISEASES**

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Alzheimer's disease (AD) is characterized by presence of insoluble aggregates of hyperphosphorylated tau proteins (1,2). Several therapeutic monoclonal antibodies (mAbs) have been approved for clinical use, their efficacy is limited by the blood-brain barrier (BBB), restricting their passage due to its selective nature (3,4). In this study, we aim to develop a single-chain variable fragment (scFv) with binding affinity to surface receptor proteins of rat endothelial cells, such as low-density lipoprotein receptor-related protein 8 (LRP8) or angulin-1, facilitating receptor-mediated transcytosis (RMT) for improved BBB penetration. The Recombinant LRP8 and angulin-1 proteins were produced using mammalian cell expression system and was used to immunize mice to generate mAbs. mAbs were fragmented to produce scFv. Specificity and efficacy of the antibodies were evaluated using Western blotting, ELISA, immunostainings and flow cytometry. BBB transcytosis of the antibodies was analysed using an in vitro 2D-BBB model. **Results**: We have successfully produced a LRP8 receptor specific scFv significantly recognizing primary rat endothelial cells and capillaries in rat brain tissue as shown in rat endothelial and brain capillary staining's (fig 1). Permeability experiments demonstrated that the scFv crosses the BBB. **Conclusion:** Through this study, we aim to develop an efficient drug delivery system capable of crossing the complex BBB, which can also serve as a diagnostic tool for detecting tau pathology. Additionally, we will assess the permeability of the LRP8-specific scFv in transgenic rat model for tauopathy. Furthermore, we will evaluate the ability of the generated scFv to recognize tau pathology in brain tissue.



**Keyword 1**: Blood-brain-barrier
**Keyword 2**: Endothelial cells receptor proteins
**Keyword 3**: LRP8

**STRUCTURAL ORGANIZATION OF THE AXON INITIAL SEGMENT IN HUNTINGTON DISEASE**

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Huntington disease (HD) is a heritable, autosomal dominant neurological disorder. In HD mice, the development of the cortex is characterized by abnormal neuronal morphology, with shorter axons and a transient simpler dendrite arborization. While these dendritic abnormalities have been associated to neuronal hyperexcitability, whether abnormalities of the axon also contribute is unknown. To address this, we focus on the Axon Initial Segment (AIS), specialized axonal domain critical for neuronal excitability regulation. Spanning 20-60µm at the proximal axon, it harbors a unique assembly of proteins and cytoskeletal elements, enabling its core functions: generation of action potential, axonal-dendritic polarity maintenance, and adaptive plasticity essential for homeostatic control of neuronal excitability. Staining for two AIS marker proteins, AnkG (master organizer of the AIS) and TRIM46 (microtubule associated protein involved in bundling) revealed a distal shift and significant reduction in the length of AIS in HD. These parameters are known to change in an activity-dependent manner due to the AIS plasticity. In HD, we observed that the AIS exhibits less plasticity in response to activity changes. Additionally, we found that TRIM46 was downregulated in HD leading to an aberrant compartmentalization of the soma-axonal domains. Overall, our findings suggest that the disbalanced expression of TRIM46 may lead to defective AIS assembly during development, ultimately resulting in AIS structural alterations. As AIS depends on its precisely organized structure for proper function, this structural alteration could contribute to its dysfunction. Specifically, the decreased AIS plasticity may play a role in the transient hyperexcitability previously identified in HD.

**Keyword 1**: Axon Intial segment
**Keyword 2**: Huntington disease
**Keyword 3**: Plasticity

**SERUM PROTEOME SHIFTS INDUCED BY REHABILITATION ON SPINAL CORD INJURY PATIENTS AND ASSESSMENT OF THEIR NEURONAL IMPACT**

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Spinal cord injury (SCI) is an untreatable neuropathology impairing motor, sensory, and autonomic functions. Aiming to identify recovery-associated proteins, mass spectrometry was applied to serum from 30 traumatic SCI patients (subacute/intermediate phase), pre- and post-rehabilitation, and 30 age/gender-matched controls. The resulting proteomic data were correlated with patients' clinical data, particularly recovery-associated outcomes.

Before rehabilitation, 94 deregulated proteins distinguished SCI patients' sera proteomes from controls. Proteins downregulated by SCI mainly relate to lipid metabolism and protein phosphorylation, while upregulated proteins mostly associate with immune pathways. Motor complete injuries (AIS A/B) induced more pronounced proteomic changes than motor incomplete ones (AIS C/D).

Rehabilitation partially restored SCI-induced alterations, deregulating 76 proteins and turning post-rehabilitation proteomes more like control ones. Time under rehabilitation predominantly downregulated immune-related proteins while upregulating many proteins associated with lipid metabolism. Notably, proteins downregulated by rehabilitation correlated negatively with clinical recovery (lower protein levels, higher recovery), while upregulated ones correlated positively (higher protein levels, higher recovery).

Selected 'beneficial' and 'detrimental' post-rehabilitation sera, categorized by recovery outcomes and proteome evolution, were incubated on human induced pluripotent stem cell-derived neuronal cultures. 'Beneficial' sera, from patients with better recovery and proteome shifts toward controls, promoted neuronal differentiation (decreased SOX2 and increased TUBB3 mRNA). Contrarily, 'detrimental' sera, from patients with poorer recovery and proteome shifts away from controls, delayed neuronal differentiation while increasing TP53 mRNA, suggesting apoptosis.

Our study supports that rehabilitation partially normalizes SCI-induced proteome alterations, supporting neuronal recovery (survival, regrowth). Recovery-related pathways provide a foundation for targeted therapies to improve SCI recovery.

**Keyword 1**: spinal cord injury
**Keyword 2**: serum proteomics
**Keyword 3**: rehabilitation
**Keyword 4**: iPSC-derived neuronal cultures

**UNCOVERING NOVEL KCC2 REGULATORY MOTIFS THROUGH A COMPREHENSIVE TRANSPOSON-BASED MUTANT LIBRARY**

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The neuron-specific K-Cl cotransporter KCC2 maintains low intracellular chloride levels, which are crucial for fast GABAergic and glycinergic neurotransmission. KCC2 also plays a pivotal role in the development of excitatory glutamatergic neurotransmission by promoting dendritic spine maturation. The cytoplasmic C-terminal domain (KCC2-CTD) plays a critical regulatory role in the molecular mechanisms controlling the cotransporter activity through dimerization, phosphorylation, and protein interaction. To identify novel CTD regulatory motifs, we used the Mu transposon-based mutagenesis system to generate a library of KCC2 mutants with 5 amino acid insertions randomly distributed within the KCC2-CTD. We determined the insertion positions in 288 mutants by restriction analysis and selected clones with a single insertion site outside known KCC2 regulatory motifs. We analyzed the subcellular distribution of KCC2-CTD mutants in cultured cortical neurons using immunocytochemistry and selected ten mutants with ectopic expression patterns for detailed characterization. Two groups of mutants were identified based on 3D structural analysis. The first group, located near the interface of transmembrane and cytoplasmic domains, may affect interactions with the N-terminal inhibitory peptide regulating KCC2 activity. The second group, situated on the external surface of the cytoplasmic domain, may disrupt interactions with regulatory proteins. Analyzing CTD mutations that modulate KCC2 activity enhances our understanding of its function and is essential for developing novel anti-seizure therapies.

**Keyword 1**: KCC2
**Keyword 2**: SLC12A5
**Keyword 3**: GABA
**Keyword 4**: Chloride homeostasis
**Keyword 5**: KCC2-CTD mutations

**UNDERSTANDING HOW EPILEPSY AFFECTS BREATHING AND SUDDEN DEATH RISK**

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Introduction: Breathing is essential for life, controlled by the brain. Some neurological disorders disrupt this process, making breathing difficult. Epilepsy, affecting 70 million people globally, can lead to Sudden Unexpected Death in Epilepsy (SUDEP). The exact cause of SUDEP remains unclear, but breathing problems during seizures may play a key role.
Our Study: We studied the DEPDC5 gene, which is linked to epilepsy, using mice with a modified version of this gene. These mice had seizures and, in some cases, experienced SUDEP. We found that their breathing slowed down before their hearts stopped, suggesting breathing difficulties contribute to SUDEP. To explore this, we focused on the Nucleus of the Solitary Tract (NTS) in the brainstem, a key breathing control center. Removing DEPDC5 from this region caused breathing irregularities even without seizures, proving DEPDC5's critical role in breathing stability.
Key Findings: Breathing problems appear before seizures and SUDEP in DEPDC5-deficient mice. These mice required lower doses of seizure-inducing drugs to trigger SUDEP. Removing DEPDC5 from the brainstem disrupted breathing but did not trigger seizures.
Why This Matters: Monitoring breathing patterns in epilepsy patients may help predict and prevent SUDEP. Understanding how DEPDC5 affects breathing could lead to better treatments, reducing SUDEP risk.
Conclusion: This study connects breathing and seizures, emphasizing the brainstem's role in epilepsy. Future research aims to develop therapies that stabilize breathing and prevent SUDEP.

**Keyword 1**: Epilepsy
**Keyword 2**: Breathing
**Keyword 3**: SUDEP

**ACCUMULATION OF THE PATHOLOGICAL TAR DNA-BINDING PROTEIN 43 IN THE SPINAL CORD IS EVIDENT AFTER REPETITIVE MILD BUT NOT SINGLE MODERATE TRAUMATIC BRAIN INJURY**

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Traumatic brain injury (TBI) is a leading cause of mortality and long-term disability, posing a significant public health challenge. While TBI primarily causes direct damage to brain tissue, secondary injury processes can also impact distant regions of the central nervous system. Repetitive brain injuries are strongly associated with chronic traumatic encephalopathy (CTE) and amyotrophic lateral sclerosis (ALS), whereas a single TBI is more commonly linked to Alzheimer's disease (AD). The pathological accumulation of TAR DNA-binding protein 43 (TDP-43) is a hallmark feature of both CTE and ALS, while in AD, it represents a secondary pathological event. This experimental study in mice aimed to determine whether single moderate or repetitive mild TBI (rmTBI) induces alterations in TDP-43 localization, fragmentation, and phosphorylation at acute and subacute time points, in the spinal cord. A single moderate TBI was induced over the left parietal cortex using the lateral fluid percussion injury (LFPI) model, while rmTBI was administered using a weight-drop apparatus, delivering two mild impacts per day over five consecutive days. Sham-injured mice served as controls for each injury model. Spinal cord samples were collected at 1-, 3-, and 14-days post-injury for western blot and immunohistochemical analysis. In the cervical spinal cord, rmTBI led to acute phosphorylated TDP-43 formation, followed by pronounced subacute cytoplasmic translocation and the accumulation of toxic phosphorylated TDP-43 species—changes absent in single moderate TBI. This research was supported by the University of Rijeka, Croatia (uniri-biomed-18-199 and uniri-mladi-biomed-23-38).

**Keyword 1**: traumatic brain injuries
**Keyword 2**: TDP-43 proteinopathies
**Keyword 3**: spinal cord

**THE ROLE OF ASTROGLIA-NEURON INTERACTIONS IN NETWORK HYPEREXCITABILITY AND SEIZURES**

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Astroglial cells play a pivotal role in multiple brain processes. These range from maintaining metabolic homeostasis to regulating key aspects of brain development, fostering connectivity and computations within neural circuits. Accumulating evidence indicates that aberrant astroglial functioning contributes to the pathophysiology observed across diverse forms of epilepsy. Previously we demonstrated distinct calcium dynamics in neurons and astroglia transitioning from pre-ictal to ictal activity and upon photic stimulation in hyperexcitable networks. In pre-ictal periods neurons exhibited local synchrony, whereas astroglia were highly active with global synchrony. Generalized seizures, however, were marked by release of astroglial glutamate as well as a drastic increase of astroglial and neuronal activity and synchrony across the brain. Knocking out astroglial glutamate transporters led to recurrent spontaneous seizures, accompanied by massive astroglial glutamate release, overall resembling a neonatal form of epileptic encephalopathy. Currently, we are using a combination of genetic and pharmacological approaches to perturb astroglial calcium and glutamate signalling and glia-neuron interactions to further investigate their role in generation and spread of epileptic seizures. To achieve this, we utilize functional dual channel recording in zebrafish expressing neuronal and astroglial calcium indicators, as well as glutamate sensors in controls and seizure prone animals. Our results demonstrate altered astroglia-neuron interactions with changes in the spatiotemporal dynamics of astroglial calcium signals as brain excitability increases. Our perturbations of astroglial glutamate transporters alter these dynamics and the animals' seizure susceptibility, suggesting astroglia as potential targets in the development of novel therapeutical approaches.

**Keyword 1**: Excitability
**Keyword 2**: Seizures
**Keyword 3**: Neuron-glia interactions
**Keyword 4**: Glutamate
**Keyword 5**: Seizure therapeutics

**THE ROLE OF CAV1.2 IN ASTROCYTES DURING EPILEPTOGENESIS**

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L-type Cav1.2 calcium channels play essential roles in central nervous system function and various physiological processes. In the brain, Cav1.2 is expressed in both glutamatergic and GABAergic neurons, localizing to dendrites, spines, and somas postsynaptically. Additionally, Cav1.2 is present in astrocytes, where it contributes to astrocyte activation and astrogliosis. Given that astrocyte activation and glial scar formation are key features of epilepsy, astrocytic Cav1.2 may play a critical role in epileptogenesis. However, research on Cav1.2 in epilepsy, particularly in astrocytes, remains limited.
Using a kainic acid-induced mouse model of mesial temporal lobe epilepsy, we observed a significant increase in Cav1.2 expression in astrocytes within the CA1 region 21 days after kainic acid administration. Moreover, Cav1.2 was rapidly upregulated in hippocampal astrocytes during epileptogenesis, suggesting its potential involvement in seizure development. To further explore the functional contribution of astrocytic Cav1.2 to epileptogenesis, we applied nisoldipine, a specific Cav1.2 inhibitor, to block its activity in CA1 and assessed the resulting epileptic changes. As a result, epileptic activity was significantly suppressed following nisoldipine treatment, indicating that astrocytic Cav1.2 contributes to seizure generation and progression.
This study demonstrates that astrocytic Cav1.2 calcium channels are significantly upregulated during epileptogenesis and actively participate in seizure development. Our findings provide new insights into the role of astrocytes in epilepsy and suggest that Cav1.2 may serve as a potential therapeutic target for epilepsy treatment.

**Keyword 1**: Astrocytes
**Keyword 2**: Epilepsy

**MICRO-COIL NEUROMODULATION FOR SUPPRESSING SEIZURES**

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Neural interfaces (NIs) enable therapeutic modulation of dysfunctional neural networks through targeted stimulation [1,2]. We developed a MEMS micro-coil-based NI system delivering micromagnetic stimulation (μMS) to mouse visual cortex in vivo [3], achieving localized neuronal suppression [4]. This study evaluates μMS efficacy in suppressing pathological hyperactivity during seizures.

Using two-photon microscopy and wide-field imaging, we compared μMS with micro-electrode stimulation (μES). μMS elicited stronger, faster somatic responses than μES-induced activation (Fig.1a). Dual-probe μMS/μES experiments demonstrated complete neutralization of μES-driven excitation (Fig.1b-d).

To test pathological suppression, we pharmacologically induced seizures via high-concentration bicuculline application in V1 cortex, generating neural bursts every 5-20s. μMS application prior to activity bursts reduced response magnitude, particularly near micro-coils (Fig.1e). Temporal analysis revealed μMS effectiveness depended on stimulation timing relative to burst onset. Spatial mapping showed suppression gradients extending 200-300μm from coil tips.

These findings demonstrate μMS's capacity to counteract both electrically induced and pathological neural hyperactivity, highlighting its potential for clinical translation in seizure management through precise spatiotemporal neuromodulation.

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**Keyword 1**: Neurointerfaces
**Keyword 2**: Micromagnetic
**Keyword 3**: Suppression
**Keyword 4**: MEMS
**Keyword 5**: TPM

**THE MITO-NUCLEAR CROSSTALK IN THE FATE OF AXOTOMIZED NEURONS AFTER SPINAL CORD INJURY**

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Spinal cord injury (SCI) is a debilitating condition characterized by permanent motor, sensory, and autonomic dysfunction. The primary insult entails destructive secondary events in remote brain areas which are functionally connected to the primary injury site, including inflammation, excitotoxicity and oxidative stress. In this scenario, the higher energy demand to respond to injury is covered by mitochondria, which regulate metabolites production, redox signalling, and, as recently described, the epigenetic status of the cells.
By using an experimental SCI model in mice, we disentangled the mito-nuclear crosstalk in axotomized neurons, and identified the key molecular players involved in these processes as well as their functional role, both in the acute and in the chronic phase of the injury.
By multi-omical and biochemical analysis on the red nucleus (RN) contralateral to injury we showed that the downregulation of the histone demethylase KDM5A, which plays a fundamental role in mitochondria biogenesis and function, and increased mitochondrial metabolism are key events in the early neuronal response to remote damage possibly for the activation of a pro-survival response. Furthermore, we observed that mitochondrial metabolites that significantly regulate KDM5A are modulated after SCI both 7- and 28-days post injury.
Dissecting these mechanisms will not only contribute to the understanding of the molecular and cellular mechanisms regulating neuronal survival and regeneration, a hot topic of regenerative medicine, but will be also crucial to circumventing the potential challenge of treatment to a broad spectrum of diseases.

**Keyword 1**: Spinal cord injury
**Keyword 2**: Mitocondria
**Keyword 3**: Epigenetics

**TRANSPLANTATION OF BRAIN ORGANOIDS FOR STROKE REPAIR IN MICE**

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Stroke is one of the leading causes of adult death, disability, and dementia. A challenge in improving stroke recovery is the limited regenerative capacity of neurons. Brain organoids, 3D self-organizing and self-renewing stem cell cultures, are promising tools for developing regenerative therapies. However, the lack of chemical signals and other supportive factors from adjacent cells restricts the long-term survival and maturation of these organoids in vitro. We suggest that intracerebral transplantation of organoids provides essential environmental cues that enhance their survival, maturation, and integration into the host, thereby aiding in brain injury repair. To investigate this, we implanted brain organoids into mice after ischemic stroke. Stroke was induced in adult non-obese diabetic severe combined immunodeficient mice using the photothrombosis method. The mice exhibited motor deficits in the grid-walk test. Triphenyl tetrazolium chloride (which marks viable tissue) staining, Nissl staining, and Evans blue leakage confirmed extensive brain damage. Seven days post-stroke, we performed a craniotomy over the damaged area to transplant a 50-day-old human brain organoid before staining the brains for various human cell types, host immune responses, and scar tissue formation. The transplanted organoids survived for up to three weeks with residual human proliferating and progenitor cells, significant microglial infiltration, and scar tissue formation around the graft. Besides, the mice experienced spontaneous sensorimotor recovery, which was not attributed to the transplantation. We observed significant atrophy of the transplanted organoid four weeks after transplantation. Thus, substantial challenges must be overcome for brain organoid transplantation to be feasible in improving stroke outcomes.

**Keyword 1**: Organoids
**Keyword 2**: Stroke
**Keyword 3**: Photothrombosis
**Keyword 4**: Transplantation

**PERIRHINAL CORTEX REGULATES HIPPOCAMPAL SEIZURES VIA EXTENSIVE DOWNSTREAM CIRCUITS**

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Temporal lobe epilepsy (TLE) is a neurological disorder characterized by an imbalance between excitation and inhibition, affecting various structures within the temporal lobe. Among these, the fasciola cinerea (FC), a previously overlooked region in the hippocampus, has recently emerged as a critical node and potential target for intervention in TLE. In this study, we identified the perirhinal cortex (PRh) in the mesial temporal lobe as the primary excitatory input to FC and investigated how PRh excitatory neurons and their projections contribute to the progression and recurrence of hippocampal seizures. Our findings revealed that PRh excitatory neurons and their projections to multiple brain regions were highly activated during seizures. Inhibition of PRh-FC, ILA, or LEnt projections slowed the progression of seizures and alleviated seizure severity. Notably, inhibition of PRh excitatory neurons suppressed the activity of several downstream regions through these extensive projections, leading to a more pronounced anti-seizure effect. These results highlight PRh as a potential target for effective seizure control in TLE.

**Keyword 1**: temporal lobe epilepsy
**Keyword 2**: perirhinal cortex
**Keyword 3**: fasciola cinerea

**AN INNOVATIVE APPROACH TO REPAIR BRAIN INJURIES: CELL-BASED THERAPY WITH 3D HYDROGEL**

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Intrinsic properties of enteric glial cells (EGCs), make them interesting candidates for brain repair. We have previously shown that intranasal delivery of human EGCs (hEGCs) is safe and can regenerate injured brain tissue (Colitti et al., 2024). Following this, we propose an innovative approach combining the biocompatible GalC9 hydrogel with hEGCs to test whether the use of a scaffold can implement the effect of hEGCs on tissue regeneration and functional recovery after brain injury. Brain injury was induced by malonate injection into the motor cortex of female rats. Two weeks later, $1*10^6$ hEGCs were delivered intranasally (n=21), followed by the intracerebral implantation of the GalC9 hydrogel a month later (n=14). Magnetic resonance imaging (MRI) is carried out to longitudinally and non-invasively follow hydrogel evolution and brain tissue evolution over time. In parallel, sensorimotor and cognitive tests are conducted to evaluate the effect of the therapy on functional recovery and memory. The first findings of the study showed that injured rats treated with hEGCs combined with hydrogel or with hydrogel alone had 14% and 16% functional recovery between 1 and 3 months, respectively, compared with only 8% for rats receiving no therapy. Moreover, our injury model generates an impairment in recognition memory while spatial memory remained unaffected. MRI analyses revealed that the GalC9 hydrogel remained present one month after implantation. This work explores GalC9's potential as a support for hEGCs to enhance their regenerative capacity in brain injury treatment, offering a potentially more effective strategy than existing approaches.

**Keyword 1**: Brain injury
**Keyword 2**: Cell therapy
**Keyword 3**: 3D hydrogel
**Keyword 4**: Neuroregeneration

**DEVELOPMENT AND PRECLINICAL EVALUATION OF A 3D BIODEGRADABLE NEUROIMPLANT FOR ENHANCING POST-STROKE BRAIN REGENERATION: INVESTIGATING CELLULAR INTEGRATION, INFLAMMATORY MODULATION, AND FUNCTIONAL RECOVERY**

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Ischemic stroke results from the obstruction of a cerebral artery, often the middle cerebral artery (MCA), leading to cell death and inflammation in sensorimotor regions causing major functional deficits. In response, the brain tries to reestablish connections through neuroplasticity, but neuronal cell replacement remains limited to the periphery and non-existant at the heart of the lesion. The development of biomimetic neuroimplants could enhance tissue regeneration. This study builds on preliminary research that evaluated the biocompatibility of seven different biomaterials: polyethylene glycol gelatin methacryloyl (PEGDA200/GelMA), polyethylene glycol diacrylate (PEGDA 200), PEGDA 500, gelatin methacryloyl (GelMA), polycaprolactone (PCL), poly(trimethylene carbonate) (PTMC), and polypropylene fumarate (PPF). Among these materials, the PEGDA200/GelMA blend (Cellink), manufactured using photopolymerization-based 3D printing, emerged as a promising candidate for a biodegradable neuroimplant.After cortical lesion induction in the sensorimotor area, we performed behavioral assessments and MRI analyses, along with histological examinations three months post-lesion. Motor tests showed significant functional recovery in implanted rats. MRI and histological analysis confirmed the integration, the presence of cellular migration pathways toward the lesion site and colonization of inside the implants. Immunofluorescence studies further revealed a modulation of the inflammatory response towards the M2 phenotype (CD206+ cells), fostering a pro-regenerative environment. These findings validate the biocompatibility of this implant and open up new perspectives for the development of therapeutic neuroimplants aimed at long-term brain regeneration, with potential applications for stroke and brain injury recovery

**Keyword 1**: Tissue bioengineering
**Keyword 2**: Brain injury
**Keyword 3**: Inflammation
**Keyword 4**: Astrocyte
**Keyword 5**: Central nervous system

**BRIDGING THE GAP TO CLINICAL REALITY: EFFICACY OF NOVEL EXPERIMENTAL THERAPIES IN AGED RATS FOLLOWING ISCHEMIC STROKE**

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Stroke is the second leading cause of death and disability worldwide and predominantly affects older populations. However, preclinical studies of neuroprotective interventions often rely on young animal models, which may limit the translatability of findings. In a rat model of middle cerebral artery occlusion (MCAO), we tested two experimental neuroprotective strategies in elderly rats (12 months old): remote ischemic post-conditioning (RIPC) and the post-stroke administration of blood cell secretome derived from young, healthy individuals (Sec). Our investigation encompassed infarct size, neurological deficit, and several key indicators of post-ischemic damage and oxidative stress. These included circulating glutamate, glutathione (a crucial cellular antioxidant), and lymphocyte DNA damage assessed via the Comet assay, reflecting the impact of ischemia-generated free radicals and reactive oxygen species. Our results revealed significant changes in the impact of ischemia and the effectiveness of the above-mentioned treatments compared to our experience in young animals. While Sec therapy significantly reduced infarct size ($p=0.017$ vs. MCAO) and lowered blood glutamate levels ($p=0.0079$ vs. MCAO), RIPC treatment failed to reduce infarct volume, modulate blood glutamate, or restore the oxidative balance. Although Sec therapy did not prevent lymphocyte DNA damage ($p=0.385$ vs. MCAO), it effectively restored glutathione levels to control values ($p=0.012$ vs. MCAO), suggesting a role in antioxidant defense. Age significantly influences the efficacy of neuroprotective therapies, impacting their clinical translation. Meanwhile, young individuals are emerging as a promising source for secretomes that induce neuroprotection in older subjects.
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**Keyword 1**: ischemic tolerance
**Keyword 2**: remote ischemic post-conditioning
**Keyword 3**: blood cell-derived secretome

**DIFFERENTIAL EFFECTS OF SINGLE MODERATE AND REPETITIVE MILD TRAUMATIC BRAIN INJURY ON SPINAL CORD CHOLINERGIC FUNCTION**

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Traumatic brain injury (TBI) is a major public health concern, leading to both immediate and long-term neurological consequences. While the primary injury is irreversible, secondary injury mechanisms can progress over time, affecting regions of the central nervous system beyond the initial trauma site. Notably, TBI has been linked to neurodegenerative diseases such as chronic traumatic encephalopathy (CTE) and amyotrophic lateral sclerosis (ALS). This study aims to investigate the cumulative pathological effects of single moderate versus repetitive mild TBI (rmTBI) on the spinal cord by analyzing changes in choline acetyltransferase (ChAT) expression, a key enzyme in cholinergic neuron function. A single moderate TBI was induced over the left parietal cortex using the lateral fluid percussion injury (LFPI) model. In a separate cohort, repetitive mild TBI was administered using a weight-drop apparatus, delivering two mild impacts per day over five consecutive days. Sham-injured mice served as controls for both injury models. Spinal cord samples were collected at 14 days post-injury for western blot and immunohistochemical analysis. Western blot and immunofluorescence analyses showed no significant difference in ChAT expression in the cervical spinal cord post-LFPI compared to controls. However, in rmTBI mice, western blot revealed a significant increase in ChAT protein levels, which was further confirmed by immunofluorescence. This upregulation suggests a compensatory response in cholinergic neurons following repetitive mild TBI, potentially indicating early neurodegenerative changes. In contrast, single moderate TBI did not alter ChAT expression, highlighting the distinct impact of repetitive injuries on spinal cord cholinergic function.

**Keyword 1**: Traumatic Brain Injury
**Keyword 2**: Spinal Cord
**Keyword 3**: Choline Acetyltransferase
**Keyword 4**: Neurodegenerative Diseases

**THE NEUROPROTECTIVE EFFECT OF THE CANNABINOID URB447 AFTER INFECTION-SENSITIZED NEONATAL BRAIN INJURY IS MEDIATED BY REDUCED GLIAL ACTIVATION AND DECREASED INFLAMMATION**

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**Aims:** Perinatal asphyxia in the newborn can lead to severe brain damage, often exacerbated by inflammation. Recent studies suggest that cannabinoids may offer neuroprotection by reducing inflammation, modulating immune responses and mitigating cell death in neonatal brain injury models. We wanted to explore the capacity of URB447, a CB1-antagonist/CB2-agonist, on decreasing glial activation and inflammation after infection-sensitized neonatal brain injury.
**Methods:** 7-day-old neonatal rats were exposed to 0.1 mg/kg lipopolysaccharide (LPS) injection to induce systemic inflammation 4h prior to unilateral hypoxia-ischemia (HI). Half of the animals received 1 mg/kg of URB447 i.p. 2 hours after HI. Pups were sacrificed seven days later to immunohistochemically evaluate astroglial (GFAP) and microglial (Iba-1) activation and BBB permeability (IgG). In addition, RAW 264.7 cells were cultured in the presence of LPS and treated with URB447 to analyze the cannabinoid anti-inflammatory potential.
**Results:** The ipsilateral hemisphere of non-treated LPS-HI animals showed relevant infarction, strong inmunohistochemical pattern for GFAP and Iba-1 and presence of IgG. URB447 reduced brain injury ($p<0.05$), hippocampal astrogliosis ($p<0.05$), cortical and hippocampal microglial activation ($p<0.01$) and IgG immunoreactivity ($p<0.05$). In cell cultures, URB447 decreased LPS-induced NF-kB activation ($p<0.01$) and inducible nitric oxide synthase expression ($p<0.01$).
**Conclusions:** Systemic inflammation intensifies neuronal damage in the neonatal rat brain. Treatment with the cannabinoid URB447 reduced brain damage and inflammation, ameliorated glial activation and maintained BBB integrity.
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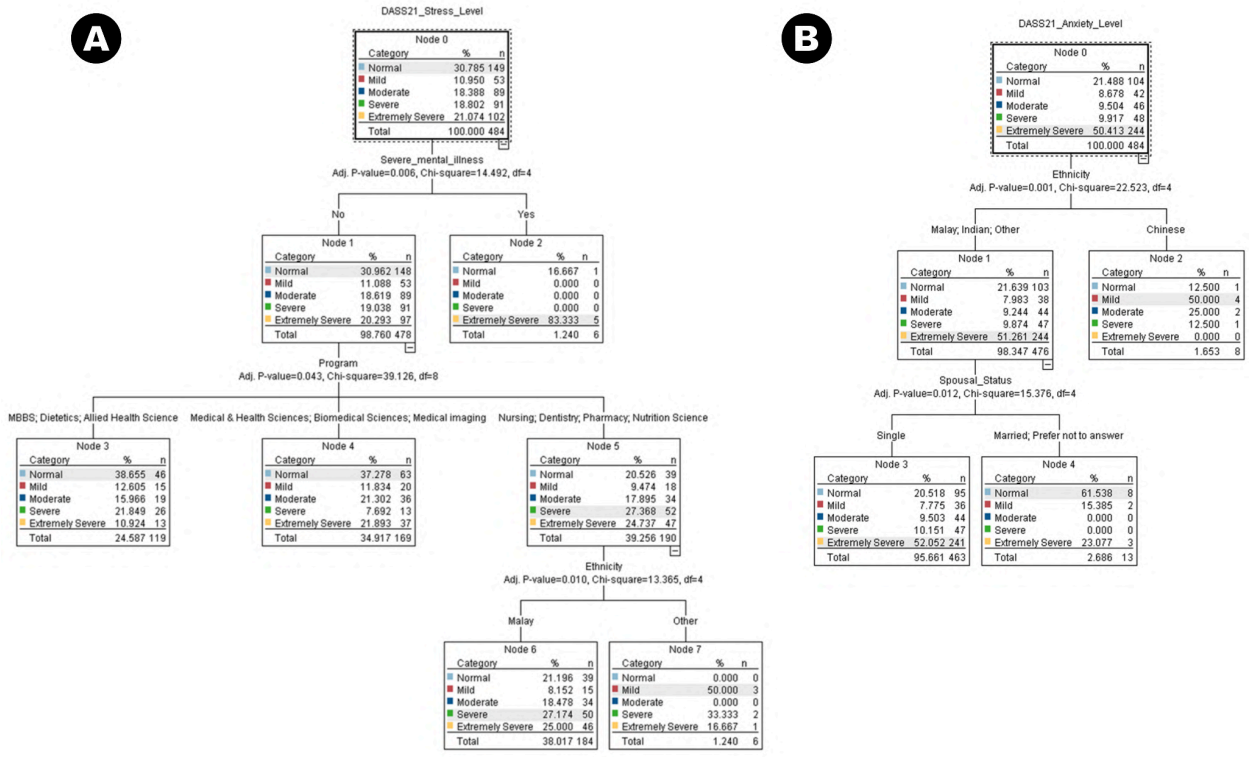
**Keyword 1**: neonatal brain injury
**Keyword 2**: infection-sensitized hypoxia-ischemia
**Keyword 3**: cannabinoid
**Keyword 4**: neuroprotection

**INTERPLAY OF SOCIO-DEMOGRAPHIC FACTORS AND CARDIO-CEREBROVASCULAR RISK IN UNIVERSITY STUDENTS' MENTAL HEALTH**

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The intricate relationship between mental health and cardio-cerebrovascular disease (CCVD) is well recognised, yet the influence of socio-demographic factors remains inadequately explored. This study examines how depression, anxiety, and stress correlate with socio-demographic characteristics and CCVD risk among undergraduate students. A cross-sectional analysis was conducted among 484 students enrolled in medical and health sciences programs at universities on Malaysia's east coast. Mental health was evaluated using the Depression, Anxiety, and Stress Scale (DASS-21), while CCVD risk factors were assessed through the QRISK3 platform. Socio-demographic and clinical data were also collected to identify key determinants of mental health outcomes and CCVD vulnerability. Findings indicate a substantial prevalence of severe anxiety (50.4%), depression (29.3%), and stress (21.1%) within the student population. Significant associations emerged between these psychological conditions and socio-demographic variables such as ethnicity, religion, and marital status. Notably, Malay and Muslim students demonstrated a marked link between religious affiliation and heightened anxiety and stress. Moreover, academic programs and a history of severe mental illness were significant predictors of anxiety and stress levels. These insights emphasise the necessity for holistic health strategies in university settings, integrating mental health support with CCVD risk mitigation. By pinpointing high-risk groups, targeted interventions can be designed to prevent long-term health repercussions. This study highlights the importance of culturally responsive and comprehensive student health programs to foster both mental and physical well-being.

**Figure:** Decision tree analysis (DTA) for the association and predictor importance between DASS-21 (A) stress and (B) anxiety level with socio-demographic and clinical proforma.




**Keyword 1**: Mental health
**Keyword 2**: Cardio-cerebrovascular disease
**Keyword 3**: DASS-21
**Keyword 4**: Risk factors
**Keyword 5**: University students

**MARRIAGE AND DEPRESSIVE SYMPTOMS: THE ROLE OF SEX, AGE AND SOCIOECONOMIC STATUS**

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Marriage rates are declining globally, particularly in East Asia, where cultural and socioeconomic shifts are reshaping traditional family structures. Existing research suggests that unmarried individuals may be at greater risk for depressive symptoms, yet national-level studies focusing on young adults are limited. This study examines the relationship between marital status and depressive symptoms among young adults in South Korea and explores the factors contributing their mental health. This study utilized data from the Korean National Health and Nutrition Examination Survey(KNHANES) spanning 2014 to 2022, analyzing a nationally representative sample of 5,628 adults aged 25-39. Depressive symptoms were assessed using the Patient Health Questionnaire-9(PHQ-9), with a score of ≥5 indicating depression. Logistic regression analysis was performed to explore factors influencing depression. Subgroup analyses explored variations across age, income, occupation, and lifestyle behaviors. Unmarried individuals had significantly higher odds of depressive symptoms compared to their married counterparts(men: OR=1.84, 95% CI: 1.48–2.31; women: OR=1.34, 95% CI: 1.11–1.61). The association was strongest among men aged 30–34 and women aged 35–39. Income-related patterns differed by gender, with lower-income unmarried men and higher-income unmarried women exhibiting the highest risk. White-collar workers and urban residents experienced heightened vulnerability. Lifestyle factors such as alcohol consumption and physical inactivity further influenced the relationship between singlehood and depression. Singlehood is associated with an increased risk of depressive symptoms, with effects varying across demographic and socioeconomic groups. These findings underscore the need for targeted mental health interventions addressing the psychological challenges faced by unmarried individuals.

**Keyword 1**: Depressive symptoms
**Keyword 2**: Marriage

# Neural Circuits and Behavior

**IMPACT OF REPETITIVE TRANSCRANIAL MAGNETIC STIMULATION ON CLINICAL AND COGNITIVE OUTCOMES, AND BRAIN-DERIVED NEUROTROPHIC FACTOR (BDNF) LEVELS IN TREATMENT-RESISTANT DEPRESSION**

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Treatment-resistant depression (TRD) affects approximately 30% of patients with major depressive disorder (MDD), posing a significant clinical challenge due to limited therapeutic options. Repetitive transcranial magnetic stimulation (rTMS) has demonstrated efficacy in reducing depressive symptoms, yet its impact on cognitive function and biological markers, such as brain-derived neurotrophic factor (BDNF), remains unclear. This study investigates the effects of rTMS on depression severity, cognition, and serum BDNF levels, while exploring the moderating role of lifetime suicide attempts (LSA) on cognitive performance and the predictive value of baseline BDNF for clinical response. Twenty-five TRD patients were assessed, with 13 receiving rTMS (20 sessions over four weeks) and 12 serving as controls. Depression severity (HAM-D, CGI), cognitive function (MMSE, Verbal Fluency, Digit Span), and serum BDNF levels were measured pre- and post-treatment. Mixed-effects linear regression models evaluated clinical and biological associations. rTMS significantly reduced HAM-D ($p < 0.001$) and CGI ($p < 0.001$) scores, while cognitive improvements were observed in MMSE ($p = 0.049$) and Digit Span ($p = 0.04$). However, BDNF levels remained unchanged ($p = 0.39$), LSA did not moderate cognitive outcomes, and baseline BDNF did not predict symptom improvement ($p = 0.68$). These findings suggest that rTMS effectively alleviates depressive symptoms in TRD patients with modest cognitive benefits, yet neuroplasticity mechanisms, as reflected by BDNF dynamics, require further investigation. Larger-scale studies with refined biomarker assessments are needed to elucidate the neurobiological underpinnings of rTMS response.

**Keyword 1**: Treatment-resistant Depression
**Keyword 2**: rTMS
**Keyword 3**: BDNF
**Keyword 4**: Cognitive Performance

**ACUTE AND CHRONIC CHEMOGENETIC VHPC MODULATION: NEUROCHEMICAL AND BEHAVIORAL INSIGHTS INTO SCHIZOPHRENIA –ASSOCIATED ALTERATIONS**

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Schizophrenia is a complex psychiatric disorder characterized by a range of symptoms, including hallucinations, delusions, diminished drive, and cognitive impairments. One of the key factors in the pathophysiology of schizophrenia is neurotransmitter dysfunction. Abnormalities in neurotransmitter systems, particularly dopamine, glutamate, and serotonin, have been implicated in the development and progression of the disorder. Dysregulation of these neurotransmitters can lead to the characteristic symptoms of schizophrenia by affecting neural circuits and brain regions involved in cognition and perception. Animal models (e.g., gestational treatment with MAM or sub chronic treatment with NMDA receptor antagonists) not only reveal schizophrenia-related symptomatology but also point to the ventral hippocampus (vHPC) to play a significant role in this dysregulation. We used inhibitory and excitatory chemogenetic approaches to demonstrate a causal relationship between local changes in neuronal activity within the vHPC and changes in anxiety-like behavior, sociability and amphetamine-induced hyperlocomotion. The protocol includes animal cohorts of acute and chronic chemogenetic manipulation through CNO (Clozapine-N-Oxide) administration either via systemic injection or via drinking water respectively. Acute modulation provides insight in the dynamic influence of vHPC on the underlying neuronal circuits; chronic manipulation contributes to the interpretation of prolonged alterations to behavioral and cognitive findings over time, aligning with clinical data on early prodromal symptoms and progressive cognitive disruptions observed in medicated patients. Subsequently, we set behavioral changes into relationship to neurochemical signatures within amygdala, medial prefrontal cortex, and nucleus accumbens, assessed postmortem.

**Keyword 1**: schizophrenia
**Keyword 2**: vHPC
**Keyword 3**: chemogenetic modulation

**HIGH RESOLUTION, TWO-PHOTON, ACOUSTO-OPTICS BASED SIMULTANEOUS IMAGING AND OPTOGENETICS THROUGH ALL CORTICAL LAYERS FROM UP TO 300 CELLS**

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Investigating the correlation between network dynamics and behaviour using an "all-optical" method is one of the hot topics in neuroscience. This is achieved by using two-photon acousto-optic (AO) stimulation technique, as it allows finer spatial resolution of photo-stimulation, compared to holographic or one-photon solution when measuring large neuronal networks. Our utilization of AO scanning enabled **3D photo-stimulation of a volume measuring 500 × 500 × 500 µm3**, successfully demonstrates that we can efficiently **stimulate and measure up to 300 cells concurrently**, while preserving subcellular spatial resolution to prevent inadvertent off-target stimulation. We found that the duration of the neuronal response relies on both the power of the laser and the length of the photo-stimulation, even at depth of 700 µm from the brain surface.

**Keyword 1**: photostimulation
**Keyword 2**: all-optical

**NOVEL TRANSGENIC ZEBRAFISH LINES TO STUDY THE CHRNA3-B4-A5 GENE CLUSTER**

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Acetylcholine (ACh), a vital neurotransmitter for both the peripheral and central nervous system, signal through nicotinic acetylcholine receptors (nAChRs) and muscarinic acetylcholine receptors (mAChR). Here, we explore the expression patterns of three nAChR subunits, *chrna3*, *chrnb4*, and *chrna5*, which are located in an evolutionary conserved cluster. This close genomic positioning, in a range of vertebrates, may indicate co-functionality and/or co-expression. Through novel transgenic zebrafish lines, we observe widespread expression within both the peripheral nervous system and central nervous system. In the PNS, we observed expression of *chrna3*$^{tdTomato}$, *chrnb4*$^{eGFP}$, and *chrna5*$^{tdTomato}$ in the intestinal enteric nervous system; *chrna5*$^{tdTomato}$ and *chrnb4*$^{eGFP}$ in sensory ganglia of the lateral line; and *chrnb4*$^{eGFP}$ in the ear. In the CNS, expression of *chrnb4*$^{eGFP}$ and *chrna5*$^{tdTomato}$ was found in the retina, all three expressed in diverse regions of the brain, where a portion of *chrna3*$^{tdTomato}$ and *chrnb4*$^{eGFP}$ cells were found to be inhibitory efferent neurons projecting to the lateral line. Within the spinal cord, we identify distinct populations of *chrna3*$^{tdTomato}$, *chrnb4*$^{eGFP}$, and *chrna5*$^{tdTomato}$ expressing neurons within the locomotor network, including *dmrt3a*-expressing interneurons and *mnx1*-expressing motor neurons. Notably, 3-4 primary motor neurons per hemisegment were labelled by both *chrna3*$^{tdTomato}$ and *chrnb4*$^{eGFP}$. Interestingly, we identified a sl-type secondary motor neuron per hemisegement that strongly expressed *chrna5*$^{tdTomato}$ and co-expressed *chrnb4*$^{eGFP}$. These transgenic lines provide insights into the potential roles of nAChRs within the locomotor network and open avenues for exploring their role in nicotine exposure and addiction in a range of tissues throughout the nervous system.

**IMPACT OF PERINATAL STROKE ON MOTOR AND COGNITIVE FUNCTIONS IN A MOUSE MODEL**

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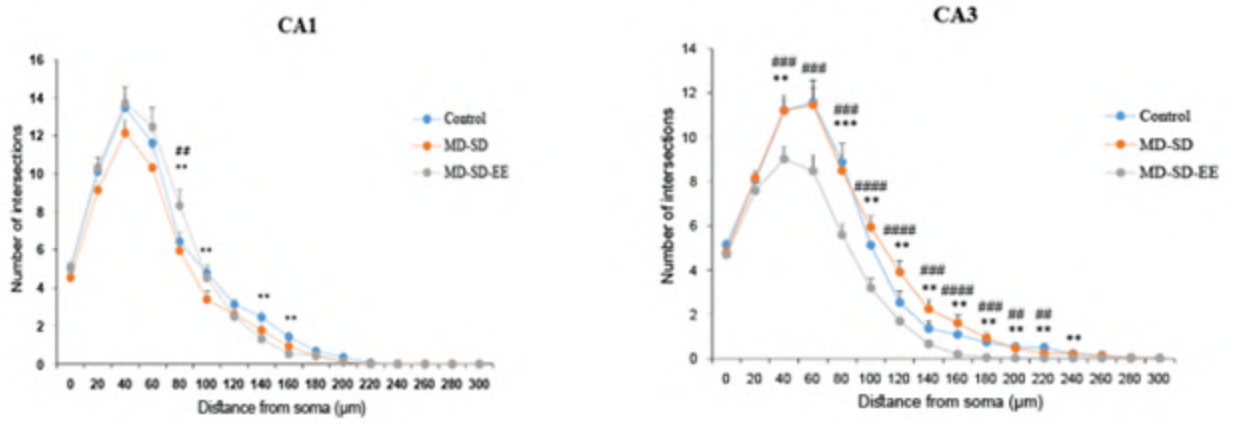
Adult cerebral ischemia (stroke) is a neurological injury caused by the occlusion of cerebral blood vessels and it is one of the leading causes of long-term disability. Notably, an ischemic stroke can occur also during brain development with the early perinatal period being characterized by critical period windows when experience provides essential information for the proper maturation of sensory, motor, and cognitive functions. Despite the large amount of evidence on adult stroke, very little it is still known about the impact of a stroke occurring during perinatal ages in the developing brain. To address this question, we performed the middle cerebral artery occlusion (MCAO, a mouse model of ischemic injury) at perinatal age (P14) in mice pups and investigated the impact on motor and cognitive outcomes employing behavioral tests (gridwalk test, rotarod test, grip strength test, Y-maze test) longitudinally at different days (D) after stroke induction till adulthood. Overall, we did not find any general motor impairment assessed using the rotarod and grip strength tests. The gridwalk test revealed motor deficits at D2, and D9 in stroke mice compared to sham (control) mice. To investigate the impact on cognition we used the Y-maze test to assess working memory and the forced Y-maze to assess spatial novelty. Stroke mice showed spatial novelty memory motor deficits at D37. Histological analysis revealed that the brain lesion volume is on average 3 mm at D79. Our findings revealed that perinatal stroke impacts on motor and cognitive functions later in life.

**Keyword 1**: Perinatal stroke
**Keyword 2**: motor impairments
**Keyword 3**: cognitive impairments
**Keyword 4**: Plasticity

**ENVIRONMENTAL ENRICHMENT PROTECTS AGAINST SOCIAL DEFEAT-INDUCED DEPRESSION AND MEMORY IMPAIRMENT IN MATERNALLY DEPRIVED RATS: RELEVANCE TO STRUCTURAL ALTERATIONS IN CA1 AND CA3 HIPPOCAMPAL REGIONS**

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Early life stress exerts long-lasting cognitive and emotional deficits, which are often linked to structural alterations in the associated brain regions. In this study, we investigated the effects of maternal deprivation (MD) and environmental enrichment (EE) on behavioral and structural outcomes in the hippocampus following social defeat stress (SD) in rats. MD pups were separated from their mothers for 3 hours/day from postnatal days (PND) 1–21 and later subjected to social defeat stress on PND 35–38. From PND 23 to PND 60, rats were housed in either standard (SE) or enriched (EE) cages. Behavioral assessments, including the forced swim test and eight-arm radial maze, were conducted between PND 61–99. Brains were collected on PND 100 for stereological analysis and Golgi-Cox staining to evaluate the structure of CA1 and CA3 hippocampal neurons. Social defeat stress induced depressive-like behavior and spatial memory impairments, while EE promoted resilience to these behavioral deficits. MD and SD stressors led to a reduction in CA1 and CA3 volumes, whereas EE counteracted these effects. Interestingly, EE had region-specific effects on dendritic morphology: it increased dendritic complexity in CA1 but reduced it in CA3. These findings suggest that environmental enrichment enhances resilience to stress-induced behavioral impairments and mitigates hippocampal structural alterations, highlighting its potential as an intervention for stress-related neuropsychiatric disorders.



**HISTAMINERGIC SYSTEM'S ROLE IN DEPRESSION**

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Depression is a chronic and recurrent neuropsychiatric disorder, in which patients often have persistent symptoms such as low mood and anhedonia, even accompanied by suicidal behavior. The pathogenesis of depression is extremely complex, and the effectiveness of existing antidepressant treatment is limited.Using a chronic restraint stress (CRS) paradigm to establish a mouse model of depression, we observed a significant upregulation of histamine H3 receptor expression in the basolateral amygdala (BLA) alongside. Additionally, we seek to elucidate the effects and underlying mechanisms of histamine and histamine receptors on neuronal activity in depression-related brain regions. This study will provide a comprehensive understanding of the histaminergic system's role in depression and identify potential therapeutic targets for modulating histamine signaling to alleviate depressive symptoms.

**Keyword 1**: depression
**Keyword 2**: histamine

**MAGNETIC MICRO-COIL STIMULATION FOR INHIBITING NATURAL NEUROACTIVITY**

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Intracortical micromagnetic stimulation (μMS) is an innovative approach to therapeutic neuromodulation that provides spatial precision, long-term functionality and improved biocompatibility[1]. While previous studies have focused on expanding μMS applications[2], its capacity to suppress spontaneous neural activity remains underexplored. Selectively suppressing abnormal neural activity is specifically beneficial for developing therapeutic interventions for conditions like epilepsy, where targeting hyperactive neurons is critical[3]. Here, we investigated whether μMS can consistently and effectively suppress spontaneous neural firing.

We developed a novel MEMS based micro-coils compatible with in-vivo two-photon microscopy (TPM)(Fig.1a-b)[4], to enable flexible fabrication processing and high-resolution imaging of cellular and subcellular responses in living brain tissue. The micro-coils were implanted in the visual cortex of transgenic mice carrying cell-type specific fluorescent indicators (neuronal calcium) to achieve visualization of stimulation effects.

Initially, wide-field imaging was used to capture macroscale neural activity. We discovered that μMS induces localized suppression around the micro-coil tip(Fig.1c,d), which was consistent among multiple stimulation trials, mice, and micro-coil devices. Furthermore, TPM measurements of single neuron responses revealed that most were suppressed (n=12/14) during μMS, with suppression magnitude depending on stimulation intensity(Fig.1e,f). Conversely, only a small proportion of neurons were excited by μMS. Furthermore, increasing the stimulation intensity did not affect the proportion of excited cells while it increased the number of cells being suppressed, emphasizing that the main effect of μMS is suppression[5*].

5*-Kim, Kayeon, et al.,“Micro-Coil Neuromodulation at Single-Cell and Circuit Levels for Inhibiting Natural Neuroactivity, Neutralizing Electric Neural Excitation and Suppressing Seizures.”,Advanced Science, under minor revision.

**Keyword 1**: Brain-computer interfaces
**Keyword 2**: Neural inhibition

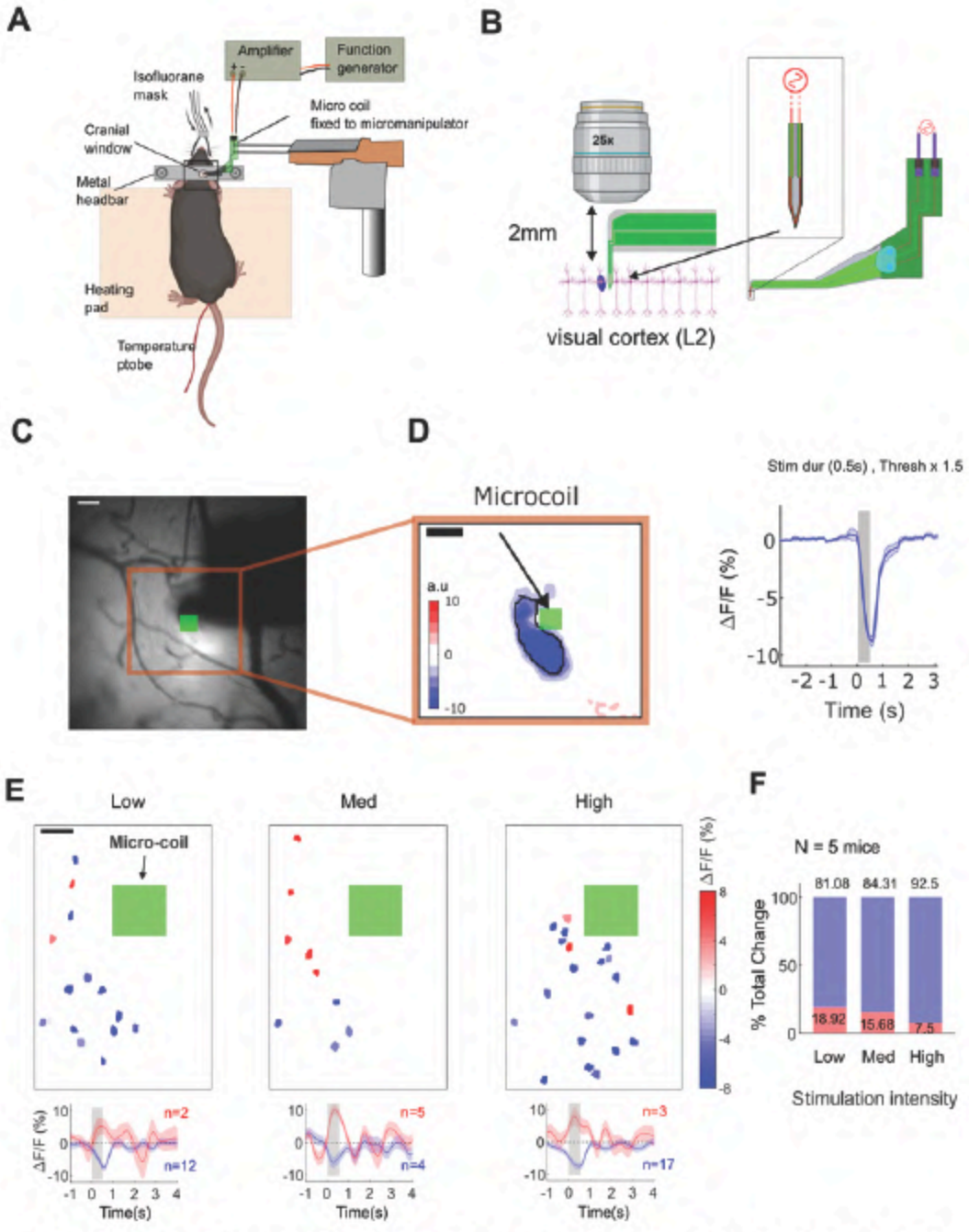



Figure 1. (A) Schematic illustration of the experimental setup, and (B) micro-coil insertion into the visual cortex layer 2 (L2) under the objective lens for two-photon imaging. (C) Wide field imaging the of micro-coil in the visual cortex under epifluorescence light, with the green square indicating the vertically inserted micro-coil, scale bar 80 µm. (D) Neuronal Ca2+ fluorescence (GCaMP8f) response to µMS to visualize cortical neuron activity in a larger area than 3 x 3 mm2 in and around V1 with high temporal resolution. Scale bar, 150 µm. Right panel: The response intensity time course, average 5 stimulation trials, shaded with ±1standard error mean (s.e.m.). (E) Measurement of somatic response amplitude in single neurons with TPM with low, medium, and high stimulation intensity (1s-post stimulation). Scale bar: 50µm. Green square indicates micro-coil cross-section. Lower panels: response traces averaged across excited (in red) and suppressed (in blue) cells, shaded with ±1s.e.m., gray line indicates stimulation duration. (F) % total change of excited (in red) and suppressed (in blue) cells against stimulation intensity.

**A NOVEL RHODOPSIN-BASED VOLTAGE INDICATOR FOR SIMULTANEOUS TWO PHOTON OPTICAL RECORDING WITH GCAMP IN VIVO**

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Genetically encoded voltage indicators (GEVIs) allow optical recording of membrane potential from targeted cells in vivo. Red-reporting GEVIs that are compatible with two photon microscopy and that can be multiplexed in vivo with green reporters like GCaMP, are currently lacking. To address this, we explored diverse rhodopsin proteins as GEVIs and engineered a novel GEVI, 2Photron-ST, based on a rhodopsin from the green algae *Klebsormidium nitens*. Using two photon ultrafast local volume excitation (ULoVE), 2Photron was compared, in several cell types, with a state-of-the-art green 2P GEVIs, i.e. JEDI2P-Kv, and a red 1P GEVI Voltron2-ST. We found 2Photron-ST enabled multiplexed recording of spiking and subthreshold voltages simultaneously with GCaMP6/8f calcium signals in visual cortical neurons of awake, behaving mice. These recordings revealed the cell-specific relationship of spiking and subthreshold voltage dynamics with GCaMP responses, highlighting the challenges of extracting underlying spike trains from calcium imaging.

**Keyword 1**: Voltage imaging
**Keyword 2**: GEVI
**Keyword 3**: multi-photon
**Keyword 4**: GCaMP

**EFFECTS OF CHEMOGENETIC INHIBITION OF THE ANTERIOR PARAVENTRICULAR NUCLEUS OF THE THALAMUS TO VENTRAL SUBICULUM PATHWAY ON CUE-INDUCED HEROIN SEEKING FOLLOWING FORCED ABSTINENCE IN RATS**

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Relapse poses a substantial obstacle to addiction treatment, and the risk for relapse is exacerbated by chronic food restriction. However, the underlying neuronal mechanisms are unknown. Our laboratory has demonstrated a role for the anterior paraventricular nucleus of the thalamus (aPVT) in cue-induced heroin seeking following forced abstinence in male and female rats. The aPVT preferentially projects to the ventral subiculum (v.sub), an important area for drug seeking. A role for aPVT to v.sub in relapse following forced abstinence has not yet been explored. Here, we chemogenetically inhibited the aPVT to v.sub pathway by infusing an inhibitoryDesigner Receptor Exclusively Activated by Designer Drug (DREADD) into the aPVT and deschloroclozapine (DCZ; 0.1 mM/.5ul) into v.sub via a bilateral guide cannulation to examine cue-induced heron seeking in food restricted and sated rats. Rats were trained to self-administer heroin (0.1mg/kg/infusion) for 10 days, followed by 16 days of forced abstinence. During forced abstinence, rats were either sated or food restricted. On day 15 of food restriction, v.sub was infused with DCZ or vehicle. Rats underwent a heroin-seeking test under extinction conditions. We expect that food-restricted rats will have greater heroin-seeking than the sated rats, and that inhibiting the aPVT to v.sub will reduce cue-induced heroin seeking in the food restricted rats.

**Keyword 1**: heroin-seeking
**Keyword 2**: food restriction

**KISSPEPTINS, KEY REGULATORS OF THE REPRODUCTIVE AXIS, MIGHT MODULATE AMYLOIDB INDUCED NEUROLOGIC DEFICIT IN FEMALE RATS**

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Previously, Kisspeptins, modulators of the reproductive system, have been shown to have a beneficial effect on learning and memory function and amyloidβ neurotoxicity. Thus, we aimed to investigate the acute effect of Kisspeptin-13, an endogenous derivative, on amyloidβ(1-42)-induced learning and memory deficit and the possible mechanism of its action in female rats.
First, intracerebroventricular cannula were implanted into adult female Wistar rats, through which amyloidβ(1-42) (4μg/4μl) was injected. After a recovery, Morris Water Maze test was performed to assess spatial memory. On the last day, Kisspeptin-13 (2μg/2μl) was administered. Afterwards, the relative gene expression of *Egr-1, Erk1/2, p38, Bdnf, Il-6, Casp2* and *Serca2b* was determined in hippocampal samples to assess how Kisspeptin-13 affects the amyloidβ-induced inflammation and apoptosis. In another part of the samples glutamate and acetylcholine release was measured via a superfusion system.
Our results showed that amyloidβ significantly decreased learning of female rats, which was antagonised by Kisspeptin-13. Kisspeptin-13, based on our preliminary results, also significantly blunted the amyloidβ-induced alterations of *Egr1, Erk1/2, p38*, and *Bdnf*, whereas in the case of *Il-6, Casp2* and *Serca2b* it had no effect. The superfusion study revealed that Kisspeptin-13 blocked the increased glutamate secretion caused by amyloidβ in the hippocampus, whereas it had no effect on acetylcholine release.
In conclusion, our results indicate that in female rats, Kisspeptin-13 might has a positive effect on amyloidβ induced neurotoxicity.
SZAOK-KKA-SZGYA: 2024.02.01-2026.01.31.; SZAOK-KKA-PKP: 2025.02.01-2027.01.31.; EFOP-3.6.2-16-2017-00006 (LIVE LONGER)

**KISSPEPTIN-8 REDUCES LOCOMOTOR ACTIVITY BY MODULATING THE EXPRESSION OF DOPAMINE RECEPTORS IN THE NUCLEUS ACCUMBENS AND STRIATUM IN RATS**

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Previously, kisspeptin-8 (Kp-8) reduced locomotor activity in the open field test and increased GABA release from the nucleus accumbens (NAc) in rats. We aimed to investigate the effects of icv. Kp-8 on spontaneous locomotion, and gene and protein expression in the dopaminergic systems of the ventral tegmental area (VTA), NAc and striatum.
Male Wistar rats were implanted with an icv. cannula and an E-mitter, then their spontaneous locomotor activity was recorded by telemetry, with daily icv. treatment (0.1; 0.5; 1 or 2 µg Kp-8 or 0.9% NaCl solution). The gene expression of *Fos, Drd1r, Drd2r* (dopamine receptor D1, D2), *Gad65* (glutamic acid decarboxylase 65kD), and *Th* (tyrosine hydroxylase) was determined in the VTA, NAc and striatum 2 hours after icv. treatment (0.1 or 1 µg Kp-8). The protein expression of D1 receptors 4 hours after icv. treatment was determined in the NAc and striatum by ELISA.
In the telemetry study, a dose-dependent reduction in locomotion was observed, the 1 and 2 µg doses induced significant hypolocomotion. 0.1 µg Kp-8 induced a downregulation of *Drd2r* in the NAc, while 1 µg Kp-8 reduced the gene and protein expression of D1 receptors in the NAc, and downregulated *Drd2r* in the NAc and striatum.
Kp-8 dose-dependently reduced spontaneous locomotion, possibly by inhibiting the VTA-NAc dopaminergic pathway through downregulation of D1 receptors and activation of GABAergic interneurons. The downregulation of *Drd2r* suggests that Kp-8 may also affect motivation and addiction, but further studies are needed to clarify this.
Funding: SZAOK-KKA-SZGYA: 2024.02.01-2026.01.31.; SZAOK-KKA-PKP: 2025.02.01-2027.01.31.; EFOP-3.6.2-16-2017-00006

**BEHAVIOURAL EFFECTS OF OBESTATIN IN THE FORCED SWIMMING TEST**

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Obestatin and ghrelin are two neuropeptides derived from the same pre-proghrelin precursor. Ghrelin is the endogenous ligand for the GHS-R1A (growth hormone secretagogue) receptor and plays a role in regulating appetite, energy homeostasis, depression, anxiety, and addiction. Obestatin, identified first as an antagonist to ghrelin's orexigenic effects, is now recognized as a regulator of glucose and fat metabolism. Its behavioural effects, however, are still largely unknown. Our previous research demonstrated that obestatin exerts both anxiogenic-like and depressive-like effects in CFLP mice.
In this study, we examined the impact of acute intracerebroventricular injections of obestatin on depression-like behaviour in C57BL/6 mice, using the Forced Swimming Test (FST), which is a widely utilized rodent model and assay.
Obestatin was administered in various doses (0.5, 1, and 1.5 µg in 2 µl of artificial cerebrospinal fluid [aCSF]). Before administering obestatin, other groups were pretreated with the corticotropin-releasing factor 1 (CRF1) receptor antagonist antalarmin (0.1 µg in 2 µl aCSF) or the ghrelin receptor antagonist [D-Lys3]-GHRP6 (1 µg in 2 µl aCSF).
Obestatin increased immobility during the FST, indicating depression-like behaviour. . However, this effect was reversed by pretreatment with both antalarmin and [D-Lys3]-GHRP6.
The current findings align with our previous results, indicating that the acute central obestatin injection can induce depression-like behaviour, regardless of the mouse strain used. In conclusion, obestatin is a pleiotropic peptide of the brain-gut axis. Both the hypothalamic-pituitary-adrenal axis and ghrelin signalling pathways likely play a role in its mechanism of action, warranting further in-depth research.
Funding: SZAOK-KKA-SZGYA: 2024.02.01-2026.01.31.

**STRUCTURAL CONNECTOME CHANGES AS BIOMARKERS OF STROKE RECOVERY: A LONGITUDINAL 7T MRI STUDY**

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Stroke remains the second leading cause of death worldwide and is a major contributor to long-term disability. From a neuroimaging perspective, stroke manifests as a network disorder, where the focal damage extends to distant brain regions through large-scale structural connections, disrupting global neural communication. Post-stroke recovery and the underlying mechanisms at the network-level recovery remain poorly understood. Longitudinal examination of the structural network reorganization following stroke can help identify the adaptive and maladaptive recovery processes. This study used 7T MRI to investigate structural connectome changes in five first-time ischemic stroke patients (4M, 1F, age range: 50-69) at critical timepoints associated with recovery: within a week of stroke onset, at one month and three months post-stroke. Structural T1 and T2-FLAIR images were used to quantify gray matter damage, white matter disconnections and direct and indirect neural path disconnections across the entire structural network. Preliminary findings demonstrated that reductions in gray matter parcel damage and canonical white matter tract disconnections were associated with improvements in clinical stroke severity scales and Fugl-Meyer motor assessment scores. Further analyses will assess the changes in the shortest structural path length among intermediary neural connections and the number of spared connections across the structural network, and their relationship to clinical stroke severity scales and motor assessments. These preliminary findings suggest that the longitudinal impact of a lesion on the neural network could serve as a potential biomarker for patient-specific adaptive and maladaptive recovery processes.

**Keyword 1**: stroke
**Keyword 2**: high-resolution MRI
**Keyword 3**: structural connectivity
**Keyword 4**: network analysis

**CALNEURON-1 IS AN ACCESSORY SUBUNIT OF MUSCARINIC M1 RECEPTORS THAT CAUSES FRONTOTEMPORAL DYSCONNECTIVITY IN SCHIZOPHRENIA**

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Calneuron-1 is a Ca2+-sensor that has been linked in several genome-wide association studies (GWAS) to schizophrenia (SCZ). Here we show that Calneuron-1 expression is elevated in the dorsolateral prefrontal cortex of schizophrenia patients and that viral overexpression of Calneuron-1 in the medial prefrontal cortex (mPFC) of mice elicits SCZ-related behavioral disabilities. Increased expression of Calneuron-1 disrupts rhythmogenesis in the mPFC, impairs functional connectivity between the hippocampus and the mPFC and causes deficits in muscarinic synaptic plasticity. These neurophysiological signatures of SCZ are intimately linked to the role of Calneuron-1 as accessory subunit of muscarinic M1 receptors (M1R). We found that Calneuron-1 displaces Gαq11 from the third intracellular loop of M1R at elevated [Ca2+]i and thereby disrupts downstream G-protein signaling. Moreover, we found that the M1R agonist xanomeline disrupts the interaction between M1R and Calneuron-1, leading to the restoration of G-protein coupling and, consequently, muscarinic synaptic plasticity. Xanomeline has demonstrated in phase III clinical trials to reduce positive and negative symptoms of SCZ patients. Collectively, our data reveal a potential causative pathomechanism of SCZ.

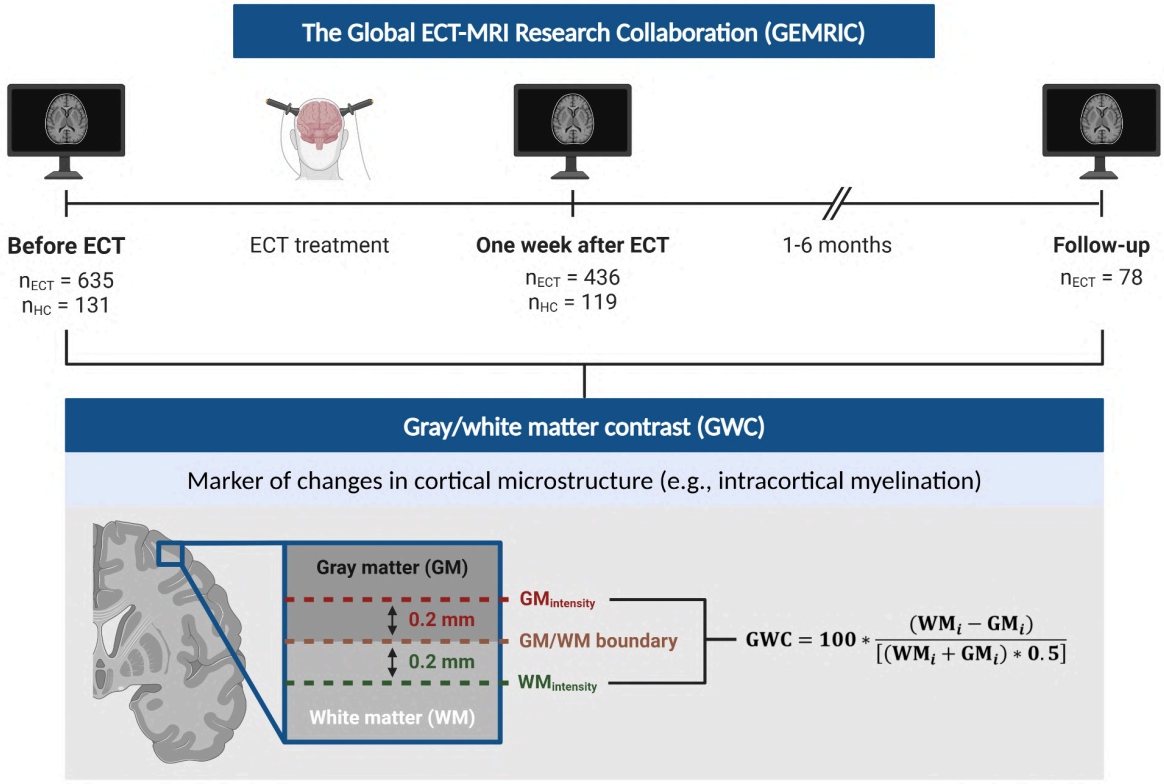
**Keyword 1**: Schizophrenia
**Keyword 2**: Calneuron-1
**Keyword 3**: muscarinic M1 receptors
**Keyword 4**: medial prefrontal cortex
**Keyword 5**: xanomeline

**CHANGES IN CORTICAL MICROSTRUCTURE AFTER ELECTROCONVULSIVE THERAPY IN PATIENTS WITH DEPRESSION**

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Electroconvulsive therapy (ECT) for depression induces broadly distributed increases of cortical grey matter volume. The clinical relevance of these macrostructural changes remains unclear and may be better understood by focusing on microstructural processes. This study evaluated the effect of ECT in the magnetic resonance imaging (MRI) grey/white matter contrast (GWC), sensitive to intracortical myelination. As part of the GEMRIC ECT-MRI Research Collaboration (GEMRIC), MRI images were obtained from patients treated with ECT for depression and healthy controls (HC) before treatment ($n_{ECT}$=635, $n_{HC}$=131), one week after treatment ($n_{ECT}$=436, $n_{HC}$=119) and at long-term follow-up ($n_{ECT}$=78). The images were processed in Freesurfer; GWC was calculated by sampling T1w intensities ± 0.2 mm from the gray/white matter border before segmenting the brain based on the Desikan-Killiany atlas. Linear mixed models were used while adjusting for age, sex and sites. At baseline, GWC was lower for ECT patients than HC in the right caudal middle frontal gyrus ($p$=.016, $d$=-0.15). Significant decreases in GWC pre-post ECT were found for multiple cortical regions one week after ECT (vs HC, $p$<.04, $d$=-0.25) and at follow-up ($p$<.05, $d$<-0.10), including prefrontal areas (e.g., bilateral superior frontal and rostral anterior cingulate gyri). No significant results survived false discovery rate correction for multiple testing. ECT led to decreased GWC, a proxy of intracortical myelination. Further contrasts will explore the association between GWC and other clinical variables, such as depressive symptoms. Overall, measures of intracortical myelination provide an innovative perspective for the pathophysiology and treatment of depression.

**Keyword 1**: Electroconvulsive therapy
**Keyword 2**: Myelin
**Keyword 3**: Depression
**Keyword 4**: Major depressive disorder
**Keyword 5**: Intracortical myelination




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**METHODS TO INVESTIGATE THE PLASTICITY OF THE ADULT HUMAN BRAIN *EX VIVO*: FROM PHYSIOLOGICAL SYNAPTIC FIRING TO VIRO-INDUCED CIRCUITRY INTERFERENCE**

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The unique complexity of the adult human brain makes it a particularly challenging organ to model. With the extensive use of animal models and advent of stem cell derived brain organoids, tremendous progress occurred in the past decade, but the lack of *in vitro* relevant models *has* hampered the study of the molecular determinants of developed human neurocognition. Recently, our lab developed the Organotypic culture of *Post-mortem* Adult Brain explants (OPAB), and showed that it is a relevant approach to study neurocognitive physiopathological conditions *in vitro*. Indeed, we previously demonstrated that OPAB can be cultured for weeks, while retaining robust electrical activity and plastic neural circuitry. Here, we used OPAB to assess how Brain-Derived Extracellular Vesicles (BDEVs) and neurotropic viruses impact basic learning processes using innovative methodological developments, including an automated microfluidics platform and customized machine learning frameworks. Using proteomics analyses and electrophysiology, we found that BDEVs and viruses both profoundly affected neural plasticity in OPAB, likely by modulating synaptic homeostasis. To assess neurocognitive-like functions *in vitro*, we optimized Theta Burst-based Stimulation (TBS) and revealed that OPAB learning capabilities were significantly disrupted upon infection with the neurotropic Tahyna virus (TAHV). Finally, we further validated the impact of TAHV on learning functions using an original setup mimicking basic sensorimotor and learning functions *in vitro*, based on the anthropomorphic embodiment of OPAB. This seminal work represents a technical *tour de force* combining interdisciplinary expertise, with a potential to foster our understanding of cognition in developed human model systems.

**DOES POOR SLEEP DURING ADOLESCENCE LEAD TO METABOLIC DISORDERS LATER IN LIFE? – TARGETED CHEMOGENETIC MODELS OF SLEEP DEPRIVATION IN MICE**

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Poor sleep and obesity are concurrent health epidemics in adolescents. This is especially concerning given that adolescents are getting less and less sleep as technology invades their bedrooms. Several studies point to adolescence as a critical period for sleep and adipose tissue development, implying that poor sleep during this period might alter the normal development of adipose tissue and cause metabolic imbalance. To investigate whether adolescent sleep quality contributes to the emergence of metabolic dysfunctions later in life, we propose to establish new rodent models of reversible sleep deprivation for which we can turn on and off sleep quality/quantity at specific developmental windows. Here we present preliminary data of our new chemogenetic model for which we can disturb sleep at specific time points between postnatal day P21 and P54 by targeting brain nuclei promoting wakefulness through the stimulation of GABAergic neurons in the ventral pallidum. We assessed the sleep amounts and quality of these animals by recording their neural (EEG) and muscular (EMG) activity. Metabolic parameters during and after sleep disturbances were assessed with metabolic cages (energy expenditure, respiratory exchange rate, food intake, weight, activity), adipose tissue histology and plasma collection (hormones and proteins). Our preliminary results indicate successful sleep deprivation concomitant with decreased respiratory exchange rate indicating a shift toward lipid oxidation as a primary energy source.

**GENDER DIMORPHISM UNDERLYING THE VULNERABILITY OF DEVELOPING CANNABIS USE DISORDER**

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Cannabis is one of the most commonly used drugs in the EU and USA. Cannabis Use Disorder (CUD) is a substance use disorder (SUD) defined by the Diagnostic and Statistical Manual of Mental Disorders (DSM-5) as an inability to quit consuming even if it causes psychological and/or physical harm to health. Similar to other SUD, CUD is a multifactorial disorder whose vulnerability is affected by the interaction between environmental, and genetic factors.

Human studies reported that women tend to have a greater risk of having a faster transition to addiction after the first substance intake, together with experiencing more significant difficulties remaining abstinent. Gender dimorphism in CUD can be associated with inherent variations in brain structure as well as the effects of circulating hormones on motivation, decision-making, reward, and cognitive processes among others. However, studies in both humans and rodents have revealed a considerable lack of data in the female cohort, leaving the processes underlying the development of addiction in women unclear.

Our study is focused on exploring the neurobiological and behavioural differences in vulnerability to CUD, which is explained by sex differences. Using a mouse model of cannabis addiction in a drug self-administration protocol, we got significantly different results for the number of addicted individuals in favour of the female group. In addition, females scored higher in the study of compulsivity and persistence to response, two hallmarks of addiction. In conclusion, our results provide evidence of gender dimorphism that can pave the way for new differential therapeutical perspectives.

**Keyword 1**: Cannabis Use Disorder
**Keyword 2**: Gender dimorphism
**Keyword 3**: Self-administration protocol

**NEUROBIOLOGICAL MECHANISMS OF CANNABIS ADDICTION: RETRO-DREADDS APPROACH IN A NOVEL SELF-ADMINISTRATION MODEL**

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Cannabis is one of the most widely consumed psychoactive substances worldwide, with increasing prevalence due to greater accessibility and changing legal regulations. While many users consume cannabis recreationally, a significant subset develops Cannabis Use Disorder (CUD), now recognized in the DSM-5 as a complex, multifactorial disease. CUD involves neuroadaptations across three major circuits: (i) the binge/intoxication phase, mediated by the basal ganglia; (ii) the withdrawal/negative affect phase, regulated by the extended amygdala; and (iii) the preoccupation/anticipation phase, governed by the prefrontal cortex. Among these, glutamatergic cortico-striatal projections play a crucial role in addiction-related neuroplasticity.

To investigate the neurobiological mechanisms underlying CUD, we developed a novel operant self-administration model for synthetic cannabinoids using WIN 55,212-2, a full cannabinoid receptor agonist. This model revealed distinct behavioral endophenotypes of vulnerability to addiction.

Our aim was to examine the role of glutamatergic projections from the basolateral amygdala (BLA) and hippocampus (HPC) to the nucleus accumbens (NAc) using a Retro-DREADD approach. Significant reductions in neuronal excitability of these pathways, via hM4Di activation by CNO, affected cannabis-seeking behavior and compulsivity.
These findings provide critical insights into the neural mechanisms underlying **CUD** and highlight the potential of targeted **neuromodulation** as a therapeutic strategy.

**Keyword 1**: Cannabis Use Disorder (CUD)
**Keyword 2**: Glutamatergic cortico-striatal projections
**Keyword 3**: Retro-DREADD
**Keyword 4**: Operant self-administration model
**Keyword 5**: Neuroplasticity

**INVESTIGATING PERSISTENT COGNITIVE DEFICITS FOLLOWING TRANSIENT GLOBAL AMNESIA**

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Transient Global Amnesia (TGA) is a neurological condition characterized by a patient's sudden loss of ability to form any new memories for a temporary time, while personal identity remains intact. Patients often experience partial retrograde amnesia and confusion regarding their surroundings.
Previously TGA was thought to be completely reversible, with no lasting cognitive impairments. This study challenges the assumption of complete recovery by assessing whether former TGA patients experience persistent cognitive deficits months and years after the episode.
Given its transient nature, TGA offers a unique insight in isolated memory dysfunction. Studying the aftermath of TGA can broaden theories of memory retrieval and resilience in memory loss. Identifying the specific cognitive domains that might remain impaired could refine our understanding of the cognitive mechanisms involved in TGA compared to those for non-reversible memory loss.
Participants in this study completed a series of online cognitive tasks developed by neuroscientists at the University of Oxford and a game designed for assessing spatial navigation abilities.
Preliminary data indicate no significant differences in behavioral task performance between the acute and post-acute phases (2 weeks later). However, a larger sample size and more sensitive cognitive assessments may reveal subtle deficits at a later stage. Previous research has suggested that TGA patients exhibit impaired spatial navigation abilities even months post-episode. Data collection from our international cohort is ongoing, with the potential to yield novel insights into the long-term cognitive consequences of TGA.

**Keyword 1**: Transient Global Amnesia
**Keyword 2**: Cognitive function
**Keyword 3**: Spatial navigation
**Keyword 4**: Episodic memory

**CHRONIC EFFECTS OF WHITE NOISE ON EMOTIONAL MEMORY IN RATS: PLUS MAZE ASSESSMENTS**

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Chronic exposure to loud white noise has been implicated in various neurobehavioral alterations, yet its impact on emotional memory remains underexplored. This study investigated the long-term effects of white noise on emotional memory in adult male Wistar rats, using the open and closed plus maze paradigms. Emotional memory was assessed based on the animals; ability to recall and adapt to aversive and non-aversive experiences in these mazes, reflecting their anxiety-like behavior and spatial memory retention.
Adult male rats were exposed to white noise (100 dB) for 1 hours daily over a period of 40 days. Behavioral testing was conducted post-exposure, with performance measures including time spent in open versus closed arms, entries into each arm type, and risk assessment behaviors. Comparisons were drawn against a control group maintained in standard laboratory conditions without white noise exposure.
Our findings revealed significant alterations in the emotional memory of noise-exposed rats. In the open plus maze, treated animals displayed a reduced preference for the open arms, indicative of heightened anxiety and impaired habituation to non-aversive conditions. Similarly, in the closed plus maze, white noise exposure was associated with diminished exploratory behavior and altered memory retrieval in response to aversive cues.
The results suggest that chronic loud noise disrupts the balance between anxiety and memory processes, potentially mediated by stress-induced neuroplastic changes in limbic and cortical regions. Understanding these effects could inform strategies for mitigating environmental noise impacts on cognitive and emotional health.
This work is support FR-21-1501

**Keyword 1**: Plus mase
**Keyword 2**: Emotional Sphere
**Keyword 3**: White Noise

**A FLEXIBLE TWO-PHOTON ENDOSCOPE FOR IN-DEPTH ALL-OPTICAL CIRCUIT MANIPULATION IN FREELY BEHAVING MICE**

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A key question in neuroscience is to unravel causal relations between neuronal circuits and behavior. The precise study of neuronal circuits requires to measure and manipulate neuronal activity with high spatial and temporal resolution within large ensembles (Marshel et al., Science 2019). All-optical experiments using two-photon (2P) calcium imaging and optogenetic photostimulation offer a promising approach to studying neuronal circuits in vivo in mice (Emiliani et al., Nat. Rev. 2022). However, they have so far focused on experiments in head restrained mice (Robinson et al., Cell 2020). We recently developed a flexible 2P microendoscope (2P-FENDO) capable of all-optical brain investigation at near cellular resolution in the L2/3 of the barrel and visual cortex of freely moving mice. The system performs fast two-photon (2P) functional imaging and 2P holographic photostimulation of single and multiple cells using axially confined extended spots (Accanto et al., Neuron 2023). Here, we exploited the characteristics and advantages of the 2P-FENDO in the hippocampus, a crucial brain structure involved in spatial navigation and memory formation. Proof-of-principle experiments were performed in freely moving mice co-expressing GCaMP8s and the opsin ChRmine in the CA1 region of the hippocampus. In a field of view of 250 µm in diameter, we demonstrate functional imaging at a frame rate of up to 100 Hz and precise photostimulation of single and multiple neurons. 2P-FENDO enables single-cell resolution imaging and control of hippocampal neurons in freely behaving animals, helping to reveal networks involved in memory and spatial navigation, essential for adaptive behavior and survival.

**ANXIETY CORRELATES POSITIVELY WITH SYNAPSE DENSITY IN THE ANTERIOR CINGULATE CORTEX IN A RAT MODEL OF PTSD**

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Anxiety is usually increased in individuals with post-traumatic stress disorder (PTSD), co-occurring with core symptoms. Such anxiety is often challenging to treat. We know little about the underlying synaptic causes of this anxiety and its resistance. Using the predator scent model of PTSD, we have investigated the relationship between anxiety-like behavior and brain microstructure and synaptic glutamate receptors in rats, using Golgi-staining, behaviour testing (elevated plus maze), and immune-electron microscopy. Our results show positive correlation between high anxiety-like behaviour and high dendritic spine density in the anterior cingulate cortex (ACC), but not in the primary sensory cortex or hippocampus. This correlation was even stronger for mushroom spines in the ACC, but not significant for other spine types or regions. Cortical thickness did not correlate with spine density or anxiety scores. Three samples of high anxiety, low anxiety, and control animals were further investigated with immuno-electron microscopy for synaptic concentration of NMDA-receptors. This analysis showed significant lower concentrations of GluN2B receptor subunits in the high anxiety group, indicating lower potential for synaptic change. Using resting state fMRI recordings performed prior to trauma and behavioral testing after trauma showed that resting state functional connectivity (RSFC) between ACC and both retrosplenial cortex and bed nucleus of stria terminalis (BST) correlated with anxiety. ACC spine density and ACC-BST RSFC mediated anxiety independently of each other. This study provides a basis for understanding the interplay between anxiety and synapse regulation in the ACC.

**EFFECTS OF AUDIENCE ON BUDGERIGAR (*MELOPSITTACUS UNDULATUS*) SOCIAL BEHAVIOR**

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Humans communicate differently with distinct individuals, a phenomenon known as the audience effect. Budgerigars (*Melopsittacus undulatus*), a highly social parakeet, imitate one another's vocalizations even as they maintain sufficient subtle acoustic differences to preserve individuality. Here, we examine for a possible audience effect by testing if distinct known social partners elicit individually-targeted vocalizations. We established three bonded pairs (each pair consisting of one female and one male) of unfamiliar birds with different contact calls. The pairs were placed in a behavioral arena equipped with directional microphones and cameras to record call convergence and courtship behavior. As expected from past work (Brockway, 1964; Farabaugh et al, 1994; Striedter et al, 2003), initial pairs converged their calls, and in this experiment, to different degrees over ten weeks. The males then underwent this same process a second time with different females. After the second bonding stage, they were swapped between the two females to determine if they could instantaneously change their call type between the females. Based on preliminary analysis, males tailored their calls to each female, indicating that they were integrating social information into the song system. This result resembles how humans change their language or behavior with distinct individuals and highlights the increased cognitive capacity of these animals, more than was previously known. More broadly, these initial behavioral studies set the stage for future neuroscientific studies to test which neural circuits and signals enable an animal to transform a social recognition signal into a vocal motor output.

**Keyword 1**: bird
**Keyword 2**: social
**Keyword 3**: vocalization
**Keyword 4**: song
**Keyword 5**: audience

**EFFECTS OF SOCIAL PARTNER FAMILIARITY ON THE SOCIAL BEHAVIORS OF SINGLE-HOUSED FEMALE MICE**

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Like humans, mice are intrinsically motivated to engage in social interactions with conspecifics. Interactions involve both vocal and non-vocal social behaviors. Mice are also sensitive to periods of social isolation, making them an excellent model to study how social isolation influences the brain and behavior. Short-term (3-day) social isolation upregulates social behavior in female mice that subsequently engage in same-sex social interactions with a novel female mouse. Here, we investigate the role of social partner familiarity in regulating the social behaviors of female mice following short-term isolation. Each single-housed (isolated) subject female was placed in her home cage within a sound-attenuating behavior chamber, and one of her group-housed (non-isolated) sisters was introduced to the cage for a 30-minute social interaction. During the interaction, an ultrasonic microphone was used to record ultrasonic vocalizations (USVs), and a modified webcam and infrared light source were used to record non-vocal social behaviors. Preliminary results suggest that social partner familiarity attenuates the effects of short-term isolation on female social behaviors, including social investigation and the production of USVs. This ongoing study sheds light on social factors that regulate mouse behavioral responses to social isolation. The results serve as a foundation for ongoing work aimed at delineating the mechanisms through which social isolation acts on the mammalian brain to influence social behavior.

**EFFECT OF ACUTE HEAT STRESS ON NEURONAL ACTIVITY IN THE BRAIN OF LARVAL ZEBRAFISH**

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Heat stress, such as that experienced during heat waves, disrupts brain function, leading to symptoms like dizziness, impaired coordination, and loss of equilibrium. While temperature fluctuations influence neuronal excitability, the rate of heating may critically shape network responses. Yet, the dynamics of neuronal activity during and after acute heat stress remain poorly understood. Investigating these mechanisms is key to understanding thermal resilience and its relevance to neurological disorders like heat stroke, epilepsy, and ischemic injury.
We investigated how heating rate affects neuronal activity in the brainstem of 5-day-old *elavl3:GCaMP6s* zebrafish larvae using epifluorescence microscopy. By applying different heat ramp speeds, we quantified the frequency and amplitude of population $Ca^{2+}$ events and identified the onset temperature of spreading depolarization (SD)—a wave of near complete neuronal and glial depolarization. Our results show that heating rate significantly influences pre-SD activity, SD onset temperature, and recovery. Faster heating shifts SD onset to higher temperatures, while slower ramps introduce distinct pre-SD suppression phases.
To further investigate cellular responses, we are employing confocal microscopy to record $Ca^{2+}$ activity at single-cell resolution under controlled temperature steps (28°C, 32°C and 36°C). This will enable us to determine e.g. whether change in populational $Ca^{2+}$ events amplitude as temperature increases result from decreased activity, or decreased correlation between individual neurons.
Our findings demonstrate that heat-induced alterations in neuronal activity do not occur at a fixed temperature threshold but depend on heating rate. This study may also have implications for neurological disorders involving SD-like events.

**Keyword 1**: Heat stress
**Keyword 2**: Spreading depolarization
**Keyword 3**: Zebrafish larval brain
**Keyword 4**: calcium imaging
**Keyword 5**: Calcium dynamics

**FUNCTIONAL AND NEUROCHEMICAL CHARACTERIZATION OF CAMKIIA-EXPRESSING NEURONS IN THE VENTRAL PALLIDUM**

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The ventral pallidum (VP) is a basal forebrain nucleus composed of GABAergic, cholinergic and glutamatergic neurons, playing a crucial role in processing both rewarding and aversive stimuli. Here, we investigated the role of CaMKIIa-expressing (+) neurons in the VP in affective behaviors of adult Wistar rats using chemogenetic inactivation. Inhibiting VP CaMKIIa+ neurons reduced locomotor activity and exploratory behavior in the open field test, elevated plus maze, and social preference avoidance test. Experimental animals also spent less time in the social interaction zone and displayed increased anxiety-like behavior, as indicated by reduced open-arm exploration in the elevated plus maze. While sucrose preference remained unchanged, total liquid consumption was lower in the experimental group after two hours in the sucrose preference test. In the novelty-suppressed feeding test, approach latency to food did not differ between groups, but experimental animals took longer to begin eating in their home cage, with no differences in total food consumption. In more aversive tasks, including the forced swim test and auditory fear conditioning, no significant behavioral differences were observed. To characterize the neurochemical profile of CaMKIIa+ neurons in the VP, we examined their co-expression with molecular markers. Although CaMKIIa is typically associated with glutamatergic principal neurons in the cortex, recent evidence suggests its expression may extend to GABAergic interneurons. Consistent with this, we found that a subset of CaMKIIa+ cells in the VP co-express parvalbumin, calbindin, and choline acetyltransferase, highlighting the heterogeneity of this neuronal population.

**STRIATAL DOPAMINE AND SEROTONIN ENCODE BILATERAL SENSORIMOTOR DECISION MAKING**

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The dorsolateral striatum (DLS) plays a central role in integrating motor and sensory information, yet the neuromodulatory interactions that shape its function remain poorly understood. While dopamine (DA) innervation in the striatum has been extensively studied and is well established in motor control and reward, serotonin (5-HT) innervation has received comparatively less attention. Notably, 5-HT terminals are positioned in close proximity to DA terminals, hinting at a potential but overlooked interaction between these systems. Here, we used fiber photometry to record bilateral DA release in the DLS during a sensorimotor decision-making task in which mice learned to turn a steering wheel based on visual cues. Our findings reveal that while DA signals are largely correlated across hemispheres, interhemispheric asymmetry is directly linked to movement onset. Additionally, and consistent with previous reports, DA responses in naïve animals were reward-related but shifted to stimulus presentation after task proficiency was achieved. To further investigate neuromodulatory dynamics, we recorded 5-HT release during the same task. Our preliminary findings indicate 5-HT signals related to stimulus presentation, movement onset, and trial response time, suggesting a broader role in sensorimotor processing. These preliminary results provide new insight into the dynamics of DA and 5-HT release in the striatum and their respective contributions to sensory, motor, and reward-related functions. Moving forward, we will simultaneously record DA and 5-HT release and use cell-type-specific perturbations to further dissect their functional interactions.

**Keyword 1**: Serotonin
**Keyword 2**: Dopamine
**Keyword 3**: Fiberphotometry

**NOVEL BRAINSTEM REWARD CENTER INHIBITS LATERAL HABENULA AND INDUCES REWARD SEEKING BEHAVIOR**

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The lateral habenula (LHb) regulates behavioral flexibility that is essential for effective decision-making. LHb activity can facilitate aversion or reward-seeking behavior as a function of its excitatory/inhibitory inputs. However, despite LHb abundant connections with hindbrain areas the role of brainstem inputs are still unclear. Using viral tract tracing, in vivo electrophysiology, single cell sequencing and optogenetic behavioral experiments in transgenic mice, we investigated a previously unrecognized gamma-aminobutyric acid (GABA)-ergic cell population in the pontine central grey, named subventricular tegmental nucleus (SVTg) and its role in reward seeking behavior. We found that their stimulation induced place preference. Moreover, mice self-stimulate their SVTg, which can also be activated directly by the orbitofrontal cortex, resulting in fast and effective inhibition of the LHb. Conversely, inhibition of SVTg caused place avoidance, and disrupted reward location memory formation. By mapping the genetic profile of SVTg neurons and identifying their specific markers (Satb1, Pax6, Reln), we successfully identified them in the brainstem of both primates and humans. Our results suggest that a novel GABAergic pontine nucleus that innervates the LHb has a major role in processing positive valence and reward-seeking behavior and they might have a role in mood-related disorders.
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**Keyword 1**: Brainstem
**Keyword 2**: Lateral Habenula
**Keyword 3**: Reward

**DIFFERENTIAL REPRESENTATION OF VARIOUS REWARDS IN DOPAMINERGIC NEURONS OF THE VENTRAL TEGMENTAL AREA**

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The ventral tegmental area (VTA) encompassing dopaminergic (DA) neurons, is a brain region crucial for processing information about various rewards and guiding appetitive behaviours directed towards them. Importantly, how the VTA DA neurons encode different rewards and to what extent this information overlaps within different DA cells is poorly understood.
For that reason, we recorded the electrophysiological activity of VTA neurons in freely-behaving male mice using silicon probes while the animals were free to spontaneously explore different rewards. The arena contained several natural rewards including water, food, a toy, a female conspecific and a running wheel.
The activity of a majority of putative DA neurons was modulated by at least one reward. Despite the heterogeneity of responses, some patterns could be observed. For example, two subpopulations with different firing patterns were modulated by different types of rewards – one of them responded more strongly to the running wheel while the other to food. Encoding of speed in the running wheel and during free locomotion was uncorrelated, suggesting that voluntary exercise is represented differently in the VTA DA neurons than the spontaneous exploratory behaviour.
Overall, our results show that the putative VTA DA neurons code different natural rewards heterogeneously. Some encoding patterns were present as, for example, we observed that separate subpopulations of DA neurons represent food and voluntary exercise. This suggests that VTA DA neurons are able to distinguish between different rewards, including the competing ones.
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**Keyword 1**: dopamine
**Keyword 2**: ventral tegmental area

**A BINARY DOPAMINERGIC SWITCH FOR CANNABIS INDUCED HYPOLOCOMOTION AND ENDOCRINE DISRUPTION**

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Cannabis consumption impacts daily activity, metabolic, and endocrine parameters in humans. These pathobiological features are recapitulated in mice, with locomotor activity and circulating prolactin levels being significantly affected. However, neither the neuroendocrine center(s) nor the cell types that are adversely modulated by cannabis – and most notably delta9-tetrahydrocannabinol (THC) – are known. The hypothalamus with its many neuroendocrine command modules is the interface for brain-body hormonal communication, and could be vulnerable to THC exposure. Here, we used single-cell RNA-sequencing, nanoscale neuroanatomy, and pharmacogenomics to show that molecularly and functionally distinct subtypes of dopamine neurons of the periventricular hypothalamic nucleus (A14) mediate THC-induced hypolocomotion and endocrine disruption. We reveal that chemogenetic activation of dopamine neurons co-expressing dopamine transporter 1 (*Slc6a3*) and CB1 cannabinoid receptors (*Cnr1*) that preferentially innervate the lateral septum rescued THC-induced catalepsy-like behavior. Alternatively, THC-induced changes in the activity of *Slc6a3*-negative, *Cnr1*-positive dopamine neurons that project to the median eminence led to altered prolactin levels in the general circulation, which could be reinstated through cell-type-specific chemogenetic manipulation. Cumulatively, we distinguish novel subtypes of hypothalamic dopamine neurons, and characterize the periventricular nucleus as a hypothalamic center for cannabis-induced behavioral and endocrine vulnerability.

**Keyword 1**: dopaminergic neuron
**Keyword 2**: periventricular nucleus of the hypothalamus
**Keyword 3**: cannabinoid rceceptor
**Keyword 4**: dopamine-prolactin signaling

**NOVEL BRAINSTEM REWARD CENTER ACTIVATES DURING REWARD AND BALANCES NEGATIVE EXPERIENCES**

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The lateral habenula (LHb) is an evolutionarily well-preserved brain structure involved in motivation and cognition. Using viral gene delivery in transgenic mice, we identified a novel inhibitory pontine cell population that projects to the LHb, which we named the subventricular tegmental nucleus (SVTg). Fiber photometry revealed that rewarding and reward-predicting cues increased SVTg activity. Optogenetic stimulation of the SVTg induced dopamine (DA) release in the nucleus accumbens, further suggesting a role in reward processing. Surprisingly, aversive airpuffs also activated the SVTg, raising the question of whether it plays a broader role in emotional regulation. Indeed, optogenetic inhibition of the SVTg during fear memory acquisition led to an increase in contextual and generalized fear. Additionally, SVTg activation decreased anxiety-related behavior in the elevated plus maze. In vitro electrophysiological recordings showed uniform spontaneous excitatory postsynaptic potentials (EPSPs) and burst firing across all SVTg neurons, with serotonin further exciting them. These findings suggest that the SVTg modulates both rewarding and aversive experiences, potentially acting as a buffer against excessive negative emotional states. Given this role, dysfunction in the SVTg may contribute to mood disorders such as depression and anxiety.

**Keyword 1**: brainstem
**Keyword 2**: lateral habenula
**Keyword 3**: reward

**NEUROMODULATION OF THE CORTICOSTRIATAL PATHWAY DRIVES ACTIVITY-DEPENDENT PURINERGIC-LIKE SIGNALLING IN THE VENTRAL STRIATUM**

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Recent literature has highlighted that medial prefrontal cortex (mPFC) inputs can modulate terminal dopamine (DA) release in dorsal regions of the striatum. However, this effect is poorly described in the ventral striatum, consisting of the Nucleus Accumbens (NAc), where DA neurons differentially originate from the Ventral Tegmental Area (VTA). This work focusses on corticostriatal driven signalling within the NAc, examining how the infralimbic region (IL) of the mPFC modulates signal integration in the NAc. To explore this, we provided *in vivo* electrical stimulation to the IL (500uA, 10Hz, 1ms PW, 2-6s) and VTA (120-200uA, 60Hz, 2ms PW, 0.4-2s) in urethane anaesthetised Wistar rats, sampling co-release of transient dopaminergic and purinergic signals within the NAc using fast-scan cyclic voltammetry (FSCV). In a second experiment, we assessed signal responses to a bidirectional adenosine transporter inhibitor S-(4-nitrobenzyl)-6-thioinosine (NBTI) [RB1] using optogenetic stimulation of IL terminals in the NAc in *ex vivo* brain slices. It was observed that IL stimulation in isolation did not induce measurable DAergic release within the NAc, and that co-stimulation of the IL and VTA did not significantly modulate DAergic release in comparison to VTA-evoked DAergic release. However, IL stimulation was observed to induce a purinergic-like response. This signal was replicated *ex vivo*, with purinergic release dynamics altered by the application of 10μM NBTI. This data points towards an activity-dependent release of ATP/adenosine downstream of IL inputs within the NAc, constituting a novel aspect of local signal integration important in driving changes in a suite of behaviours.

**Keyword 1**: Corticostriatal
**Keyword 2**: Purine Signalling
**Keyword 3**: Dopamine

**CENTRAL CONTROL OF OPIOID-INDUCED MECHANICAL HYPERSENSITIVITY AND ANALGESIC TOLERANCE**

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Morphine and other opioids still remain clinically indispensable for treating severe chronic pain. However, chronic use of morphine and other opiates could cause two major side effects: opioid-induced hypersensitivity (OIH) and analgesic tolerance. Otherwise, the central mechanisms underlying mechanical OIH/tolerance remain unresolved. Here, we use behavioral tests and whole-cell patch-clamp electrophysiological recordings of spinal cord and brain slices, combined with research methods such as virology, chemical genetics, brain stereotaxy, and in situ hybridization, to report that a brain-to-spinal opioid pathway, starting from μ-opioid receptor (MOR)-expressing neuron in the lateral parabrachial nucleus ($lPBN^{MOR+}$) via dynorphin (Dyn) neuron in the paraventricular hypothalamic nucleus ($PVH^{Dyn+}$) to κ-opioid receptor (KOR)-expressing GABAergic neuron in the spinal dorsal horn ($SDH^{KOR\text{-}GABA}$), controls repeated systemic administration of MF-induced mechanical OIH and tolerance in mice. The above effect is likely mediated by disruption of dorsal horn gate control for MF-resistant mechanical pain via silencing of the Dyn-positive GABAergic neurons in the SDH ($lPBN^{MOR+}\rightarrow PVH^{Dyn+}\rightarrow SDH^{KOR\text{-}GABA}\rightarrow SDH^{Dyn\text{-}GABA}$). Repetitive binding of MF to MORs during repeated MF administration disrupted the above circuits. Targeting the above brain-to-spinal opioid pathways rescued repetitive MF-induced mechanical OIH and tolerance.

**Keyword 1**: morphine
**Keyword 2**: OIH

**DOPAMINE SIGNALS REFLECT RULE-SHIFT LEARNING PROCESS IN MICE**

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Context-dependent and flexible learning is a crucial cognitive ability for adapting to changing environments. Deficits in this function are observed in schizophrenia, Alzheimer's, Parkinson's, and other neurological disorders. While cognitive flexibility is assessed clinically using tests like the Wisconsin Card Sorting Test, rodent paradigms for evaluating context-dependent rule shifts remain underdeveloped. These paradigms are often challenging, and prolonged training limits the ability to record neural signals throughout learning. Thus, developing tasks that are both efficient and cognitively demanding is essential for neurobehavioral research. To address this, we designed a rule-shift task assessing behavioral flexibility in mice. The task alternates between two simple rules: a place-rule and a light-rule, enabling measurement of rule-learning, rule-reversal, and rule-shift processes. The task exhibits asymmetrical difficulty—mice acquire and reverse the place-rule quickly but struggle more with the light-rule, making reversals and shifts more challenging. This distinction helps differentiate neural correlates and assess the effects of neurological disorders of varying severity. As an initial study, we examined mesolimbic dopamine (DA) signals throughout different task phases. We found that VTA DA neuron firing and DA release in the nucleus accumbens tracked performance changes across all rules. Additionally, DA signals correlated with task difficulty, particularly in rule-reversals and shifts. These findings provide valuable insights into the neurobiological mechanisms underlying cognitive flexibility, offering a promising framework for future studies of learning deficits in neurological disorders.

**NEUROSCIENCE OF SCENTS APPLIED TO BEHAVIOR CHANGE: AN EXPERIENCE REPORT**

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**Objective:** To describe the experience of olfactory clinical consultations aimed at behavior change, based on hedonic and pharmacological mechanisms. **Methods:** This is a professional experience report on behavioral aromatherapy clinical consultations conducted in Brazil by a pharmacist aomatherapist between 2019 and 2024. The consultations lasted for a minimum period of three months, during which patients applied aromatherapy practices to achieve behavior change or formation goals. Individualized olfactory prescriptions guided the use of aromas in two specific moments: (1) During the execution of the behavior, a perfume or essential oil (optional according to the patient's hedonic preference) was used for immediate inhalation to activate the reward system. This aroma stimulate the system of goal-directed behaviors, facilitating its execution. (2) During sleep, ambient aromatization was applied in the sleeping environment, allowing the patient to inhale the bioactive components of essential oils. This aimed to stimulate neuroplasticity, promoting long-term memory consolidation of the experience, thereby facilitating the creation of the desired behavior. **Results:** The personalized strategies consisted of two olfactory interventions, based on the action mechanisms previously described. The table below presents some of these strategies:

**Conclusions:** The individualized behavioral olfactory strategy, grounded in the Neuroscience of scents, promotes behavior formation through a dual neural mechanism. This report aims to encourage clinical studies on the combined use of these two mechanisms, providing behavioral professionals with a more effective tool for intervention.

**Keyword 1**: #neuropharmacology
**Keyword 2**: #scents
**Keyword 3**: #goaldirectedbehaviors
**Keyword 4**: #behaviorchange
**Keyword 5**: #aromatherapy

| Individualized behavioral olfactory strategy | | |
|---|---|---|
| **Case:** | **Hedonic mechanism used at moment 1:** | **Pharmacological and hedonic mechanism used at moment 2:** |
| 1 | Botanical perfume Black pepper (*Piper nigrum*) and Cardamom (*Elettaria* cardamomum). | Sicilian Lemon Essential Oil (*Citrus limon*) – bioactive components limonene and pinene – pharmacological mechanisms: acetylcholinesterase inhibition, acetyltransferase activation, and stimulation of hippocampal neurogenesis via GAP-43 activation. |
| 2 | Synthetic perfume (Alien Mugler). | Black pepper essential oil (*Piper nigrum*) – bioactive components: pinene, limonene, and beta-caryophyllene – pharmacological mechanisms: acetylcholinesterase inhibition, acetyltransferase stimulation, hippocampal neurogenesis stimulation via GAP-43 activation, and beta-amyloid inhibition.<br><br>Vanilla essential oil (*Vanilla plantifolia*) – bioactive component: vanillin – pharmacological mechanisms: acetylcholinesterase inhibition, hippocampal neurogenesis stimulation, and reduction of oxidative stress. |
| 3 | Aromatic spray Atlas Cedar (*Cedrus atlantica*) – hedonic mechanism + pharmacological mechanism: activation of the dopaminergic system via D1 receptor agonism in the ventral tegmental area. | Copaiba essential oil (*Copaifera langsdorffii*) – bioactive component: beta-caryophyllene – pharmacological mechanisms: beta-amyloid inhibition and neuroprotection.<br>Orange essential oil (*Citrus sinensis L.*) – B<br>bioactive component: limonene – pharmacological mechanisms: acetylcholinesterase inhibition and acetyltransferase activation. |

Table 1. Neuroscience of scents applied to behavior change: an experience report. Nathânia Sousa Frutuoso

**FUNCTIONAL CONNECTIONS MODULATING EXTINCTION OF LEARNED TASTE-IMMUNE RESPONSES**

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Paradigms of taste-immune associative learning are suggested elegant strategies aiming at a controlled drug dose reduction while maintaining efficacy of treatment. It is well established in rats, that a conditioned taste avoidance paradigm (CTA) using a novel sweet taste (saccharin) as the conditioned stimulus (CS) together with injections of immunosuppressive drugs as unconditioned stimulus (UCS) leads to altered T cell functioning and cytokine production upon sole re-exposure to the CS at later time points. These observed conditioned immune responses, which mimic the actual effects of the immunopharmacological agent used as UCS indicate that the immune system is now under CS-control. Given that conditioned responses gradually weaken over time following repeated exposure to the CS without the UCS, it is important to understand how such learned immune responses may be protected from extinction and how they can maintain on demand by CS re-exposure. Despite data highlighting the insular cortex' prominent role in taste-immune associative learning, findings indicate that within this paradigm, learning differs at the behavioral level (CTA) and at the level of the immune system with respect to neuronal correlates and/or circuits, respectively. Building on this, and to gain deeper insight into the neurobiological underpinnings of taste-immune associative learning and its extinction, the present work revealed reciprocal connections of the insular cortex with the nucleus of the solitary tract via fluorescence labeling. Subsequent work, using chemogenetic manipulations, demonstrate that this anatomical relationship is functionally relevant during extinction of learned taste-immune responses.

**Keyword 1**: Taste-immune associative learning
**Keyword 2**: Extinction
**Keyword 3**: Insular Cortex
**Keyword 4**: DREADD
**Keyword 5**: Conditioned taste avoidance

**PUPILLOMETRY REVEALS AUTONOMIC ADJUSTMENTS DURING DIVING REFLEX IN FACE IMMERSION APNEA**

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The human diving reflex (DR), an innate defensive reflex triggered during periods of apnea, concurrently activates both the sympathetic and parasympathetic branches of the autonomic nervous system to regulate physiology against challenging demands. Despite pupil dilation and constriction being antagonistically controlled by the two autonomic branches, the effect of the diving reflex on pupil diameter fluctuations is still unknown. Thus, we compared participants' pupil diameter fluctuations while breathing or performing apnea either with (Wet) or without (Dry) face immersion in cold water. We found that pupil diameter fluctuations in both apneic conditions are associated with lower power in the low-frequency band (< 0.25 Hz) while in the Wet condition, a reallocation of power towards higher frequencies (> 0.25 Hz) occurs, together with an increased entropy, contrary to the remaining conditions. This indicates a shift in autonomic balance and an increased complexity of pupil fluctuations during the diving reflex. Our findings present pupil dynamics as a valuable entry point to the exploration of autonomic adjustments for this reflex.

**Keyword 1**: Pupillometry
**Keyword 2**: Diving response
**Keyword 3**: Autonomic balance

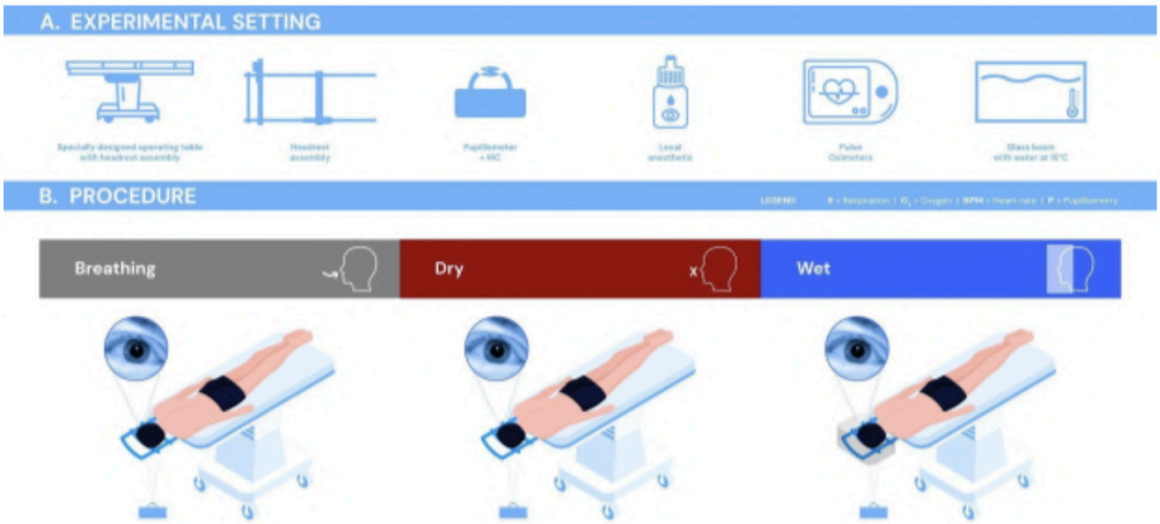



**Fig 1. Experimental setup.** a) Overview of the setup showing, from left to right, a custom-designed operating table with a headrest for head stabilization, a pupillometer with an infrared camera (IRC), an oximeter for measuring oxygen saturation and heart rate, and a transparent glass basin filled with water maintained at a constant temperature of 15°C, monitored via an internal thermometer. Anesthetic eye drops were used for the Wet condition. b) Illustration of the positioning of subjects, who were instructed to lie face down on the operating table

**HIPPOCAMPAL FUNCTIONAL CONNECTIVITY TO REGIONS OF INTEREST WITHIN THE PREFRONTAL CORTEX AND COGNITIVE FLEXIBILITY**

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Psychiatric disorders are primarily diagnosed through symptom-based clinical assessments, which often lack biological specificity. Recent research suggests that Network Neuroscience approaches focusing on neural connectivity may offer a framework to deepen our understanding of brain function and mental health.
An essential component of adaptive behavior that is impaired in multiple psychiatric and neurological disorders is the ability to flexibly adapt cognitive and emotional states to changing environments.
We hypothesize that the hippocampus plays a key role in this process through its interactions with brain regions such as the medial prefrontal cortex, as it encodes knowledge about the structure of the world that must be accessed flexibly in a context-appropriate manner. Dysregulation in hippocampal connectivity may thus contribute to maladaptive responses and increased vulnerability to psychopathology.
In this study, we used resting-state functional magnetic resonance imaging data from the Young Adult Cohort of the Human Connectome Project to investigate how hippocampal connectivity with key regions within the prefrontal cortex relates to cognitive flexibility and mental health scores by fitting linear regression coefficients. We also compare subregion-specific hippocampal connectivity, estimated by gradientography, to whole-hippocampus measures. Additionally, we assess whether resting-state functional connectivity can reliably predict cognitive flexibility in an independent dataset. This research aims to deepen our understanding of hippocampal involvement in psychopathology and its potential to inform connectivity-based diagnostic and therapeutic approaches.

**Keyword 1**: hippocampus
**Keyword 2**: prefrontal cortex
**Keyword 3**: functional connectivity
**Keyword 4**: cognitive flexibility

**FNIRS INSIGHTS INTO SOCIAL COGNITION AND SCHIZOPHRENIA: BRIDGING NEURAL AND CLINICAL OUTCOMES**

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Social cognition deficits are critical in schizophrenia, profoundly affecting interpersonal functioning and quality of life, yet their neural mechanisms remain incompletely understood. It is striking how prefrontal cortex (PFC) activity differs in schizophrenia patients during real-time social coordination tasks and to what extent inter-brain neural synchronization (INS) correlates with social cognitive performance. This review aims to synthesize findings on neural correlates of social cognition in schizophrenia, using the PRISMA model to focus on studies utilizing fNIRS, published since 2010 in PubMed, Web of Science, and Scopus databases. Ten experimental studies, selected via PRISMA guidelines with keywords "schizophrenia," "social cognition," "fNIRS," "prefrontal cortex," and "neural synchronization," explored paradigms such as facial affect recognition, theory of mind (ToM), social coordination, and emotional-cognitive interactions. These studies used fNIRS to measure PFC activity, INS, and dynamic functional connectivity (dFC) during these tasks. Results indicate that schizophrenia patients exhibit hyperfrontality during facial processing, reduced INS in cooperative tasks, and altered fronto-temporal activation compared to healthy controls, often linked to symptom severity. While some studies found no association between cortical activity and social cognitive performance, others identified dFC-based biomarkers, achieving 75.5–82.5% accuracy in distinguishing schizophrenia from controls. As a result, fNIRS-based assessments revealed disrupted neural mechanisms underlying social and cognitive deficits in schizophrenia, supporting the utility of personalized interventions. These findings highlight the potential of integrating fNIRS-derived metrics into clinical practice to enhance diagnostic precision and therapeutic outcomes for schizophrenia patients.

**Keyword 1**: Schizophrenia
**Keyword 2**: Social Cognition
**Keyword 3**: Fnirs

**BEYOND AGGRESSION: UNCOVERING THE PHENOTYPIC IDENTITY OF SOCIAL RANK IN MALE MICE**

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Aggression and dominance are defining features of how male mice interact socially. A common tacit assumption is that these are phenotypically parallel traits, reflecting a shared advantage in resource acquisition, sometimes also presupposing shared neural substrates. This thinking often leads to conflation of the two concepts when interpreting the behavioral effects of social circuit manipulations.

Here, we tested whether social rank predicts territorial aggression in male C57BL6 mice. Contrary to expectation, dominant animals (as identified by tube test) were significantly less likely to attack, regardless of the intruder's rank. When measuring rank via access competition in the tube test, we observed that dominant mice made regular concessions primarily to familiar lower-ranked individuals, effectively granting them "free wins". This pattern of dyad interaction still guarantees a high win performance by dominants.

Given that rank best predicts a social strategy, we hypothesized that it represents a phenotypic identity which may persevere outside social interactions. To examine this possibility, we employed unsupervised machine learning (deep variational embeddings of animal motion, VAME) to deconstruct behavior into fundamental elements. During open-field exploration, mice exhibited a flexible representation of their own rank, which was further modulated by contextual cues, revealing a surprising degree of specificity between exploratory behavior and social status (longitudinal decoding analysis).

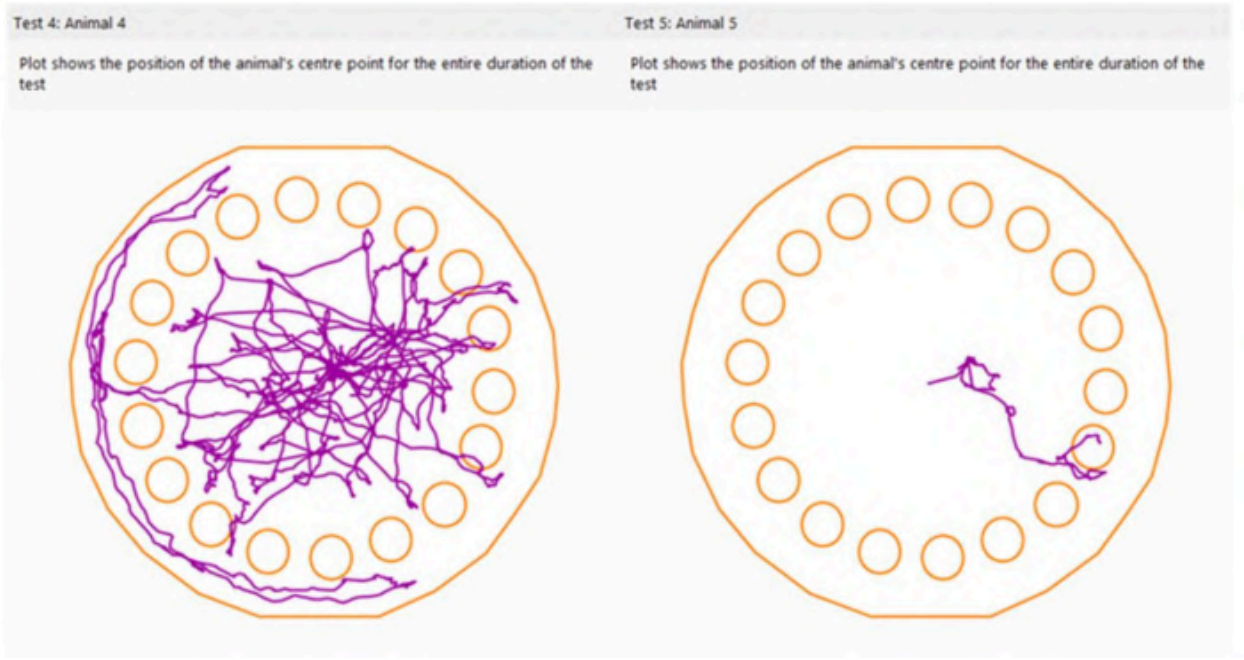
These findings suggest strategies for social dominance which do not necessarily engage neural circuits that drive aggression. We also provide empirical support for a phenotypic identity, by showing that a male mouse's exploratory pattern is predictive of its social rank.

**Keyword 1**: Hierarchy
**Keyword 2**: Internal state
**Keyword 3**: Computational Neuroethology
**Keyword 4**: Decoder

**COMPARATIVE RESEARCH ANALYSIS OF LEARNING IN ACTIVE AVOIDANCE AND BARNES MAZE TASKS IN RATS**

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Present study is a comparative research of correlation between two widely employed behavioral paradigms – two-way active avoidance and the Barnes maze.To correctly asses this correlation we worked with two experimental designs. In the first design experimental animals were tested first in active avoidance task and then in Barnes Maze. In the second design the behavioral tasks order was reversed. In both cases experimental animals were adult albino rats of both genders. During active avoidance task rats were subjected to total of 100 consecutive pairings of conditioned (tone) and unconditioned (mild foot shock) stimuli, with 1min interval between each pairing. Barnes maze was performed with three separate trials: 2 learning trials with 1hr interval between them and 1 test trial, 24 hr later. In both experimental designs learning protocols of active avoidance and Barnes maze remained unchanged.
Acquired results demonstrate that rats who excel in active avoidance learning perform poorly in Barnes maze and vice versa. Our data suggest that fear likely plays a role in this reciprocal relationship.
We think that our results can act as a helpful tool for screening and selecting good and bad learner experimental subjects prior to selected learning task, especially when researching effects of memory enhancing drugs or working with inhibited memory in various animal models.



**FUNCTIONAL ANALYSIS OF AXO-AXONIC CELLS IN THE BLA DURING FEAR LEARNING**

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Neuronal competition plays a central role in selecting neurons involved in memory encoding. While the properties of excitatory neurons in competitive mechanisms of memory encoding have been extensively studied, the role of inhibitory neurons remains poorly understood. In this study, using fear conditioning as a model for fear memory encoding, we found that an imbalance in the strength of inhibition by axo-axonic cells (AACs) plays a crucial role in selecting the neurons that encode fear memories during learning. In vivo calcium imaging of AACs in the basolateral amygdala (BLA) reveals that AACs are synchronously activated by footshock but not by conditioned stimuli. Attenuating neurotransmission from AACs increased the number of activated neurons and disrupted fear conditioning, indicating that AAC-mediated inhibition is essential for maintaining proper neuronal competition during fear learning. To further investigate how AAC-mediated inhibition affects neuronal competition, we combined the Robust Activity Marking (RAM) system with patch-clamp recordings. The results showed that neurons inactive during learning (RAM-negative) received stronger inhibitory input from AACs compared to activated neurons (RAM-positive). Furthermore, monosynaptic rabies virus tracing revealed that AACs have distinct local and long-range input properties compared to parvalbumin-positive and somatostatin-positive cells, suggesting that AACs integrate distinct input signals that may contribute to their role in neuronal selection. Taken together, these findings highlight the involvement of AACs in the neuronal selection process for memory encoding and provide novel insights into their functional role in the BLA.

**Keyword 1**: fear conditioning
**Keyword 2**: Amygdala
**Keyword 3**: axo-axonic cell
**Keyword 4**: neuronal compitition
**Keyword 5**: interneuron

**THE INVOLVEMENT OF CHOLINERGIC LATERAL SEPTUM NEURONS IN PROCESSING ANXIETY AND NOXIOUS STIMULI IN MALE AND FEMALE MICE**

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The lateral septum (LS) plays a crucial role in regulating emotional states like anxiety and aggression and mediates social behaviors, potentially in a sex-specific manner. Although traditionally thought to consist solely of GABAergic cells, our research has identified a subset of LS cells that express cholinergic markers.
This study investigates the function of LS cholinergic neurons (LSCNs) by manipulating their activity through optogenetics and assessing behavioral correlates in anxiety-related tasks, including the open field test and place preference test, and by using fiber photometry to measure neuronal responses to noxious stimuli. Cellular composition and anatomical tracing studies have also been conducted.
Our findings indicate that optogenetic activation of LSCNs induces an avoidance bias in the stimulation-coupled chamber in the place preference test, and anxiety-like behavior in the open field test, more prominent in males. Both footshock and air puff increased LSCN activation, while acetylcholine levels in the anterior hypothalamus, their main target area, fluctuated. We also observed substantial input from somatostatin (SOM) terminals to LSCNs.
We plan to employ various techniques such as chemogenetic manipulation and electrophysiology to further elucidate the functional role of LSCNs in the LS and their implications for emotional, social behavior, and cognitive function in both physiological and dementia mouse models.
This research was supported by the MTA-HUN-REN, the National Research, Development and Innovation Office (NKFIH K135561, NKFIH K147097), the Hungarian Brain Research Program 3.0 (NAP2022-I-1/2022) and LP2024-8/2024 from the Hungarian Academy of Sciences.

**Keyword 1**: Septum
**Keyword 2**: Cholinergic
**Keyword 3**: Anxiety
**Keyword 4**: Optogenetics

**INHIBITION MEDIATED BY TYPE III MGLUR'S REGULATES HABENULAR ACTIVITY AND DEFENSIVE BEHAVIORS**

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Habenula is an evolutionary conserved brain region that was shown to play important roles in adaptive and defensive behavior. Habenula received excitation and inhibitory intputs from the forebrain and relays this information to monoaminergic brain nuclei. We observed that several members of group 3 metabotropic glutamate receptors (mGluRs) expressed in zebrafish habenula. Moreover, targeting type III mGluR's pharmacology, alters the membrane potential and the connectivity of habenular neurons. Targeting type III mGluRs with both, genetic and pharmacological perturbation directly modulates the ongoing and sensory driven activity in habenula and alters defensive and adaptive behaviors in juvenile zebrafish. Altogether, our results propose an important role for type III mGluR's for regulating habenular circuits and animal behavior.

# VIOLIN MAJOR MUSIC ALLEVIATES UNIVERSITY STUDENTS' ANXIETY MAYBE THROUGH THE DACC AND DLPFC CIRCUITS

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## INTRODUCTION

State anxiety is a prevalent emotion that, if left unmanaged, can escalate into severe disorders. Music therapy has shown promising potential in recent years. However, existing studies have insufficiently explored the impact on characteristics of music, with the neural circuit underlying remains unclear. This research aims to examine and offer new insights into music therapy's effects on anxiety relief, focusing on the impact of different musical tonalities and timbres with sLORETA completing the brain localization.

## METHODS

This research selected major and minor pieces from Bach's repertoire to compile a library named Piano Major (PA), Piano Minor (PI), Violin Major (VA), and Violin Minor (VI). The anxiety data was recorded, with Wavelet transform and Fast Fourier Transform being conducted. The SLORETA-KEY is employed to pinpoint signal origins in low-resolution brain imaging, which will be applied to complete brain localization.

## RESULTS

Violin major key music had the strongest anxiety-relieving effect (Figure 1A, 1B), aligning with anxiety's high-energy nature (Juslin & Laukka, 2004). Anxious individuals, influenced by high-energy emotional states, responded better to high-energy music. Violin's higher frequency and rougher timbre (Figure 1D, 1E) may disrupt cognitive focus, redirecting attention and reducing tension (Mogg & Bradley, 1998).

Neural analysis via sLORETA showed violin major music significantly activated the dorsal anterior cingulate cortex (dACC) and dorsolateral prefrontal cortex (DLPFC) (Figure 1F, 1G). The dACC is crucial in anxiety genesis, while DLPFC activation, often reduced in depression, is linked to antidepressant effects (Fox et al., 2012), supporting these findings.

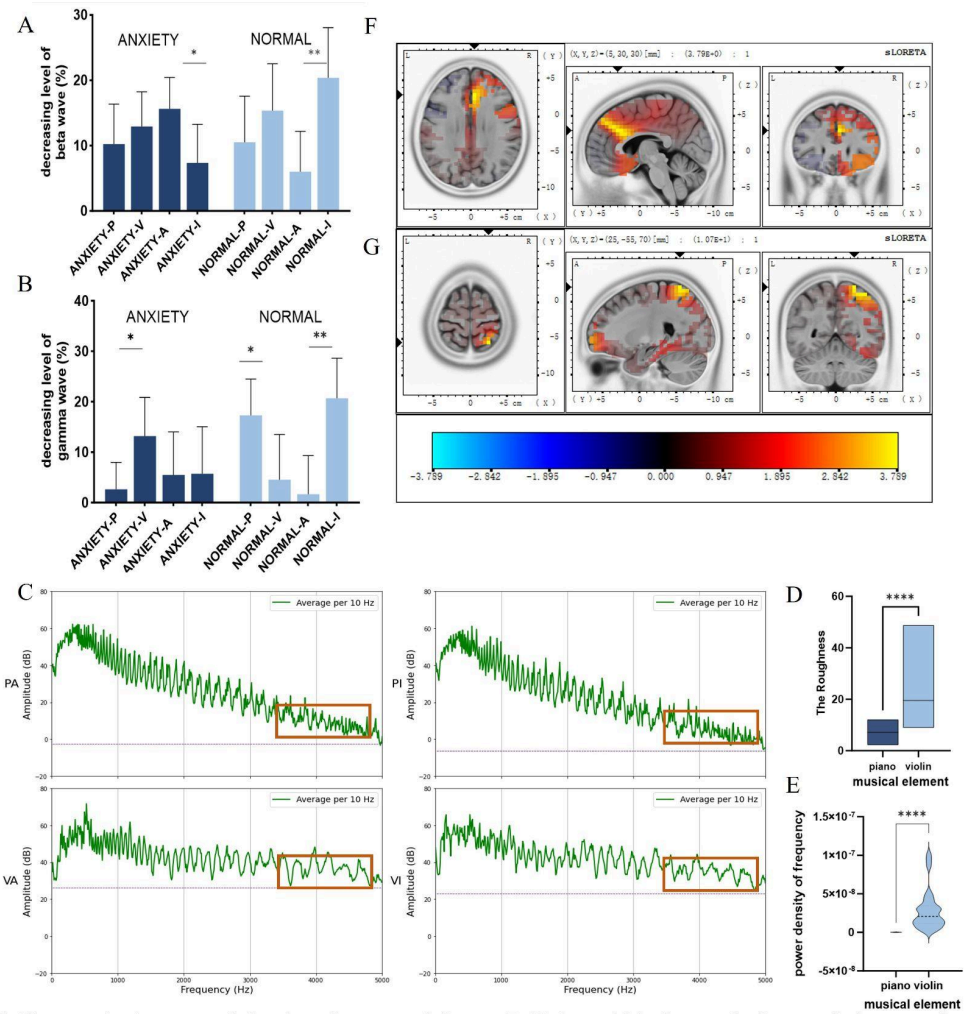



Fig 1. (A-B) Changes in the spectral density of separated factors P, V, A, and I before and after music intervention in beta-brain waves (A) and gamma-brain waves (B). (C) Spectrograms of the PA, PI, VA, and VI experimental tracks averaged every 10 Hz. (D-E) Roughness (D) and power density of frequency (E) between piano pieces and violin pieces in the 4000 Hz--5000 Hz range. (F-G) Changes in the spectral density of separated factors P, V, A, and I before and after music intervention in beta-brain waves (F) and gamma-brain waves (G). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$. P = PA + PI; V = VA + VI; A = PA + VA; I = PI + VI. Abbreviations: PA, piano major; PI, piano minor; VA, violin major; VI, violin minor; P, piano; V, violin; A, major; I, minor. FFT: fast Fourier transform.

**Keyword 1**: Anxiety
**Keyword 2**: Music therapy
**Keyword 3**: EEG
**Keyword 4**: sLORETA

**DIFFERENTIAL NEURAL CORRELATES OF SIGN- AND GOAL-TRACKING IN THE NUCLEUS ACCUMBENS AND LOCUS COERULEUS: IMPLICATIONS FOR OUR UNDERSTANDING OF RESILIENCE AGAINST THE DEVELOPMENT OF COMPULSION**

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The biobehavioural factors contributing to the individual vulnerability to lose control over coping behaviours and develop compulsion have not been fully elucidated. The development of a polydipsic coping response under a schedule-induced polydipsia (SIP) procedure, and the propensity to attribute incentive value to reward cues (ie. sign-tracking), both depend on dopaminergic mechanisms in the nucleus accumbens (NAc), which are regulated by local acetylcholine. The vulnerability to develop compulsive coping under SIP is instead associated with alterations in the noradrenergic locus coeruleus (LC). The role of LC-dependent noradrenergic mechanisms to sign-tracking, or how sign-tracking relates to the vulnerability to switch from controlled to compulsive coping remain unknown.
We demonstrated that Sprague Dawley sign-tracker rats developed polydipsic coping behaviour more readily than goal-trackers, which instead were protected against the transition to its compulsive manifestation, hyperdipsia. We then used fibre photometry to measure dopamine and acetylcholine release in the NAc alongside calcium transients in NAc-projecting noradrenergic LC neurons in sign- and goal-tracker rats. Goal-trackers and sign-trackers exhibited distinct and sex-dependent NAc DA/ACh release and LC-NAc activation signatures to the reward and reward-predictive cue that predictably evolved over training, thereby suggesting that early interactions between NAc dopamine, acetylcholine and noradrenaline may shape the development of sign- vs goal-tracking. Since these signatures evolve within the first autoshaping session, catecholaminergic and cholinergic processes were shown jointly to track learning of the cue-reward association. Our data reveal differences in NAc and LC dynamics across Pavlovian learning between rats with distinct approach tendencies and vulnerabilities to compulsion.

**Keyword 1**: Compulsivity
**Keyword 2**: Nucleus accumbens
**Keyword 3**: Sign-tracking
**Keyword 4**: Locus coeruleus
**Keyword 5**: Catecholamine

**SEX-DEPENDENT DIFFERENTIAL CONTROL OF ALCOHOL DRINKING UNDER NEGATIVE REINFORCEMENT BY MEMORY AND INTEROCEPTIVE SYSTEMS IN RATS**

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We are not equally prone to engage the same memory system to acquire tasks such as spatial navigation. Spatial learning by place or response strategies depends on dissociable hippocampus- or dorsolateral striatum-dependent substrates. Response learning in humans is associated with greater lifetime addictive drug use. However, the direction of any causal relationship remains unclear. Using a rodent model of alcohol drinking to cope with stress, which exacerbates the vulnerability to alcohol use disorder, we investigated whether the individual tendency to rely on response versus place memory systems shaped the vulnerability towards compulsive alcohol drinking, the neural systems engaged by alcohol drinking, or both. Twenty-one (11 female) Sprague-Dawley rats were identified as place/response learners in a spatial navigation task and subsequently characterised for their tendency to rely on alcohol to develop a polydipsic response under a schedule-induced polydipsia (SIP) procedure, the compulsive manifestation of which was assessed as the persistence of drinking despite quinine adulteration. Micro-punches extracted from animals sacrificed 45min after a SIP-alcohol session were processed for quantitative polymerase chain reaction to measure *c-fos* mRNA levels. Individual differences in adjunctive or compulsive alcohol drinking were not predicated on a prevailing navigation strategy. However, in males, *c-fos* mRNA levels in the anterior and posterior insula were higher in response than in place learners. In females, diametrically opposite differences were restricted to the posterior insula. This suggests a sex-dependent relationship between the individual tendency to utilise dissociable memory systems and the recruitment of interoceptive processes controlling alcohol drinking as a coping strategy.

**Keyword 1**: Alcohol
**Keyword 2**: Spatial Learning
**Keyword 3**: Interoception
**Keyword 4**: Stress

**SEX DIFFERENCES IN THE STRIATAL ASTROCYTIC TRANSCRIPTOME ARE ASSOCIATED WITH A SEXUALLY DIMORPHIC ASTROCYTE-MEDIATED RECRUITMENT OF INCENTIVE HEROIN-SEEKING HABITS**

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Compulsive opioid seeking, a hallmark feature of opioid use disorder (OUD), arises from the loss of control over incentive drug-seeking behaviours. In males, incentive heroin-seeking habits (IHSHs) develop when protracted cue-controlled heroin seeking engages a dopamine-dependent functional coupling between the nucleus accumbens core (NAcC) and the anterior dorsolateral striatum (aDLS), likely involving alterations in the striatal astrocytic dopamine transporter (DAT). Because males and females exhibit profound differences in their vulnerability to develop OUD, we investigated whether the mechanisms underlying the development of IHSHs differ between the sexes.

In female rats, cue-controlled heroin seeking became sensitive to intra-aDLS dopamine receptor blockade following the same time course as observed in males. However, males and females develop IHSHs via different cellular mechanisms, as revealed by a causal bidirectional modulation of NAcC astrocytes (using a virally transduced membrane calcium pump or Gq DREADD). Activation of NAcC astrocytes expedited the functional recruitment of the aDLS in females but prevented it in males, who instead showed a more rapid transition to aDLS control of behaviour after inhibition of NAcC astrocytes.

Cell type-specific spatial transcriptomics revealed that, across the striatum, males expressed more astrocytic DAT mRNA than females, while females expressed more connexin 43 in the aDLS than males. In females only, high levels of astrocytic estrogen receptor 1 mRNA were associated with lower levels of astrocytic DAT. Altogether, these findings demonstrate that striatal astrocytes regulate the ventral-to-dorsolateral striatum functional shifts that underlie the development of IHSHs through molecular mechanisms that differ between the sexes.

**Keyword 1**: Addiction
**Keyword 2**: Habits
**Keyword 3**: Astrocytes
**Keyword 4**: Sex differences
**Keyword 5**: Dopamine

**REPEATED NON-CONTACT EXPOSURE TO PUPS INHIBITS INFANTICIDAL AND FACILITATES PATERNAL BEHAVIOR IN VIRGIN ADULT MALE MICE (C57BL6)**

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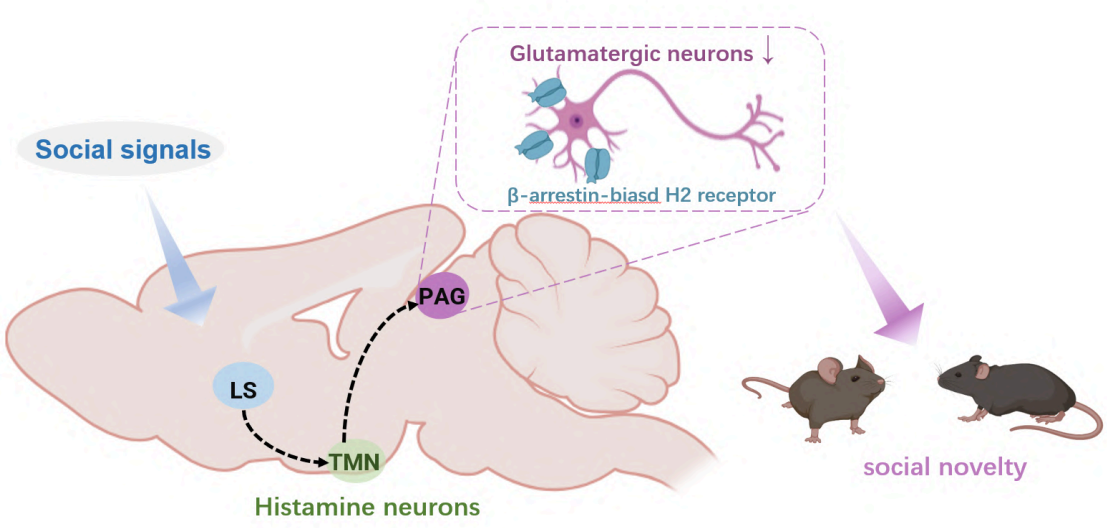
Pup-naïve virgin adult male C57BL6 mice are mainly infanticidal when exposed to pups for the first time. The processes underlying pup-directed aggression and the transition toward parental care are poorly understood. Social isolation has been shown to inhibit infanticidal behavior in some strain of mice. However, it is unclear if highly infanticidal male CB57BL6 mice can sensitize after repeated exposures to pups. The aim of this study was to determine whether repeated non-contact exposure to pups (to prevent immediate attack), with or without movement restriction and social isolation, can inhibit infanticidal behavior in male mice. We also investigated whether pup-directed aggression was associated with male-male aggression in a resident-intruder test. We found that repeated non-contact exposure to pups, in socially isolated males or in males with movement restraint, significantly reduced the incidence of aggression towards pups and increased the incidence of parental behavior. Social isolation or movement restraint alone had no significant effect. Finally, the frequency of pup-directed aggression was not associated with the levels of male-male aggression. This study shows that the experience of being exposed to newborns without contact with them can inhibit the highly driven impulsive-like attacking behavior towards pups and facilitate parental behavior. Our results suggest that aggressive behavior towards pups can be blocked in naïve male mice and that this behavior differs from male-male aggression.

**Keyword 1**: Infanticidal
**Keyword 2**: Impulsivity
**Keyword 3**: aggression
**Keyword 4**: restraint
**Keyword 5**: Social isolation

**A DEDICATED HYPOTHALAMUS-MIDBRAIN HISTAMINERGIC CIRCUIT MEDIATES SOCIAL NOVELTY**

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Social behavior is an inherent and primitive behavior in animals including human beings, wherein social dysfunction leads to a variety of neuropsychiatric disorders such as autism and schizophrenia. Social novelty preference (SNP) represents an intrinsic behavioral paradigm that animals exhibit a pronounced propensity toward socially novel conspecifics over familiar ones, a phenomenon critical for evolutionary adaptation and reproductive success. Histamine, a pivotal neurotransmitter, has recently been implicated in the modulation of social behaviors. Nonetheless, the neural circuits that integrate the histaminergic system with social novelty seeking remain elusive, thereby hindering the development of histamine-targeted therapies for neuropsychiatric disorders. In this study, we identify a neural circuit projecting from the tuberomammillary nucleus (TMN) to the periaqueductal gray (PAG) that is intricately associated with social behavior in mice. We elucidate that the PAG-projecting histamine circuit precisely mediates social novelty, independent of social memory mechanisms. Histamine acts as a reinforcement signal for social novelty within the core of the PAG, exerting its effects through glutamatergic neurons via a β-arrestin-biased H2 receptor signaling pathway. Furthermore, we demonstrate the therapeutic potential of H2 receptor modulation in a sodium valproate-induced autism model. Additionally, we reveal that histaminergic neurons integrate social signal inputs from the lateral septum (LS), an upstream brain region. Collectively, our findings delineate a novel hypothalamus-midbrain circuit that fine-tunes non-memory-based social novelty, offering new insights into the neural mechanisms underlying social behavior and potential avenues for treating neuropsychiatric conditions.




**Keyword 1**: Social novelty
**Keyword 2**: histamine
**Keyword 3**: circuit
**Keyword 4**: periaqueductal gray
**Keyword 5**: autism

**SOCIAL MEMORY DEFICITS INDUCED BY A BRAF HYPERACTIVATING MUTATION IN AN INTERNEURON-SPECIFIC MOUSE MODEL OF RASOPATHY AND THEIR RESCUE BY CHRONIC DENTATE GYRUS INTERNEURON INHIBITION**

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RASopathies are a family of developmental disorders that affect approximately 1 in 1000 individuals and are caused by germline mutations in genes encoding components of the Ras-ERK pathway. Patients have a higher probability of experiencing neuropsychological impairments such as cognitive, social, and emotional challenges. Although cognitive alterations have been considerably characterized in different mouse models of RASopathies, social and emotional dysregulations and their underlying neuronal mechanisms have not been rigorously explored so far. Therefore, a conditional mouse line was established with a selected knock-in hyperactivating mutation of the BRAF gene (central component of the Ras-ERK pathway) exclusively in forebrain GABAergic interneurons ($BRAF^{Q241R\,floxed/wt}$-$DLX5\text{-}6^{Cre/+}$ or DBRAF mice) and its effects on social, emotional, and cognitive behaviors were characterized. DBRAF mice exhibited an increased sociability phenotype followed by impaired social recognition memory in the three-chamber sociability test. Additionally, they showed a pronounced anxiety phenotype in the elevated plus maze and open field tasks, and a deficit in working memory in the Y-maze. A significantly increased number of somatostatin-positive interneurons in the dorsal hilus accompanied by a facilitated long-term potentiation in the dorsal dentate gyrus (dDG) in DBRAF mice led us to explore a potential involvement of this hippocampus subregion in the observed behavioral phenotypes. We found that chronic chemogenetic inhibition of dDG interneurons rescues the social memory deficits in DBRAF mice without affecting their anxiety and working memory behaviors. These results uncovered a critical role of BRAF signaling in dDG GABAergic interneurons in the emergence of social memory dysfunctions.

**Keyword 1**: BRAF
**Keyword 2**: RASopathy
**Keyword 3**: Social memory
**Keyword 4**: Dentate gyrus

**ACTIVATION OF NEURONAL ENSEMBLES ENHANCES PATTERN SEPARATION**

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The dentate gyrus (DG) is critical for memory encoding and retrieval of episodic memories and has been established as a site of cellular engrams during contextual memory formation. Computing pattern separation, the DG helps to distinguish environmental inputs and to minimize interference between memories. Here, investigated the role of a cellular engram in the dorsal DG in differentiating contextual features during fear memory retrieval. To this end, young adult (4-month-old) C57Bl/6 mice underwent fear conditioning (three unconditional stimuli US foot shock: 0.4mA for 1s, 20s aftershock interval) and were examined for their fear response 1-day after the training in the originally trained or in an altered context. Engram cells were labelled with virally (AAV) delivered doxycycline controlled tTA/TRE Robust Activity Marking (RAM) vectors and immunohistochemical cFos detection and were selectively stimulated during fear memory retrieval with RAM-dependent-DREADD (Designer Receptors Exclusively Activated by Designer Drugs). cFos labelling confirmed the stimulation of engram cells by DREADD activation regardless of context. Behaviorally, mice after engram activation show improved discrimination of altered and original context, with reduced freezing in the altered context and unaltered freezing level in the original context. These results are in line with a role of DG ensemble activation in pattern separation during retrieval of contextual fear memories. Further, an increase in cFos activation in the hilus of DREADD activated animals suggests a potential role for the recruitment of local interneurons in the differential behavioral response.

**Keyword 1**: engram
**Keyword 2**: pattern separation
**Keyword 3**: novelty detection
**Keyword 4**: neuronal ensembles
**Keyword 5**: engram reactivation

**TOPOGRAPHIC ORGANIZATION OF THE DIVERSITY OF CORTICAL FAST-SPIKING GABAERGIC NEURONS**

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GABAergic neurons represent about 15% of the neuronal population of the cortex but show a wide diversity. The largest group of GABAergic neurons is represented by Parvalbumin (Pvalb)-expressing fast-spiking interneurons, accounting for 40-50% of cortical interneurons. This GABAergic class, transcriptionally characterized by the expression of Pvalb, includes basket cells and chandelier cells, targeting the perisomatic compartment of excitatory neurons. Although Pvalb neurons represent the largest population, the distribution of Pvalb-expression is not homogeneous in the cortex, being much lower in cortices if the lateral association network. We demonstrated that despite the low density of Pvabl-neurons in association cortices, most fast-spiking GABAergic neurons do not express Pvalb. We also show that fast-spiking neurons in association cortices have slower biophysical properties as compared to those in primary somatosensory cortex. Our results suggest that the Pvalb transcriptomic class shows a topographically organized diversity that might tune the biophysical properties of these neurons to the computational demands of association cortices. Our in vitro electrophysiological experiments suggest that these neurons provide feedforward inhibition in response to optogenetic stimulation of sensory inputs. In the lateral association network, these cells might be involved in gating sensory inputs along the cortico-hippocampal network.

**Keyword 1**: Interneurons
**Keyword 2**: Cortex
**Keyword 3**: parvalbumin
**Keyword 4**: inhibition
**Keyword 5**: neuronal diversity

**DIFFERENTIAL CONTRIBUTIONS OF INHIBITORY NEURONAL SUBTYPES TO FORMS OF SHORT- AND LONG-TERM HABITUATION**

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Mismatch negativity (MMN) and 1/f slope are phenomena measured in the electroencephalogram (EEG). MMN is an increase in the magnitude of cortical event-related potentials (ERPs) in response to an unexpected stimulus within a sequence of familiar stimuli, revealing the effects of short-term habituation. Recent work has shown that MMN serves as a readout of neocortical GABAergic inhibitory neurons, which are often affected in neurodevelopmental disorders, but little is known about the specific types of neurons involved. Stimulus-selective response plasticity (SRP) is a robust form of cortical potentiation observed by an increase in local field potential (LFP) ERPs to highly familiar visual stimulus in the mouse primary visual cortex (V1). GABAergic inhibitory neurons have been shown to play a key mechanistic role in SRP; however, this form of long-term habituation produces seemingly opposite LFP response to MMN.

We used genetic tools in mice to inactivate parvalbumin-expressing (PV+) neurons or somatostatin-expressing (SST+) neurons within V1 during orientation-specific, visual MMN as well as multi-day repeated stimuli (SRP) paradigms in mice.
Inactivating PV+ neurons leaves MMN unaffected but significantly alters the ability to distinguish familiar from novel in long-term habituation, whereas inactivating SST+ interneurons impairs both forms of habituation, suggesting differential contributions of inhibitory subtypes across timescales.
We also present future work that aims to investigate the translation of these habituation mechanisms across timescales into humans using infant EEG paradigms. This study is being conducted in 5–7-month-old infants and aims to assess if SRP can be reliably observed in infant scalp EEG.

**SPATIAL MULTIOMIC ASSAY FOR STUDYING INTERNEURON HETEROGENEITY IN THE BRAIN**

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Interneurons are a diverse group of inhibitory neurons that play an essential role in maintaining the delicate balance and function of neural circuits within the brain. Any disruptions of the balance between excitatory and inhibitory signals can lead to complex neurological disorders, age-related cognitive decline, and psychiatric disorders. Neurological and aging-related disorders lead to structural and functional changes, such as alterations in synaptic plasticity, neurotransmitter levels, and neural connectivity. Interneurons, particularly within the hippocampus, the cortex, and the striatum, are integral to memory formation, learning, and sensory processing. Therefore, valuable insights can be gained from understanding the interneuron diversity in the brain.

To enable optimum protein detection with RNA, we have developed a new protease-free workflow for the RNAscope™ Multiplex Fluorescent V2 assay that can detect up to 4 RNA targets with proteins. This assay utilizes a TSA-based amplification strategy that boosts the signal for both RNA and protein targets. We utilized 3 RNA probes against three widely studied interneuron types: *Pvalb* for Parvalbumin neurons, *Vip* for Vasoactive Intestinal Peptide, and *Sst* for Somatostatin interneurons. An antibody against NeuN was used to stain the nuclei of all mature neurons.

RNAscope™ offers a powerful technique for studying cellular diversity by visualizing target RNAs with protein markers in brain tissue sections. The protease-free workflow enables optimal protein detection by preserving antigen integrity and tissue morphology. This multiomic workflow valuable insights required to study complex tissues like the brain and aids in understanding interneuron heterogeneity at different ages and in various neurological disorders.

**Keyword 1**: Spatial Multiomics
**Keyword 2**: Cellular diversity
**Keyword 3**: Interneuron heterogeneity
**Keyword 4**: Neurological disorders
**Keyword 5**: RNA - protein markers

**CONDITIONAL KNOCKOUT OF NDR2 IN FOREBRAIN GABAERGIC INTERNEURONS INCREASES CONTEXT-DEPENDENT ACTIVITY LEVELS AND REDUCES SHOCK-INDUCED FREEZING BEHAVIOR IN MICE**

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The nuclear Dbf2-related protein kinase 2 (Ndr2) belongs to the family of AGC protein kinases and plays an important role in the differentiation and migration of neuronal cells and the control of neurite growth and branching, as well as synapse formation and synaptic plasticity. Increased expression of Ndr2 in the mouse amygdala during fear-memory consolidation has also suggested its involvement in the regulation of emotional behavior. Although expression of Ndr2 is prominently observed in inhibitory interneurons, the impact of Ndr2 specifically in inhibitory interneurons of the forebrain has not yet been investigated.
Here, we characterize a new mouse line with a conditional ablation of Ndr2 selectively in GABAergic interneurons of the forebrain using driver mice expressing CRE recombinase under a DLX5/6 promoter. Behavioral characterization of these mice shows that, while the baseline home cage activity is not affected by the conditional knockout of Ndr2 a strong hyperactivity, without apparent changes in anxiety-related behavior, in both the elevated plus maze and the Y maze is displayed. Moreover, the mutants display reduced shock-induced freezing during fear conditioning training and retrieval. These data indicate an increased locomotor arousal response of Dlx5/6-Ndr2 mutants; underlying effects on neuronal circuit activation and regional changes in the function of inhibitory interneurons are currently under investigation.

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**Keyword 1**: Ndr2
**Keyword 2**: Interneurons
**Keyword 3**: Context-dependent activity

**INVESTIGATING THE BRAIN MECHANISMS BEHIND LOOKING, SEEING, AND DECISION-MAKING IN A VISUAL SEARCH TASK USING EVENT-RELATED POTENTIALS (ERP)**

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In a visual search task to find a target, first, attention needed to be guided to the target, this is looking. The task often involves a subsequent perception of the target, this is seeing. Finally, a decision is made on the target, completing the task. Typically, these three processes, looking, seeing, and decision-making are temporally overlapping with each other. To dissect the processes, we adapted a visual search task in a previous study (Zhaoping & Guyader (2007)) involving four different stimulus conditions, Asimple, Bsimple, A, and B, and the target can be near or far from the center of gaze. Fixating to the display center, observers covertly searched for a uniquely oriented bar to report whether the target is in the left or right half of the search array. This target bar is more salient in conditions Asimple and Bsimple, guiding looking more strongly, as manifested by a stronger N2pc component in the ERP. As the target in conditions A and B is less salient, observers are typically compelled to see the target before decision making. However, due to crowding, perceiving the target's shape clearly is nearly impossible when the target is far from the gaze. The target in condition A, but not condition B, is part of an object that is a rotated version of other objects in the search array, confusing the observers in decision-making after they see the near target, manifested in the late components across frontal electrodes for the near but not the illegible far target.

**LAYER 5 MARTINOTTI AND PYRAMIDAL NEURONS CONTRIBUTE DISTINCTIVELY TO SPATIAL INFORMATION ENCODING IN THE MOTOR CORTEX**

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Mammals rely on external sensory cues and internal bodily signals to create a precise spatial awareness. The motor cortex is essential for this process, integrating proprioceptive and spatial information. Here, we aimed to elucidate the neural mechanisms underlying spatial processing in the motor cortex. Our study revealed an intriguing finding that both pyramidal cells and layer 5 martinotti cells (Mα2), a subset of somatostatin-positive interneurons, can be tuned to place (PC), head direction (HDC), and grid(GC) within the primary motor cortex. We recorded neuronal activity using miniscopes in freely moving WT mice expressing CaMKIIa-GCaMP8f and in Chrna2-Cre+ mice expressing DIO-GCaMP8f to examine pyramidal and Mα2 cell activity, respectively. Our analysis shows that Mα2 cells exhibit a contingency between PCs and HDCs, with an overrepresentation of cells classified as both PC and HDC compared to chance. Similarly, pyramidal cells showed an overrepresentation of cells classified as both PC and HDC compared to chance.Moreover, we found that pyramidal cells exhibit more precise tuning for spatial information than Mα2 cells. Specifically, pyramidal PCs were more sharply tuned than Mα2 PCs, and pyramidal HDCs were more sharply tuned than Mα2 HDCs. Further, Mα2 PCs showed increased activity compared to non-PCs, whereas pyramidal PCs were less active than non-PCs. Additionally, both Mα2 and pyramidal HDCs exhibited reduced activity compared to non-HDCs. These findings suggest that distinct neural coding strategies are employed by different cell types within the motor cortex for processing spatial information, highlighting the complex neural mechanisms underlying spatial cognition.

**THE ROLE OF LAYER 5 MARTINOTTI CELLS IN MOTOR LEARNING: INVESTIGATING THE NEURAL CIRCUITRY UNDERLYING COMPLEX MOTOR ACTIONS**

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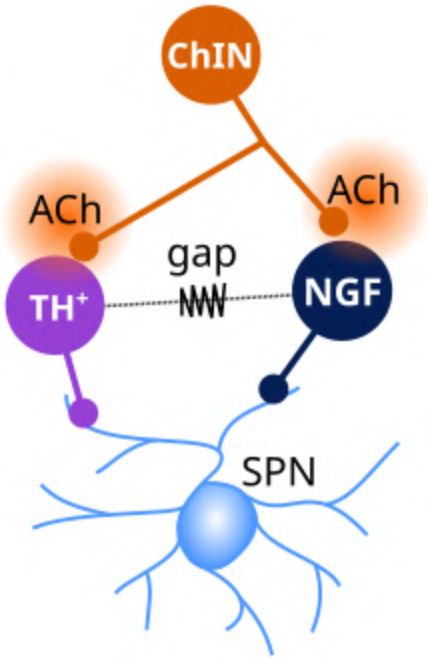
Motor actions require precise and coordinated neural processes to execute movements, such as grasping an object or playing a musical instrument. Cortical structures have been implicated in controlling forelimb movements, with activation of both cortical hemispheres being crucial for complex dexterity. This study aims to investigate the role of cortical processing, specifically layer 5 martinotti alpha 2 (Mα2) cells activity, in learning and performing complex movements. Previous studies have highlighted the importance of layer 5 Martinotti cells in synchronizing cortical pyramidal cell activity, but their involvement in motor learning remains to be investigated. Here, Chrna2-Cre mice were used to target, record and manipulate Mα2 cells activity. A reaching learning task was combined with optical imaging using miniscopes to examine Mα2 calcium activity during movement execution and learning performed over consecutive training sessions. We found that Mα2 cells increase their activity during learning, suggesting an important role for these cells in motor learning. In addition, Mα2 activity peaks earlier before successful prehensions than before failed ones, and its activity peak becomes broader with training for successful prehensions. These findings suggest that Mα2 plays a role in coordinating movement planning and execution during complex motor tasks. Furthermore, they provide new insights into the neural circuitry of motor learning, which has significant implications for understanding the neural basis of motor disorders such as Parkinson's disease and ataxia. The results may also contribute to developing novel therapeutic approaches for improving motor function in individuals affected by these conditions.

**INVESTIGATION OF THE FUNCTION OF NEUROGLIAFORM AND TYROSINE HYDROXYLASE POSITIVE INTERNEURONS IN A LARGE-SCALE BIOPHYSICALLY DETAILED MODEL OF THE STRIATAL MICROCIRCUIT**

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Recent advances in experimental techniques have enabled the creation of animal models that precisely target cell types based on specific molecular markers. This progress has led to the identification of numerous newly described neuronal subtypes and states, highlighting the remarkable complexity of neural tissue. While the functions of many newly described subtypes remain largely unexplored, emerging experiments are beginning to elucidate their roles. For example, recent studies demonstrate that co-activation of GABAergic interneurons and glutamatergic synapses on striatal projection neurons enhance glutamatergic input by amplifying dendritic spikes. In this study, we develop biophysically detailed models of neurogliaform interneurons and tyrosine hydroxylase positive interneurons and integrate them into a large-scale model of the striatal microcircuit. Using the tool Snudda we perform touch detection and pruning to create realistic connectivity patterns and investigate the mechanisms and conditions under which co-activation of these GABAergic interneurons facilitates signal integration at glutamatergic synapses.

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**Keyword 1**: striatal microcicuit
**Keyword 2**: dendritic integration
**Keyword 3**: gaba+glutamate
**Keyword 4**: thyrosine hydroxilase positive interneuron
**Keyword 5**: neurogliaform interneuron

**FEAST OR FRIENDSHIP: ROLE OF OXYTOCIN IN BEHAVIOURAL PRIORITISATION**

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In the pursuit of survival, animals constantly adapt their behaviour to environmental cues and internal states. This process involves allocating cognitive resources in the brain to promote relevant behaviours while inhibiting others. Recent advances have shed some light on the biological mechanisms underlying behavioural prioritisation. However, the neuronal and molecular mechanisms governing the capacity to prioritize competing needs remain largely unknown. We hypothesized that the neuropeptide oxytocin (OXT), mainly produced in the hypothalamus, plays a key role in behavioural prioritization. This hypothesis is supported by observations that OXT modulates social and feeding behaviours. To investigate the impact of physiological conditions on need prioritization, we examined social and food preferences in adult CD1 male mice using the Social versus Food Preference test. We manipulated their social and physiological states through housing conditions (group-housed vs. single-housed) and food deprivation. We observed that both group-housed and single-housed mice initially preferred social stimuli. However, as caloric needs increased, group-housed mice shifted toward food stimuli, while single-housed mice maintained an equal preference for both stimuli. Using immunofluorescence, we examined patterns of neuronal activity in various brain regions. Our results indicate a correlation between OXT activity in the hypothalamic Paraventricular Nucleus (PVN) and the preference strength. Additionally, we observed preference-specific activity in the bed nucleus of the stria terminalis (BNST) and the amygdala, two regions implicated in mediating food and social behaviours. Although further characterization is required, our findings suggest that OXT mediates the preference between competing social and food needs in mice.

**Keyword 1**: behavioural prioritisation
**Keyword 2**: needs
**Keyword 3**: neuropeptide
**Keyword 4**: Oxytocin
**Keyword 5**: preference

**HOW STUTTERING SHAPES BRAIN ACTIVITY: AN FMRI STUDY**

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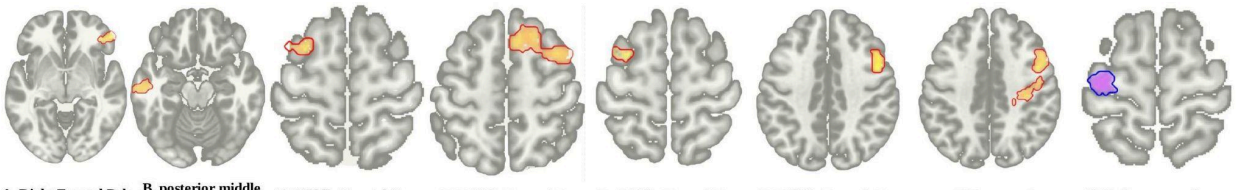
This study aimed to apply resting-state fMRI in the study of stuttering to compare brain connectivity in people who stutter (PWS) with Typically Fluent Speakers (TFS).

Thirty male participants were recruited, (PWS, n=15 and TFS, n=16). Stuttering severity was assessed using the Stuttering Severity Instrument-4 (SSI-4). MRI data were acquired on 3.0T scanner including; 3D T1-MPRAGE sequence and EPI Resting-state fMRI (rs-fMRI). Data were preprocessed and analyzed using the CONN toolbox. Connectivity associated with stuttering was identified using a seed-based approach.

Initial analyses revealed that the TFS group exhibited increased functional connectivity in several regions. The increased connectivity in networks such as the salience (left supramarginal gyrus, bilateral insula, anterior cingulate cortex), default mode (right lateral parietal cortex), and dorsal attention (intraparietal sulcus, middle frontal gyrus) networks in TFS may explain the efficient integration of self-monitoring, attention, and error detection during speech production. We found increased connectivity in the left putamen among PWS which plays a central role in motor planning and execution, particularly in coordinating sequential motor actions necessary for fluent speech. This aligns with a growing body of evidence implicating basal ganglia dysfunction in the neural basis of stuttering. However, these differences did not survive correction for multiple comparisons, warranting cautious interpretation and further investigation with larger samples.

Although our results align with prior evidence implicating basal ganglia and attentional control dysfunction in stuttering, our methodological approach provides a broader network-level perspective, enabling simultaneous characterization of interactions among regions critical for speech fluency and self-monitoring.

**Keyword 1**: Stutter
**Keyword 2**: Speech
**Keyword 3**: Stammer
**Keyword 4**: fMRI
**Keyword 5**: Neurobiology



A. Right Frontal Pole | B. posterior middle temporal gyrus | C. Middle Frontal Gyrus | D. Middle Frontal Gyrus | E. Middle Frontal Gyrus | F. Middle Frontal Gyrus | G. Middle Frontal Gyrus | H. Left precentral gyrus

| | Connection | Connectivity | Coordinates (X.Y,Z) | Cluster size | Peak p-unc |
|---|---|---|---|---|---|
| **A** | Dorsal Attention (IPS) - Right Frontal Pole | Increased | +541 +40 -02 | 158 | 0.000006 |
| **B** | Salience network (SMG) - posterior middle temporal gyrus | Increased | +02-60 -42 | 624 | 0.000031 |
| **C** | Dorsal Attention (L.IPS) - Middle Frontal Gyrus | Increased | -26 +12 +66 | 217 | 0.000001 |
| **D** | Salience network (ACC) - Middle Frontal Gyrus | Increased | +48 +16 +44 | 244 | 0.000014 |
| **E** | Dorsal Attention (R.IPS) - Middle Frontal Gyrus | Increased | -28 +04 +62 | 137 | 0.000039 |
| **F** | Salience network (ACC) - Middle Frontal Gyrus | Increased | +48 +16 +44 | 244 | 0.000014 |
| **G** | Right insula - Middle Frontal Gyrus | Increased | +42 +04 +40 | 548 | 0.000025 |
| **H** | Putamen - Left precentral gyrus | Decreased | -28 -16 +74 | 473 | 0.000063 |

**ROLE OF NICOTINIC ACETYLCHOLINE RECEPTORS EXPRESSED BY CHOLINERGIC INTERNEURONS IN THE CONTROL OF STRIATAL ACTIVITY**

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The striatum is the main input nucleus of the basal ganglia that plays an important role in the control of motor functions, behavior and cognition. Its activity is greatly influenced by striatal cholinergic interneurons (CINs) which express nicotinic acetylcholine receptors (nAChRs) containing beta2 subunit (beta2* nAChRs). We have previously shown that the deletion of beta2* nAChRs in CINs leads to behavioural changes in mice and alterations of cFos expression in the striatum. The aim of the present project was to investigate the effects of beta2* nAChRs deletion on electrophysiological properties of CINs and signaling of local striatal circuits. To this aim, we deleted beta2* nAChRs in CINs by crossing beta2-flox/flox mice with ChAT(IRES)-Cre mice expressing Cre recombinase selectively in cholinergic neurons. Then we determined the passive and active electrophysiological properties of striatal CINs and medium spiny neurons (MSNs) in the control and conditional knock-out (KO) mice. Although the deletion did not have a profound effect on passive properties of striatal CINs as well as the passive and active properties of MSNs, we detected a less pronounced sag in CINs of the KO mice. Furthermore, to explore possible associations between the electrophysiological changes and behavioural phenotype, we evaluated social and anxiety-like behaviour and the sensitivity to stimulants in the mutated animals. The project will help us to understand the functional role of beta2* nAChRs expressed by CINs and their effect on the CINs' activity and the activity of striatal circuits.

**Keyword 1**: Striatum
**Keyword 2**: Mice
**Keyword 3**: Nicotinic acetylcholine receptors
**Keyword 4**: Cholinergic interneurons

**ADMINISTRATION OF NEUROPEPTIDE QRFP INTO THE LATERAL HYPOTHALAMUS AFFECTS SOCIAL MEMORY IN WISTAR RATS**

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The involvement of the hypothalamus and its projections in innate social behavioral patterns, including aggression, reward, dominance, experience exchange, and others, was recognized long ago. The emerging role of the hypothalamic neuropeptides in these mechanisms led to increased research interest in the possible involvement of pyroglutamylated arginine-phenylalanine amide peptide (QRFP), a member of the RF-amide family. Multiple brain regions involved in social cognition e.g. medial prefrontal cortex, anterior cingulate cortex, anterior insular cortex, amygdala, etc., have well-described connections towards the hypothalamus. Our previous studies suggest a pivotal role of the lateral hypothalamic area (LHA) in the cognitive effects of QRFP, thus LHA was chosen as a target area for treatment. Male Wistar rats received microinjection of neuropeptide QRFP dissolved in 0.4 µl saline. The effects of direct intraparenchymal administration of QRFP on social behavior were studied in a Three-chamber paradigm. The test involved three subsequent sessions, tending to uncover the animal's interest in a live subject (session A), interest towards a newly presented conspecific (session B), and ability to distinguish between the familiar and a newly presented animal (session C). NPY/NPFF receptor antagonist BIBP3226 was applied to elucidate the mechanism of possible changes and involvement of NPY and NPFF systems. Applying QRFP in dose 400 ng directly into the LHA did not affect sociability or social novelty, nevertheless, social memory was significantly improved. Pretreatment with the NPY/NPFF receptor antagonist, BIBP3226 did not abolish this effect, suggesting that other mechanisms are involved in the observed phenomenon.

**Keyword 1**: hypothalamus
**Keyword 2**: social memory
**Keyword 3**: neuropeptide

**DISCERNING NATURALISTIC BEHAVIORS AND THEIR CORTICAL REPRESENTATIONS BY TRACKING BODY POSTURE AND WHISKER DEPLOYMENT IN RATS**

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One of the central interests of neuroscience is to understand how the brain produces and orchestrates behavior. Our group studies these topics in freely moving rats and has previously demonstrated robust postural and behavioral representations in the posterior parietal cortex (PPC), frontal motor cortex and primary sensory cortices (Mimica et al., 2018, Mimica, Tombaz et al., 2023). We now focus on active sensing, with the goal of understanding how neuronal populations integrate sensory inputs to inform and update ongoing posture. To do this we have included tracking of whiskers and other facial features in our recording paradigm. Our custom-made head mounted setup combines two facial-tracking cameras, housing for Neuropixels recording probes and a retroreflective rigid body for 3D posture tracking. With this system we gain better insight into the animals' behavioral repertoire by adding high-speed facial tracking data to our time-series behavioral clustering pipeline (adapted from Berman et al., 2014 and Mimica, Tombaz et al., 2023). We present how including whisker dynamics affects the segmentation of behavioral motifs, with specific focus on rearing and other behaviors relevant to active exploration. We will use the expanded postural kinematics data and improved behavior segmentation to further investigate sensory-motor integration in two relevant cortical areas. In the whisker area of the somatosensory cortex (S1) our preliminary analyses confirm stable encoding of whisker position. Our second area of interest remains the PPC where we are extending our knowledge on behavioral representation by investigating the combined representations of head posture and whisker deployment.

**Keyword 1**: posture
**Keyword 2**: whiskers
**Keyword 3**: facial tracking
**Keyword 4**: posterior parietal cortex
**Keyword 5**: barrel cortex

**TOPOGRAPHICALLY ORGANIZED SENSORY-MOTOR ACTIVITY OF DORSAL RAPHE AND ITS FOREBRAIN INNERVATIONS MODULATE RESTING-STATE AND SENSORY-EVOKED FOREBRAIN ACTIVITY AND ANIMAL BEHAVIOR**

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The dorsal raphe nucleus (DRN) is crucial for regulation of various behaviors such as mood, motivation, appetite, sleep, and social interactions. This diversity is mirrored in its structure, with distinct molecular groups of neurons organized topographically and targeting diverse brain regions. However, key questions persist: How do DRN neurons process information? How do they communicate with the forebrain? And what impact do DRN inputs have on forebrain activity and animal behavior? To investigate these questions, we examined the activity patterns of DRN neurons, their axons, and their targets in the juvenile zebrafish forebrain. Our study uncovered a notable topographic organization of ongoing activity and sensory-motor responses within the DRN. We discovered that many DRN neurons are primarily driven by the animal's movement. Additionally, a specific group of DRN neurons, identified by the marker Gad1, showed unique activity patterns during rest, movement, and sensory stimulation. DRN axons, which extensively innervate the forebrain, exhibited organized excitatory and inhibitory responses to sensory stimuli and motor activity. We observed significant and rapid covariation between the activity of DRN axons and nearby forebrain neurons. Chemogenetic ablation of DRN resulted in a significant reduction in forebrain sensory-motor responses, and synchrony, accompanied by notable deficits in adaptive behaviors. In summary, our findings emphasize the functional diversity of DRN neurons and their role in transmitting locomotor signals through organized projections. These projections regulate forebrain activity and are crucial for modulating animal behavior.

**Keyword 1**: Dorsal Raphe Nucleus
**Keyword 2**: Forebrain
**Keyword 3**: Sensory-motor responses
**Keyword 4**: Topographic Organization
**Keyword 5**: Chemogenetic Ablation

**THE INTERNAL FUNCTIONAL STATE OF THE THALAMUS EXPLAINS VARIABILITY IN ONGOING CORTICAL ACTIVITY AND ITS RESPONSE TO STIMULI**

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The thalamus is the largest subcortical structure in the human brain, facilitating sensory processing, multiple aspects of cognition, network orchestration and coordination, and the generation and control of the alpha rhythm. Despite this central role in a wide range of functions, the thalamus is often referred to as a simple 'relay station'. We collected EEG and fMRI data concurrently whilst participants viewed visual stimuli either passively or as part of an attention task to address the question: does the internal functional state of the thalamus explain variability in ongoing cortical activity and its response to stimuli? We employed Hidden Markov Modelling to decompose activity within the thalamus into spatiotemporal states, and examined their link with visual response variability, cortical evoked fMRI and EEG activity, and the power of the alpha rhythm. This identified five separate states, each of which showed significant correlations with cortex, unique temporal relationships with the posterior alpha rhythm, and which were associated with significant modulations in the amplitude of both fMRI and EEG responses when they were active during the pre-stimulus period. These results clearly demonstrate a widespread impact of the internal functional state of the thalamus on cortical activity, both in terms of ongoing oscillatory activity and the response to stimuli.

**Keyword 1**: Thalamus
**Keyword 2**: EEG-fMRI
**Keyword 3**: Attention
**Keyword 4**: Brain state

**COLLATERAL CONNECTOMES OF *ESR1*-POSITIVE HYPOTHALAMIC NEURONS MODULATE DEFENSIVE BEHAVIOR PLASTICITY**

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The ventromedial hypothalamus (VMH) projects to the periaqueductal gray (PAG) and anterior hypothalamic nucleus (AHN), mediating freezing and escape behaviors, respectively. We investigated VMH collateral (VMH-coll) neurons, which innervate both PAG and AHN, to elucidate their role in postsynaptic processing and defensive behavior plasticity. Using all-optical voltage imaging of 22,151 postsynaptic neurons *ex vivo*, we found that VMH-coll neurons engage inhibitory mechanisms at both synaptic ends and can induce synaptic circuit plasticity. *In vivo* optogenetic activation of the VMH-coll somas induced escape behaviors. We identified an *Esr1*-expressing VMH-coll subpopulation with postsynaptic connectome resembling that of wild-type collaterals on the PAG side. Activation of Esr1+VMH-coll neurons evoked freezing and unexpected flattening behavior, previously not linked to the VMH. Neuropeptides such as PACAP and dynorphin modulated both Esr1+VMH-coll connectomes. In vivo κ-opioid receptor antagonism impaired Esr1+VMH-coll-mediated defensive behaviors. These findings unveiled the central role of VMH-coll pathways in innate defensive behavior plasticity.

**Keyword 1**: Voltage imaging
**Keyword 2**: Ventromedial hypothalamus

**SAFETY OF LONG-TERM PI3K DELTA EXPRESSION IN TREATMENT OF RAT SPINAL CORD INJURY**

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The PI3K/Akt pathway plays a critical role in neuronal survival, regeneration, and synaptic plasticity. PI3Kδ, a class I PI3K isoform, has been shown to enhance axonal regeneration, making it a promising candidate for CNS repair strategies. However, its involvement in oncogenic signalling raises concerns regarding its long-term safety. This study aimed to determine whether sustained neuronal PI3Kδ expression induces tumorigenesis or other pathological changes in rodents. Adult male and female Wistar rats (220–400 g) received stereotaxic AAV1-hSYN-PIK3CD injections into the sensorimotor cortex, while control animals were not injected. Animal weight was monitored weekly for 12 months. At study completion, blood samples were collected for biochemical analysis, and rats underwent MRI for tumour detection, followed by the collection of brains, liver, spleen, and kidneys. These organs were sectioned at 40 or 20 µm and processed with fluorescent immunohistochemistry (IHC) and haematoxylin-eosin (HE) staining. Iba-1 IHC was performed to assess microglial activation, and peripheral organs were weighed and analysed histopathologically. MRI scans showed no tumour formation in the AAV1-hSYN-PIK3CD-treated group; however, one uninjected control developed an obliterating tumour in the aqueductus mesencephali. Histopathological analysis revealed no abnormalities beyond expected physiological variations. Iba-1 staining showed no significant differences in microglial activation between groups, indicating no neuroinflammatory response. Our findings suggest that long-term neuronal PI3Kδ expression does not induce tumorigenesis, neuroinflammation, or other pathologies in rodents. These findings reinforce the safety profile of long-term PI3Kδ expression and support its continued development for CNS repair.

**Keyword 1**: Spinal Cord Injury
**Keyword 2**: Gene Therapy
**Keyword 3**: PI3K
**Keyword 4**: Axonal Regeneration

**THE ROLE OF PI3K DELTA ON PROTEIN EXPRESSION IN THE CONTEXT OF SPINAL CORD INJURY**

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CNS neurons lose regenerative ability with maturity, meaning damage to the spinal cord causes permanent loss of motor function due to lack of corticospinal tract (CST) axonal regeneration. We have recently shown that hyperactive PI3Kδ enables functional recovery and long-distance regeneration of adult rat CST. Additionally, we observed in rats injected with a mixture of AAV1-hSYN-eGFP and AAV1-hSYN-PIK3CD, GFP signal is strikingly higher compared to rats transduced with same dose of GFP vector alone. We expect that vector delivered PI3Kδ is directly influencing mTOR driven protein translation, but it could also regulate transcription. In this study, we express GFP under CAG or hSYN, in the presence or absence of hSYN-PIK3CD for 6 weeks. Our aim is to understand PI3Kδ regulation of translation/transcription, to describe its role in boosting protein levels during regeneration and whether this is promoter dependent. We injected AAVs into rat motor cortex and produced C4 spinal cord injury (SCI). Brain cortices were studied with immunohistochemistry and western blot (WB) to confirm high levels of GFP driven by PI3Kδ, with more pronounced GFP increase when both promoters were the same. Preliminary WB data shows that PI3Kδ increases LysRS, a tRNA synthetase, and decreases REST, a transcriptional repressor. We are working on RT-qPCR to compare mRNA levels of transcription factors (STAT3, FOXO3, SOX11) and proteins (Rab11, TrkB) known to be upregulated during peripheral nerve or CNS regeneration. We will further assess the promoter's effect at labelling spinal axons, as well as if this GFP increase is neuronal specific.

**Keyword 1**: PI3Kδ
**Keyword 2**: Spinal cord injury

**AN INTACT LATERAL ORBITOFRONTAL CORTEX IS REQUIRED FOR CONTINGENCY DEGRADATION LEARNING BUT NOT FOR CONTINGENCY DEGRADATION RETRIEVAL**

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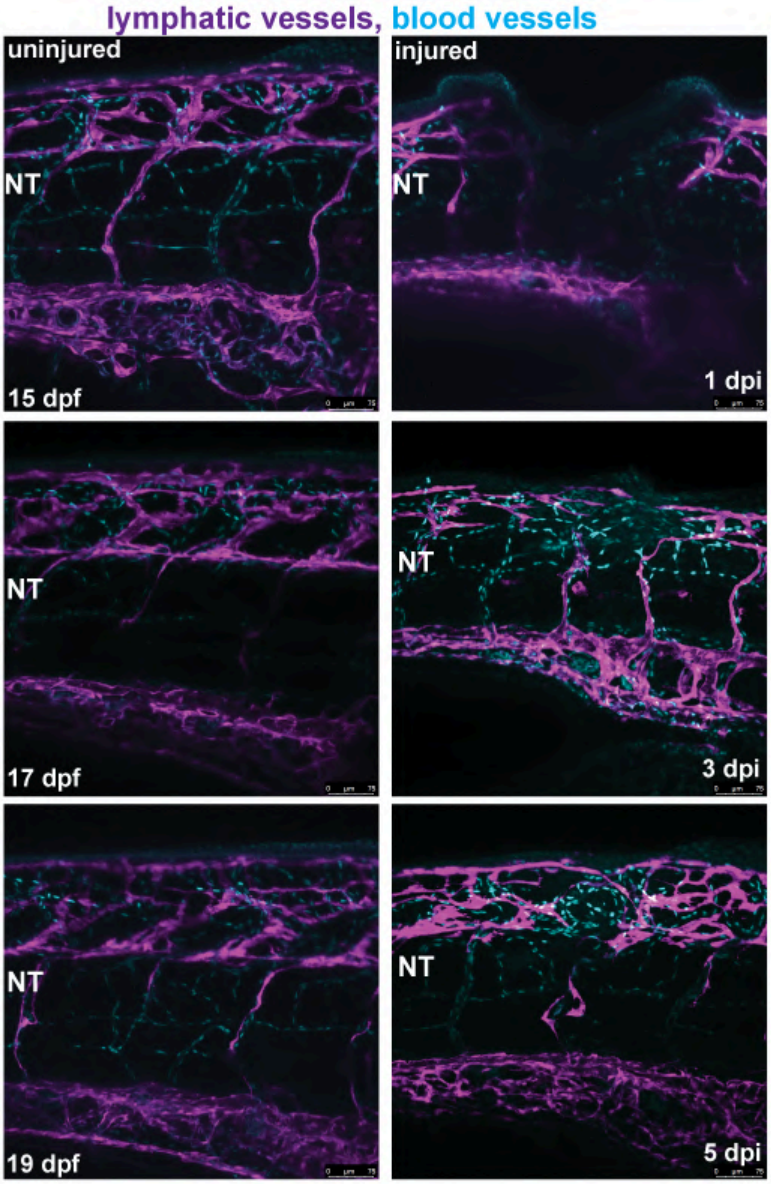
Extinction (i.e. the nonreinforcement of a previously rewarding cue or action) has been shown to reduce conditioned responding, but only temporarily, because such responding 'spontaneously recovers' over time. Here we investigated whether degrading the contingency between action and outcome might produce a longer-lasting reduction in instrumental (i.e. lever press) responding. In Experiment 1, rats were first trained to press a right and left lever for pellet and sucrose outcomes respectively (counterbalanced), after which one of these outcomes was delivered non-contingently, in the absence of a lever press. As a result, rats reduced responding on the degraded relative to the non-degraded lever, and this pattern (nondegraded > degraded) was observed whether animals were tested 1 day (immediate test) or 2 weeks (delayed test) after training. Moreover, in a subsequent reinstatement test, rats underwent 15 minutes of extinction followed by unearned presentations of each outcome and were given a choice between levers. Whereas rats reinstated normally following presentations of the nondegraded outcome when presented with the degraded outcome rats reinstated on the opposite lever, the one that *did not* earn that outcome during training (i.e. nonreinstated > reinstated). This pattern was preserved on both the immediate and delayed reinstatement tests and suggested that degradation produced inhibitory responding that remained inhibitory over time. In Experiment 2, we demonstrated that chemogenetic silencing of the lateral orbitofrontal cortex (lOFC) prevented contingency degradation learning. In Experiment 3, we showed that chemogenetic inhibiting of the lOFC does not affect contingency degradation retrieval.

**Keyword 1**: Lateral Orbitofrontal Cortex
**Keyword 2**: Contingency degradation learning and retrieval
**Keyword 3**: Goal-directed actions
**Keyword 4**: Spontaneous recovery
**Keyword 5**: Chemogenetic

**DEFINING THE ROLE OF LYMPHATIC VESSELS IN REGENERATION AFTER SPINAL CORD INJURY**

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Upon spinal cord injury (SCI), the lymphatic vessels expand, however, their role in spinal cord regeneration remains elusive. We will investigate if lymphatic vessels supply lymphangiocrine factors to support spinal cord healing. We hypothesise that lymphatics can stimulate axonal growth and cellular reprogramming to improve spinal cord regeneration. As mammals have poor regenerative capacity, we take advantage of the highly regenerative zebrafish, which are able to regenerate and restore function after SCI. As lymphatic vessels surround the spinal cord from 14 days-post-fertilization (dpf), we used this timepoint for injury as larvae size and transparency facilitate *in vivo* imaging. A complete transection approach is used for the injury at the level of the 15th somite. We performed *in vivo* imaging with the reporter lines labelling lymphatic vessels and blood vessels. The number of lymphatic cells after SCI was significantly lower than uninjured age-matched siblings at 1 day-post-injury (dpi) and 3 dpi. At 5 dpi, we saw a significant recovery in terms of comparable lymphatic cell numbers. We observed that new lymphatic endothelial cells emerge and new vessels form by sprouting following SCI. Moreover, preliminary data using hypomorphic *vegfc* mutants, which have delayed lymphangiogenesis, showed an impaired response of lymphatic vessels to the injury and delayed functional/behavioural recovery. This work aims to uncover the molecular and cellular mechanisms governing neuronal regeneration in relation to lymphatic plasticity. Understanding the nature of tissue-tissue interactions is a pivotal step towards new therapeutic avenues to promote spinal cord regeneration in humans.




**Keyword 1**: Spinal cord injury
**Keyword 2**: Lymphatic vessels
**Keyword 3**: Regeneration
**Keyword 4**: Zebrafish

**TRANSIENT AND SIDE-INVARIANT RESPONSES TO TACTILE STIMULATION IN THE PERIRHINAL CORTEX**

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Hierarchical processing has been shown to underlie complex functions such as invariant object coding. The perirhinal cortex (PER) sits at the apex of such processing streams and is involved in object recognition. Although it receives tactile information from the barrel cortex (wS1) and secondary somatosensory cortex (S2), it is unclear how PER neurons respond to such stimuli. To address this, we performed population recordings from the barrel and perirhinal cortices of head-fixed mice while an object deflected whiskers on either side of their face. The object moved in a square-wave pattern at 1Hz for 5 seconds, therefore providing 10 stimulations per trial.

For both regions, single-unit activity and PCA illustrated a distinct response profile to the first stimulation in a trial compared to subsequent ones. Further, K-means clustering revealed that the first stimulation evoked transient and primarily inhibitory responses in the majority PER neurons, with no responses to subsequent stimulations. This adapting response pattern was less representative of wS1 units, which instead featured a more uniform distribution of response profiles. Moreover, in PER there were no readily apparent differences in responses to the side being stimulated, which was not the case in wS1. These observations were recapitulated through three decoding approaches, namely stimulus-baseline decoding, a cross-conditional variant, and stimulation side decoding. Together, these results demonstrate that PER responses to simple tactile stimulation are both side-invariant and transient.

**Keyword 1**: Hierarchical processing
**Keyword 2**: Perirhinal
**Keyword 3**: Representations
**Keyword 4**: Invariance
**Keyword 5**: Population

**SPATIO-TEMPORAL ORGANIZATION OF RESTING STATE AND SENSORY EVOKED ACTIVITY IN THE ZEBRAFISH TELECEPHALON**

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Cognitive processes and adaptive behaviors in zebrafish rely on temporally coordinated activity, implying the existence of analogous networks across phyla. Previous studies have identified structured resting-state activity in telencephalic cortico-limbic circuits; however, the temporal dynamics and their relation to spatial organization remain unexplored. In this study, we present topographically organized neuronal ensembles within the zebrafish telencephalon that are functionally distinct and stereotyped across individuals. Using volumetric two-photon calcium imaging, we identified telencephalic nuclei with structured ongoing activity that are conserved across animals. Functional connectivity analysis suggests distinct circuit architectures, with specific forebrain regions exhibiting characteristic temporal dynamics along the anterior-posterior axis. These findings suggest a hierarchical organization of activity patterns that may underlie sensory processing and adaptive behaviors.
Ongoing work aims to characterize the features and modulatory factors of these temporal structures, including their relationship to behavioral states and sensory stimuli.

**Keyword 1**: resting
**Keyword 2**: coordinated
**Keyword 3**: hierarchical
**Keyword 4**: phyla
**Keyword 5**: dynamics

**LATERAL HYPOTHALAMIC INPUT TO ANTERIOR THALAMUS REGULATES FEEDING MOTIVATION**

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In the lateral hypothalamus (LH), distinct cell populations play critical roles in regulating innate behaviors that ensure survival. Motivation is essential to direct navigation towards need-appropriate goals, such as food, water or conspecifics. Using anterograde tracing, we identified the anterior thalamus (ATN) as an output target of Neurotensin- expressing (Nts)-LH neurons. Here, we investigated the role of these Nts neurons in coordinating innate behaviors across different internal states that modulates food motivation through projections to the ATN. Using single-cell calcium imaging in freely behaving mice, we identified a neurotensinergic (Nts) subpopulation of the lateral hypothalamus (LH) that preferentially encodes accessible and inaccessible food. Optostimulation of these cells increased preference for exploration of food over other innately rewarding stimuli. Optogenetic stimulation of the Nts projections to ATN dynamically modulated food-seeking behaviors and enhanced food consumption in a state-dependent way. When the food was placed in the middle of a novel arena, activation of the Nts projections in the ATN resulted in a higher food consumption and a shorter latency to feeding onset.
Together, these results demonstrate that projections of food-excited $Nts^{LH}$ subpopulations into anterior thalamus convey food motivation, directing navigation towards food sources and increasing the likelihood of consumption.

**Keyword 1**: Lateral Hypothalamus
**Keyword 2**: Innate Behaviors
**Keyword 3**: Neurotensin
**Keyword 4**: Anterior Thalamus

**THE ROLES OF THE CHOROID PLEXUS IN REGULATING BRAIN HOMEOSTASIS, NEURONAL ACTIVITY AND ANIMAL BEHAVIOR**

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The choroid plexus (ChP), which is a specialized tissue located within the brain ventricles, secretes cerebrospinal fluid (CSF) by transporting ions and water from adjacent blood vessels. The ChP plays also crucial roles in maintaining brain homeostasis, acting as a CSF-blood barrier and an immune interface. To date, the mechanisms by which the ChP influences brain physiology and animal behavior remain largely unexplored. To gain novel insights into the physiological roles of the ChP, we utilize zebrafish as a model system. First, we showed that the zebrafish ChP shares evolutionarily conserved cellular and molecular features with human and rodent ChPs. Next, we generated genetic lines capable of ablating the ChP epithelial cells and revealed that the ChP is required for the maintenance of the brain ventricles. We are currently investigating how the ChP contributes to brain physiology by measuring animal behaviors and neuronal activity in ChP-ablated animals. Our long-term goal aims to understand how the ChP regulates brain physiology in both health and diseases.

**Keyword 1**: choroid plexus
**Keyword 2**: cerebrospinal fluid
**Keyword 3**: zebrafish
**Keyword 4**: behavior
**Keyword 5**: brain ventricles

**EXPLORING CONNECTIVITY ALONG THE ANTERIOR-POSTERIOR AXIS OF THE PIRIFORM CORTEX**

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Olfactory processing involves various brain regions, with the piriform cortex (PC) being a key region. This brain area is mainly responsible for odor identity, odor contextualization, and olfactory memory. Its subdivisions - anterior PC and posterior PC (aPC and pPC) - are known to process distinct functional aspects of olfaction. Other factors that distinguish these sub-regions include their morphologies, electrophysiological properties, and connectivity with other brain areas. It has been hypothesized earlier that aPC and pPC might form specific, separate loops with particular brain regions. To examine how connectivity patterns differ along the anterior-posterior axis of the PC, specifically between aPC, pPC, and the aPC-pPC transition zone, we applied neuronal tracing. We injected neuronal tracers at multiple locations along the anterior-posterior axis of the PC, enabling the study of brain-wide connectivity. The comprehensive post-processing approach, based on the Quint workflow with additional adjustments, aided in dissecting the details of PC connectivity patterns. This approach involved registering microscopy images with anatomical reference atlases, segmenting labeled regions, and quantifying the intensity and distribution of tracer signals across the entire brain. Through pixel intensity analysis of brain sections, we identified the exact sites of tracer injections and their respective projection patterns to specific brain areas. Our findings emphasize the differences between aPC and pPC connectivity patterns and the distinct loop systems they form, which will be further explored in relation to their functional distinctions.

**Keyword 1**: olfactory
**Keyword 2**: piriform cortex
**Keyword 3**: connectivity

**INTRINSIC PROPERTIES OF EXTERNAL GLOBUS PALLIDUS NEURONS IN DOPAMINE DEPLETED MICE**

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Parkinson´s disease (PD) is a neurodegenerative motor disorder that has been associated with the progressive loss of midbrain dopaminergic neurons in the substantia nigra *pars compacta* and their projection in the basal ganglia (BG). The globus pallidus externa (GPe) is a central part of the BG that has been reported to be affected by dopamine loss. This nucleus is composed of two cell populations, that differ by electrophysiological properties, molecular markers, developmental origin, and projection targets. The majority of GPe cells are the prototypic neurons which are highly active, while the arkypallidal neurons, are a smaller group of less active neurons. Preliminary data from our lab recorded in vivo, have shown cell-type-specific alterations in the membrane properties of GPe cells between healthy and parkinsonian mice. In this study, we aim to characterize the intrinsic properties of the two GPe neuronal populations and study their possible alteration in the unilateral 6-hydroxydopamine (6-OHDA) model of dopamine-depleted mice in the ex-vivo slice preparation. This study will generate new knowledge that will progress our understanding of PD and give rise to new possible treatments.

**NEURONAL ENSEMBLES ENCODING SENSORY BEHAVIOR ARE DEFINED BY ASTROCYTE EXCITABILITY**

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How the primary somatosensory cortex (S1) discriminates and processes simultaneous information from four different modalities (touch, temperature, pain and proprioception) remains a longstanding question in neuroscience. Here, we hypothesize that cortical astrocytes fine-tune the spatially and temporally organized activity of neuronal ensembles encoding sensory modalities, thereby influencing behavior. To investigate this, we employed *in vivo* chemogenetic manipulation of astrocytes and behavioral assessment combined with microendoscopic calcium imaging (miniscope), to record the simultaneous activation of neuronal sensory ensembles across S1 layers. Our *in vivo* imaging data, obtained from freely moving animals, showed that peripheral tactile and thermal stimulation activate distinct neuronal ensembles across the cortical layers, with a small subset of multimodal neurons participating in both tactile and thermal ensembles. Furthermore, *in vivo* modulation of astrocyte $Ca^{2+}$ activity using Gq-DREADDs enhanced the number of multimodal neurons, indicating increased flexibility of single neurons to shift from ensemble to ensemble in the presence of a disrupted astrocytic network. However, rather than being beneficial, the induced multimodality impaired texture and temperature discrimination in behavior tests, suggesting that neuronal ensembles recruited under disrupted astrocyte activity are broadly tuned and less specialized to discriminate the arriving inputs. Thus, our results demonstrate that S1 ensures the activation of the appropriate neuronal ensembles to process sensory modalities, thereby enabling accurate behavioral responses.

**Keyword 1**: neuronal ensemble
**Keyword 2**: astrocyte
**Keyword 3**: sensory discrimination
**Keyword 4**: sensory behavior

**COMBINATORIAL BIOMATERIAL AND MOTOR TRAINING THERAPY PROMOTES SUPRASPINAL PLASTICITY AND FUNCTIONAL RECOVERY AFTER SPINAL CORD INJURY**

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Piezo4Spine aims to develop a multifactorial therapy to promote axonal regeneration and brain-spinal cord connectivity after spinal cord injury (SCI). Our first step was the development of a 3D-biomimetic collagen-based matrix with iron oxide nanoparticles (COL-CHI), combined with a motor training (MT) protocol. After *in vitro* characterization of the matrix components, the biomaterial was implanted *in vivo* in a cervical hemisection model (C6) across different groups: SCI, SCI + COL-CHI and SCI +COL-CHI + MT (combined therapy). These animals underwent longitudinal assessment up to 120 days post-injury to evaluate sensorimotor functions and thalamocortical connectivity using electrophysiology, behavior and anatomical approaches. Tactile and thermal behavioral tests showed altered sensory threshold responses in COL-CHI rats, particularly in the dorsal column-lemniscal (tactile) pathway. Additionally, COL-CHI rats showed improved forelimb motor abilities in a task assessing corticospinal tract function. Both sensory and motor improvements were observed up to 60 days post-injury but decreased at long-term. However, the chronic changes at behavioral level were reverted in animals submitted to MT protocols, suggesting that the combinatorial therapy counteracts biomaterial degradation effects. At circuit level, COL-CHI and COL-CHI + MT rats showed increased sensorimotor plasticity evidenced by enhanced magnitude of cortical sensory evoked potentials in *in vivo* electrophysiology and increased thalamic inhibition by immunofluorescence staining of GABAergic afferents in NRT, POm and VPL**.** Together, these results demonstrate that biomaterial degradation should occur only after the full recovery or stabilization of spinal cord circuits and that combinatorial therapy promotes supraspinal plasticity that persists even after biomaterial degradation.

**Keyword 1**: Spinal cord injury
**Keyword 2**: Biomaterial
**Keyword 3**: Combinatorial therapy
**Keyword 4**: Sensory system

**MANIPULATION OF ASTROCYTES WITH NEUROTOXIC POTENTIAL IN SPINAL CORD AS A THERAPEUTIC TARGET FOR MOTOR AND FUNCTIONAL RECOVERY AFTER INCOMPLETE SPINAL CORD CONTUSION INJURY**

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Spinal cord injury (SCI) is a pathology that still lacks effective treatments to improve the quality of life of patients. Previous studies have shown that after SCI, microglia adopt a protective phenotype by eliminating dead cells, as well as participating in synaptic remodelling. However, microglia, through the joint release of IL-1$\alpha$, TNF$\alpha$ and C1q, are also the trigger for the polarization of astrocytes towards a neurotoxic phenotype capable of decreasing axonal regeneration and decreasing synaptic connectivity.

Based on this, the present study hypothesized that inhibition of neurotoxic astrocytes (positive for complement component C3) could favour the recovery of neuronal connectivity after SCI by modulating the death of axotomized neurons. Therefore, the main objectives of this work are focused on 1) initial characterization of the presence of $C3^+$ astrocytes in the spinal cord after moderate contusion, 2) determining their anatomical relationship with the ascending sensory and descending motor tracts, 3) determining how pharmacologic inhibition of $C3^+$ astrocytes contribute to functional recovery of locomotion, balance, and motor coordination in SCI mice.

Our results indicate that moderate SCI increases the number of $C3^+$ astrocytes near corticospinal tract axons in the rostral zone, whereas in the caudal zone, they are primarily localized to the reticulospinal tracts. These findings suggest a heterogeneous spatial distribution of $C3^+$ astrocytes in the spinal cord. At the behavioral level, inhibition of $C3^+$ astrocytes led to motor function improvements, highlighting their potential as a therapeutic target for mitigating axonal damage after SCI.

**Keyword 1**: Spinal cord injury
**Keyword 2**: Astrocytes
**Keyword 3**: Behaviour

**DECODING THE CEREBELLO-STRIATAL PATHWAY: CELLULAR AND SYNAPTIC MECHANISMS FOR SENSORIMOTOR FUNCTION**

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The basal ganglia (BG) and the cerebellum are highly interconnected, and have been proposed to control motor- and non-motor behaviors. Except for the traditional cerebllo-thalamo-cortical pathway, the deep cerebellar nuclei (DCN) connect with dorsal striatum (DS) via thalamus (TH). The DCN-TH-DS pathway is vital in contributing to execution of motor commands and goal directed behaviors. Although this pathway innervates medial spiny neurons (MSNs) and other non-MSNs *ex vivo*, the specific neuronal subtypes receiving cerebellar inputs and their impact on the striatal microcircuitry remain unknown. In addition, sensory and motor processes are tightly linked at the cortical level, however, it is unknown how the convergent sensory inputs from neocortex and cerebellum integrate at the BG level and affect sensorimotor processes. To investigate the cellular and network mechanism, we performed *in vivo* whole-cell patch clamp recordings in head-restrained animals, combining optogenetic manipulation to the cerebello-striatal pathway with sensory stimulation. We found that optogenetic activation of DCN elicited subthreshold excitatory postsynaptic potentials (EPSPs) or action potentials in dorsal MSNs. The latency between light pulse onset and EPSPs initiation ranged from tens to hundreds of milliseconds, suggesting disynaptic and polysynaptic pathways. These findings demonstrate that cerebellar output robustly influences dorsal striatum *in vivo*, and may modulate striatal sensory processing. Future studies will focus on elucidating the functional integration of cerebellar output and sensory inputs at the striatal level.

**Keyword 1**: cerebellum
**Keyword 2**: striatum
**Keyword 3**: sensory perception

**PRE- AND POSTSYNAPTIC DETERMINANTS OF CLAUSTRO-CORTICAL CONNECTIVITY**

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The claustrum (CLA) is characterized by its extensive synaptic connectivity with multiple cortical regions. While CLA projection neurons are glutamatergic, several studies have shown an inhibitory impact of CLA on its cortical targets, suggesting the involvement of inhibitory cortical interneurons. We employed *in vivo* and *ex vivo* electrophysiology and optogenetics in mice to dissect the synaptic organization of projections from CLA to the anterior cingulate cortex (ACC), a robust claustro-cortical pathway implied in cognitive functions. Optogenetic stimulation of CLA neurons expressing CaMKIIa or VGLUT2 evoked distinct multiphasic excitatory and inhibitory responses in the ACC that depended on the stimulated CLA population and the recipient layer in the ACC. Stimulation of CLA-ACC axons evoked monosynaptic responses in pyramidal neurons and four types of molecularly defined interneurons. Synaptic responses were uniquely determined by the presynaptic CLA projection type, the recipient cortical layer, and the type of postsynaptic neuron, with interneurons displaying the highest specificity. This intricate organization of the CLA-ACC pathway explains the diverse and complex influence of CLA on cortical activity.

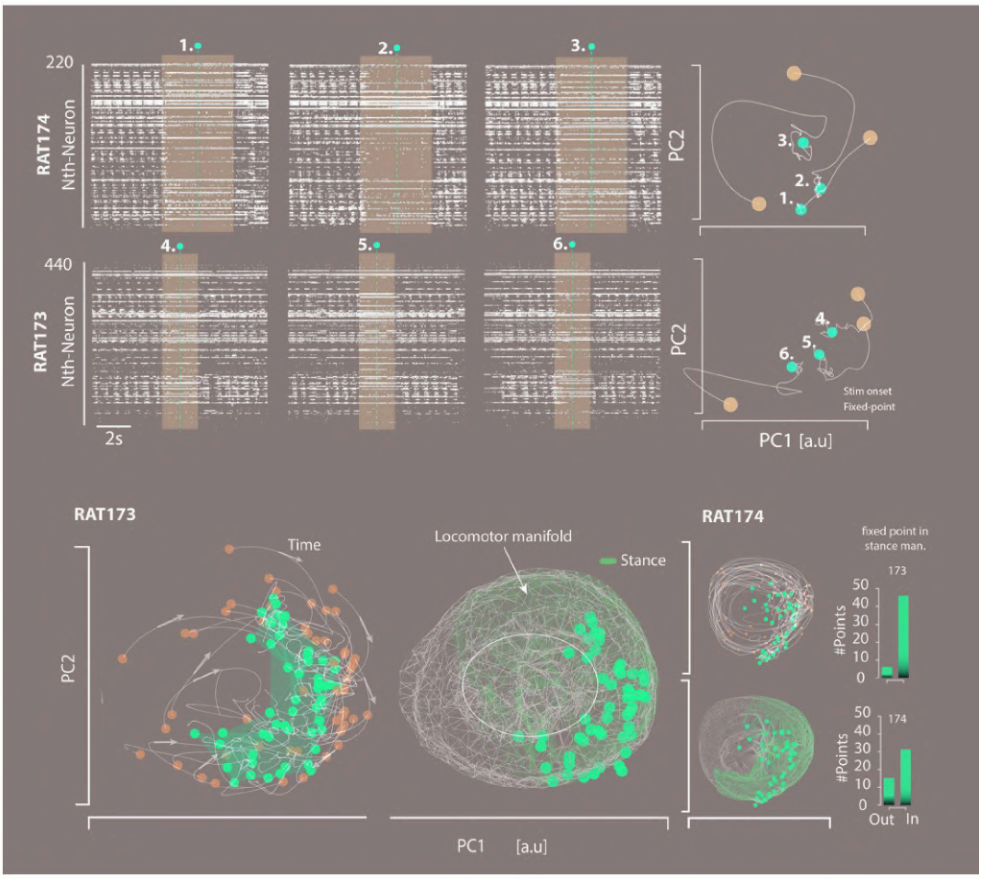
**Keyword 1**: Claustrum
**Keyword 2**: Anterior Cingulate Cortex
**Keyword 3**: Feed-forward inhibition
**Keyword 4**: Interneurons
**Keyword 5**: In vivo

**THE ART OF THE STOP: THE NEURAL POPULATIONS THAT BOTH GENERATE AND PAUSING MOVEMENT FORM A CONTINUOUS ATTRACTOR NETWORK IN THE SPINAL CORD**

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How does a cat gracefully walk and suddenly freeze when spotting a mouse? So far, it has been difficult to contemplate a CPG capable of both autonomous generation of movement and pausing that movement at any point. To better understand the generation and pausing of motor activity, we use Neuropixels probes in the rat spinal cord during voluntary locomotion. To control the pausing of movement, we utilize optogenetic perturbation of the pedunculo-pontine nucleus, a known regulator of movement arrest (Goni-Erro 2023). We find that during locomotion, the neuronal manifold activity exhibits robust rotational patterns invariant to speed (Linden 2022). Further, this trajectory converges to a stable point-attractor precisely at the moment of arrest, which persists until the movement is resumed. Through computational modeling, we argue that the network is a Continuous Attractor Network, as seen elsewhere in the nervous system (Gardner 2022), and suggest a structural mechanism of physical implementation.

**Keyword 1**: motor control
**Keyword 2**: spinal cord
**Keyword 3**: CPG
**Keyword 4**: movement
**Keyword 5**: attractor



**CORTICO-SPINAL DYNAMICS DURING RODENT LOCOMOTION**

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Spinal circuits possess the ability to generate locomotor rhythms independently of the brain, as evident by experiments of spinalized animals [1]. When necessary, cortical systems interfere with locomotor rhythms and adjust them as necessary to navigate obstacles, dangers or accommodate the demands of other brain networks [2]. To achieve this successfully, cortical and spinal circuits have to interact fluently to share the control of locomotion. However, the dynamics of this interaction remains vaguely understood. In this work, we present a neuromechanical model of a rat hindlimb where spinal circuits generate rhythm independently of the motor cortex, and the cortex interferes as necessary to adjust gait during obstacle avoidance. We show, in simulation, that transient input from the motor cortex can perturb the locomotor cycle to achieve obstacle avoidance. We investigate the role of feedforward and feedback interactions from the cortex to the spinal cord and vice versa. We further present preliminary behavioral and neurophysiological data to investigate the interaction between the motor cortex and the spinal cord during an obstacle navigation task. Our results suggest that interaction between the motor cortex and the spinal cord is likely two-way and more dynamic than previously thought.

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**Keyword 1**: Locomotion
**Keyword 2**: Spinal Cord
**Keyword 3**: Motor Cortex
**Keyword 4**: Skilled Movement

**LOCAL ACTIVITY OF DEEP CORTICAL LAYERS PREDICTS THE INFORMATION PROPAGATION AMONG DISTANT CORTICAL AREAS**

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The information processing of the brain is modulated by the ongoing activity state and consciousness level. During wakefulness, the electroencephalographic (EEG) response to a stimulation is long-lasting and propagates across the whole cortical surface with a complex pattern. Conversely, during dreamless sleep, general anaesthesia or vegetative state, the response is simplified or remains local. Complexity metrics, such PCI (perturbational complexity index), have indeed promising clinical implications for disorders of consciousness and brain damages. However, a mechanistic description of this state-dependent modulation remains uncertain. Also, the exact contribution that local cortical circuits have in the propagation of information among areas at larger scale is unclear.

To shed light on this topic, we simultaneously measured large-scale cortical responses and layer-specific activity in mice, during wakefulness and general anaesthesia (sevoflurane), combining: *i)* electrical stimulation of right secondary motor cortex, *ii)* electrocorticography (ECoG) from 5 spatially distant cortical areas, and *iii)* local laminar recordings from cortical layers in left somatosensory cortex (S1).

We found that a layers-specific evoked activity emerged only during wakefulness: neurons from the deepest cortical layer 6 showed the highest increase in firing rate, following the distant cortical stimulation. Moreover, the neuronal firing of L6 was coherently associated with the local field potential, which in turn correlated the most, together with L5, with the superficial ECoG activity. Coherently, the local activity of such deep layers strongly predicted the complexity level (PCI) of large-scale activation patterns, suggesting a mechanistic role in the propagation of information among distant cortical areas.

**Keyword 1**: Perturbational complexity index, PCI
**Keyword 2**: Cortical layer 6
**Keyword 3**: Cortical layer 5
**Keyword 4**: Anaesthesia
**Keyword 5**: Consciousness

**PTEN-DELETED GRANULE CELLS PROPAGATE MTOR HYPERACTIVITY ACROSS DENTATE GYRUS, DISRUPTING ITS GATING AND MEMORY CODING FUNCTIONS**

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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 −70mV resting potential but regained excitability upon depolarization, suggesting a positive shift in AP threshold and potential ion channel dysfunction. Video-EEG recordings detected spontaneous seizures with behavioral automatisms, while histopathological analysis confirmed hippocampal sclerosis and mossy fiber remodeling, consistent with TLE pathology. Crucially, PTEN-KO GCs not only exhibited intrinsic hyperactivity but also propagated mTOR dysregulation across 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 PP→DG/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 PP→DG and DG→CA3 transmission 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.

**Keyword 1**: Epilepsy and cognitive impairment
**Keyword 2**: mTOR hyperactivity
**Keyword 3**: Dentate gyrus dysfunction
**Keyword 4**: Hippocampal network plasticity
**Keyword 5**: Conditional PTEN deletion model

**SPATIAL REPRESENTATION IN THE RETROSPLENIAL CORTEX AND HIPPOCAMPAL FORMATION OF FREELY MOVING MICE**

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For decades, the study of rodent navigation primarily focused on the hippocampal and parahippocampal regions in isolation. However, recent studies suggest that a broader cortical network contributes to the generation of our sense of space. Within this network, the retrosplenial cortex (RSC) appears to serve as a convergence zone in linking the neocortex and the hippocampal formation. We sought to understand the retrosplenial contribution to spatial coding and navigation by recording simultaneously from this region as well as the hippocampus and the subiculum of adult mice.

We used Neuropixels 2.0 probes to identify hundreds of simultaneously active cells in the agranular and granular RSC, subiculum, and the hippocampus. Our standard experimental set-up involved foraging for treats in one or several 80 x 80 cm open field arenas. We investigated how novelty, object interaction, and spatial remapping between environments influenced retrosplenial activity.

Our data reveals clear spatial tuning in the granular RSC, characterised by a wide variety of firing patterns in two-dimensional arenas. Some showing aspects of egocentricity and some leaning more towards an allocentric representation of space. When moving between different environments high-firing, non-spatial retrosplenial cells showed signs of rate remapping, whilst a subset of spatially modulated cells in the granular RSC showed classic global remapping responses similar to what is observed in the hippocampus. We are currently trying to quantify both spatial and non-spatial dynamics of the recorded retrosplenial population and relating that to what is already known of the population within the (para)hippocampal loop.

**Keyword 1**: retrosplenial cortex
**Keyword 2**: navigation
**Keyword 3**: hippocampus
**Keyword 4**: spatial cells

**TOPOGRAPHICAL ORGANIZATION OF FUNCTIONAL CELL TYPES IN THE MEDIAL ENTORHINAL CORTEX**

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The medial entorhinal cortex (MEC) forms a map of local space and local landmarks. This is achieved through canonical feature cells like grid, object vector, head direction and border cells. These functional cell types are all located in the superficial layers of MEC but their exact anatomical organization remains to be determined.

Previous work has shown that the grid cell network consists of discrete modules of increasing scale along the dorsal-ventral axis of MEC. In addition, imaging studies have hinted at a clustering of grid cells towards the dorsomedial part of MEC. However, former studies were restricted to limited subdivisions of MEC in individual animals, precluding the imaging of all cell types simultaneously in the same animal.

Here, we combine miniaturized two-photon microscopy (MINI2P) in freely moving mice with multiple field-of-views to image activity in layer II of the dorsomedial area of MEC and parasubiculum (PaS). The preparation allowed us to map the canonical features of cells over several hundreds of micrometers in both directions mediolaterally from the PaS-MEC border and dorsal-ventrally within individual animals.
Our data show that each grid cell module forms an anatomically organized cluster at the very dorsomedial part of MEC close to the PaS border. While other cell types do not show such a clustering, we find an anticorrelation of their localization to the grid cell cluster.
These findings can have implications not only for where the grid signal is generated but also on our understanding of the functional interplay within the grid cell network.

**Keyword 1**: Entorhinal Cortex
**Keyword 2**: Spatial cells
**Keyword 3**: Topography
**Keyword 4**: mini2p

**ONTOGENY OF SPACE CODING: TOROIDAL MANIFOLDS PRECEDE GRID CELLS**

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The ability to navigate the environment is essential for survival and is therefore expected to emerge early in life. Grid cells in the medial entorhinal cortex (MEC) are considered the backbone of the mammalian neural map of space and, at the population level, organize into low-dimensional toroidal manifolds, consistent with continuous attractor network (CAN) theories. However, the developmental timeline of the grid network remains elusive. Using Neuropixels 2.0 probes, we performed high-density population recordings (600–1200 simultaneously recorded units) from the entorhinal cortex of freely moving rat pups as early as postnatal day P8. We uncovered and quantified the presence of toroidal manifolds as early as P10—preceding eye and ear canal opening and the onset of active exploration outside the nest. The abrupt emergence of the toroidal manifold around P10–P11 coincided with an increase in inhibitory drive within the network and the desynchronization of entorhinal firing dynamics, enabling the entorhinal network to disengage from external sensory inputs. From P10 to P12, distinct toroidal modules gradually emerged along the dorsoventral axis of the MEC, decorrelating from an initially single, coherent module. Once the animals began actively exploring their environment from P15 onward, we observed the gradual emergence of grid fields in individual cells, reflecting the progressive mapping of space onto the toroidal manifold. Our findings suggest that the neural networks underlying spatial coding possess strong experience-independent components, with self-organizing principles guiding the formation of low-dimensional manifolds onto which spatial information is subsequently mapped through experience.

**Keyword 1**: Entorhinal cortex
**Keyword 2**: Development
**Keyword 3**: Spatial cells

**POPULATION-LEVEL REMAPPING ACROSS THE HIPPOCAMPAL-ENTORHINAL-SUBICULAR CIRCUIT**

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The hippocampal-entorhinal circuit encodes and retrieves representations of distinct spatial environments. When an animal enters a new space, hippocampal place cells globally remap, while entorhinal grid cells, and head direction/ border cells of the subicular structures shift/rotate but largely maintain coherent representations.

Despite extensive research, most remapping studies have focused on single regions, recorded few neurons, and examined only a limited number of environments. Additionally, a key integrative node, the subiculum, remains understudied despite its strong reciprocal connectivity within the HPC-EC loop, where it may propagate or transform remapping signals and interface with the cortex.

Here, we assess population-level representations of distinct environments across the hippocampus, MEC, and subicular structures. We examine whether these regions encode environments using orthogonal, overlapping, or other structured representations and analyze the fine-timescale dynamics governing transitions between them.

We record from 3-4 NPX2.0 probes across the circuit as rats explore 2–5 distinct environments. CA1 representations of distinct rooms are orthogonal but destabilize across repeats when intervening rooms are experienced, while MEC representations expectedly overlap in low-dimensional space. Interestingly, subiculum forms a continuous, structured manifold, where timepoints from different sessions smoothly vary along a gradient reflecting the sequence in which rooms were sampled, despite minimal random population drift.

Moving forward, we aim to identify which subpopulations are unique across rooms, the sequence in which these ensembles remap, and whether transitions occur smoothly or as discrete shifts. This study will provide a high-resolution, circuit-wide analysis of remapping while clarifying the subiculum’s role in spatial representation.

**Keyword 1**: hippocampus
**Keyword 2**: remapping
**Keyword 3**: entorhinal
**Keyword 4**: subiculum
**Keyword 5**: dynamics

**DEEP-TISSUE TWO PHOTON IMAGING IN FREELY MOVING MICE**

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To uncover the neuronal mechanisms underlying complex cognitive functions, neuroscientists need to understand how information is encoded in different brain areas. This includes measuring neuronal activity from deep brain regions at cellular resolution in animals exhibiting natural behaviors. Currently, the state-of-the-art technologies are silicon probes and miniaturized two-photon microscopes (2P miniscopes). While 2P miniscopes offer significant benefits over silicon probes in terms of subcellular spatial resolution and the ability to record from the same neuronal population over extended periods, their imaging capacity is limited to superficial layers. Although three-photon (3P) miniscopes can overcome this limitation, their complexity has hindered applications in most behavioral studies. Therefore, calcium imaging in freely moving animals remains restricted to superficial brain regions, limiting our ability to study neurocomputational mechanisms within deep brain microcircuits.

In this study, we enhance the performance of our recently developed and commercial 2P miniscope (Mini2P-Thorlabs) by integrating a custom low-repetition-rate laser (20 MHz, Spark laser). This simple modification improves over 2-fold the signal-to-noise ratio for neuronal recordings in deep-brain tissue, enabling the detection of over 200 visual cortex neurons at 400µm depth, which was not achievable with a standard 80 MHz repetition-rate laser. Additionally, we demonstrate the suitability of this approach to record activity from medial entorhinal cortex (MEC) axons terminating in deep layers of CA1 and MEC layer V cell bodies in freely moving mice.

In conclusion, we present an effective solution to extend Mini2P's imaging depth, filling a critical gap in miniscopes technology for investigating deep-brain neural circuits.

**Keyword 1**: Two photon miniscope
**Keyword 2**: Deep tissue imaging
**Keyword 3**: Entorhinal cortex and Hippocampus

**NEURAL CONTROL OF HIPPOCAMPUS-DEPENDENT MEMORY CONSOLIDATION DURING SLEEP**

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The hippocampus plays a crucial role in memory consolidation during sleep. However, the exact cell types controlling this process are largely unknown. Here, we identified a new type of GABAergic interneuron (GINX) in the hippocampus that is selectively active during non-rapid eye movement (NREM) sleep, which is the major sleep state for memory consolidation. Inhibiting the activity of GINX neurons during NREM sleep impaired the formation of hippocampus-dependent spatial memory. Furthermore, GINX neurons had widespread projections within the hippocampus and controlled the generation of sharp-wave ripple activity, a hippocampal neural signature for memory reactivation. Together, we have uncovered a potential key cell type in the control of hippocampus-dependent memory consolidation.

**Keyword 1**: memory
**Keyword 2**: sleep
**Keyword 3**: hippocampus

**ENTORHINAL SINGLE-NEURON OUTPUTS TO THE TELENCEPHALON ARE DIVERSE AND EXHIBIT A SPATIAL GRADIENT**

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Long-term storage of everyday experiences relies on dynamic interactions between the hippocampus and the neocortex. The entorhinal cortex (EC) is thought to facilitate this hippocampal-neocortical dialogue, yet the organisational principles governing its output pathways remain poorly understood. To address this, we used MAPseq, single-neuron axonal reconstructions, and complementary viral tracing approaches to map the projection architecture of EC output neurons at single-cell resolution, testing competing models of global versus selective cortical targeting. Our findings reveal a striking mediolateral gradient in projection specificity, with neurons at the medial and lateral extremes preferentially targeting distinct, restricted cortical areas, while those in intermediate zones exhibit the highest broadcasting capacity. Furthermore, this gradient in projection selectivity aligns with a transcriptional continuum among L5a neurons, suggesting that EC output neurons are not confined to discrete functional compartments but instead follow a continuous organisational axis. These results support a model in which the EC flexibly integrates selective and widespread projection strategies, allowing for dynamic modulation of neocortical engagement based on behavioural demands.

**Keyword 1**: Entorhinal cortex
**Keyword 2**: MAPseq
**Keyword 3**: Single-neuron tracing
**Keyword 4**: Neural circuitry of memory

**HIERARCHICAL BAYESIAN MIXTURES FOR THE DISCOVERY OF PROJECTION NEURON SUBTYPES FROM MAPSEQ DATA**

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The mapping of single neuron projection patterns (motifs) reveals important organizational principles underlying inter-region communication, and informs our understanding of how brain regions distribute information to downstream targets.
Multiplexed Analysis of Projections by Sequencing (MAPseq) extracts single-cell resolution, brain-wide projection patterns from large numbers of neurons, enabling the discovery of the diverse projection neuron subtypes which can exist within a single brain region. MAPseq allows for high throughput data collection, but suffers from inherent noise induced by sequencing artefacts, contamination, and the common practice of pooling data across multiple brains.
The clustering of neurons into projection pattern based subtypes via MAPseq data, henceforth referred to as motif analysis, requires appropriate methods capable of both accommodating the aforementioned variability and effectively combining information across different brains.
We developed HBMAP - Hierarchical Bayesian Mixtures for Axon Projections - a novel model-based approach for motif analysis. HBMAP directly models axonal projections via the Dirichlet Multinomial. This avoids the need for extensive data transformations, accommodates the over-dispersion characteristic of MAPseq data, and provides a clear biological interpretation of the resulting motifs. Furthermore, the hierarchical approach employed by HBMAP enables the interrogation of inter-brain variability. Here we demonstrate the utility of HBMAP on two datasets describing axon projections from the visual cortex and auditory cortex respectively.

**Keyword 1**: MAPseq
**Keyword 2**: Computational Model

**REUNIENS-HIPPOCAMPUS BETA COUPLING DURING NAVIGATION IMPLICATES NUCLEUS REUNIENS IN SPATIAL MEMORY RECALL**

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Successful spatial navigation relies on the coordinated activity across a large-scale neural network including the hippocampus (HC) and prefrontal cortex (PFC). Based on anatomical connectivity, the nucleus reuniens (RE), a midline thalamic nucleus, has been hypothesized to act as a hub for coordinating the interactions between PFC and HC. The network interactions occur at different frequency ranges (e.g., theta, beta, gamma), which are thought to reflect different steps of information processing; however, the detailed mechanisms of task-specific cross-regional synchronization within the RE-HC-PFC network remain poorly understood.
We recorded extracellular activity (local field potential, LFP) from RE, HC, and medial PFC in rats learning a spatial navigation task in a large maze. Based on rat behavior in the maze, we distinguished two behavioral phases, early spatial exploration and later (food-motivated) goal-oriented behavior. Analysis of the LFP on directed cross-regional interactions revealed strong RE to HC 20-30 Hz beta synchrony during the goal-oriented task phase only, which increased with task performance. Besides the reversal of information flow from RE to HC during goal-oriented spatial navigation, we were able to confirm 6-10 Hz theta synchrony from HC to RE and mPFC to RE, as well as RE to mPFC coupling in the 30-120 Hz gamma range throughout all phases of the task. The synchronization between RE and HC implicates the RE in spatial memory recall, in line with the hypothesis of RE acting as a bidirectional information relay between mPFC and HC.

**Keyword 1**: Spatial memory
**Keyword 2**: Thalamus
**Keyword 3**: Navigation

**HOW ARE SPATIAL MAPS IN CA1 STABILIZED? EVALUATION OF NMDA RECEPTORS REQUIREMENT FOR SPATIAL MAP CONSOLIDATION**

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Spatial orientation is crucial for animal's survival. It is well established that place cells generate a cognitive map of an environment, enabling animals to determine their spatial location. Nevertheless, we still do not fully understand how these cells form and maintain spatial maps of novel environments over time. Previous work has shown that the formation of a stable hippocampal map of a novel environment requires NMDA receptor (NMDAR) activity, as the blockade of these receptors during exposure to a novel environment prevents the stabilization of the place fields of that environment, but has no effect on the stability of place fields of a familiar environment. This suggests that a place cell map becomes consolidated over time and this consolidation depends upon NMDAR, but as of yet we know little about the mechanisms underlying such consolidation. Many theories of memory consolidation posit their reactivation during offline states or subsequent sleep periods, which could also involve NMDAR activity. To study this, we familiarized the animals to one environment, and on the test day exposed them first to the familiar environment A and then a novel environment B. We injected the NMDAR antagonist CPP immediately after (rather than before) exposure to a novel environment, and then tested the long-term stability of the newly formed map of environment B and the already established map of environment A.

**Keyword 1**: hippocampus
**Keyword 2**: place cells
**Keyword 3**: spatial map
**Keyword 4**: NMDA
**Keyword 5**: consolidation

**DOWNREGULATION OF HIPPOCAMPAL TRPA1 CHANNEL EXPRESSION LEADS TO A HYPERMNESIC PHENOTYPE**

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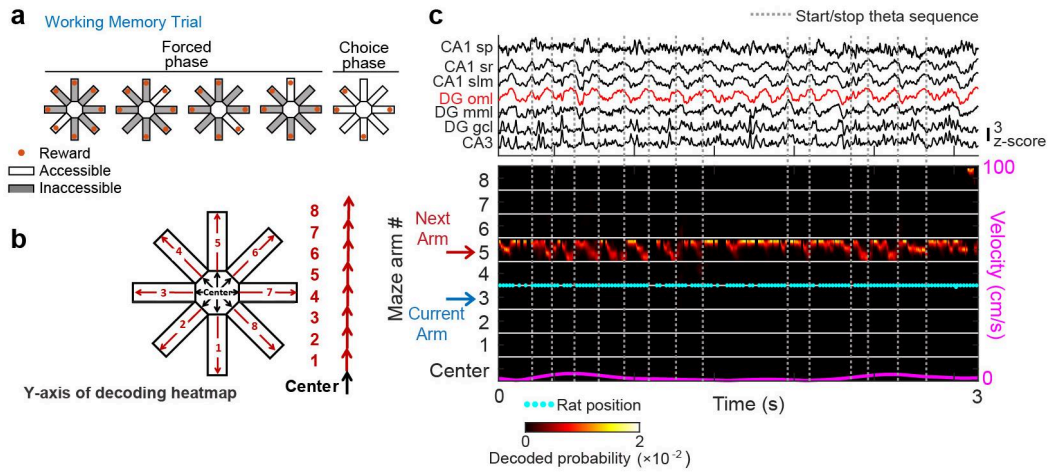
Transient Receptor Potential Ankyrin 1 (TRPA1) is a $Ca^{2+}$-permeable channel expressed in neuronal and non-neuronal cells of the peripheral and central nervous systems. Recent studies suggest a role for TRPA1 in cognition, as genetically modified mice lacking the TRPA1 gene (*Trpa1*$^{-/-}$) perform better than wild-type (*Trpa1*$^{+/+}$) mice in memory tasks. However, the specific cell types and memory phases involved remain unclear. Here, we investigated TRPA1 expression in neuronal populations by *in situ* hybridization, revealing its expression in GABAergic and glutamatergic cells of the hippocampus and frontal cortex, two key memory-related areas. Next, we assessed the impact of TRPA1 loss of function on recognition memory using novel object recognition and social discrimination paradigms, employing different methodological approaches: (i) *Trpa1*$^{-/-}$ mice, (ii) viral-mediated neuronal TRPA1 knockdown (AAV8-hSyn-sh-TRPA1-GFP) in the hippocampus and frontal cortex of *Trpa1*$^{+/+}$ mice; (iii) pharmacological antagonism via treatment with A967607 (100 mg/kg i.p.) in *Trpa1*$^{+/+}$ mice. Our findings confirm the previously reported hypermnesic phenotype of *Trpa1*$^{-/-}$ mice, extending this observation to recognition memory. Notably, we found that hippocampal but not cortical neuronal TRPA1 knockdown also induced a hypermnesic phenotype. Pharmacological studies revealed that TRPA1 antagonism produced different effects depending on the memory phase: it enhanced memory consolidation but had no effect on retrieval. Currently, we are investigating the impact of viral-mediated TRPA1 downregulation in specific hippocampal neuronal populations through the targeted injection of viral vectors into hippocampal GABAergic and glutamatergic neurons to further elucidate its role in cognition.

**MENTAL EXPLORATION OF FUTURE CHOICES DURING IMMOBILITY THETA OSCILLATIONS**

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Imagining the future is a fundamental cognitive function supported by the hippocampus. However, the neural mechanisms that sustain mental exploration in the service of working memory (WM) remain poorly understood. Although awake-sharp wave ripples (SWRs) have previously been proposed to support similar functions, recent evidence has made a strong case for SWRs as the key mechanism for memory consolidation rather than ongoing decision making. For example, disruption of SWRs does not impair immediate future choices in WM (Deceuninck & Kloosterman, 2024). We confirm these findings and identify novel network mechanisms in the rodent hippocampus in support of mental exploration while rats (*n* = 8) performed a hippocampal-dependent spatial WM task on an 8-arm radial maze. Using Neuropixels probes, we recorded local field potentials and neural population activity across the dorsal hippocampus. We observed theta oscillations that emerged independent of movement, were characterized by distinct current source density profiles in CA1 from standard sensory theta, and persisted during immobility. During bouts of immobility theta, hippocampal neuronal activity was organized into theta sequences, similar to movement. Yet, immobility theta sequences differed from movement sequences that represent short virtual path segments sweeping ahead of or behind the rat's current location, and instead, occurred at a shifted theta phase and coded for remote locations. They represented multiple arm choices, with frequent representation of the next choice. Our findings suggest that recurring bouts of theta oscillations during immobility, which are also observed in primates and humans, support the cognitive demands of mental exploration.

**Keyword 1**: Theta oscillations
**Keyword 2**: Hippocampus
**Keyword 3**: Mental exploration
**Keyword 4**: Theta sequences



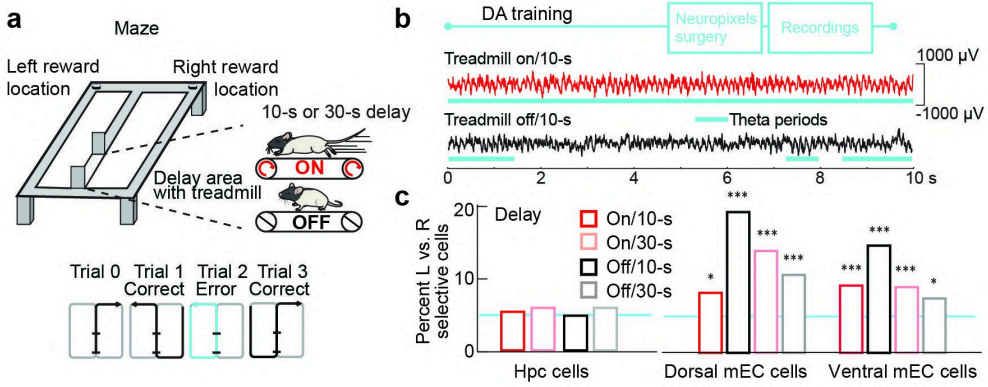

**Theta sequences during immobility preferentially represented the upcoming choice. a,** Hippocampa-dependent working memory task. **b**, Schematic of linearized heatmap organization. **c**, Local field potential (top) and Bayesian decoded probability of simultaneously recorded hippocampal population activity (bottom).

**DISTINCT HIPPOCAMPAL AND ENTORHINAL FIRING PATTERNS DURING MEMORY RETENTION**

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Working memory (WM) enables the temporary retention and manipulation of information, playing a crucial role in everyday decision-making and goal-directed behavior. Sequential firing patterns of cells in the hippocampus (HPC) and medial entorhinal cortex (MEC) are possible neural mechanisms for WM computations during delay intervals. We used a delayed spatial alternation task in which rats were either forced to run during the delay, which resulted in continuous theta oscillations, or not forced to run during the delay, which resulted in discontinuous theta and frequent non-theta states. With or without sustained theta oscillations over 30-s delay periods, we found that most hippocampal time cells (n = 6 rats) were only active during the first few seconds. In contrast, dorsal MEC time cell sequences (n = 9 rats) lasted ~15 s with running, but only ~5 s without running. As expected for grid cell-associated networks, these patterns strongly represented distance rather than time. Moreover, we observed periodic firing patterns during the delay period, but these seemed to be internally driven rather than related to delay onset time or distance in obvious ways. Interestingly, the periodic firing patterns did not occur on every trial, but rather in subsets of consecutive trials within a session. It was also MEC cells, but not hippocampal cells that showed memory-related firing patterns during the delay, and we will describe to what extent memory-related and attractor-like activity patterns are related. Taken together, our findings identify MEC, and to a lesser extent, hippocampus as critical for working memory retention.

**Keyword 1**: Time cells
**Keyword 2**: Working memory
**Keyword 3**: Place cells
**Keyword 4**: Grid cells




**Delay period neural activity patterns in a hippocampal-dependent working memory task. a**, Schematic of the delayed spatial working memory task (DA training). **b**, (top) Experimental timeline, (bottom) LFP from the same recording site during treadmill-on and off delay conditions. Blue line indicates theta periods. **c**, Proportion of turn-selective cells in hippocampus (Hpc) and mEC during the delay conditions.

**INVESTIGATING THE ROLE OF AGE-RELATED GENE BGLAP3 IN NEURON EXCITABILITY AND SPATIAL CODING IN THE MEDIAL ENTORHINAL CORTEX**

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The medial entorhinal cortex (MEC) is a key component of the spatial network that supports navigation and memory. Impaired function of the MEC is associated with cognitive decline, such as deficits in path integration during ageing and in Alzheimer's disease. However, whether impaired MEC function is correlated with changes in the expression of specific genes remains elusive. In this study, we found that Bglap3, a gene specifically expressed in MEC layer III, exhibited decreased expression in old mice (>22 months old) compared to young mice (3-4 months old). By knocking down or overexpressing Bglap3 in the MEC of either young or old mice, we identified common differentially expressed genes associated with neuronal excitability and synaptic transmission signaling pathways. Knocking down Bglap3 in the MEC of young mice decreased neuronal excitability, miniature EPSCs, and dendritic spine density of MEC layer II stellate cells. Furthermore, in vivo two-photon calcium imageing revealed that knocking down Bglap3 impaired spatial coding in the MEC, particularly in grid cells, head direction cells, and spatial cells. Additionally, knocking down Bglap3 impaired spatial learning performance in a path-integration-dependent water maze task. Our results demonstrate that Bglap3 is critical for the cellular function of the MEC and suggest its potential role in restoring ageing-related dysfunction in spatial learning and memory.

**Keyword 1**: Medial entorhinal cortex
**Keyword 2**: Ageing

**UNRAVELING THE ROLE OF OLMA2 CELLS IN THE REGULATION OF APPROACH-AVOIDANCE BEHAVIOR**

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The hippocampus exhibits functional distinctions along its dorsal-ventral axis, yet the connectivity patterns of specific interneuron populations remain incompletely understood. Among these, OLMα2 cells, identified by the *Chrna2* gene, serve as regulators of hippocampal processing through their gating properties. Using monosynaptic rabies tracing, we mapped the input connectivity of OLMα2 cells across the dorsal, intermediate, and ventral hippocampus, revealing region-specific differences in both intrinsic and extrinsic circuit integration. These findings underscore the functional heterogeneity of OLMα2 cells in shaping hippocampal network dynamics and behavioral output.
The ventral hippocampus plays a pivotal role in resolving conflicting information to modulate cortical and subcortical regions implicated in proactive behavioral responses. Notably, OLMα2 cells in the intermediate and ventral hippocampus receive inputs from projection neurons targeting the medial prefrontal cortex and nucleus accumbens core structures critically involved in approach-avoidance behavior. To elucidate the functional role of OLMα2 cells in behavioral regulation, we modulated their cholinergic responsiveness and assessed their involvement in escape behavior. Additionally, we examined the effects of the cholinergic modulation on local field potential activity in the ventral hippocampus to explore its impact on network oscillations. Our findings provide insights into the region-specific connectivity and neuromodulatory regulation of OLMα2 cells, highlighting their contribution to hippocampal circuitry and adaptive behavioral responses. This study further contributes to the understanding of interneuron-specific connectivity along the hippocampal axis and its implications for cognitive and affective processing.

**Keyword 1**: Hippocampus
**Keyword 2**: OLM-cells
**Keyword 3**: Chrna2
**Keyword 4**: Acetylcholine
**Keyword 5**: Approach-avoidance

**BEHAVIOURAL EFFECTS OF OLMALPHA2 CELLS ACROSS THE DORSOVENTRAL AXIS OF THE HIPPOCAMPUS**

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Oriens lacunosum-moleculare cells are GABAergic interneurons situated to control the inputs to CA1 pyramidal neurons. A subgroup of these neurons mostly in the ventral hippocampus express the *chrna2* gene (OLMa2 cells), and have been demonstrated to control theta type II oscillations. We found that such cells can have anxiogenic and anxiolytic effect if inhibited or stimulated respectively, which was coupled to theta II oscillations. In parallell studies, we found that intermediate OLMa2 cells affected object recognition, again in a bidirectional manner. However, dorsal OLMa2 cells had opposite effects on fear learning compared to intermediate cells and were not involved in object learning. We here investigate additional functional differences between intermediate and ventral cells. We use optogenetics to modulate either intermediate or ventral cells during behaviours such as object recognition, predator odour task and neutral odor association task. Our preliminary data and ur previous work indicate that intermediate OLMa2 cells are responsible for processing of object-related inputs while ventral OLMa2 cells are more involved in the anxiety related behaviours. Hence, we also investigate whether there are differences in connectivity with either intermediate or ventral hippocampus and external structures by retrograde viral tracing. We have identified a number differences in the connectivity patterns comparing intermediate and ventral hippocampus. Therefore, we suggest that intermediate and ventral OLMa2 cells have distinct functions in behaviour.

**Keyword 1**: Plasticity
**Keyword 2**: Synapse
**Keyword 3**: CREB
**Keyword 4**: Transport
**Keyword 5**: Jacob/NSMF

**OSCILLATORY RHYTHMS IN HIPPOCAMPUS AND ENTORHINAL CORTEX DURING A SOCIAL RECOGNITION TASK**

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Social memory is a fundamental aspect of behavior, enabling the recognition and recall of conspecifics, which is important for animal survival and reproduction. Hippocampal area CA2 is essential for social recognition memory including encoding, consolidation, and recall of social interactions. Indeed, principal cells in CA2 exhibit both spatial and social tuning. Interestingly, the lateral and medial entorhinal cortices provide rich contextual and spatial information to the hippocampus, but the involvement of the entorhinal cortex in social information processing remains unclear.

The local field potential (LFP) reflects the synchronized activity of local neurons, and specific oscillatory rhythms are hypothesized to contribute to information processing. For instance, theta (6-12Hz) and gamma (25-100Hz) oscillations synchronize neural activity within the medial temporal lobe and are considered important for learning and memory processes. However, the contribution of oscillatory rhythms to social processing is still unknown.

To address this, we recorded LFP from the dorsal hippocampal subfields CA1, CA2, and CA3, and the lateral and medial entorhinal cortices during a social recognition task. The task included a baseline session where the subject rat explored an open field, followed by sessions where a familiar conspecific was moved between perforated corner boxes. Lastly, a novel animal was introduced in the opposing corner.

The average power of the theta oscillations was of similar magnitude during the different trials. We are currently comparing the LFP between the different conditions, including whether interactions with familiar or novel conspecifics are reflected in changes in the LFP of the hippocampus or entorhinal cortex.

**Keyword 1**: Social recognition memory
**Keyword 2**: CA2
**Keyword 3**: Hippocampus
**Keyword 4**: Lateral entorhinal cortex
**Keyword 5**: Medial entorhinal cortex

**VISUAL CUE REPRESENTATIONS IN THE POSTRHINAL AND MEDIAL ENTORHINAL CORTEX DURING SPATIAL NAVIGATION**

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The integration of visual information during navigation remains incompletely understood. Emerging evidence indicates that neurons in the medial entorhinal cortex (MEC) exhibit a generalized encoding of sensory cues, with neurons anchoring to visual cues, creating a cohesive representation of space. While the MEC integrates self-motion and environmental sensory information to encode position, the postrhinal cortex (POR) enhances the visual details necessary for recognizing specific features within different contexts. However, it remains unclear how the spatial environment and visual cues are represented in the MEC and POR and how these representations evolve when animals learn to navigate.
Here, we investigated the extent to which visual computations during virtual reality navigation are shared or unique between the POR and MEC. Our findings confirm that MEC neurons exhibit spatially anchored, generalized visual representations, and specifically represented distinguishable visual cues. In the POR, neurons exhibited generalized visual representations but showed more selective tuning to the cues. Significant changes occurred in the MEC population following repeated exposure to the same environment. This was reflected by an increase in neurons with tuning curves that collectively tile the entire track once and a decrease in neurons with multiple firing fields. In contrast, the POR neurons showed more stable visual tuning from naive to expert. When exposed to novel cue configurations, neurons with multiple cue fields showed greater stability across tracks with varying cue spacing in both regions. The POR maintained higher stability across different environments, while MEC neurons exhibited more dynamic changes during learning and across environments.

**Keyword 1**: postrhinal cortex
**Keyword 2**: visual association
**Keyword 3**: entorhinal cortex

**LOCAL INHIBITORY CIRCUITS MEDIATE CORTICAL REACTIVATIONS AND MEMORY CONSOLIDATION**

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Highly salient events activate neurons across various brain regions. During subsequent rest or sleep, the activity patterns of these neurons often correlate with those observed during the preceding experience. Growing evidence suggests that these reactivations play a crucial role in memory consolidation, the process by which experiences are solidified in cortical networks for long-term storage.

Here, we employ longitudinal two-photon Ca2+ imaging alongside paired LFP recordings in the hippocampus and cortex, to show that targeted manipulation of PV+ inhibitory neurons in the lateral visual cortex *after* daily training selectively attenuates cue-specific reactivations and learning, with only minute effects on spontaneous activity and no apparent effect on normal function such as visual cue-elicited responses during training. In control mice, reactivations were biased towards salient cues, persisted for hours after training had ended, and the prevalence of reactivations was aligned with the learning process. Overall, our results underscore a crucial role for cortical reactivations in memory consolidation.

**Keyword 1**: postrhinal cortex
**Keyword 2**: visual association
**Keyword 3**: memory consolidation

**TASK AND BEHAVIOR-RELATED VARIABLES ARE ENCODED BY THE POSTRHINAL AND MEDIAL ENTORHINAL CORTEX DURING NON-SPATIAL ASSOCIATIVE LEARNING**

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The medial entorhinal cortex (MEC) is pivotal in spatial computations and episodic memory. However, it remains elusive whether MEC could play a general role in different types of associative learning and how this develops with training. It has been shown that the postrhinal cortex (POR), upstream to MEC, integrates visual stimuli with salient outcomes. Here, we use a non-spatial visual association task to investigate whether MEC neurons represent visual cues during learning. We recorded neural activity in MEC and POR throughout training as mice associated drifting gratings with rewarded, aversive, or neutral outcomes.

Our findings reveal that the neural tuning curves in both the POR and MEC change with task learning. POR neurons exhibited robust cue responses from the start as expected. In contrast, MEC neurons did not initially respond to visual cues but developed a robust tuning toward rewarded trials, forming distinct functional clusters that were either activated or suppressed by reward-related behavior. These clusters segregated anatomically in MEC and maintained strong within-cluster correlations before and after training. Strikingly, information about choice to lick emerged with learning in both areas, and most significantly within the functional cell clusters representing reward consumption and plus-cues. Our results demonstrate that although neurons in MEC and POR develop behavior-modulated tuning during learning, and that the MEC exhibits strong within-cluster correlations and anatomical organization. Conversely, the POR population exhibits less structural organization and more specific stimulus-tuning. Our findings reveal that the MEC can encode task- and behavior-related variables beyond spatial information.

**Keyword 1**: entorhinal cortex
**Keyword 2**: visual association
**Keyword 3**: postrhinal cortex

**NEUROMODULATORY DYNAMICS DURING MEMORY FORMATION (REMAPPING) AND RECALL IN RSC**

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The reorganization of place cell's activity, known as remapping, is crucial for the brain to adapt to new experiences and represent novel spatial environments. For successful remapping, the network needs to dynamically adapt to the continuous stream of sensory information. In addition, novelty causes the release of several neuromodulators, often associated with arousal, that likely play a permissive role in forming new memories. Here, we investigate the role of several key neuromodulatory systems, including acetylcholine (ACh), dopamine (DA), noradrenaline (NE) and serotonin (5-HT). These modulators have a widespread influence and may alter place cell dynamics by regulating synaptic plasticity, thereby promoting remapping. Recent work has shown that neuromodulators facilitate plasticity in the hippocampus, yet its role in the modulation of neocortical place-like cells remains unexplored. The recent development of so-called GRAB protein sensors now enables measuring ACh, DA, NE or 5-HT using microscopy with subsecond resolution. We expressed these sensors in the retrosplenial cortex of mice that were trained to perform a simple navigation task in a virtual environment. We found that several neuromodulators demonstrate a transient increase in fluorescence when mice enter a novel environment. Distinct patterns of neuromodulatory release were also correlated with locomotion. These preliminary data suggest that ACh, DA, NE and 5-HT are central for organizing cell activity to represent new spatial environments in the retrosplenial cortex.

**Keyword 1**: Spatial memory
**Keyword 2**: Place cells
**Keyword 3**: Neuromodulators

**PLASTICITY PARAMETERS OF ORIENTATION SELECTIVE RETINAL CIRCUITS**

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*[1]King's College London, Centre for Developmental Neurobiology, London, United Kingdom, [2]King's College London, MRC Centre for Neurodevelopmental Disorders, London, United Kingdom*

Interactions with the environment influence how the brain processes sensory information. In the visual system, it is well established that altering visual input to the eyes early in life has significant effects on brain plasticity in the cortex and subcortical areas. Recently our lab discovered that the retina, as the sensory organ itself, possesses its own capacity for plasticity in response to varying visual environments during development. These changes were apparent for a specific visual functionality, orientation selectivity, which allows the retina to detect elongated object in the visual field. We found that raising zebrafish under different visual conditions changes the morphology and function of specific retinal cells associated with orientation-selective circuits and alters the visual behaviour of animals.

I will demonstrate evidence of plasticity relevant to orientation selectivity in the retina. I will outline my work, which focuses on the parameters of this plasticity and specifically the possible existence of critical period during which it occurs. I will present my findings on how proportions of different orientation selectivity populations change over time in development and how they are differently affected when exposing animals to altering visual environments at different time windows or for different time lengths.

My results show that the parameters of plasticity in the sensory organ vary over time, possibly affecting horizontal and vertical circuits differently at distinct developmental stages.

**Keyword 1**: Retina
**Keyword 2**: Orientation
**Keyword 3**: Plasticity

# Cognitive Neuroscience and Decision Making

**PUPIL CONSTRICTS DURING MINDFULNESS MEDITATION**

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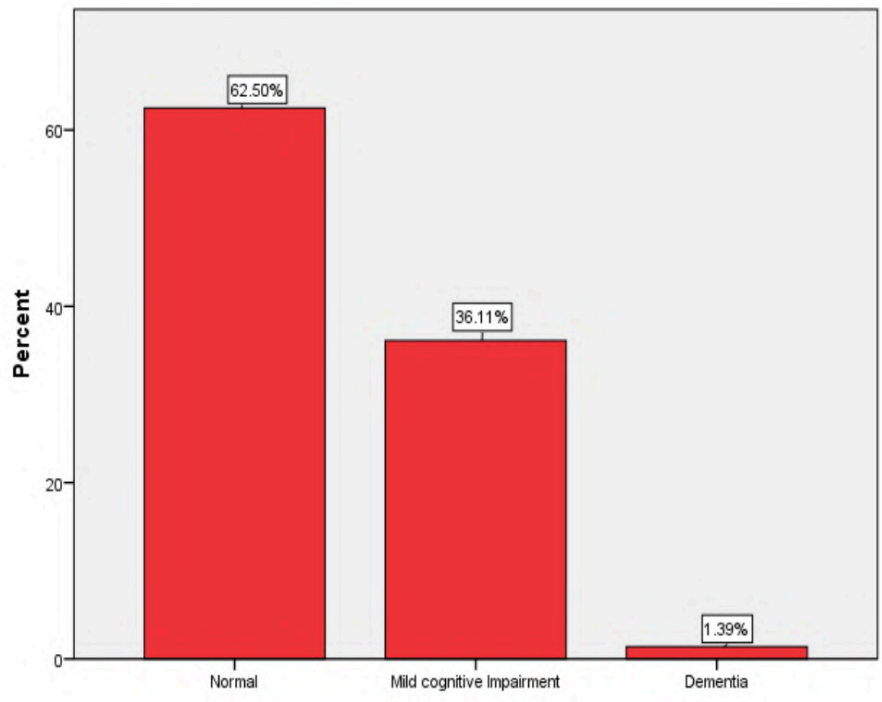
Mindfulness meditation is widely practiced by people all over the world and has been both theorized and scientifically validated. Many clinical studies have shown that mindfulness meditation helps mitigate psychiatric and behavioral issues, such as depression and attention-deficit/hyperactivity disorder. The depth and quality of meditation are often evaluated physiologically using measures such as heart rate variability and electroencephalography. The pupil is another useful physiological measure for this purpose, as it changes in response to both autonomic activity levels and ambient light intensity.
College students were recruited for the study and were dark-adapted before counting their breaths silently (breath-counting method). During meditation, they were instructed to keep their eyes open to measure pupil diameter. The pupil constricted significantly during meditation but began to dilate soon after the practice. This pupillary change suggests that meditation increases parasympathetic nervous activity.
In a separate study, other college students were instructed to focus on their spontaneous breath cycles (breath meditation method). Their pupils also constricted during meditation, indicating that parasympathetic nervous activity is activated during meditation.
Some psychological studies have reported that two personality traits—conscientiousness and neuroticism—correlate with the ease of mindfulness meditation practices, as measured using Big-Five personality inventories. We also found that the magnitude of pupillary constriction varied depending on these traits in the breath meditation method, whereas no such effect was observed in the breath-counting method. These contradictory findings suggest that the breath-counting method may be easier for inducing meditation.

**Keyword 1**: mindfulness
**Keyword 2**: meditation
**Keyword 3**: pupil
**Keyword 4**: autonomic nervous system
**Keyword 5**: personality

**AGE-RELATED COGNITIVE CHANGES IN ELDERLY MALES**

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**Background and Aim:** Early detection and intervention for mild cognitive impairment (MCI) may improve older adults' quality of life. Validated cognitive screening methods, such as the Montreal Cognitive Assessment (MoCA) tests, are advised for the early detection of cognitive impairment in the most recent guidelines.
**Methods:** Convenience sampling method was used to collect data from 100 healthy elderly (above 60 years age) males of Biratnagar metropolitan city in Nepal. MoCA test was performed to assess the cognitive impairment of the subject. The cut off score for mild cognitive impairment was set less than 26 and dementia less than 21 out of 30 score test. +1 score was added to all the subjects having less than 12 years of education to minimize procedural error.
**Results:** The data was analyzed in IBM SPSS version 23 software. The sample's mean age was 71.14 + 6.8 years, and its mean MoCA test score was 26.18 + 2.40. Among 72 participants, 36.1% had mild cognitive impairment and 1.4% had dementia.
Pearson's correlation test was performed to observe the relationship between age, education level and MoCA score. There was strong correlation (-0.558) between age and MoCA score which is statistically significant at (<0.05). Whereas there was statistically significant medium correlation (+0.384) between education level and MoCA score.
**Conclusion:** Healthy individuals, age and educational attainment have an impact on cognitive deterioration. The quality of life and outcomes for those who are afflicted by cognitive impairment can be enhanced by timely management of risk factors.

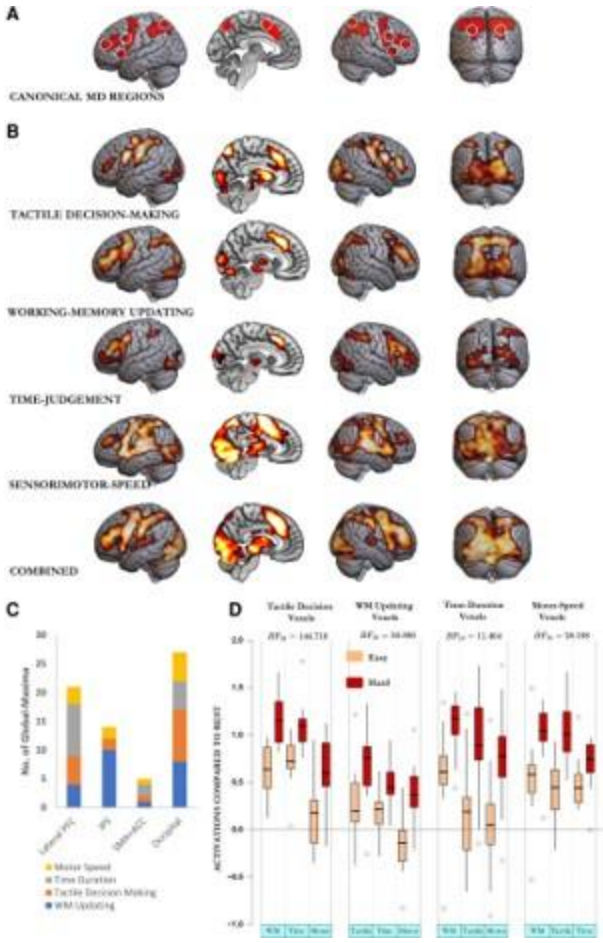



**Keyword 1**: MoCA test,
**Keyword 2**: Cognitive impairment,
**Keyword 3**: Elderly males

**THE VISUAL CORTEX IN THE BLIND BUT NOT THE AUDITORY CORTEX IN THE DEAF BECOMES MULTIPLE DEMANDS REGIONS**

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Sensory deprivation alters cortical activation patterns, yet the functional roles of these changes remain unclear. In congenitally blind individuals, the visual cortex activates to a wide range of non-visual tasks, while in the congenitally deaf, the auditory cortex responds to visual and tactile tasks. Activating to any task related events and various sorts of cognitive demands involving different task-modalities is a sign of being frontoparietal multiple-demand (MD) regions. In the present study, we investigated whether deprived sensory cortices in the blind and deaf become MD regions.Our study tested this by examining 22 congenitally and early blind, 20 sighted, and 10 early deaf individuals performing four diverse fMRI tasks with alternating easy and hard blocks, involving tactile judgments, working memory updating, time-duration estimation, and rapid motor responses. In blind individuals, nearly the entire occipital cortex activated in response to increased control demands and showed stronger connectivity with MD regions compared to sighted controls. Crucially, the same occipital voxels in blind individuals responded robustly to all task demands (BF10 > 28), whereas the auditory cortex in the deaf showed no such response (BF01 > 3). These findings indicate that the deprived visual cortex in blindness integrates into the MD network, whereas the deprived auditory cortex in deafness does not.

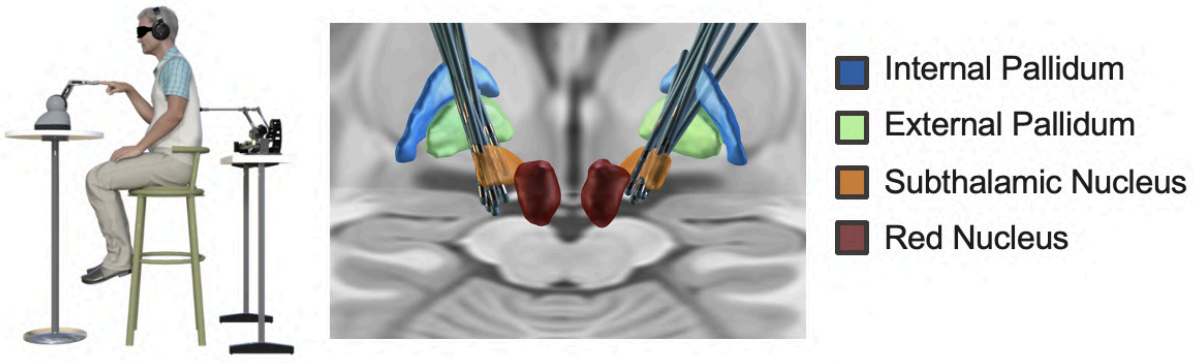



**Keyword 1**: Neuroplasticity
**Keyword 2**: blind
**Keyword 3**: multiple-demands network
**Keyword 4**: cognitive control
**Keyword 5**: deaf

**SUBCORTICAL AND CORTICAL NEURAL SIGNATURES OF HALLUCINATIONS IN PARKINSON'S DISEASE**

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Parkinson's disease (PD) is a progressive neurodegenerative disease characterized by a wide range of motor symptoms. Growing evidence indicates that PD is also affecting non-motor circuits, resulting in numerous non-motor symptoms. **Hallucinations, affecting about 50% of PD patients,** are linked to rapid disease progression, including **cognitive decline and psychosis**. Despite their high clinical relevance, the brain mechanisms, especially the subcortical ones, underlying hallucinations in PD are currently poorly understood. Here, thanks to **latest DBS-recording technology**, we characterize for the first time the subcortical and cortical electrophysiological signature of a specific minor hallucination, namely the presence hallucination (PH), in PD patients. One of the challenges in studying hallucinations is their unpredictable and subjective nature. To overcome this issue, we employed **a robotic sensory-motor stimulation device**, to induce on-demand PH in PD patients. During the robotic procedure, we recorded brain activity from **Subthalamic Nuclei (STN)** and cortical regions. Subcortical activity was recorded through chronically implanted DBS devices (Medtronic Percept) that allow acquisition of the local field potential in the target region, while cortical activity was acquired using scalp EEG. We found that the activation of the STN in the alpha band (8-12 Hz) was higher during robot-induced hallucinations than in the control condition, suggesting that abnormal activation of this frequency band could be linked to the induction of PH. However, the difference in cortical evoked responses between PH-inducing and control condition was dampened for PD patients already suffering from hallucinations in their daily lives, indicating a possible mechanism underlying symptomatic hallucinations.




**Keyword 1**: presence hallucination
**Keyword 2**: subthalamic nucleus
**Keyword 3**: Parkinson's Disease

**AT THE BEGINNING OF EMOTIONAL BURNOUT: ANXIETY TENSION STAGE FORMATION**

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Burnout develops gradually and often goes unnoticed, with symptoms that may take years to manifest and lead to significant mental and behavioral changes. Despite its impact, the processes underlying burnout remain largely unknown. Early detection of burnout is essential. This study aimed to examine EEG frequency changes associated with the severity of the Anxiety-Tension stage of Emotional Burnout. A total of 752 participants, including students and staff from Taras Shevchenko National University of Kyiv (209 males, mean age = 19.2; 543 females, mean age = 18.28), were assessed using the 84-item Boyko's Syndrome of Emotional Burnout Inventory. EEG data were recorded during a 3-minute resting state with eyes closed, and artifact-free segments from all frequency bands (0.2–45 Hz) were analyzed. Normalized power spectral densities (PSD) and coherence were calculated, focusing on the 61–70 second segment of the recordings. The results revealed burnout-related changes in spectral characteristics associated with the Anxiety-Tension stage, with significant alterations observed in the theta 2 band (frontal and left temporal-parietal cortex), alpha 2 band (right parietotemporal cortex), and beta 1 band (left frontal-central-right parietal axis). Coherence analysis complemented the spectral findings, highlighting alpha band synchronization in frontal and parietal areas, which supports visuospatial attention and memory processes. In contrast, desynchronization in high beta and gamma bands indicated disrupted connectivity, affecting higher-order cognitive functions. These findings suggest that the Anxiety-Tension stage primarily impacts processes related to short-term memory and focused attention, providing a potential framework for the early identification of burnout through neurophysiological markers.

**Keyword 1**: Emotional burnout
**Keyword 2**: Anxiety Tension
**Keyword 3**: EEG
**Keyword 4**: Power Spectral Density
**Keyword 5**: Coherence

***Fig. 1.*** Frontal cortex. The significant difference in rank values between independent samples (normalized power spectral densities) for two conditions ("not formed (Anxiety Tension 1)" and "in progress (Anxiety Tension 2)"; "not formed" and "formed (Anxiety Tension 3)"; "in progress" and "formed").

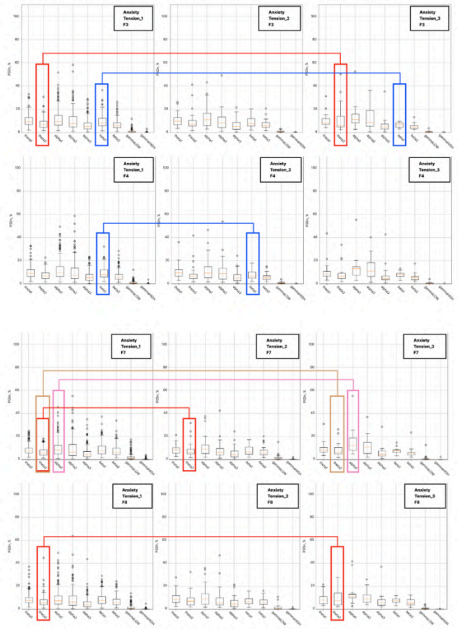


***Fig. 2***. Coherence between the brain areas for the considered frequency ranges in resting state (alpha subbands)

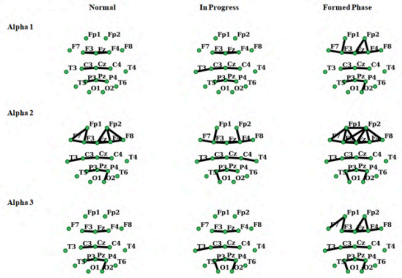
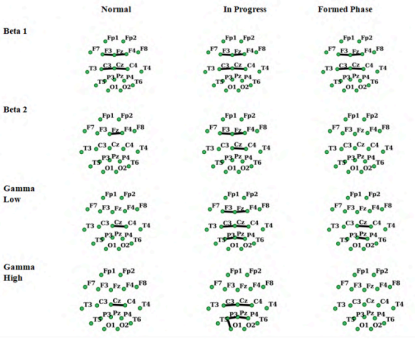



***Fig. 3***. Coherence between the brain areas for the considered frequency ranges in resting state (beta, gamma subbands)



**STUDY OF PSYCHOLOGICAL INTERVENTION AND EEG IN ADHD CHILDREN**

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Introduction: Attention Deficit/Hyperactivity Disorder (ADHD) is complex neurodevelopmental condition in children. The research studied neurobiological mechanisms to applied EEG and Cognitive Computer Tests (CCT) intervention. This is preliminary data of research.
Methods: 20 Children with ADHD(predominantly inattention) aged 7-11 year participated in study. EEG was done 2 times before and after psychological training for measurement improvement of attention. CCT held twice per week and cognitive paper exercises conducted daily during 3months. Content and complexity of exercises complicated in proportion to improvement of skills. EEG registration includes resting states with closed and open eyes(3 minutes each), again with eyes closed(4 minutes). EEG spectral analysis done with different frequency. EEG data in presented research are uploaded to first online EEG dataset(in Georgia) -EEGEEGHUB.GE. Big EEG data collected in Georgia uploaded for first time and integrated in EOSC under „Horizon“ grant.
Summary: 3 months CCT training decreased the average reaction time($P<0.05$) but no correct responses. Preliminary EEG revealed diffuse pattern of baseline recording(61%); elimination power spectra predominantly frontal-parietal delta(44%) and theta(53%) and less modulate alpha(43%).
Conclusion: Improvement with CCT(decreased reaction time) might not be translated into possible outcomes of CCT training in ADHD children.
Different EEG pattern might correlate delay of brain maturation in ADHD children. More research needs to reveal the objective and definitive markers of ADHD. Identification of distinctive EEG profiles can be used as a less costly biomarker and has prognostic implications for correct diagnosis and/or treatment of ADHD. Study supported by Shota Rustaveli National Science Foundation Research grant #FR-22-1991

**Keyword 1**: EEG
**Keyword 2**: ADHD
**Keyword 3**: CCT

**RISK, REWARD AND RESTRAINT: INVESTIGATING IMPULSIVITY ON A RODENT GAMBLING TASK**

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*[1]The University of British Columbia, Vancouver, Canada, [2]SDPR Government of British Columbia, Victoria, Canada*

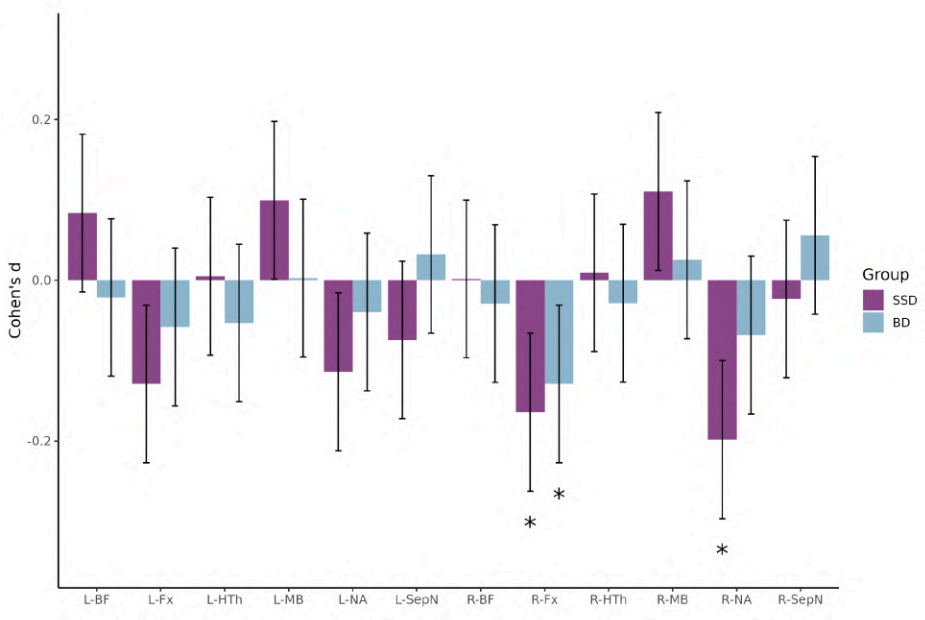
Gambling disorders involve persistent risk-taking despite adverse consequences. Similar to other addictive behaviours, pathological gambling is reinforced by rewarding cues like lights and sounds in casinos. The cued-rat gambling task (crGT) has demonstrated the impact of win-paired cues in reinforcing risky choices in rats, promoting an addictive-like phenotype. Compared to optimal options, risky options result in larger per-trial rewards but diminished returns long-term. Addiction scientists constantly seek to improve animal models to promote voluntary abstinence, but gambling disorder research has experienced limited innovation. To address this gap, this current work manipulates how long animals must wait before either the risky or safe options are presented on the crGT, taking advantage of animals' natural tendency to discount the value of delayed rewards. In this "delay discounting" version of the crGT (dd-crGT), presenting optimal options 2-8 seconds sooner than risky options for 15 sessions significantly increased optimal choice in most male and female rats. Furthermore, these beneficial effects were evident even after wait times were equalized across options, thereby encouraging voluntary abstinence from risk-taking. However, risk-preferring female rats failed to show long-term improvement, demonstrating a resistance to the optimal-phenotype manipulation ($p$= 0.903). Our findings suggest that risky female rats show a unique vulnerability to persistent addictive behaviour, replicating human studies in which women experience more difficulty breaking addiction patterns. In finding that modifying wait times discouraged persistent gambling behaviour in risky males($p$<0.001), our results offer promising translational potential for treating gambling disorders in these individuals, which historically have proven difficult to address.

**Keyword 1**: Gambling Disorder
**Keyword 2**: Delay-Discounting
**Keyword 3**: Decision-Making

**STRUCTURAL BRAIN ABNORMALITIES OF THE SUBCORTICAL LIMBIC SYSTEM IN PATIENTS DIAGNOSED WITH SCHIZOPHRENIA AND BIPOLAR DISORDER**

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Schizophrenia spectrum disorders (SSD) and bipolar disorders (BD) are severe mental disorders linked to alterations in the limbic system of the brain. While larger limbic structures like the amygdala and hippocampus are well-studied, smaller subcortical brain structures remain less explored. We examined case-control differences in MRI-derived brain volumes of the fornix, nucleus accumbens, basal forebrain, septal nuclei, hypothalamus, and mammillary bodies, and their associations with negative symptoms, age of onset, sex, and medication status. A cross-sectional sample of 1,604 age- and sex-matched participants (802 healthy controls (HC), 462 patients with SSD, and 340 BD patients) aged 17-65 years were included for volumetric analysis of T1-weighted MR images using the ScLimbic segmentation tool from FreeSurfer. ANCOVA and multiple regression models, adjusted for age, age², sex, and intracranial volume, assessed volumetric case-control differences and associations with clinical variables. We observed smaller right nucleus accumbens volumes in SSD (Cohen's d = -0.19, p = 0.04), and smaller right fornix volumes (p = 0.008) in SSD (d = -0.16) and BD (d = -0.12) compared to HC (Fig1), driven by schizophrenia and bipolar I disorder subgroups. Additionally, SSD patients using antipsychotic medication had smaller bilateral basal forebrain volumes than non-users (left, p = 0.003, right, p = 0.005). No significant associations were found between subcortical limbic volumes and negative symptoms, age of onset, or sex. The findings highlight region-specific volumetric differences in subcortical limbic structures in SSD and BD. Observed basal forebrain differences suggest a potential impact of AP use, warranting further investigation.




**Keyword 1**: Psychotic disorders
**Keyword 2**: Limbic system
**Keyword 3**: Negative symptoms
**Keyword 4**: Structural MRI

**ENHANCING COGNITIVE PERFORMANCE THROUGH OLFACTORY STIMULATION: THE IMPACT OF LAVENDER ODOR ON PROBLEM-SOLVING AND MEMORY ACROSS DIFFERENT AGE GROUPS**

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Background:
Olfactory stimulation has been increasingly recognized for its potential cognitive benefits. Lavender odor has been linked to improved cognitive function, including problem-solving and memory. This study investigates the effects of lavender exposure on problem-solving, short-term memory (STM), and long-term memory (LTM) across three educational levels: Primary (ages 12–15), Middle (ages 16–18), and High (ages 18+).
Methods:
A Cluster Randomized Controlled Trial (RCT) was conducted across multiple institutions, with 60 students per category divided into experimental (lavender exposure) and control groups. Cognitive performance was assessed using category-specific tasks
Descriptive statistics (Mean, SD, IQR) were computed, and data normality was assessed using Shapiro-Wilk tests. Independent t-tests for normal distributions, while Mann-Whitney U tests for non-normal data were used. Effect sizes were calculated using Cohen's d (0.5–0.9) and Rank-biserial correlation (r = 0.3–0.6).
Results:
Lavender exposure significantly improved cognitive performance across all categories. In the Primary category, problem-solving scores were notably higher in the experimental group ($M = 14.18$, $SD = 4.49$) than in the control ($M = 10.90$, $SD = 4.32$, $p < 0.05$, $d = 0.68$). Similar enhancements were observed in the Middle category ($p < 0.05$, $r = 0.52$) and High category ($p < 0.01$, $d = 0.75$). STM and problem-solving showed the most substantial improvements across all groups.
Conclusion:
Lavender odor significantly enhances cognitive function with the strongest effects in middle and high school students. These findings support the use of olfactory stimulation as a cognitive enhancement strategy in educational settings.

**Keyword 1**: Cognitive enhancement
**Keyword 2**: lavender odor
**Keyword 3**: problem-solving
**Keyword 4**: short-term memory

**CHANGES IN MISMATCH NEGATIVITY (MMN) AND ERROR RELATED NEGATIVITY (ERN) DURING A HEALTHY PREGNANCY**

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Pregnancy is marked by dramatic social, psychological and physiological changes and is a time of increased risk for mental illnesses, such as postpartum psychosis. Recent research has further shown that human pregnancy is associated with heightened structural neuroplasticity. The functional brain changes during pregnancy and how this leaves women vulnerable to mental illnesses are however still poorly understood.
The predictive coding theory of psychosis proposes that disruptions to the updating of mental models of the world in light of sensory information underlies positive symptoms. Two components of the event-related potential that are hypothesized to be related to such updating are the mismatch negativity (MMN), representing the detection of surprising changes in our environment, and the error related negativity (ERN), which signals when we have made a mistake. Characterizing changes to these components during pregnancy may be relevant to understanding the heightened risk for psychosis associated with the postpartum period.
In this longitudinal EEG study, we investigated the ERN and MMN amplitudes and subclinical psychotic symptoms, in 112 healthy women before, during and after pregnancy. We hypothesized that the amplitude of both ERPs would be altered during pregnancy and that the amount of change would correlate with changes in symptoms.
We found significant decreases of the ERN amplitude after childbirth, $t(162)=2.231$, $p=0.02$, but no change in the MMN amplitude. Future directions include assessing whether these changes in the ERN amplitude are related to symptoms of psychosis.

**NEUROPHYSIOLOGICAL FEATURES OF MENTAL STRESS AND THEIR SEX-SPECIFIC DIFFERENCES**

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Mental stress is a common experience in nearly everyone's life and affects individuals differently. It impacts health in various ways, including elevated blood pressure and the development of conditions such as depression and neurodegenerative diseases. However, identifying reliable markers for mental stress is challenging due to its variability.
This work aims to identify consistent correlates of mental stress using both Electroencephalography (EEG) and Electrocardiography (ECG), with a particular focus on sex differences. Twenty-five volunteers were subjected to stress by solving mental arithmetic tasks under time pressure. To enhance the stress levels experienced, participants were additionally exposed to ambient workplace noise and received negative feedback.
Band power (BP) features and frontal alpha asymmetry (FAA) were extracted from the EEG signals. The mean theta BP was significantly higher during the stress phase in frontal, central, and parieto-occipital brain regions. The mean alpha BP indicated higher values predominantly in the left hemisphere of the brain. In contrast, the mean beta BP and FAA did not reveal significant results. No significant differences in heart rate variability (HRV) were found. Positive correlations were observed between self-reported stress levels, based on the Primary Appraisal Secondary Appraisal (PASA) questionnaire, and EEG as well as ECG features. The results highlighted significant sex differences: Males reported higher stress levels and exhibited greater activity in the right hemisphere, while females demonstrated more pronounced activity in the left hemisphere and a stronger increase in mean theta band power during periods of stress.

**Keyword 1**: mental stress
**Keyword 2**: Neural correlates
**Keyword 3**: sex differencies
**Keyword 4**: EEG
**Keyword 5**: ECG

**REWARD PREDICTION ERROR IN COMPULSIVE SEXUAL BEHAVIORS - AN ERP STUDY**

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Previous studies suggest that problematic pornography users exhibit increased activation of the ventral striatum in response to erotic but not monetary cues compared to controls, indicating potential alterations in reward processing. Reward Prediction Error (PE), representing the difference between expected and received rewards, is typically studied using the Monetary Incentive Delay Task (MIDT). During feedback processing, the Feedback-Related Negativity (FRN), an Event-Related Potential (ERP) reflecting PE, appears approximately 250 ms after feedback onset over frontocentral areas. To investigate whether alternations observed in previous studies extend to PE mechanisms, we examined PE using a modified paradigm incorporating sexual stimuli. Thirty-four individuals with Compulsive Sexual Behavior Disorder (CSBD) and 34 healthy controls (HCs) participated in an EEG study using a modified Incentive Delay Task (IDT) with addiction-specific (erotic) and non-specific (monetary) rewards. ERP results revealed an interaction in the FRN component between performance evaluation (gain vs. loss) and reward evaluation (incentive vs. neutral), with a significant reward effect in the erotic condition but not in the monetary condition. A similar interaction appeared in P300 component, a positive peak around 300-450 ms post-feedback in parietal areas. However, amplitudes in both components did not significantly differ between groups. These findings challenge models suggesting reward hypersensitivity or deficits in CSBD. The results highlight the role of context-dependent reward processing in PE learning. Future research should address potential limitations–such as increasing sample sizes, refining tasks, and incorporating multimodal approaches–to improve the detection of neural alterations in CSBD and inform targeted interventions.

**Keyword 1**: Event-Related Potentials
**Keyword 2**: Reward Prediction Error
**Keyword 3**: Compulsive Sexual Behavior Disorder
**Keyword 4**: Incentive Delay Task
**Keyword 5**: behavioral addiction

**MEMORY LANE: MAPPING THE HUMAN BRAIN'S TRAFFIC DURING ENCODING AND RETRIEVAL**

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Both the formation and retrieval of episodic memories underlie complex and diverse brain network dynamics that place the hippocampal complex and medial temporal lobe (MTL) at the center of their hub. This does not describe the full picture, as more recent studies repeatedly have demonstrated that while necessary, MTL structures are not sufficient by themselves to support most episodic memory functions. To explore and capture the more distributed memory encoding and retrieval dynamics of the human cortex, we recorded intracranial electrophysiological brain activity with 10 drug-resistant epilepsy patients while performing an image recognition task. Over several consecutive days, participants were presented with up to 3000 images and had to indicated whether an image was new or a repetition of a previous trial. Correct trials were then grouped into three categories: New, Recent (Repetition: Same Day), and Old (Repetition: Different Day). We applied both machine learning and advanced effective connectivity analyses to explore distributed intra- and inter-regional activity dynamics across several frequency bands. By running a Random Forest Classifier on the Correct Trial Data, we managed to identify category-distinct spectral profiles across seven frequency bands that were especially prominent in early to mid-visual stream areas, MTL regions and the inferior frontal lobe. Afterwards, we performed a state-space Granger Causality analysis for each pair of sampled regions to further describe the timing and potential information flow of these spectral patterns.

**Keyword 1**: sEEG
**Keyword 2**: encoding
**Keyword 3**: retrieval
**Keyword 4**: machine learning
**Keyword 5**: effective connectivity

**EXAMINING PREDICTION ERROR IN ALCOHOL ADDICTION: AN EEG STUDY ON EVENT-RELATED POTENTIALS**

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Previous studies suggest that substance addiction influences reward processing and prediction error (PE), affecting decision-making. This study examined PE in individuals with alcohol use disorder (AUD, n = 24) and controls (n = 34) using EEG-derived Event-Related Potentials (ERPs): Feedback-Related Negativity (FRN), reflecting early PE processing (~250 ms post-feedback), and P300, associated with later cognitive evaluation (~300–450 ms). Participants completed a modified Incentive Delay Task (IDT) with addiction-specific (alcohol) and non-specific (monetary) rewards delivered with 75/25% reward probabilities. ERP results revealed a significant interaction between reward type (alcohol vs. monetary) and outcome (win vs. loss) in the FRN ($p = 0.013$). A similar interaction emerged in the P300 ($p = 0.018$). However, no generalized group differences in FRN or P300 amplitudes were observed between AUD and controls, indicating comparable PE processing across stages overall. These findings challenge models positing generalized reward deficits or hypersensitivity in AUD. Instead, they highlight context-dependent adaptations between P300 responses to non-specific rewards (monetary) and FRN for addiction-specific wins (alcohol), aligning with predictions of Reward Deficiency Syndrome (RDS) and Incentive Sensitization Theory (IST). Reward type and outcome dominated neural encoding, with no generalized group effects, emphasizing stimulus-driven adaptations over broad deficits. These results refine frameworks like Reward Deficiency Syndrome and Incentive Sensitization Theory, challenging models positing global reward-processing impairments.

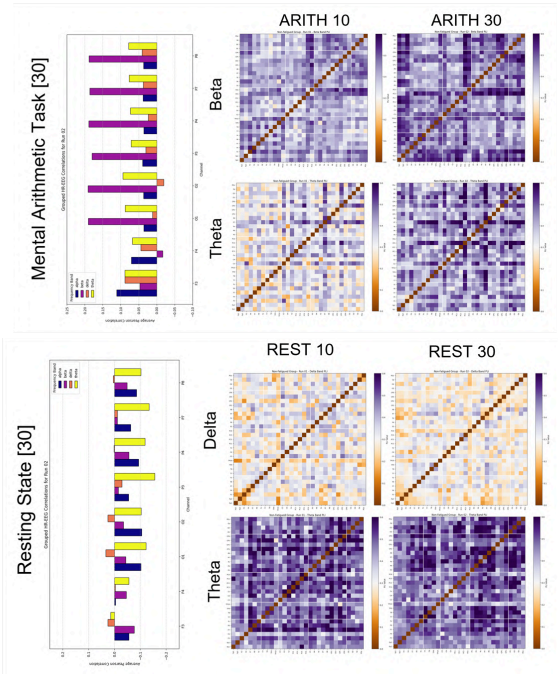
**Keyword 1**: event-related potentials
**Keyword 2**: reward prediction error
**Keyword 3**: substance addiction
**Keyword 4**: incentive delay task
**Keyword 5**: alcohol use

**MULTIMODAL ANALYSIS OF CAFFEINE'S EFFECTS ON CEREBRAL, CARDIAC, AND FATIGUE DYNAMICS ACROSS COGNITIVE TASKS AND RESTING STATES: AN EEG-FMRI STUDY**

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This study examines caffeine's effects on cerebral and cardiac dynamics using EEG-fMRI modalities. Simultaneous EEG and fMRI data were collected using a 3T scanner with GRE-EPI settings (FA = 90°, TR = 3 s, TE = 36 ms, in-plane resolution = 2.5 mm). Nine subjects completed resting-state scans with their eyes open and a cognitive test that included mathematical equations; the task sequences were [45-second: fixation, 9-second: task, 36-second: rest]. fMRI data was preprocessed by applying the following steps: spatial normalization, motion correction, slice-timing, and signal drift, using 'afni_proc' pipeline from AFNI. We assessed heart rate using a pulse oximeter that synced with the fMRI data. EEG preprocessing included the elimination of MRI gradient and cardio-ballistic artifacts, along with filtering (0.5-35 Hz) and independent component analysis (ICA) for the removal of eyeblinks, with participants categorized into High Theta (Hθ) and Low Theta (Lθ) groups based on frontal-theta levels. Therefore, a correlation was established between EEG power spectral density analysis (PSD) and voxel-based functional connectivity maps in the prefrontal cortex (PFC) and posterior cingulate cortex (PCC), indicating that variations in EEG activity are associated with corresponding changes in fMRI responses. The findings showed that whilst (Lθ) group showed better cognitive processing, (Hθ) group suffered early cognitive impairment and significant correlations in heart rate. Negative correlations seen in (Lθ) group during rest show that caffeine reduces connectivity. Particularly in non-fatigued individuals, the results reveal that caffeine alters brain and cardiovascular reactions, thereby boosting cognitive abilities and neural coordination.

HR-EEG and PLI (Phase Lag Index) Frequency-Specific Correlations for Cognitive and Rest Groups




**Keyword 1**: Bold fMRI
**Keyword 2**: Caffeine
**Keyword 3**: Electroencephalography (EEG)
**Keyword 4**: Functional fMRI
**Keyword 5**: Multi-Modal Imaging

**COGNITIVE PERFORMANCE OVER TIME: A PILOT STUDY USING COGNISCAN**

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Objective: This pilot study explores cognitive changes using Cogniscan, a neuropsychological tool. The aim was to assess variations in attention, intelligence, and memory in a postgraduate student between two assessments one year apart. Methods: A 27-year-old woman at the first evaluation (March 2024) and 28 at follow-up (February 2025) underwent cognitive tests measuring alternating, concentrated, and divided attention, non-verbal intelligence, and recognition memory. At the first assessment, she reported attention difficulties following a COVID-19 infection in late 2023. Results: Alternating attention remained stable (percentile 80), concentrated attention improved (75 to 90), and divided attention increased (40 to 80). Non-verbal intelligence declined (70 to 40), with more comprehension errors. However, only 25% of individuals typically score above 30. Recognition memory slightly declined (90 to 80). Reaction time remained “very low” across attention tests. Conclusions: These preliminary findings suggest cognitive improvements in attention-related tasks, particularly in focused and divided attention, which may reflect adaptive cognitive strategies. The reported initial attention difficulties and the subsequent cognitive improvements highlight a potential recovery trajectory following COVID-19-related cognitive complaints. However, a decline in non-verbal intelligence scores indicates potential challenges in problem-solving efficiency. As a pilot study, these results provide initial evidence of cognitive fluctuations over time and highlight the importance of continuous cognitive monitoring. Further research with larger samples is necessary to confirm these trends and explore interventions to enhance problem-solving skills while maintaining attentional gains.

**Keyword 1**: Cognitive assessment
**Keyword 2**: Neuropsychological screening
**Keyword 3**: Post-COVID cognitive effects
**Keyword 4**: Pilot study

**THE ROLE OF THE VESTIBULAR SYSTEM IN AUDITORY-VERBAL WORKING MEMORY AND ITS PSYCHOPHYSIOLOGICAL CORRELATES: EVIDENCE FROM GALVANIC VESTIBULAR STIMULATION**

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Previous studies have shown that the vestibular system plays a role in spatial working memory (WM)[1], but its involvement in auditory-verbal WM and the associated psychophysiological correlates remain unexplored. To address these questions, three different configuration of Galvanic Vestibular Stimulation (GVS) were administered to participants performing an auditory-verbal WM task, while heart rate (HR) and perceived stress were recorded.
In the RGVS (right-anodal/left-cathodal), LGVS (left-anodal/right-cathodal), and Sham conditions, 30 participants performed an auditory-verbal 2-back task, preceded and followed by a rest period. Heart rate (HR) was measured continuously, and participants rated their perceived stress at different time points during the experiment.
Signal detection analysis of task performance revealed a significant shift of participants' response criterion towards more liberal values (more false alarms than misses) during RGVS compared to Sham ($p=0.008$). During task performance, HR was higher in RGVS compared to both Sham ($p=0.001$) and LGVS ($p<0.001$), and lower in LGVS compared to Sham ($p=0.001$). During the post-task rest period, HR in RGVS was higher compared to Sham ($p=0.006$). Stress ratings were lower in LGVS than both RGVS ($p=0.001$) and Sham ($p=0.002$).
These results demonstrate the contribution of the vestibular system to auditory-verbal WM. Previous studies have shown that RGVS and LGVS reduce the activity of right and left vestibular signal, respectively[2]. Our results show that modulation of left vestibular system increases parasympathetic activity, which is reflected in lower HR and stress, while modulation of right vestibular system increases sympathetic activity, which is reflected by higher HR and stress.

**Keyword 1**: vestibular system
**Keyword 2**: working memory
**Keyword 3**: galvanic vestibular stimulation
**Keyword 4**: psychophysiology

**ELUCIDATING THE NEUROBIOLOGICAL MECHANISMS UNDERLYING HUMAN HUNTING BEHAVIOUR USING MINECRAFT - AN FNIRS STUDY**

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Many species, including rodents, bats, monkeys, and humans, can neurally encode the distance to a stationary goal. In humans, this extends to moving goals, with the entorhinal cortex and its cortical connections able to track the location of a moving human avatar. However, the role of the prefrontal cortex (PFC) in tracking moving goals, especially in dynamic, group-based behaviors, remains underexplored. A classic example of goal tracking that requires coordination among individuals is group hunting. This study investigated the PFC's role in group hunting using a virtual environment (Minecraft). Two participants acted as predators hunting a human prey (a confederate) in Hunt trials, while in Follow trials, they followed the path of a human experimenter. Functional near-infrared spectroscopy (fNIRS) recorded predators' brain activity. Behaviourally, successful hunting was associated with maintaining a closer proximity to the prey, exhibiting adaptability in adjusting this distance, and achieving greater coordination with one's co-hunter. Neurally, at the single subject-level, less alignment in the heading direction of the predator with that of the prey's location was associated with greater activity in dorsolateral PFC region during hunt trials compared with follow trials. At the dyadic level, hunting was associated with significantly greater brain-to-brain synchrony than following in dorsolateral and frontopolar regions. This preliminary finding suggests a possible role for the PFC in coordinating goal-tracking behaviour in group contexts, warranting further investigation with larger samples to understand the underlying neurobiological mechanisms.

**Keyword 1**: Human Spatial Navigation
**Keyword 2**: Human Social Neuroscience
**Keyword 3**: fNIRS (Functional Near Infrared Spectroscopy)
**Keyword 4**: Human Brain Imaging

**INFLUENCE OF VISUAL MOTION AREA V5/MT ON THE LATERAL GENICULATE NUCLEUS DURING VISUAL-SPEECH RECOGNITION**

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Sensory thalamus responses are modulated when humans recognize auditory and visual speech. For example, blood oxygen level-dependent (BOLD) responses in the visual thalamus, specifically the lateral geniculate nucleus (LGN), are higher when participants recognize visual speech compared to a control task. This effect occurs even when the stimulus material remains identical. Here, we test the hypothesis that this task-dependent modulation of the LGN relies on the processing carried out by the cerebral cortex area V5/MT. V5/MT is a key region for visual motion processing – including visual speech – with direct connections to the LGN. We applied offline inhibitory transcranial magnetic stimulation (TMS) over bilateral V5/MT or the vertex region (as an active control stimulation) in 26 healthy human adults. TMS was followed by 3 Tesla functional magnetic resonance imaging (fMRI) while participants watched muted videos of a speaker uttering short syllables. Depending on task instructions, participants performed either a one-back visual speech recognition task or a one-back facial colour recognition task. Both tasks were matched in difficulty. Our results revealed a significant difference in BOLD signal between the two tasks, indicating a task-dependent modulation of the LGN, specifically with a more negative response for the visual speech task than the colour task. Most importantly, inhibitory stimulation of V5/MT reduced this task effect compared to the active control stimulation. These findings provide novel insights into the role of cerebral cortex in modulating sensory thalamus functions.

**Keyword 1**: Speech
**Keyword 2**: Thalamus
**Keyword 3**: fMRI
**Keyword 4**: TMS

**ASSESSMENT OF PUPIL FEATURES AS A POTENTIAL BIOMARKER FOR DETECTION OF TRAIT ANXIETY DISORDER: A STUDY FROM SUBCLINICAL ANXIETY POPULATION**

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Anxiety influences brain regions involved in attentional and emotional processing, particularly the anterior cingulate cortex , amygdala, and prefrontal cortex . Trait anxiety, a stable predisposition to experience anxiety, has been shown to affect emotional face recognition, a task engaging both emotional and attentional networks. It has been shown that anxiety disorder induces an attentional bias towards negative valence emotions. Recent literatures have shown emotion-specific differences in pupil diameter. The locus coeruleus, a brainstem region involved in arousal, attentional control, and modulating pupil diameter, is a part of attentional and emotional network. This study examines how trait anxiety impacts pupil dynamics during an emotion recognition task. We recorded pupillary responses from 65 participants, categorized into high- and low-trait anxiety groups based on STAI scores. Participants completed an emotion detection task (happy, angry, neutral) and a control task identifying the spatial location of a red dot overlaid on the same stimuli. The control task was designed to redirect attention from emotional face to non-emotional feature(red dot). Pupil responses were analyzed to extract six features, i.e. latency of constriction onset, constriction rate, constriction amplitude, dilation rate and full width at half maxima (FWHM) . Significant differences in the features among high and low-anxiety groups highlight that emotional and attentional processing differences can also be quantified through pupillary response. A machine learning model trained on pupil features achieved 91% accuracy in classifying anxiety conditions, suggesting that pupil dynamics may serve as a biomarker for anxiety screening and advancing understanding of anxiety disorders.

**Keyword 1**: Emotion Perception
**Keyword 2**: Pupillometry
**Keyword 3**: Trait Anxiety
**Keyword 4**: Predictive Model
**Keyword 5**: Attention

**CHOLINERGIC ACTIVITY IN THE BASAL FOREBRAIN DECREASES WITH AGING AND ALZHEIMER'S DISEASE**

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The basal forebrain cholinergic neurons (BFCNs) play a crucial role in regulating cognitive functions through the release of acetylcholine (ACh) in several brain areas. During aging and Alzheimer's disease (AD), BFCNs undergo different physiological and morphological changes, which can drastically alter neuronal behavior. The goal of our study was to address uncertainties regarding the relationship between cholinergic activity and age-related neurodegeneration, as well as to explore the impact of neurodegeneration on cognitive processes.
We conducted experiments with head-fixed young, old and AD model mice performing an auditory Pavlovian conditioning task. First, we optogenetically inhibited cholinergic neurons in the horizontal band of Broca (HDB) during the presentation of conditioned stimuli (CS). Next, we performed electrophysiological recordings of HDB cholinergic neurons. Finally, we measured ACh release in the basolateral amygdala (BLA) and the medial prefrontal cortex (mPFC) using fiber photometry.
Our findings showed that optogenetic inhibition of cholinergic neurons in the HDB during CS presentation led to learning deficits. Electrophysiological data revealed that BFCNs increased their activity in response to reward-predicting CS in young animals, but not in old animals. In older mice and AD models, ACh levels in the BLA and mPFC showed diminished responses to reward-predicting CS as well, which was also reflected in learning difficulties.
Overall, our results suggest that ACh release from BFCNs is critical for forming CS-US associations during Pavlovian conditioning. Both BFCN activity patterns and ACh release were altered in aged mice and AD models, indicating an age-related decline in cholinergic function.

**SEX DIFFERENCES IN FEAR GENERALIZATION AND ACTIVITY OF DENTATE GYRUS GRANULE CELLS IN MICE**

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**INTRODUCTION:** The ability to differentiate between similar environments is essential for adaptive behavior. Inadequate pattern separation can lead to pathological states like post-traumatic stress disorder, which is twice as likely in females compared to males. The hippocampal dentate gyrus (DG) plays a role in spatial learning and pattern separation, and running is believed to improve these functions.
**METHODS:** We studied the association between DG granule cell activity and behavior during discrimination contextual fear conditioning in adult C57/black male and female mice, and how running affects this in females (n=6/group). A fluorescent calcium indicator (GCaMP6s) was delivered to the DG using a viral vector, and a lens was implanted to enable imaging with a microendoscope. DG granule cell calcium activity and behavior were recorded during pre-exposure and discrimination fear conditioning phases, each lasting 10 days, with mice exploring each context for 10 minutes daily. Electrical shocks were delivered in one context from day 11 onwards.
**RESULTS:** Consistent with previous reports, females generalized fear more than males. Contrary to expectations, running did not improve discrimination in females; instead, running capacity was inversely associated with discrimination. Approximately one-third of measured granule cells showed context-specific activity, with a slight increase from pre-exposure to conditioning, especially in males, where this proportion also correlated with behavioral discrimination.
**CONCLUSION:** Fear generalization in females persists even after a running intervention, and DG granule cell activity during learning does not straightforwardly predict behavioral discrimination.

**Keyword 1**: hippocampus
**Keyword 2**: dentate gyrus
**Keyword 3**: in vivo calsium imaging
**Keyword 4**: mouse
**Keyword 5**: fear learning

**ADULT BRAIN NEURONS' BACK REMODELING AND MEMORY FORMATION**

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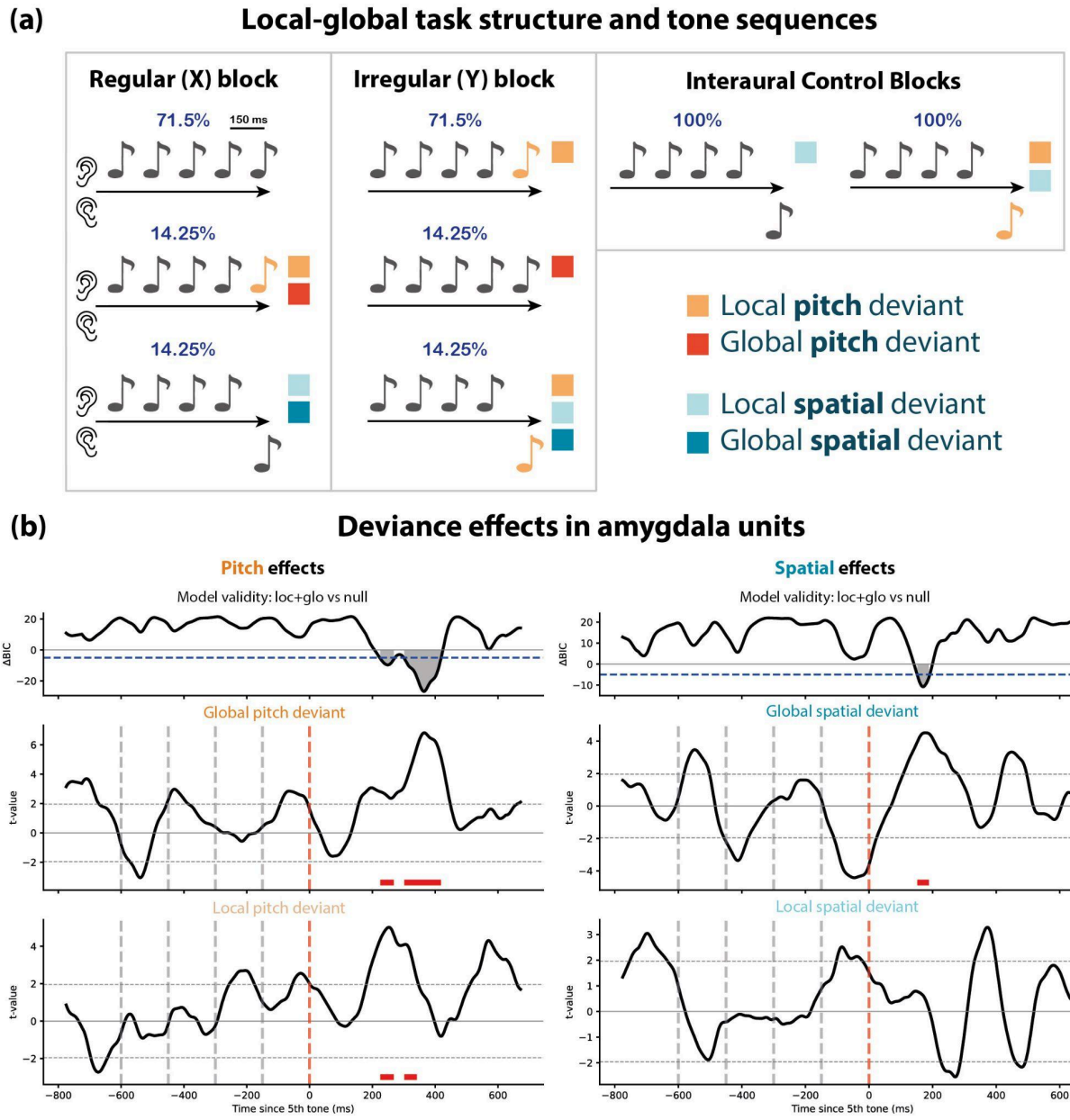
Last years an increase of interest and research of the role of brain neurogenesis in the adult organisms in memory processes is noted. In this study the effects of polyclonal antibodies to serotonin-modulating anticonsolidation protein (SMAP) complex and its constituent collapsin-response mediator protein 2 (CRMP2) have been analyzed. Intra-cerebral administration of polyclonal anti-SMAP antibody significantly facilitated the task elaboration by shortening duration of learning sessions and strengthened memory through increasing correct trials ratios in two difficult-to-learn behavioral conditioned models (alternative running and two-lever operant differentiation) beginning since 72 h later of antibody administration. Furthermore, intra-cerebral administration of anti-SMAP antibody resulted within 72 h timeframe in an increase of the level of nerve growth factor (NGF) in the protein fraction of the hippocampus. Concomitantly, intra-cerebral administration of anti-CRMP2 antibody induced within 72 h timeframe a downregulation of β-III tubulin (a marker of differentiated neurons) in the hippocampus and left parietal cortex of the naive rats evaluated through indirect ELISA test. The obtained results indicate to possible positive regulation by CRMP2 of the back remodeling of the differentiated nerve cells of the adult organisms' brains, occurring during learning of the rats in the behavioral paradigm used in this study under the effects of anti-SMAP and anti-CRMP2 antibodies. The conclusion is made that the back remodeling (dedifferentiation) of the mature nerve cells in the brain, apparently, is generally engaged in memory formation in novel task acquisition.

**Keyword 1**: differentiated neurons' back remodeling
**Keyword 2**: anti-CRMP2 antibody
**Keyword 3**: difficult-to-learn conditioned tasks
**Keyword 4**: memory formation acceleration and strengthening
**Keyword 5**: beta III tubulin downregulation

**AMYGDALA RESPONSES TO AUDITORY PREDICTION VIOLATIONS: EVIDENCE FROM HUMAN SINGLE-NEURON RECORDINGS**

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Oddball paradigms are widely used to study hierarchical sensory processing and prediction error signaling across diverse methodologies. However, single-neuron responses to auditory oddballs in humans remain underexplored, limiting the understanding of how the brain processes unexpected sensory information at its most basic functional level.
We investigated whether amygdala and hippocampus units show sensitivity to local (within-sequence) and global (across-sequence) tone deviants (Chennu,2013; Fig.1A) using intracranial recordings obtained from eight patients with depth electrodes. Unit firing rates were analyzed using generalized linear models (GLMs), modeling the effects of local, global, and inter-aural deviancy on neuronal activity.
Amygdala units (15 SU, 29 MU) exhibited late responses (300-450ms) to global deviants and early responses to local (200-300ms) and inter-aural (75-125ms) deviants (Fig.1B). Surprisingly, hippocampal units (17 SU, 18 MU) showed no consistent sensitivity to deviance type, despite this region's established role encoding the temporal structure/sequence of events and in detecting (contextual) violations in predicted event sequences.
This dissociation suggests differential processing mechanisms (and functional specialization) between these interconnected structures in auditory processing: Amygdala neurons may prioritize and respond rapidly to unexpected, salient acoustic changes, whereas the hippocampal contributions may be limited to higher-order statistical learning not fully engaged by our paradigm. Hippocampal contributions may also involve alternative coding schemes not captured by our analyses.
Our findings provide novel insights into how prediction violations in the auditory environment are processed at the single-neuron level in the human medial temporal lobe structures, advancing our understanding of the neural mechanisms underlying auditory deviance detection.




**Keyword 1**: Deviance detection
**Keyword 2**: Mismatch negativity (MMN)
**Keyword 3**: Hippocampus
**Keyword 4**: Amygdala
**Keyword 5**: Single-neurons

**ACUTE SLEEP DEPRIVATION DISRUPTS SPATIAL CODING AND NETWORK ORGANIZATION IN THE MEDIAL ENTORHINAL CORTEX**

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Sleep is essential for memory consolidation, and sleep deprivation (SD) is known to impair spatial memory. The medial entorhinal cortex (MEC) plays a key role in spatial navigation by encoding spatial representations at both the single-cell and network levels. However, how SD affects MEC neuronal firing properties and network organization remains unclear. This study examines the impact of acute SD on MEC spatial coding and functional network structure.
Mice underwent 6 hours of SD followed by behavioral testing in an open field and an open field with objects to assess locomotion and object-related spatial processing. Two-photon calcium imaging recorded neuronal activity in the MEC. We analyzed firing rates, spatial tuning properties of various cell types (e.g., grid cells, object-vector cells), population coding stability, as well as functional network connectivity using graph theory.
Following SD, MEC neurons exhibited increased firing rates but reduced spatial tuning in grid cells, head direction cells, and object-vector cells. Elevated firing extended beyond stable spatial fields, reducing spatial precision. At the network level, SD enhanced neuronal synchronization, increased functional connectivity density, and reduced modularity, lowering the dimensionality of network representation.
These findings suggest that acute SD disrupts spatial coding in the MEC by altering neuronal activity, impairing population stability, and reorganizing network connectivity. This may underlie spatial memory deficits associated with sleep disorders.

**Keyword 1**: Sleep deprivation
**Keyword 2**: Medial entorhinal cortex (MEC)
**Keyword 3**: Spatial coding
**Keyword 4**: Neuronal synchronization

**HBK-15, A MULTIMODAL COMPOUND, IMPROVES COGNITIVE FLEXIBILITY IN A TOUCHSCREEN-BASED REVERSAL LEARNING MODEL IN MICE**

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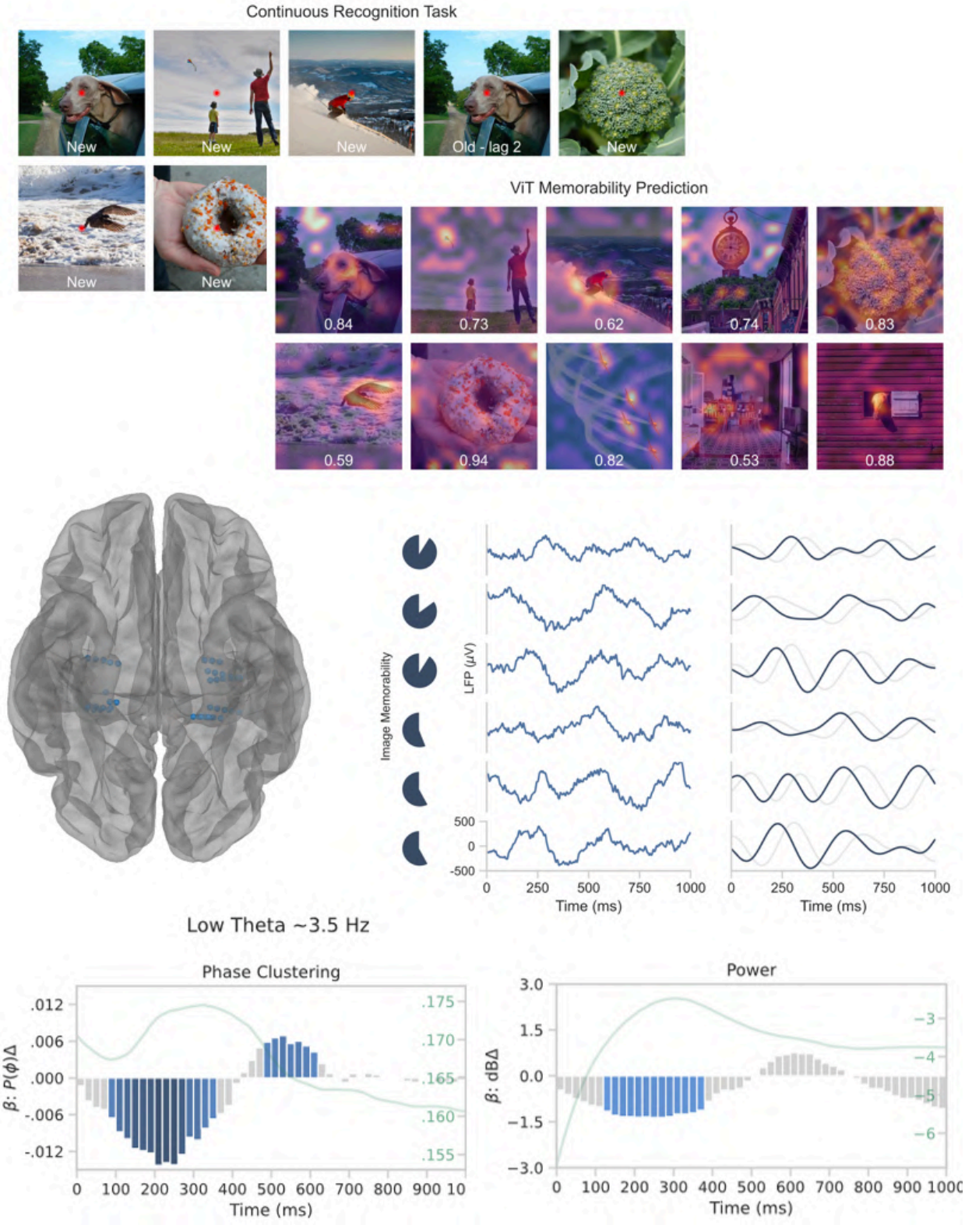
Cognitive dysfunction remains a major challenge in neuropsychiatric disorders, necessitating the development of novel therapeutic strategies. In addition to impairments in basic memory functions, deficits in higher executive functions—such as cognitive flexibility—are critically affected in these conditions. HBK-15, a multimodal compound, has demonstrated promising cognitive-enhancing properties in preliminary studies, particularly in enhancing various domains of basic memory. However, its influence on higher cognitive functions remains unexplored. Our ongoing research investigates the effects of HBK-15 in MK-801-induced cognitive deficits model. We use a highly translative pairwise visual discrimination task with reversal learning to assess cognitive flexibility alongside evaluations of its impact on hippocampal neurogenesis and synaptic architecture. Early results indicate that HBK-15 positively influences cognitive flexibility supporting its potential role in improving both fundamental and executive cognitive processes. More specifically all tested doses improved accuracy by increasing the percentage of correct responses during reversal task. Additionally, the two highest doses (1.25 mg/kg and 2.5 mg/kg) also shortened the time taken to complete the task. However, the compound did not influence the error correction or perseverative aspect of the task suggesting that HBK-15 may differently affect various learning processes involved in the reversal pairwise visual discrimination task. Ongoing experiments aim to delineate the underlying mechanisms by integrating molecular and cellular approaches. These findings position HBK-15 as a promising candidate for cognitive enhancement, although further studies are required to confirm its mechanistic underpinnings and translational relevance.
Support ERASMUS+ Programme Strategic Partnerships
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**Keyword 1**: HBK-15
**Keyword 2**: cognitive flexibility
**Keyword 3**: neurogenesis
**Keyword 4**: executive functions
**Keyword 5**: mice

**HIPPOCAMPAL THETA AND HIGH-FREQUENCY ACTIVITY TRACKS IMAGE MEMORABILITY IN HUMANS**

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Some visual images are more readily remembered than others, a phenomenon captured by image memorability—a visual stimulus' a priori probability of being remembered. Although population-level memorability can be reliably predicted from images, the underlying neural mechanisms behind his systematic prioritisation remain largely unexplored, particularly regarding direct hippocampal recordings in humans. We investigated whether hippocampal activity tracks image memorability using stereotactic EEG recordings from 11 patients with refractory epilepsy. Participants viewed a continuous stream of up to 1,000 unique images while instructed to detect repeats. The images' memorability scores were derived using a dedicated neural network. Our preliminary results reveal that image memorability predicts event-related potential amplitudes in the hippocampus, most notably between 300–600 ms post-image onset. This effect is accompanied by modulations of lower theta (~3.5 Hz) power and phase clustering, along with an overall reduction in high-frequency (70–150 Hz) activity for more memorable images.These findings show that memorable visual stimulus properties systematically influence hippocampal electrophysiological responses, describing a potential neural signature of image memorability. By capturing how hippocampal dynamics respond differently to images of varying memorability, this study contributes to a novel perspective, bridging perceptual and memory processes, through the study of neural responses to prioritized visual inputs.


Continuous Recognition Task
New
New
New
Old - lag 2
New
New
New
ViT Memorability Prediction
0.84
0.73
0.62
0.74
0.83
0.59
0.94
0.82
0.53
0.88
Image Memorability
LFP (μV)
500
0
-500
0
250
500
750
1000
Time (ms)
Low Theta ~3.5 Hz
Phase Clustering
β: P(ϕ)Δ
.012
.006
.000
-.006
-.012
.175
.170
.165
.160
.155
0 100 200 300 400 500 600 700 800 900 10
Time (ms)
Power
β: dBΔ
3.0
1.5
0.0
−1.5
−3.0
−3
−4
−5
−6
0 100 200 300 400 500 600 700 800 900 1000
Time (ms)

**ERP INSIGHTS INTO THREAT PERCEPTION: NEURAL RESPONSES TO HUMAN, ROBOT, AND PAREIDOLIA FACES IN THE UNCANNY VALLEY**

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This study investigates the neural and behavioral responses to human, robot, and pareidolia faces in the context of threat perception and the Uncanny Valley effect. Using event-related potentials (ERP), we examined differences in neural activity associated with face processing, particularly focusing on the N170 and P1 components. A total of 21 participants (10 female, 11 male, aged 18-27) took part in an experimental paradigm where they were presented with images of human, humanoid robot, mechanical robot, and pareidolia faces, both in motion and static conditions. Participants rated the perceived threat level of each stimulus on a 7-point Likert scale while EEG data were recorded.

Results indicated that humanoid robot faces elicited significantly higher threat ratings compared to mechanical robots, pareidolia, and human faces ($p < .001$). ERP analysis revealed that motion increased N170 amplitudes across all conditions ($p < .001$), with humanoid robot faces producing the highest N170 response. In contrast, mechanical robot faces generated significantly lower N170 amplitudes, suggesting reduced face-like processing. P1 amplitudes were greater for static stimuli compared to moving ones ($p = .045$), implying early visual processing differences. Additionally, static humanoid robot faces were rated as more threatening than even moving human faces, further supporting the Uncanny Valley hypothesis.

These findings suggest that humanoid robots, due to their near-human resemblance, evoke stronger neural and behavioral responses associated with threat perception. The results contribute to understanding the neural mechanisms underlying human-robot interaction and highlight the importance of design considerations in social robotics.

**Keyword 1**: face perception
**Keyword 2**: uncany valley
**Keyword 3**: robot faces
**Keyword 4**: paredolia
**Keyword 5**: ERP

**ERP MARKERS OF AUDITORY PERCEPTION ABNORMALITIES IN A WORD RECOGNITION TASK**

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The non-clinical population frequently experiences auditory perceptual abnormalities (APA), which can, in some circumstances, lead to the development of hallucinations. Researchers have made numerous attempts to investigate the production of voices in a laboratory setting. However, there is still a debate as to whether the results are due to the experience of voice hearing or a higher tendency for mistakes.
This study examines APA in a non-clinical population, comparing false alarms (FA) to correct rejections (CR) and hits (correct recognitions) in auditory (A) and audiovisual (AV) conditions. Event-related potentials (ERPs), specifically the P300 and mismatch negativity (MMN) components of the auditory and motor areas and prefrontal cortex, were analysed using electroencephalography (EEG) to investigate the underlying mechanisms of FA. Participants were asked to identify whether they heard a word embedded in continuous background noise. The stimuli were prepared in a way that 20% of the trials were not changed at all (100% speech), 40% were scrambled, so they contained about 50% of speech information, and 40% were fully scrambled (0% speech). We did this to ensure that each stimulus had the same acoustic impact on the brain.
The study contributes to a deeper understanding of the mechanisms behind APA and the role of attentional and predictive processes in perceptual accuracy. Moreover, it provides a foundation for future research on hallucinatory experiences examined in laboratory conditions in clinical and non-clinical populations.

**Keyword 1**: ERP
**Keyword 2**: Auditory Perception
**Keyword 3**: Auditory Processing

**BRAIN-WIDE NEURAL MECHANISMS OF CROSS-TASK INFORMATION GENERALIZATION**

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Cognitive flexibility is the cornerstone of adaptive behavior, enabling humans and other animals to rapidly learn the associations between external stimuli and to appropriately generalize the learned knowledge to solve different problems for continual task demands. Many studies have shown that both frontal cortex (PFC, OFC) and hippocampus play important roles in representations of task states. However, little is known about the neural mechanisms of cross-task information generalization through the whole brain. To address this question, we trained mice learning to associate sequential odors with rewards in tasks involving 2 working memory tasks and 1 non-working memory task. High-throughput electrophysiological recordings showed that neurons exhibit both task-specific and cross-task generalization encoding abilities. At the single-cell level, neurons showed diverse encoding patterns within the same task. At the population level, orbitofrontal cortex neurons demonstrated differentiated encoding during the learning stage but evolved cross-task generalization for structurally similar tasks during the expert stage. Whole-brain analysis revealed that while individual regions like the anterior olfactory nucleus showed distinct generalization patterns, overall neural populations exhibited more task-specific representations during learning and more consistent generalization during expertise. Using support vector machine analysis, we found that cross-task encoding abilities increased with learning, especially for structurally similar tasks. Neural activity trajectories in a three-dimensional decoding space showed increased convergence for similar tasks and divergence for distinct tasks during the expert stage. The above research has expanded our global understanding of cognitive flexibility, making an important contribution to further in-depth understanding of this advanced function in the field.

**Keyword 1**: Cognitive flexibility
**Keyword 2**: Large-scale electrophysiological recording
**Keyword 3**: Learning dependent dynamic encoding
**Keyword 4**: Cross-task generalization of neural encoding

**DIFFERENTIAL EXPRESSION OF SPATIOTEMPORAL SLEEP SPINDLE CLUSTERS IN AGEING**

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Sleep spindles have been proposed as potential early biomarkers for memory decline in the aging population. However, significant within-person variability in spindle attributes complicates their utility in predicting age-related cognitive deterioration. This study aimed to uncover distinct spindle types and their and functional relevance to memory decline during healthy ageing using a data-driven clustering approach. We collected polysomnographic data from young (n = 43, ages 20-45) and older adults (n = 34, ages 60-81). Spindles were clustered into four groups using multiple features and spatiotemporal context, irrespective of participant age. Resulting clusters were hierarchically defined by the sleep stage, slow oscillation concurrence, and hemisphere. Stage N3 spindles (15%) formed a distinct group, followed by N2 spindles coinciding with slow oscillations (27%). Remaining N2 spindles were categorized into unilateral (41%) and bilateral clusters (17%). In older adults, there was a reduced proportion of N2 bilateral spindles and an increased proportion of N2 spindles concurrent with slow oscillations. Elderly also showed a decline in spindle frequency and power, as well as changes in post-spindle power spectrum and complexity, regardless of cluster belonging. Our results indicate differing expression of spatiotemporal spindle clusters in older age. However, no evidence was found for distinct spindle types relevant for memory processing. Instead, spindle diversity rather aligned with global sleep stage dynamics. These results emphasize the interconnectedness of spindle activity with overall sleep patterns, underscoring the importance of spatiotemporal context within and across sleep stages, alongside changes in global sleep dynamics with aging.

**Keyword 1**: sleep
**Keyword 2**: episodic memory
**Keyword 3**: ageing

**EXPLORING THE INTERPLAY BETWEEN REINFORCEMENT LEARNING, WORKING MEMORY, AND ATTENTION DURING LEARNING: INTEGRATING AN EEG MEASURE OF ATTENTION WITH COMPUTATIONAL MODELING**

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Instrumental learning lies at the core of goal-directed decision-making and adaptive behavior, with deficits linked to various neuropsychiatric disorders such as ADHD, bipolar disorder, and schizophrenia. Cognitive computational modeling approaches have advanced our understanding of the cognitive processes involved in instrumental learning, such as reinforcement learning and working memory. The Reinforcement Learning Working Memory (RLWM) task and computational model have been designed to disentangle the contributions of each cognitive system in instrumental learning. However, it is likely that other higher-order functions not accounted for in this model, such as attention, also play an important role. In the present study, we aimed to assess the interactions between working memory, reinforcement learning, and attention in an instrumental learning task, the RLWM task. As the RLWM task does not include an explicit behavioral measure of attention, we used trial-by-trial variations in parieto-occipital alpha power, which has previously been shown to be associated with sustained attention, as an indicator of attentional processes. Future directions include implementing the EEG-derived measure of attention into the RLWM computational model to explore the interplay between reinforcement learning, working memory, and attention during learning.

**THE ROLE OF THE MEDIAL PREFRONTAL CORTEX IN A SHORT- AND LONG-TERM SPATIAL MEMORY TASK IN MICE**

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The medial prefrontal cortex (mPFC) is involved in numerous cognitive functions, including memory processing. Spatial memories are thought to initially be encoded in the entorhinal-hippocampal circuit, and consecutively be transferred into the cortex via memory consolidation. In the mPFC, space is represented in a generalized/ abstract form, as opposed to the spatial representation by so-called place cells in the hippocampus. A substantial proportion of mPFC cells exhibits activity related to rewards and reward expectancy. Our work aims to understand the prefrontal involvement in a spatial short- and long-term memory task in mice. We examine at what stage place-related activity emerges in relation to the spatial learning, and test whether goal locations are disproportionally represented in the mPFC at different times. For this we perform extracellular recordings in the prelimbic area of the mPFC in freely moving mice with permanently implanted arrays of movable tetrodes. Electrophysiological and position tracking data is collected throughout the entire learning phase on the cheeseboard maze, and short- and long-term memory retrieval phases.

**Keyword 1**: Memory
**Keyword 2**: Spatial memory

# Neuroimmunology and Inflammation

**THE INHIBITION OF NLRP3 INFLAMMASOME ACTIVATION PROMOTES THE MORPHOLOGICAL AND FUNCTIONAL REGENERATION OF THE INJURED SPINAL CORD**

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Neuroinflammation plays a critical role in both acute and chronic neurodegenerative processes. Damage to the central nervous system triggers the assembly and activation of inflammasomes, leading to pro-inflammatory responses and the release of interleukin 1-beta (IL-1β), a major pro-inflammatory cytokine that can result in chronic neuroinflammation. We used female Sprague-Dawley rats aged 10 to 12 weeks, weighing 230 to 260 grams to induce a contusion spinal cord injury at the thoracic segment 10 level. One hour after the injury, the animals received a specific NLRP3 inflammasome inhibitor (MCC950) via intraperitoneal injection, with subsequent treatments every 24 hours until the third postoperative day. We examined changes in the expression of various inflammatory genes using qPCR one and three days after the injury. In the long-surviving groups (12 weeks post-injury), we evaluated the microglia/macrophage response and glial scar formation at the injury site using Iba-1, CD68, CS56, and FAP immunohistochemistry. Additionally, we placed Fast Blue crystals in the L3 spinal cord segment to map propriospinal and supraspinal neurons. Functional reinnervation was assessed through movement pattern analysis. The MCC950-treated group showed reduced inflammatory component expression compared to controls. Immunohistochemistry revealed a significant decrease in CD68, Iba-1, and CS56 in the injured spinal cord segment compared to the control groups. Furthermore, the animals treated with MCC950 displayed significant functional improvement, supported by morphological studies, which showed a significantly higher number of retrogradely labeled neurons in the MCC950-treated group. Our results indicate that inhibiting NLRP3 inflammasome activation can enhance neuronal survival and regeneration.

**Keyword 1**: NLRP3
**Keyword 2**: Inflammasome
**Keyword 3**: Spinal cord injury
**Keyword 4**: Contusion
**Keyword 5**: MCC950

**LOX-1 INDUCES INFLAMMATORY ACTIVATION OF MICROGLIA**

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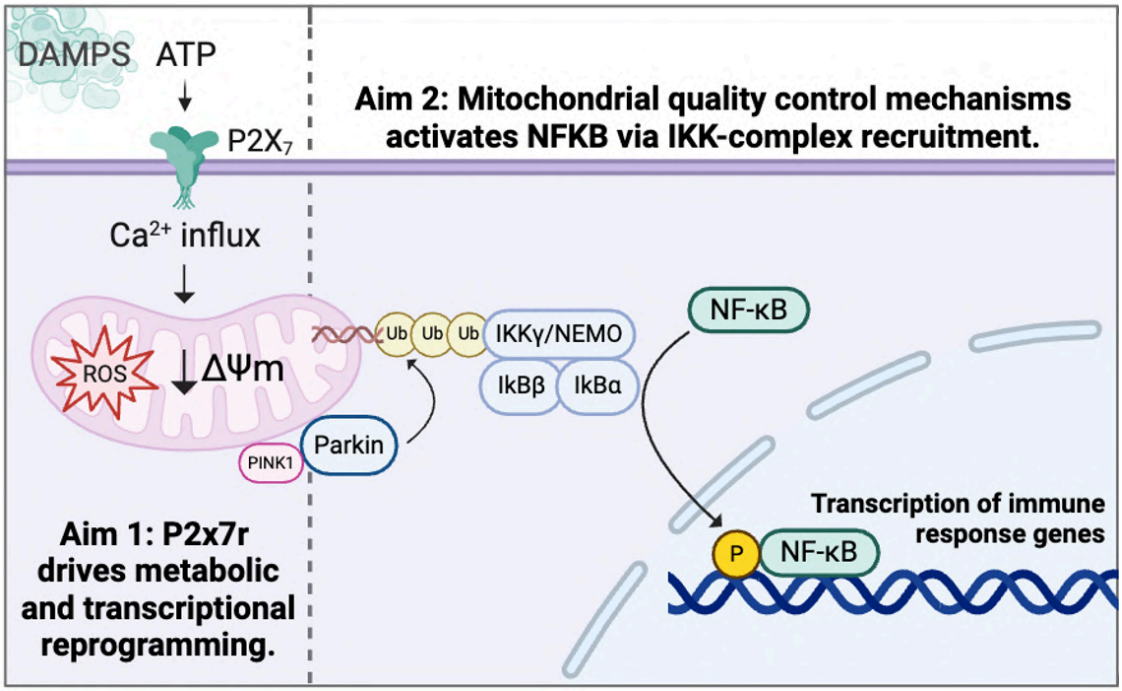
**Background and aim** Microglial cells play an important role in the immune system in the brain. Activated microglial cells are not only injurious but also neuroprotective. We confirmed marked lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1) expression in microglial cells in pathological lesions in the neonatal hypoxic-ischemic encephalopathy (nHIE) model brain. Here, we investigated a novel role of LOX-1 and the molecular mechanism of LOX-1 gene transcription microglial cells under hypoxic and ischemic conditions.
**Methods** We isolated primary rat microglial cells from 3-day-old rat brains. We treated primary rat microglial cells with oxygen glucose deprivation (OGD) as an in vitro model of nHIE. Then, we evaluated the expression levels of LOX-1, cytokines and chemokines in cells treated with or without siRNA and inhibitors. We performed a luciferase reporter assay and chromatin immunoprecipitation assay. In addition, we analyzed reactive oxygen species and cell viability.
**Results** We found that OGD treatment induced LOX-1 expression and inflammatory mediators, such as the cytokines of IL-1β, IL-6 and TNF-α and the chemokines of CCL2, CCL5 and CCL3. Then, the LOX-1 signal transduction pathway was blocked by some inhibitorssuppressed the production of inflammatory mediators. We found that NF-κB and HIF-1α bind to the promoter region of the *OLR-1* gene. Moreover, we demonstrated that LOX-1 in microglial cells was autonomously overexpressed by positive feedback of the intracellular LOX-1 pathway.
**Conclusion** The hypoxic/ischemic conditions of microglial cells induced LOX-1 expression and activated the immune system. LOX-1 and its related molecules or chemicals may be major therapeutic candidates.

**Keyword 1**: microglia
**Keyword 2**: LOX-1

**DEFINING THE CONTRIBUTION OF THE PINK1/PARKIN MITOPHAGIC PATHWAY DURING MICROGLIAL POLARIZATION FOLLOWING BZATP EXPOSURE**

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Ischemic injury is a leading cause of death and long-term disability worldwide. During ischemia, oxygen and glucose deprivation lead to mitochondrial dysfunction, contributing to neuronal death. Microglia, the brain's resident immune cells, detect damage-associated molecular patterns (DAMPs) and initiate inflammatory responses in a process termed polarization. Mitochondrial health is crucial in this process, as fragmented mitochondria are associated with the pro-inflammatory microglial phenotype and post-stroke neuroinflammation. The E3 ubiquitin ligase Parkin regulates mitochondrial quality control via the PINK1/Parkin mitophagy pathway but may also promote inflammation by facilitating IKK-complex formation, a key step in NF-κB activation. To investigate Parkin's role in microglial polarization, we exposed primary wild-type (WT) and Parkin-/- microglia to BzATP, a known post-ischemia DAMP, and assessed mitochondrial health and inflammatory markers. Live-cell imaging revealed mitochondrial depolarization and fragmentation post-BzATP exposure. Parkin-/- microglia exhibited greater accumulation of punctate mitochondria, reduced IKK-complex recruitment, and diminished NLRP3 expression and pro-inflammatory cytokine release, suggesting impaired inflammatory signaling. Preliminary studies using rapamycin indicate that autophagy induction alone is sufficient to increase iNOS expression in a Parkin-dependent manner. Additionally, Seahorse Mito Stress Test analysis revealed that while BzATP increased maximal respiration, likely due to $Ca^{2+}$ influx, mitochondrial respiration remained unchanged between genotypes. Ongoing studies will employ the genetically encoded probe MitoQC to visualize mitophagic flux following BzATP exposure. Understanding Parkin's role in microglial inflammation could uncover new therapeutic targets for post-ischemic neuroprotection.



**Keyword 1**: microglia
**Keyword 2**: stroke
**Keyword 3**: mitophagy

**HDAC1 ACTIVATION AS A THERAPEUTIC STRATEGY FOR NEUROPROTECTION AND FUNCTIONAL RECOVERY AFTER STROKE**

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Effective neuroprotective and regenerative strategies for stroke remain elusive. Histone deacetylase 1 (HDAC1), a key regulator of transcriptional repression and DNA damage repair, plays a crucial role in neurodegeneration, yet its involvement in stroke pathophysiology remains unclear. Here, we demonstrate that HDAC1 protein levels and enzymatic activity are disrupted following ischemic injury. A novel small molecule, compound 5104434, selectively enhances HDAC1 enzymatic function in neurodegenerative conditions, but its therapeutic potential in stroke is unexplored. Using an ischemic stroke model in rats, we evaluated its neuroprotective efficacy. In vitro, compound 5104434 restored HDAC1 activity following oxygen-glucose deprivation, preserved neurite integrity, and mitigated neuronal damage. In vivo, it significantly reduced infarct volume, neuronal loss, apoptosis, and DNA damage in ischemic brains. Further, we identified HDAC1 dysfunction as a driver of neuroinflammation, characterized by increased gliosis, pro-inflammatory cytokines, and matrix metalloproteinase (MMP) activity. Loss of HDAC1 function skewed microglia toward an M1 pro-inflammatory phenotype, exacerbating post-stroke inflammation. Remarkably, restoring HDAC1 activity via compound 5104434 attenuated neuroinflammation and enhanced behavioral recovery, improving neuromuscular coordination, balance, and forelimb strength. These findings establish HDAC1 as a critical modulator of ischemic pathology and highlight compound 5104434 as a promising therapeutic candidate for stroke. Further investigation into its mechanism and clinical translation is warranted.

**BEHAVIORAL AND IMMUNE RESPONSE TO MENINGEAL VENOLYMPHATIC DYSFUNCTION**

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Lymphatic vessels and veins are closely associated in the dura mater around the sinuses (perisinus), but how venous outflow can influence dural lymphatic vessels' structure and function remains unknown. To investigate the functional interactions between veins, lymphatics and the dura mater immune cells, our team developed a mouse model of cerebral venous outflow obstruction by bilateral external and internal jugular vein ligation (JVL-mice).
In the first part of the project, we demonstrated that cerebral venous outflow obstruction in JVL-mice led to transient intracerebral hypertension, regression of the dorsal meningeal lymphatic vasculature (MLV), and brain swelling due to impaired intracerebral clearance. Here, we hypothesize that these venolymphatic dysfunctions induce alterations of the central nervous system neuroimmune surveillance that may contribute to behavioral changes of JVL-mice.
Behavioral testing of JVL-mice reveals sleep disturbances concomitant with the previously observed raised intracerebral pressure, dysfunctional brain fluid drainage and impeded dorsal MLV. Additionally, chemokine and cytokine analysis of JVL-mice plasma indicates that these alterations coincide with a pronounced proinflammatory profile. Upon functional restoration of the MLV and brain fluid drainage, immune responses transition towards an anti-inflammatory state, mirrored not only in the plasma but also in the transcriptomic profiles of dural mesenchymal and immune cells, as well as in the gene expression of brain microglial cells. Interestingly, this anti-inflammatory profile aligns with a transient reduced anxiety-like behavior of JVL-mice.

**Keyword 1**: Meningeal immunity
**Keyword 2**: Venolymphatic dysfunctions
**Keyword 3**: Behavior
**Keyword 4**: Glymphatic system
**Keyword 5**: CNS immune surveillance

**MEMANTINE REDUCES THE EXPRESSION OF IFN-GAMMA AND IL-17 BY CD4$^+$ T CELLS IN AGED EAE RATS**

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The average age of patients with multiple sclerosis (MS) has risen in recent decades. The incidence of late-onset MS has also increased. More than half of people living with an MS diagnosis are over 55. Ageing affects the composition and function of N-methyl-D-aspartate receptors (NMDARs), regardless of whether they are expressed on neuronal or immune cells. The aim of this study was to investigate the effects of memantine, a non-competitive NMDAR antagonist, on CD4$^+$ T lymphocytes in young and aged rats immunized for experimental autoimmune encephalomyelitis (EAE). Memantine was administered by oral gavage to 3- and 24-month-old female Dark Agouti rats for 7 consecutive days from the first day post-immunization (dpi) or from the 7$^{th}$ dpi. Mononuclear cells were isolated from the lymph nodes draining the site of immunization or the spinal cord and analysed by flow cytometry. A histopathologic analysis of the spinal cord was performed. Memantine administration more effectively reduced the mean neurological score and histological score in aged rats. Memantine reduced the expression of IFN-gamma and IL-17 by CD4$^+$ T lymphocytes derived from draining lymph nodes or the spinal cord to a greater extent in aged rats. On the other hand, there was no difference in apoptosis of CD4$^+$ T lymphocytes between control and memantine-treated young rats, whereas apoptosis of these cells was significantly reduced in memantine-treated aged rats. NMDARs have a greater effect on IFN-gamma and IL-17 synthesis by CD4$^+$ T cells and apoptosis of these cells in aged than in young EAE rats.

**Keyword 1**: Ageing
**Keyword 2**: NMDAR
**Keyword 3**: multiple sclerosis
**Keyword 4**: memantine

**ABERRANT IL-1B-INDUCED CYTOKINE RESPONSE IN SCHIZOPHRENIA-DERIVED MICROGLIA AND ITS IMPACT ON NEURONAL AND ASTROCYTIC FUNCTION**

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Schizophrenia is a neurodevelopmental disorder, with a multifaceted etiology, involving genetic and environmental factors. The aberrant activation of microglia is one of the suggested key factors in schizophrenia manifestation, contributing to neuroinflammation, synaptic dysfunction, and metabolic disturbances. Investigating microglial activation in schizophrenia has been challenging due to the lack of appropriate brain cell models. Previously, we found that human induced pluripotent stem cell (hiPSC)-derived-microglia from patients with schizophrenia exhibit an inflammatory phenotype, suggesting that these cells are inherently primed, and display altered stress responses to the proinflammatory cytokine interleukin-1β (IL-1β). In the current study, we aim to understand the impact and mechanisms of IL-1β-induced cytokine imbalance in schizophrenia-derived microglia and its effects on excitatory neurons and astrocytes in mixed tri-cultures. Preliminary results indicate that IL-1β treatment did not compromise cell viability in our tri-culture model. Schizophrenia-derived microglia exhibited proinflammatory phenotype and high proliferation in tri-cultures. Acute IL-1β treatment altered the cytokine balance depending on the cell type composition. The most pronounced effect was observed in tri-cultures with schizophrenia-microglia, although mixing cells with only schizophrenia-derived astrocytes or neurons produced a similar effect. These findings suggest an exaggerated inflammatory response in schizophrenia-microglia, which may contribute to the chronic neuroinflammation observed in psychiatric disorders. Further investigation will clarify how this dysregulation impacts neuron-glia and glia-glia interactions, potentially revealing novel targets for therapeutic intervention in neuroimmune dysfunction.

**Keyword 1**: Schizophrenia
**Keyword 2**: Neuroinflammation
**Keyword 3**: Human induced pluripotent stem cells
**Keyword 4**: Microglia
**Keyword 5**: Tri-culture

**CAN TRANSIENT PERIPHERAL INFLAMMATION LEAD TO LONG-TERM CHANGES IN THE CENTRAL NERVOUS SYSTEM?**

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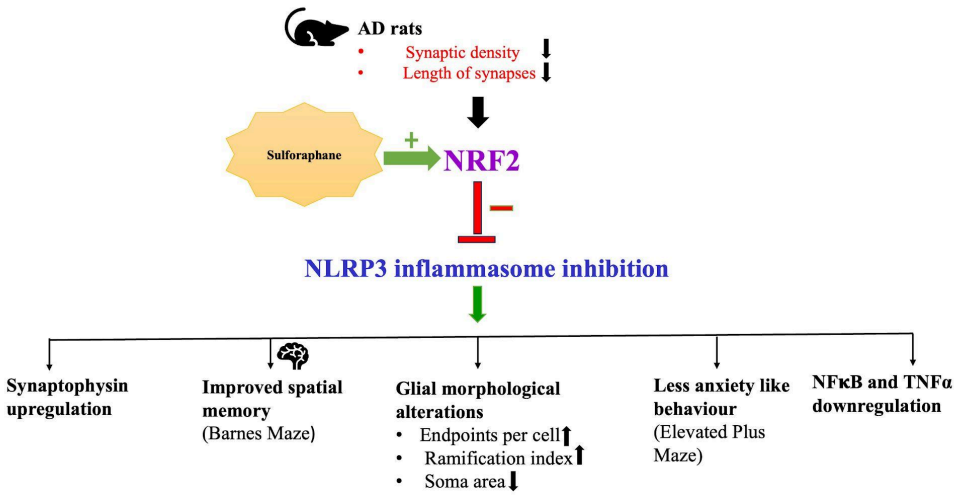
Peripheral inflammation is known to influence brain function during active episodes, but whether it leaves lasting effects after resolution remains unclear. Clinical evidence links severe infections and systemic inflammatory conditions, such as sepsis, to heightened risks of neurological disorders, including mood disorders and dementia. Using a collagen antibody-induced arthritis (CAIA) mouse model we assess cognitive function, cellular changes, and molecular markers at key time points: peak inflammation (day 9), recovery (day 60), and after repeated inflammatory events (day 120). Our pilot experiments revealed mild impairments in hippocampal-dependent spatial memory and enhanced fear learning. Imaging studies identified reactive astrocytes accumulating around blood vessels in the hippocampus. Peripheral macrophages were observed attaching to the choroid plexus during acute inflammation, suggesting persisting damage to this brain barrier under these conditions. To understand how these changes persist and whether they can be reversed, we analyze cytokine profiles and test therapeutic interventions. TNF-alpha inhibitors, which have shown promise in reducing dementia risk in rheumatoid arthritis patients, are evaluated for their capacity to mitigate inflammation-induced brain alterations. Future cohorts will explore the impact of inhibiting additional cytokines identified as key mediators of inflammation. Advanced imaging and image analysis techniques will provide insights into how inflammation reshapes neuronal circuits and glial interactions over time. This research aims to redefine the understanding of how transient inflammation affects long-term brain health. Findings may lead to improved treatment strategies for inflammation-associated neurological conditions and emphasize the importance of anti-inflammatory lifestyles.

**Keyword 1**: Neurons
**Keyword 2**: Astrocytes
**Keyword 3**: Microglia
**Keyword 4**: Inflammation
**Keyword 5**: Cytokines

**NRF2 ACTIVATION BY SULFORAPHANE MITIGATES SPATIAL MEMORY DEFICITS AND NEUROINFLAMMATION VIA SUPPRESSION OF NLRP3 SIGNALLING IN ALZHEIMER'S DISEASE RAT MODEL**

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Alzheimer's disease (AD) is characterized by cognitive impairments and neuroinflammation, with oxidative stress playing a pivotal role in its pathophysiology. Role of Nuclear factor erythroid 2-related factor 2 (NRF2), a key mediator of antioxidant responses, in mitigating AD induced neuronal dysfunctions remains poorly understood. Our study investigated the effects of NRF2 activation through Sulforaphane (SFN), a NRF2 agonist on anxiety-like behaviour, spatial memory, synaptic plasticity, neuroinflammation, glial cell morphology, and micromineral homeostasis in a $Ab_{1-42}$ induced rat model of AD. Adult male Wistar rats were divided into four groups: control with vehicle, control with SFN (10 mg/kg), $Ab_{1-42}$ induced AD with vehicle, and $Ab_{1-42}$-induced AD with SFN. SFN was administered intraperitoneally (10mg/kg) for 5 days. Behavioural assessments included the Elevated Plus maze (EPM) and Barnes maze Test. Brain tissues were analysed for synaptic ultrastructure, NLRP3 inflammasome and NRF2 activation, inflammatory cytokines, glial morphology by immunohistochemistry and micromineral levels by Inductively-coupled Plasma Mass Spectrometry (ICP-MS) in the hippocampus. NRF2 activation by SFN significantly reduced anxiety-like behaviour and ameliorated spatial memory deficits in AD rats. Expression of synaptophysin improved in the hippocampus. SFN attenuated NLRP3 inflammasome activation and modulated pro-inflammatory cytokine (Tnfa and Nf-kB) expression. NRF2 activation modulated glial cell morphology and partially restored micromineral homeostasis in the hippocampus. These findings highlight the therapeutic potential of NRF2 activation in ameliorating cognitive deficits, synaptic dysfunction, and neuroinflammation in AD. NRF2-mediated modulation of glial function and micromineral dynamics represents its protective effects in neurodegenerative disorders.




**Keyword 1**: Azheimer's Disease
**Keyword 2**: NRF2
**Keyword 3**: Sulforaphane
**Keyword 4**: Neuroinflammation
**Keyword 5**: NLRP3

**FROM HEART TO BRAIN: NEUROINFLAMMATION AND MEMORY IN RATS WITH HEART FAILURE WITH PRESERVED EJECTION FRACTION**

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Heart Failure with Preserved Ejection Fraction (HFpEF) is an increasingly prevalent condition strongly associated with cognitive impairment (CI), yet its underlying mechanisms remain unclear. This study aims to characterize
neuroinflammatory and cognitive changes in a well-established cardiometabolic model of HFpEF. ZSF1-Obese and ZSF1-Lean rats were used as experimental and control groups, respectively. HFpEF phenotype was confirmed through
echocardiography. At 29 weeks of age, animals underwent Novel Object Recognition (NOR), Object Location Recognition (OLR), and Temporal Order Recognition (TOR) tests to assess memory functions. Additionally, hippocampal
microglia and astrocyte activation were analyzed using stereological estimation of GFAP- and CD11b-immunoreactive cells, and hippocampal cytokine mRNA expression was quantified. NOR and OLR tests suggested a tendency toward
impaired recognition and spatial memory in ZSF1-Obese rats, though differences were not statistically significant. In the TOR test, no differences were observed. Furthermore, preliminary results show that the numerical density of GFAP- and CD11b-positive cells remained unchanged between groups. However, TNF-α expression was significantly increased in ZSF1-Obese rats, indicating a pro-inflammatory shift without overt glial activation. These findings suggest that in ZSF1-Obese rats, an experimental model of HFpEF, there is a potential short-term memory impairment and pro-inflammatory state. However, further studies are necessary to confirm this tendency, as we could benefit from increasing the number of animals studied. Further experiments are being carried out to also study other cognitive domains and behaviour.

**Keyword 1**: Neuroinflammation
**Keyword 2**: Behaviour
**Keyword 3**: Preserved Ejection Fraction Heart Failure
**Keyword 4**: Cognitive Impairment
**Keyword 5**: Memory

**ONCOSTATIN M ACTIVATES NOCICEPTIVE NEURONS INNERVATING THE MURINE GUT**

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The cytokine binding subunit (Osmr) of the receptor for oncostatin M (OSM) is co-expressed with the transducers of noxious stimuli transient receptor potential vanilloid 1 (TRPV1) and transient receptor potential ankyrin 1 (TRPA1) in sensory neurons innervating the gut. This is indicative of a causative role for OSM in generation of abdominal pain during colitis. We hypothesise that OSM stimulates neurons through the sensitisation of TRPV1/TRPA1 cation channels. Murine dorsal root ganglia (DRG; T12-L5) neurons were isolated, dissociated and underwent in vitro calcium imaging in response to OSM application in the absence and presence of targeted antagonists against proteins. Superfusion with OSM (30nM) elicited a rise in intracellular [Ca2+] in a greater proportion of sensory neurons than perfusion with vehicle. This response was significantly attenuated by pre-treatment with the TRPV1 antagonist (A425619, 1 mM; $p<0.01$, 39.86±5.5% vs 17.17±5.7%, Figure 1A) or the TRPA1 antagonist (AM0902, 1 mM; $p<0.01$, 26.55±3.2 vs 11.91±1.9%; Figure 1B), in separate experiments. Bath perfusion with OSM (20ml, 2nM) evoked a significant increase in the colonic afferent response to colorectal distension (CRD) compared with vehicle (Figure 1C) resulting in a peak afferent response to CRD which was 138.4±21.7% of that evoked before OSM application, compared with a peak afferent response to CRD which was 84.6±4.9% of the response obtained prior to vehicle treatments ($p=0.016$). These findings suggest a role for OSM in the activation of sensory neurons innervating the gut, and this may be mediated by modulation of TRPA1 and TRPV1.

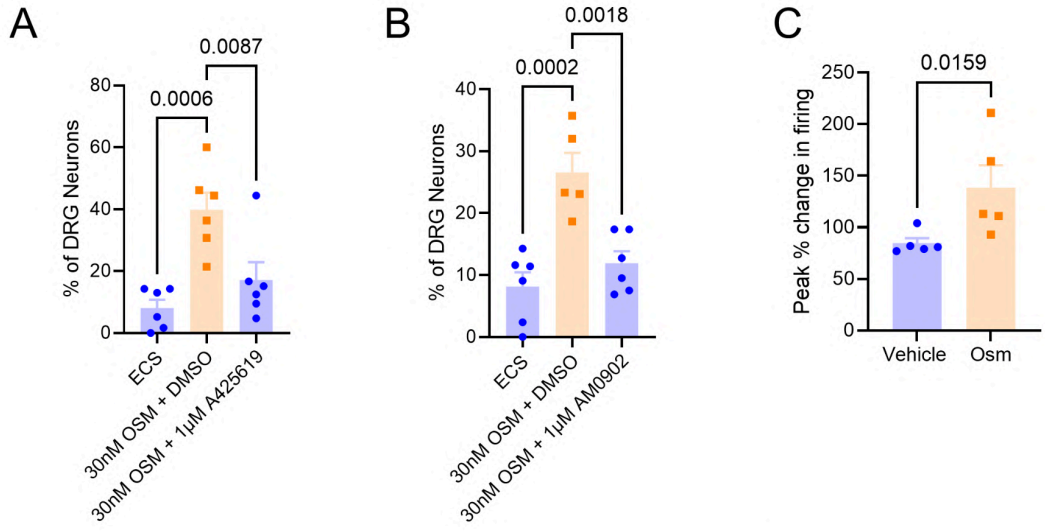



**Fig. 1.** Superfused OSM increases intracellular [$Ca^{2+}$] in DRG neurons, which is reduced by pre-incubation with a TRPV1 antagonist (A) or a TRPA1 antagonist (B). Tissue bath perfusion of OSM also increased the colonic afferent response to noxious colorectal distension compared to vehicle (C).

**A MULTIPLEXED ELECTROPHYSIOLOGY ASSAY FOR ASSESSING IPSC-DERIVED NEURON AND ASTROCYTE CO-CULTURES**

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Advances in induced pluripotent stem cell (iPSC) technology have greatly improved the fidelity of in vitro models of human physiology, including that of the brain. Many studies use iPSC-derived neurons to study neural biology and disease. However, complex neural processes and pathologies often involve other cell types, such as astrocytes. Therefore, we assessed the electrophysiological properties of a neuron-astrocyte co-culture using the Maestro Pro microelectrode array system. Briefly, 120,000 iPSC-glutanuerons were plated with or without 20,000 iPSC-astrocytes in a CytoView 48 well plate, and the cells' electrical activity was monitored on the Maestro Pro for 28 days. Recordings showed that inclusion of astrocytes increased mean firing rate (3.2 Hz for neurons and astrocytes, 1.6 Hz for neurons alone) and area under the normalized cross correlogram (0.22 for neurons and astrocytes, 0.01 for neurons alone). To further optimize the culture, we tested two media systems: a complete BrainPhys-based medium and a "tri-culture" media that included supplements designed to enable the addition of further cell types (i.e. microglia). Activity and synchrony remained robust in the complete BrainPhys media for both neurons and neuron-astrocyte co-cultures. However, culturing in tri-culture media led to sharp decreases in all electrical activity metrics. These results highlight the utility of the Maestro Pro as a system to measure differences in the electrophysiology of in vitro neuronal models. Further research can be focused on improving culture conditions to facilitate the addition of other neuronal cell types, thereby increasing the complexity of the culture and better recapitulating in vivo physiology.

**Keyword 1**: kisspeptin
**Keyword 2**: locomotion
**Keyword 3**: dopamine
**Keyword 4**: nucleus accumbens

**OPTIMIZING THE TIMING AND EFFICACY OF ANTI-INFLAMMATORY MICRORNAS FOR BEHAVIORAL RECOVERY IN MICE UNDER INFLAMMATORY STRESS**

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This study investigates the effects of intravenous administration of anti-inflammatory microRNAs (miRNAs) on behavioral recovery in two groups of mice subjected to inflammatory stress. Mice were divided into two experimental groups, each receiving a specific anti-inflammatory miRNA treatment. Behavioral assessments were conducted at four distinct time points: 3, 7, 14, and 28 days post-treatment. These assessments included a range of behavioral tests designed to evaluate anxiety-like and depressive-like behaviors, as well as cognitive function. Specifically, the open field test was used to assess anxiety, the forced swim test was employed to evaluate depressive-like behavior, and the novel object recognition task was utilized to measure cognitive performance. Our results demonstrate that both groups of mice exhibited significant improvements in behavioral recuperation over time, with the efficacy of the miRNA treatments differing between the groups. Notably, the most pronounced recovery was observed at 14 days post-treatment, suggesting that this may represent a critical window for the anti-inflammatory effects of miRNAs on behavior. Furthermore, the observed improvements in cognitive and emotional behaviors indicate that miRNA therapies may have a broader impact on neural recovery following inflammatory stress. This study underscores the potential of anti-inflammatory miRNAs as promising therapeutic agents for enhancing recovery from inflammatory conditions. Additionally, these findings provide valuable insights into the timing and effectiveness of miRNA treatments in modulating behavior in a preclinical model. Future research will be necessary to investigate the underlying mechanisms and to optimize these treatments for potential clinical application.

**GUT MICROBIOME COMPOSITION: A BIOLOGICAL MARKER OF THE VULNERABILITY TO DEVELOP AN ALCOHOL USE DISORDER**

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Alcohol Use Disorder (AUD) is a leading global cause of morbidity and mortality, yet only a subset of individuals transition to compulsive use. Current pharmacotherapies remain largely unsatisfactory, with limited efficacy and a predominant focus on late-stage AUD, neglecting early intervention strategies. Identifying biomarkers of vulnerability could enable targeted prevention before addiction becomes entrenched. Converging evidence implicates the gut-brain axis and neuroinflammation in the emergence of compulsive alcohol use, particularly following adolescent stress—a critical window of neurodevelopmental and microbial plasticity during which environmental insults may fundamentally alter long-term brain function.
We hypothesized that adolescent stress-induced gut dysbiosis precipitates a chronic low-grade inflammatory state, disrupting the maturation of impulse control circuits and heightening susceptibility to AUD. Using two established preclinical models—Chronic Mild Unpredictable Stress (CMUS) and a DSM-5-based addiction vulnerability paradigm—we demonstrate that stress-exposed rats exhibit heightened impulsivity, disinhibition, and addiction-like behaviors, alongside profound gut microbiota perturbations and persistent central and peripheral neuroimmune responses. Notably, neuroimmune alterations within the anterior cingulate cortex (ACC), a hub for executive control, were accompanied by systemic elevations in RANTES (CCL5), marking a sustained neuroinflammatory state.
To establish causality and therapeutic relevance, we employed Fecal Microbiota Transplantation (FMT) and SB3CT, a selective MMP-9 inhibitor. FMT reinstated microbial homeostasis, attenuated neuroimmune activation, and mitigated compulsive behaviors, whereas SB3CT conferred resilience against stress-induced impulsivity and neuroimmune dysregulation. These findings causally implicate neuroimmune dysfunction as a mechanistic driver of stress-induced vulnerability to AUD, underscoring the gut-brain axis as a viable therapeutic target.

**Keyword 1**: Addiction
**Keyword 2**: Inflammation
**Keyword 3**: Alcohol
**Keyword 4**: Gut microbiota

**GUT MICROBIOTA ALTERATIONS AND HYPOTHALAMIC INFLAMMATION PRECEDE OBESITY IN A RAT MODEL OF BINGE EATING**

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Overconsumption of ultra-processed food and sedentary lifestyle are the main causes of obesity, which in turn, increases the risk of developing metabolic, cardiovascular, and psychiatric diseases. Loss of control over food intake and binge eating often worsen as obesity progresses. Many studies have shown the effect of obesity on gut-microbiota composition and inflammation. However, less is known about microbiota and central adaptations occurring during the initial phases of body weight gain. To address this question, we used a rat model of binge eating, in which, rats (C/P) were fed twice a week with homemade cheesecake, as palatable food, for two months. At the end of the feeding protocol, C/P rats developed cheesecake binge eating behavior compared to chow fed rats (C/C). Despite the lack of obesity and peripheral inflammation, C/P rats showed altered microbiota composition, increased Firmicutes/Bacteroidetes ratio (F/B ratio) and α-diversity. In the hypothalamus of C/P rats, using q-PCR, we observed impaired cytokine and leptin-signaling-related protein gene expression. Additionally, we performed immunofluorescence analysis of Iba1+ microglia cells in the hypothalamus consisting in microglia counting, branches analysis and fractal analysis. Cheesecake consumption in C/P rats led to increased number of Iba1+ cells and changes in their morphology. Microglia cells showed decreased complexity and increased density, known as typical features of a pro-inflammatory state. Taken together these data suggest that changes in microbiota composition and neuroinflammation precede obesity and could contribute to overeating. Therefore, strategies able to limit these gut-brain axis adaptations may be useful in preventing excessive body weight gain.

**Keyword 1**: gut microbiota
**Keyword 2**: inflammation
**Keyword 3**: binge eating

**CANNABIMIMETIC MODULATION AS AN EARLY PREVENTIVE APPROACH AT THE ONSET OF NEUROINFLAMMATION**

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Systemic inflammation and neuroinflammation pose a significant risk of delayed neurodegenerative changes. Traumatic brain injuries, cardiovascular and infectious diseases, peripheral inflammation, mental stress, autoimmune diseases, brain aging, metabolic changes, and lifestyle, are accompanied by neuroinflammation and may compromise blood-brain barrier integrity. Therapy should include the early prevention of delayed neurological complications. Using the animal model of LPS-induced neuroinflammation, we studied the action of SEA, one of the poorly studied endogenous mediators of the expanded endocannabinoid system, on the interplay between endocannabinoid and prostaglandin signalling. Experiments were performed on C57BL/6 mice, males. Four treatment groups were analysed: vehicle-treated mice; mice that received a single LPS injection (5 mg/kg, i. p.); mice that were treated with SEA (50 mg/kg, per os) every 24 h for 7 days; mice that received a single LPS injection (5 mg/kg, i. p.) and were treated with SEA (50 mg/kg, per os).
We demonstrated that LPS-induced neuroinflammation promotes significant changes in the expression of the prostaglandin D2 receptor (DP1) in the peripheral macrophages and affects the production of several interleukins within the brain regions studied (prefrontal cortex and hippocampus). In the dentate gyrus of LPS-injected mice, we observed higher production of IL-10 and its colocalization with postsynaptic density protein PSD-95. SEA restricted the monocyte migration, which may protect the brain against infiltration of the immune cells accompanying neuroinflammatory states.
Our study demonstrated that endogenously produced SEA can mediate the early neuroprotective response, which modulates the inflammatory state and restricts its spreading within the tissue.

**Keyword 1**: neuroinflammation
**Keyword 2**: stearoylethanolamide
**Keyword 3**: prostaglandin D2 receptor
**Keyword 4**: hippocampus
**Keyword 5**: endocannabinoid system

**SYSTEMIC NEUROECTODERMAL STEM CELL TREATMENT RESCUES INJURED MOTONEURONS OTHERWISE DESTINED TO DIE BY DOWNREGULATING L-SELECTIN EXPRESSION ON LEUKOCYTES**

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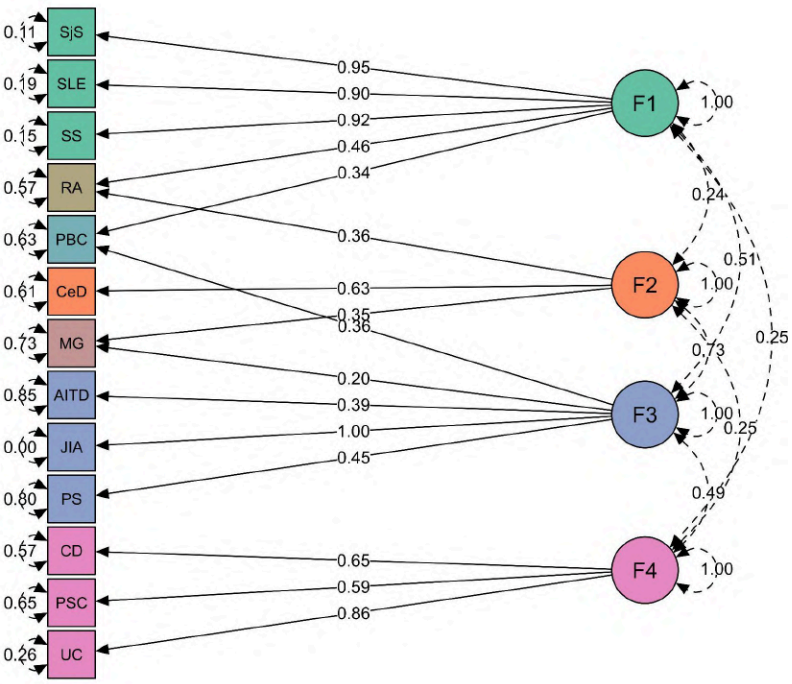
Avulsion injury results in motoneuron death due to the increased cytotoxicity developing after the injury. In this study we intended to prove the motoneuron rescuing effect of intravenously grafted NE-4C stem cells and reveal the mechanism of action used by the grafted cells.
In our experimental model the left lumbar 4 (L4) ventral root of the spinal cord was avulsed and then reimplanted into the L4 spinal segment. Treated animals received various doses of NE-4C stem cells intravenously and the survival and regeneration of the affected motoneurons was checked by morphological and functional analysis. The molecular changes within the treated cord were followed by the ELISA Proteome Profiler rat cytokine array and qPCR analysis. To mimic the effect of stem cells fucoidan treatment was applied for two weeks intraperitoneally.
High doses of intravenous stem cell treatment induced the reinnervation of the reimplanted ventral root by surviving injured motoneurons (up to 38% of the total L4 pool). Proteome Profiler analysis showed that systemic stem cell treatment downregulated the level of L-selectin, that promotes leukocyte rolling on vascular endothelium. Both systemic stem cell and fucoidan treatment reduced macrophage and microglial densities in the affected spinal segment and administration of fucoidan downregulated inflammatory cytokine and inflammasome levels along with improved morphological and functional reinnervation.
Blocking L-selectin, similarly to systemic NE-4C stem cell treatment decreases the neuroinflammation in the injured spinal cord segment after ventral root avulsion and induces significant motoneuron survival and functional reinnervation of the denervated hind limb muscles.

**Keyword 1**: motoneuron injury
**Keyword 2**: stem cells
**Keyword 3**: fucoidan
**Keyword 4**: neuroinflammation
**Keyword 5**: regeneration

**EXPLORING THE AUTOIMMUNE-AUTOINFLAMMATORY SPECTRUM: INSIGHTS FROM STATISTICAL GENETICS IN CROSS-DISORDER ANALYSIS**

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**Aims:** Immune-mediated diseases can be classified as a continuum from pure autoimmune to autoinflammatory with mixed diseases in between [McGonagle, 2006, 2021]. Here we investigate the polygenic continuum of immune-linked disorders using statistical genetics methods and define genetic architecture focusing and groups of disorders on autoimmune-autoinflammatory axis.
**Methods:** We analyzed genome-wide association studies for 15 immune-linked disorders (MS, MG, SLE, SS, SjS, PSC, PBC, AITD, UC, CD, CeD, PS, RA, JIA, T1D) using statistical genetics methods (LDSC, MiXeR, LAVA and genomic SEM) for cluster definition, characterization of overlap and genetic architecture. MAGMA according to FUMA protocol was used for the functional annotation.
**Results:** Genomic SEM suggested a continuum structure with four underlying factors from autoimmune diseases at one end to inflammatory on the opposite end (Fig.1). We observed a balanced mixture of negative and positive local correlations within the MHC region. In contrast, outside this region, they were predominantly positive. MAGMA analysis shows genes associated with monogenic immune diseases in diseases with prominent autoimmune and inflammatory components.
**Conclusion:** Our findings support a polygenic continuum with four genetic clusters across immune-mediated disorders. The "polygenic autoimmune" and "polygenic inflammatory" clusters reside on the margins of this continuum. The findings provide insights informing future research in this field and potential therapeutic strategies.




**Disclosures:** No special disclosures. This work was supported by an RCN grant 324252.

**Keyword 1**: autoimmunity
**Keyword 2**: inflammation
**Keyword 3**: autoimmune diseases
**Keyword 4**: genome-wide association studies
**Keyword 5**: polygenicity

**DYSFUNCTIONAL NATURAL KILLER CELLS IN CHILDREN WITH AUTISM SPECTRUM DISORDERS**

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Autism spectrum disorders (ASD) are characterized by abnormal neurodevelopment, genetic, and environmental risk factors, as well as immune dysfunction. In adults with high functioning ASD (hf-ASD), we previously reported the persistence of dysfunctional NK cells, expressing markers of activation and adaptive NKG2C+ NK cells, generally observed in the context of viral infectious events. These results led us to investigate profiles of NK cells in children with ASD, where preliminary data also suggest alterations in NK cells, including increased numbers and reduced cytotoxic activity.

NK cells from 22 children with ASD (6 to 16 year old) were stratified into two groups ($2C^+$ and $2C^-$) according to their expression of NKG2C. The extensive phenotype and polyfunctional capacities (i.e., degranulation and intracellular cytokine production) of NK cells were compared between the two groups, along with their association with circulating cytokines.

Compared to $2C^-$ samples, NK cells from $2C^+$ showed a high levels of cell activation ($p<0.0001$), spontaneous degranulation ($p<0.001$), and cytokine production (IFN-g and TNF-a) ($p<0.01$), whereas they were exhausted after in vitro stimulations. NKG2C expression was also significantly associated with increased levels of IFN-g, IL-12p40 and VEGF ($p<0.01$, $p<0.01$ and $p<0.001$, respectively), while chemokine levels were significantly decreased.

This study strongly suggests major NK-cell dysfunction in children with ASD with a significant proportion of samples having highly activated adaptive NK cells indicating a possible association with underlying pathogens. These findings, similar to those observed in adults with hf-ASD, suggest that NK cell dysfunction may contribute to the immune dysregulation associated with ASD.

**Keyword 1**: Autism spectrum disorders
**Keyword 2**: Natural Killer cells
**Keyword 3**: NK cells
**Keyword 4**: Innate immune system
**Keyword 5**: Immuno-psychiatry

**UNCOVERING MITOCHONDRIAL AND LYSOSOMAL ADAPTATIONS IN MICROGLIAL CELLS PRIOR TO DEPLETION IN RESPONSE TO COLONY-STIMULATING FACTOR 1 RECEPTOR INHIBITOR**

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Colony-stimulating factor 1 receptor (CSF1R), a cell-surface receptor found in myeloid cells, plays crucial roles in the survival, regulation, proliferation, and function of microglia in the central nervous system. Pharmacological inhibitors of CSF1R, such as Plexxikon 5622 (PLX5622), have been extensively used to deplete microglia in vivo, uncovering their critical functions in rodent models. However, little is known about the cellular and molecular events that occur in microglia prior to their depletion. This study investigates these early responses following exposure to 10 μM PLX5622 compared to DMSO-treated controls using a combination of live-cell imaging, RNA sequencing, and transmission electron microscopy. Our experiments reveal significant changes in microglial organelles. In rodent BV-2 cells, PLX5622 treatment induced decreased lysosomal pH and increased lysosomal mass at 6 and 24 hours, similar to human HMC3 cells, where a decrease in lysosomal pH was observed at 24 and 48 hours. Mitochondrial adaptations included increased membrane potential and mass in BV-2 cells at 24 hours and increased membrane potential in HMC3 cells at the same time point. These functional alterations were accompanied by upregulation of lysosomal and mitochondrial genes in PLX-treated cells. Ongoing ultrastructural analyses aim to elucidate mitochondrial changes (e.g., elongation, membrane disruption, cristae enlargement) and other intracellular alterations. These findings provide new insights into the early cellular responses of microglia to PLX5622 and will help refine its application in neuroimmunology research.

**Keyword 1**: Microglia
**Keyword 2**: PLX5622
**Keyword 3**: Depletion model
**Keyword 4**: Mitochondria
**Keyword 5**: Lysosome

**EFFECTS OF THE NEONICOTINOID ACETAMIPRID ON THE ADIPOSE TISSUE/MICROGLIA CROSS-TALK IN OBESITY**

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Obesity, a multifaceted condition influenced by various environmental factors, represents a critical public health challenge. Dietary choices and exposure to metabolic disrupting compounds (MDCs) play pivotal roles in shaping adipose tissue (AT) functions and its crosstalk with different organs, including the brain. The present study aimed to investigate the complex interaction between the AT and microglia, the brain immune cells, in the context of obesity and exposure to environmental toxicants, such as the insecticides neonicotinoids, which have recently emerged as harmful to human health. By employing the human microglia clone 3 (HMC3) cell line and AT-conditioned media (CM) collected from biopsies obtained from obese male and female patients, we aimed to: i) assess the effects of AT-derived signals on microglial immune profiles, ii) determine the impact of molecular signals from ATs exposed to the neonicotinoid Acetamiprid (ACE) on microglia, and iii) examine possible protective effects of the flavonoid Protocatechuic Acid (PCA). We analyzed the cytokines Interleukin-6 (IL-6) and Interleukin-1β (IL-1β), crucial in central glucose homeostasis. Research studies suggest that elevated IL-6 and IL-1β in microglia during early obesity stages may counteract metabolic dysfunction. Our findings show that AT-CM from obese patients increased IL-6 and IL-1β expression in microglia. However, ACE disrupted this protective mechanism by altering AT secretions, reducing cytokine expression. Additionally, microglia exposed to CM-ACE showed increased insulin receptor (InsR) expression, indicating altered glucose metabolism. Unfortunately, PCA did not revert these effects. Overall, our data raise concerns regarding the safety of ACE for human health.

**Keyword 1**: Microglia
**Keyword 2**: Obesity
**Keyword 3**: Neonicotinoids
**Keyword 4**: Flavonoids

**NEUROIMMUNE RHYTHMS PRIME THE OLFACTORY BULB'S RESPONSE TO AN INTRANASAL CHALLENGE**

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The interaction of immune and circadian processes in brain homeostasis is an important new area of research. To investigate neuroimmune transcriptional rhythms in different brain regions, we collected mouse olfactory bulbs (OBs) and somatosensory cortices (SSCs) over 8 times of day and extracted RNA. Using Nanostring and DiffCircaPipeline, we detected 55 rhythmic transcripts shared between brain regions, 49 distinct to the SSC, and 106 distinct to the OB. Using Metascape, we found that rhythmic OB transcripts that peaked around dusk were associated with immune processes. This suggests the OB is primed to mount greater neuroimmune responses at some times of day than others. To test this, we intranasally challenged mice with poly(I:C) or vehicle at ZT0 (dawn) or ZT12 (dusk), collected OBs at 0-, 3-, 12-, and 24-hours post-inoculation, and found that time of inoculation produced distinct OB transcriptional responses. This indicates the OB is primed to differentially respond to an intranasal challenge in a time-of-day-dependent manner. To test if microglia also show differential responses, we intranasally challenged mice with poly(I:C) or vehicle at ZT0 or ZT12, isolated OB microglia 24-hours post-inoculation, and used imaging flow cytometry. We detected two distinct microglia populations defined by the presence of intrinsically fluorescent puncta. This distinction was functionally relevant, as intranasal poly(I:C) at ZT12 produced a greater change in the proportion of these populations than at ZT0. We conclude that immune rhythms prime the OB to differentially respond to intranasal poly(I:C) in a time-of-day-dependent manner, and that microglia may contribute to these responses.

**Keyword 1**: circadian
**Keyword 2**: microglia
**Keyword 3**: neuroinflammation
**Keyword 4**: neuroimmune
**Keyword 5**: homeostasis

**DISTINCT ANTIBODY GLYCOSYLATION PROFILES REFLECT IMMUNE RESPONSE COMPARTMENTALIZATION AND DISEASE TRIGGERS IN ANTI-NMDAR ENCEPHALITIS**

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Anti-N-Methyl-D-Aspartate Receptor encephalitis (NMDARe) is a severe neurologic disorder caused by pathogenic antibodies targeting the NMDAR. Triggers include ovarian teratoma and herpes simplex encephalitis (HSE), though many cases remain unknown (idiopathic). While NMDAR-antibodies, mainly IgG1, are detected in serum and CSF, some patients have antibodies exclusively in CSF. Together with the presence of CSF-unique IgG oligoclonal bands, this supports that immune responses may be compartmentalized. Whether antibody characteristics differ between serum and CSF or based on disease trigger remains unclear..We aimed to characterize serum and CSF antibodies by analyzing their Fc-glycan composition, to identify trigger-specific profiles. Paired serum and CSF (age- and sex-matched) from patients with NMDARe (n=50) were analyzed using liquid-chromatography-mass-spectrometry to determine IgG1 antibody-glycan composition, by quantification of fucosylation, sialylation, galactosylation and bisecting n-acetylglucosamination. These glycol-profiles were then compared between compartments (CSF vs serum) and triggers (post-HSE, tumor-related, idiopathic).Compared to serum, CSF antibody-glycans had lower levels of sialylation and galactosylation, indicating a more inflammatory profile. Similarly, patients with post-HSE NMDARe displayed an enrichment of inflammatory glycoforms, characterized by reduced sialylation and galactosylation and increased bisecting N-acetylglucosamine, compared to those with tumor-related or idiopathic causes.In conclusion, our data demonstrate that antibody-glycan profiles are qualitatively different, with CSF exhibiting more inflammatory glycoforms than serum. Furthermore, glycan composition varies across triggers, with post-HSE anti-NMDARe linked to more inflammatory glycovariants than other causes. These findings highlight the role of glycans in the pathogenesis of NMDARe, which could be harnessed to develop novel compartment- and trigger-specific therapies based on glycan modifications.

**Keyword 1**: Autoimmune encephalitis
**Keyword 2**: Glycan
**Keyword 3**: antibody
**Keyword 4**: NMDAR encephalitis

**MICROGLIA IN THE INFRALIMBIC CORTEX ARE REQUIRED FOR DEPRESSIVE-LIKE BUT NOT SICKNESS BEHAVIOR FOLLOWING SYSTEMIC INFLAMMATION**

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Major Depressive Disorder (MDD) is a psychiatric disorder with rising global prevalence and significant socio-economic impact. Recent studies suggest that systemic inflammation, often associated with infections, contributes to depression. For instance, elevated inflammatory cytokine levels have been linked to the onset of treatment-resistant depression.
An acute response to infection typically induces behavioral changes characterized by decreased appetite and mobility, defined as sickness behaviors in this study. Following this response, depressive-like behaviors, characterized by behavioral despair, may emerge and can persist. Altered neuronal activity in brain regions such as the prefrontal cortex, hippocampus, and amygdala has been implicated in depressive-like behavior. In particular, the infralimbic (IL) cortex, part of the medial prefrontal cortex, exhibits reduced activity following inflammation. For example, lipopolysaccharide (LPS) administration decreases IL activity, marked by increased inhibitory synaptic transmission onto Layer 5 pyramidal neurons. Notably, chemogenetic activation of this region can mitigate depressive-like behaviors, yet the molecular mechanisms underlying inflammation-induced neuronal changes during depression remain unclear.
Microglia, the brain's resident macrophages, play a crucial role in neuroinflammation by responding to peripheral cytokines and modulating neural circuits through synaptic remodeling and cytokine release. We hypothesized that microglia mediate inflammation-driven depressive-like behavior. Our findings demonstrate that microglial ablation and functional modulation attenuate depressive-like behaviors. Moreover, targeted ablation of IL microglia also mitigates these behaviors, suggesting that microglia in this region play a key role in the neural response to systemic inflammation. These results highlight microglia as a potential therapeutic target for inflammation-associated depression.

**Keyword 1**: microglia
**Keyword 2**: infralimbic cortex

**CYTOMEGALOVIRUS AND THE CEREBELLUM IN SEVERE MENTAL ILLNESS**

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Neurotropic pathogens like cytomegalovirus (CMV) have been implicated in severe mental illness (SMI), with seropositivity linked to brain abnormalities. The cerebellum, essential for motor control, cognition, and emotion regulation, often exhibits structural alterations in schizophrenia (SZ) and bipolar disorder (BP). We investigated whether CMV seropositivity (CMV+) is associated with reduced cerebellar volumes in SMI and whether these effects are region-specific. Our sample included 528 patients with SMI and 494 healthy controls from the Thematically Organized Psychosis study cohort. T1-weighted MRI data was processed using CerebNet for cerebellar lobule segmentation, and ComBat was applied to adjust for scanner differences. ANCOVA models examined CMV effects on total and lobular cerebellar volumes, controlling for age, sex, and total intracranial volume. A false discovery rate of 5% was applied to correct for multiple comparisons. We found a significant effect of CMV+ on total cerebellar volume in SMI ($F = 5.512$; $p = 0.018$), with CMV+ patients exhibiting smaller volumes than CMV- patients. No such effect was observed in controls. In patients, CMV was associated with volume reductions in lobules I–IV and V bilaterally, as well as vermis VIII, vermis X, and lobule X bilaterally.
These findings indicate a region-specific association between CMV and cerebellar volume reductions in SMI, potentially exacerbating structural abnormalities. Certain cerebellar lobules may be particularly sensitive to pathogen-related neuroanatomical changes, highlighting the need for longitudinal studies to clarify causality and clinical implications.

**Keyword 1**: Psychiatry
**Keyword 2**: Cerebellum
**Keyword 3**: Neuropathogens

**B-LYMPHOCYTES-DERIVED EXTRACELLULAR VESICLES INFLUENCE MICROGLIAL ACTIVATION: INSIGHTS INTO EPSTEIN BARR VIRUS ROLE IN MULTIPLE SCLEROSIS**

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**BACKGROUND**
Multiple sclerosis (MS) pathophysiology begins peripherally, where immune cells migrate into the CNS, cross the blood-brain barrier, and trigger an autoimmune attack. Epstein-Barr virus (EBV) may be a key environmental trigger. Extracellular vesicles (EVs) influence CNS inflammation and immune modulation, possibly carrying EBV molecules. Understanding EV-microglia interactions is crucial for elucidating their role in MS. This study aims to develop an experimental model to investigate EVs-microglia interactions.
**METHODS**
ARH77 (EBV+) and OPM2 are B-lymphocytes derived cell lines whose EVs were isolated through ultracentrifugation. HMC3 microglial cell line were treated with 2*10e8 EVs/100000 cells for 24h. RT-qPCR was performed to assess the expression of IL-1β, IL-6, IL-8, TNFα and CD68. Mean Cq and fold change was calculated.
**RESULTS**
Characterization of EVs revealed an increase in mode and mean size of ARH77-EVs compared to OPM2-EVs ($p<0.05$). ARH77-EVs are carriers of EBV genetic material.
Inflammatory phenotype of HMC3 upon stimulation with EVs has been evaluated: there was a significant and general decrease of IL-1□, IL-6, TNFα and CD68 ($p<0.05$) in both treatments compared to untreated cells. Interestingly, stimulation with ARH77-EVs resulted in an increase of IL-8 expression compared to that of OPM2-EVs stimulation and to untreated cells ($p<0.05$).
**CONCLUSIONS**
Stimulation of HMC3 microglial cell lines with B lymphocytes-derived EVs switched microglia towards an anti inflammatory phenotype. The solely increase of IL-8 in ARH77-EVs treated HMC3 might be related to the activation of specific pathways by EBV genetic material carried by EVs. Its role, as that of anti-inflammatory cytokines, will be further investigated.

**Keyword 1**: Multiple Sclerosis
**Keyword 2**: Epstein-Barr virus
**Keyword 3**: HMC3
**Keyword 4**: Extracellular Vesicles
**Keyword 5**: Immune phenotype

**THE HUMAN INTEGRATED SENSOR SYSTEM (HISS): HARNESSING THE POWER OF PSYCHONEUROIMMUNOLOGY FOR THE EARLY DETECTION OF BIOLOGICAL THREAT EXPOSURE**

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The COVID-19 pandemic has highlighted the urgent need for early detection of biological threat exposure. This has driven the creation of the Human Integrated Sensor System (HISS) powered by a mechanistic understanding of psychoneuroimmunology (PNI). This rodent study advances our clinical program by examining the spatiotemporal pattern of the neuroimmune response and sex differences in sickness behaviour following a peripheral immune challenge. Our integrated understanding will enable the HISS mission to develop a wearable warning system capable of detecting deviations in an individual's PNI state within minutes of threat exposure. Forty-eight male and female C57BL/6 mice received two consecutive intraperitoneal injections: TLR4 antagonism (LPS-RS vs. saline) followed by TLR4 agonism (LPS vs. saline) an hour later. Peritoneal white blood cells (WBCs), nodose ganglia, and brains were collected 15 and 60 minutes post-treatment. Immunofluorescence staining assessed early response markers in peritoneal WBCs, the nodose ganglia and sickness-related brain nuclei. Generalised linear regression and ANOVA analysed treatment, sex, and time effects on p-ERK-NeuN co-expression and NF-kB localisation. Preliminary findings show significantly increased p-ERK expression within 15 minutes of LPS administration, with males engaging the nucleus tractus solitarius and females engaging cerebral nuclei related to behavioural adaptation. Ongoing analyses of nodose ganglia and peritoneal WBCs will also be presented. These findings demonstrate rapid, sex-specific central nervous system engagement following peripheral LPS administration. Our next steps involve identifying hyperacute biomarkers to differentiate the type and magnitude of threat using electrophysiological and behavioural measures in freely-moving mice, enabling the earliest-ever threat exposure warning system.

**Keyword 1**: TLR4
**Keyword 2**: Early response markers
**Keyword 3**: Peritoneal white blood cells
**Keyword 4**: Nodose Ganglia
**Keyword 5**: Nucleus tractus solitarius

**INVESTIGATING LONGITUDINAL CHANGES IN THE SYSTEMIC INFLAMMATORY RESPONSE OF APOEE4 CARRIERS VERSUS NON-CARRIERS WITHIN THE PREVENT COHORT**

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**Aims**: Chronic inflammation is implicated in the progression of Alzheimer's disease (AD), yet the extent of its influence on disease development remains unclear. This study hypothesises that C-Reactive Protein (CRP) levels, as a marker of systemic inflammation, will increase significantly in APOEε4 carriers compared to non-carriers of the PREVENT cohort between visit one and two.
**Methods**: PREVENT Dementia is a longitudinal cohort study of 700+ cognitively healthy participants aged 40-59 across five sites in the UK and Ireland, with 38% APOEε4 carriage. Participants with chronic inflammatory conditions or acute illness were excluded. Age and sex-matched propensity score matching balanced APOEε4 carriers and non-carriers. Paired t-tests and linear regression were used to evaluate changes in CRP, neutrophil-to-lymphocyte ratio (NLR) between visits, and their association with changes in Addenbrooke's Cognitive Examination-III (ACE-III) scores.
**Results**: Preliminary findings suggest no significant difference in CRP or NLR values between visits for APOEε4 carriers and non-carriers. CRP levels were significantly greater in APOEε4 non-carriers at visit one, but this difference was less marked at visit two. A higher increase in NLR was significantly associated with a greater decline in ACE scores, an effect observed in both APOEε4 carriers and non-carriers.
**Conclusions**: Although no significant differences in CRP or NLR were observed between visits, the link between increased NLR and cognitive decline suggests that chronic systemic inflammation may impact cognitive performance, warranting further investigation into the interacting effect of age and sex with APOE ε4 in this context, which will be presented at the conference.

**INVESTIGATION OF TRANSCRIPTOMIC AND EPIGENETIC LANDSCAPE IN NEURAL PROGENITORS AND HUMAN BRAIN ORGANOIDS DERIVED FROM MONOZYGOTIC TWINS CLINICALLY DISCORDANT FOR MULTIPLE SCLEROSIS**

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Multiple Sclerosis (MS) is an autoimmune disorder affecting neurons, myelin, and oligodendrocytes in the central nervous system, often following a relapsing-remitting course. While genetic predisposition contributes to MS susceptibility, environmental factors and epigenetic mechanisms, like chromatin modifications and DNA methylation, significantly influence disease onset and progression. Notably, monozygotic (MZ) twins clinically discordant for MS exhibit only a 25% concordance rate, highlighting non-genetic factors in pathogenesis. To elucidate the molecular underpinnings of MS, cutting-edge single-cell multi-omics approaches were employed to investigate gene expression dynamics and chromatin accessibility in neural precursor cells (NPCs) and human brain organoids (hBOs) derived from induced pluripotent stem cells (hiPSCs) of MZ twin pairs discordant for MS. hiPSCs generated from dermal fibroblasts were differentiated into NPCs using dual-SMAD inhibition. 3D hBOs were obtained from NPCs transfected with an hSox-10-expressing lentiviral vector under doxycycline induction to promote oligodendrocytes differentiation. Additionally, autologous microglia were derived from the same hiPSCs and incorporated into mature hBOs prior to sequencing. Both NPCs and hBOs underwent single-cell RNA sequencing (scRNA-seq) and single-cell Genome & Epigenome by Transposase sequencing (scGET-seq), enabling a comprehensive analysis of chromatin dynamics and transcriptional heterogeneity. Bioinformatics analyses using Seurat and Scanpy pipelines revealed distinct neuronal and glial populations, with MS samples exhibiting a stressed phenotype characterized by dysregulated immune signalling and metabolic pathways. Differential chromatin accessibility at MS-risk loci further underscored the interplay between gene expression and chromatin state. These findings highlight MS-specific regulatory heterogeneity and provide crucial insights into disease pathophysiology, paving the way for novel therapeutic strategies.

**Keyword 1**: Multiple Sclerosis
**Keyword 2**: Human Brain Organoids
**Keyword 3**: Transcriptome
**Keyword 4**: Epigenome

**A CASE-CONTROL STUDY - HEMORHEOLOGICAL DYSREGULATION AND SYSTEMIC INFLAMMATORY PROFILES ACROSS ACUTE PHASES OF SCHIZOPHRENIA**

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Introduction: Schizophrenia is linked to changes in proinflammatory status and disrupted blood flow, both of which may elevate cardiovascular risk. However, the effects of these changes during various acute stages of schizophrenia are not well understood. This study explores the differences in blood viscosity and systemic inflammation between individuals experiencing their first episode of schizophrenia (FES) and those undergoing acute episodes of schizophrenia (S-AE). Methods: 74 FES patients, 56 S-AE patients, and 60 healthy controls (HC) were recruited. Whole blood viscosity (WBV) at low shear rate (LSR) and high shear rate (HSR) was estimated using de Simone's formula. Systemic immune-inflammation index (SII) and systemic inflammatory response index (SIRI) were calculated from complete blood count parameters. At the same time, high-sensitivity C-reactive protein (hs-CRP) was measured at hospital admission. Analyses were conducted using ANCOVA to adjust for age. Results: After adjustment, WBV at both LSR and HSR was significantly lower in FES and S-AE groups than HC ($p<0.001$). SIRI levels were significantly elevated in FES relative to S-AE and HC ($p=0.002$). In the FES group, hs-CRP was a significant predictor of WBV at LSR ($\beta = 0.38$, $p = 0.002$), whereas age and SIRI were not significant ($p > 0.05$). Conclusion: The findings suggest that alterations in hemorheology are a common characteristic of schizophrenia, while heightened systemic inflammation in first-episode schizophrenia (FES) may signify an acute immune response. Further research is needed to clarify the mechanisms linking inflammation, hemorheology, and cardiovascular risk in individuals with schizophrenia.

**Keyword 1**: Systemic Inflammation
**Keyword 2**: Acute Schizophrenia
**Keyword 3**: Hemorheology

**CEREBROSPINAL FLUID FROM EARLY-STAGE MULTIPLE SCLEROSIS PATIENTS PROMOTES OSCILLATORY MIGRATION OF MICROGLIA IN A CONCENTRATION- AND TIME-DEPENDENT MANNER**

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As resident immune cells of the CNS, microglia are recruited in multiple sclerosis (MS) even prior to the formation of structural lesions, transitioning from pro-reparative to chronic inflammatory activity. Although their dual contribution has long been observed, understanding the context in which microglia are driven to lose their protective states and become destructive has only begun to be addressed.
To further characterize the role of microglia in MS pathogenesis, we exposed defined-medium cultures of microglial cells (serum-free) to cerebrospinal fluid (CSF) collected from five drug-naive female patients after the first attack of clinical symptoms, with relapsing-remitting MS. Here, we analyzed the effect on migration at 4% and 10% CSF-MS exposure, relative to 10% serum exposure (serum control, SC) and non-treated control (NC), using live-cell microscopy.
Microglia exposed to 10% CSF-MS showed significantly increased migration compared to 4% CSF-MS and NC microglia; there was no significant difference when compared to SC-exposed microglia. Additionally, we demonstrated that microglia exposed to 4% CSF-MS and NC microglia relied on a constant migration pattern, while 10% CSF-MS showed a peak between 4.5 and 6h after exposure. Interestingly, the SC-exposed microglia were characterized by a fast migration pattern only in the first 3 hours and remained constant beyond this timepoint.
In conclusion, the oscillatory migration pattern of microglial cells under CSF-MS exposure is concentration- and time-dependent. These findings have implications for understanding the migratory properties of microglia in MS and may help to characterize the transition of microglial states from neuroprotective to disruptive.

**Keyword 1**: multiple sclerosis
**Keyword 2**: microglia
**Keyword 3**: cerebrospinal fluid

# Developmental Neuroscience and Neurogenesis

**MTOR HYPERACTIVATION DRIVES MUTATION-SPECIFIC HYPERACTIVITY IN HIPSC-DERIVED NEURONAL NETWORKS THROUGH ALTERED GABAERGIC SIGNALING**

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The mTOR pathway is a pivotal cellular signaling pathway that impacts neuronal differentiation and function. However, disrupted mTOR signaling in neurodevelopmental disorders remains poorly understood. Genetic mutations leading to hyperactivation of the mTOR pathway, collectively termed as mTORopathies, cause rare genetic and systemic disorders with profound effects on brain development and a strong clinical association with epilepsy. Immature GABAergic signaling resulting in altered excitatory/inhibitory balance in developing neuronal networks is thought to be a key mechanism underlying the etiology of mTORopathies. Using human induced pluripotent stem cells (hiPSCs) and CRISPR/Cas9 genome editing, we generated a panel of isogenic hiPSC lines carrying mutations in key mTOR regulator genes, including gain of function mutations in *RHEB*, *MTOR*, *PIK3CA,* as well as a loss of function mutation in *TSC2*. In hiPSC-derived neuronal networks composed of glutamatergic and GABAergic neurons on microelectrode arrays, we observed that all induced varying degrees of neuronal network hyperactivity, each with a distinct functional fingerprint. Pharmacological interrogation of the networks during development suggests that these mutation-specific fingerprints may arise from delayed or incomplete maturation of GABAergic signaling. This hypothesis is further supported by our findings on chloride cotransporter expression regulation over time. Furthermore, we observed alterations in glutamatergic and GABAergic synapse density, along with changes in neuronal morphology, indicating distinct structural consequences for neuronal network organization. Our findings reveal mutation-specific hyperactive network phenotypes, undercoring the complex interplay between genetic factors and mTOR pathway signaling that may underlie the clinical complexity of mTORopathies.

**Keyword 1**: mTORopathies
**Keyword 2**: epilepsy
**Keyword 3**: iNeurons
**Keyword 4**: MEA

**THE HYPOXIA-INDUCIBLE FACTOR 1ALPHA AND PRIMARY CILIA – A FUNCTIONAL ANALYSIS OF THE INTERPLAY IN NEURONAL CELLS**

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Primary cilia, also called "the cells antenna", are centriole based sensory cellular organelles, which are present on almost all mammalian cells and became important during the last decades. They realize a variety of functions, including maintaining neuroplasticity and the adaption to hypoxic conditions via a variety of signalling pathways, including the SHH-signalling, the MEK-ERK-signalling or the PDGFRa-signalling pathway. Based on these observations, the work pursued the goal to investigate the interplay of the hypoxia-inducible factor 1alpha and primary cilia in neuronal cells. The establishment of primary neuronal *HIF1alpha*-knockout cells, based on a specific tamoxifen induced Cre/LoxP-system served as the base for this work. Thereby the knockout efficiency was determined by quantitative PCR analysis and Western-Blotting. By using the cells, we investigate a connection between the transcription factor and the “cellular antenna” and define its interplay more precisely by focussing on a variety of signalling pathways and the primary cilia morphology. In particular, influences on the expression of the Notch signalling components Notch 3 and 4, as well as on GATA3 could be observed. The significance of the interaction of both factors, as well as the transferability to diseases associated with hypoxia, such as ischemic stroke, must be investigated by further experiments.

**Keyword 1**: primary cilia
**Keyword 2**: hypoxia
**Keyword 3**: Notch signalling
**Keyword 4**: ischemic stroke
**Keyword 5**: Neurospheres

**INVESTIGATING TANGO2'S ROLE IN MITOCHONDRIA AND NEURONAL ACTIVITY USING PATIENT-DERIVED IGLUNEURONS**

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Biallelic *TANGO2* loss-of-function variants cause a multisystemic disorder with metabolic-encephalomyopathic crises, rhabdomyolysis, and neurodegeneration. We generated induced-Pluripotent-Stem-Cells(iPSCs) from two siblings with the same genotype (c.262C>T(p.Arg88*); c.338delG(p.Gly113Alafs*10)) but exhibiting different clinical symptoms. Symptomatic patient-derived neurons showed increased oxidative stress, whereas asymptomatic sibling's cells remained comparable to controls. To further investigate, we examinatedTANGO2's role in mitochondrial redox-imbalance and synaptic connectivity in induced-glutamatergic-Neurons (iGluNeurons).
Human iPSCs from TANGO2-patients and controls were differentiated into iGluNeurons via neurogenin-2-inducible overexpression. Mitochondrial membrane potential and elongation were evaluated with tetramethylrhodamine-ethyl-ester(TMRE) dye, while electrophysiological activity was recorded using High-Density Multi-Electrode-Array(HD-MEA). Neurotransmission changes was performed with glutamate assay.
At 21 days *in-vitro*(DIV), no significant differences in mitochondrial membrane potential were observed, likely because this early maturation stage is too premature to reveal TANGO2-deficiency effects. Further analyses will exhibit energy maintenance at later stages. However, mitochondrial length increased in both patient-iGluNeurons, with slightly elongation in the asymptomatic ones, highlighting enhanced fusion processes, potentially as a cellular stress response.
Electrophysiological analysis (30DIV) showed that symptomatic patient-iGluNeurons exhibited a significant reduction in firing rate, burst rate, and duration compared to controls, suggesting impaired neuronal activity, and signaling. Additionally, glutamate release was reduced at 21DIV, further confirming synaptic dysfunction. Asymptomatic patient analysis is ongoing.
Increased mitochondrial fusion suggests an initial compensatory mechanism, more effectively sustained in the asymptomatic patient. Subsequent studies will explore whether asymptomatic patient's compensation involves enhanced ATP production and mitophagy. Electrophysiological impairment and reduced glutamate release observed underscore TANGO2's role in synaptic transmission and neuronal signaling.

**Keyword 1**: hIPSCs
**Keyword 2**: Neural stem cells
**Keyword 3**: iGluNeurons

**Keyword 4**: TANGO2
**Keyword 5**: Neurogenesis

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Cajal-Retzius cells (CRs) are a glutamatergic cell type that regulates neuronal migration, lamination, and synapse formation during development. Although the majority of cortical CRs disappears in the early postnatal days, a distinct subset persists in the hippocampus for several weeks. These remaining CRs, located in the stratum lacunosum-moleculare (SLM) of the CA areas and the outer blade of the dentate gyrus, receive excitatory inputs from GABAergic interneurons while also targeting both interneurons and pyramidal cells in CA1. At this critical time when CRs still populate the hippocampus, this region undergoes maturation of specific functional cell types, such as place cells.Previous studies have indicated that postnatal ablation of hippocampal CRs leads to layer-specific effects on dendritic spines, synapse-related genes, and a decrease in SLM-specific postsynaptic guiding proteins. Give their importance for the establishment of the hippocampal circuit, we hypothesized that the persistence of hippocampal CRs might be essential also for the optimal maturation of the hippocampal function and the development of the spatial navigation system.
To test this hypothesis, we used a combination of transgenic mice and viral vectors to specifically remove postnatal CRs from the hippocampus and employed multi-shank silicon probes to perform in vivo recordings in freely moving animals of spatially modulated cells in the CA1 region of the hippocampus. Our preliminary analysis shows that early postnatal ablation of hippocampal CRs causes changes in the basic properties of place cells, such as spatial information content and remapping.

**Keyword 1**: Cajal-Retzius cells
**Keyword 2**: place cells

**DECIPHERING INTERNEURON DISTRIBUTIONS IN THE MEDIAL ENTORHINAL CORTEX**

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GABAergic interneurons (INs) are crucial for the development and modulation of cortical circuits. They are highly heterogeneous, exhibiting diverse morphological, electrophysiological, and molecular characteristics. INs are commonly classified into three major groups based on the expression of three largely non-overlapping molecular markers: parvalbumin (PV) and somatostatin (SST), both derived from the medial ganglionic eminence (MGE), and serotonin receptor 5HT3AR, derived from the caudal ganglionic eminence (CGE).
Their distribution varies across brain regions, with PV+ INs being the predominant subtype in most neocortical primary sensory areas. However, recent studies indicate variations in other regions, such as association cortices.
The medial entorhinal cortex (MEC), a key region for spatial information processing, remains understudied in terms of IN distribution. Previous research indicates a dorsoventral (DV) gradient of PV+ INs, with a higher density in the dorsal MEC, but little is known about the other interneuron subtypes.
Using GAD67-EGFP mice, we confirmed a significant DV gradient of PV+ INs in the MEC. However, our data show that the overall distribution of GABAergic cells does not differ across the DV axis. Immunohistochemical analysis for MGE- and CGE-specific markers did not show any gradient. We therefore wondered if SST+ INs showed an opposing DV gradient to PV+ INs, explaining our results. However, the lower PV+ IN density in the ventral MEC is not compensated by SST+ INs.
To elucidate the mechanisms underlying this discrepancy, we plan to employ an intersectional viral strategy, immunohistochemical stainings, and spatial transcriptomics to further characterize MEC IN development and distribution.

**Keyword 1**: interneurons
**Keyword 2**: medial entorhinal cortex
**Keyword 3**: parvalbumin interneurons
**Keyword 4**: dorsoventral gradient
**Keyword 5**: interneuron development

**DIFFERENCES IN CAJAL-RETZIUS CELL DENSITY AND POSTNATAL PERSISTENCE ACROSS CORTICAL AREAS REVEALED BY A NOVEL INTERSECTIONAL GENETIC LABELING APPROACH**

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Cajal-Retzius (CR) cells are an early born, reelin-expressing, excitatory neuronal population located in the neocortex and hippocampus that serve a critically important function during the first postnatal week (P0-P7) of cortical development in the mouse. CR cells are usually described to largely die off in the neocortex after the second postnatal week, while in the hippocampus, they are observed to persist throughout postnatal development and even into adulthood. However, a comprehensive analysis of CR cell density, distribution, and persistence across the development of multiple cortical regions has never been done. To this end, we crossed a recently developed NDNF driver line (*NDNF-flox-FlpO*) with a *Pde1c-Cre* line and the intersectional tdTomato reporter Ai65 to specifically and selectively label CR cells. Our data demonstrate that in addition to the hippocampus, several cortical areas have a prolonged persistence of CR cells beyond P14, pointing to a wider function for these cells in postnatal brain circuitry. Interestingly, areas that are highly interconnected with the hippocampus do not necessarily show the same trend in CR cells persistence. Indeed, the two subdivisions of the entorhinal cortex (EC), the medial EC (MEC) and the lateral EC (LEC), which are the main input and output areas of the hippocampus, display different trends. MEC has a CR cell persistence that is similar to the hippocampus, while the CR cells in the LEC disappear at a rate more comparable to association cortices.

**Keyword 1**: Cajal-Retzius cells
**Keyword 2**: Development
**Keyword 3**: Hippocampus
**Keyword 4**: Entorhinal cortex
**Keyword 5**: Whole-brain cell counting

**PROTEOMIC CHARACTERIZATION OF GROWTH CONES IN THE HIPPOCAMPUS AND DENTATE GYRUS DURING EARLY POSTNATAL DEVELOPMENT**

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The first postnatal weeks are critical for the establishment and maturation of the hippocampal circuit. A decisive event during this period is the innervation of entorhinal cortex (EC) layer 2 fibers into the CA3 and Dentate Gyrus (DG) subregions of the hippocampus (HP). Immunohistochemical studies show the process is completed approximately by postnatal day (P) 5, but little is known about how the axons travel through the subregions to reach their final target. To investigate this process, we analyzed the proteome of growth cones (GCs) from wild-type mice at P1, P3, and P5. Shotgun sequencing of these samples identified 4,895 proteins in hippocampal GCs (HP-GCs) and 4,897 proteins in DG-specific GCs (DG-GCs). KEGG pathway analysis of the shared proteomes revealed pathways typically associated with GCs, such as axon guidance, focal adhesion, and regulation of actin cytoskeleton. Comparative analysis of HP-GC and DG-GC showed distinct protein subsets specific to tissue origin and timepoint, with P1 samples exhibiting the highest number of unique proteins. Interestingly, gene ontology (GO) analysis highlighted a developmental shift in biological processes. P1 samples were enriched for mitochondria-associated terms, while later stages (P3 and/or P5) showed enrichment for mRNA-related processes, such as mRNA stability and splicing regulation. Differences between HP and DG GO terms further indicated delayed development of the DG compared to the whole HP. This data provides valuable insights into GC dynamics and their unique molecular signatures during hippocampal circuit maturation.

**Keyword 1**: Growth cones
**Keyword 2**: Hippocampus
**Keyword 3**: Dentate Gyrus
**Keyword 4**: Proteomics

**THE ONTOGENY OF HIPPOCAMPAL LEARNING AND MEMORY SYSTEMS IS SUPPORTED BY POSTNATAL CAJAL-RETZIUS CELLS**

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The hippocampus is a brain structure known for its role in memory formation, learning and the representation of space. The hippocampal circuitry is comprised of a diverse set of excitatory and inhibitory neurons, including a developmentally transient population of Cajal-Retzius cells (CRc). In contrast to neocortical CRc, which disappear soon after birth, hippocampal CRc persist for an extended period during postnatal development. In previous work, we showed that early postnatal ablation of hippocampal CRc leads to deficits in dendritic spine development, alterations in synapse-related genes and a complementary loss of synapse-related proteins. This demonstrates that CRc play a fundamental role in the establishment of the hippocampal circuit. However, the impact of CRc on hippocampal function remains to be determined.
To assess their functional role, we use a combination of a double transgenic mouse line and viral vectors to specifically ablate hippocampal CRc at birth and subsequently test their spatial memory in a radial-arm maze. We found that the CRc ablated animals failed to reach the same level of performance as control animals by committing more errors. The application of a reinforcement learning model showed that experimental animals make more stochastic actions than control animals, indicating that they rely less on a value structure to navigate. Furthermore, *in vivo* electrophysiological recordings revealed that the spatially modulated cells in the CA1 are highly unstable, rendering them less efficient in supporting spatial navigation.
Taken together, our findings show that CRc are crucial for the development of hippocampal spatial memory systems.

**Keyword 1**: Hippocampus
**Keyword 2**: CR cells
**Keyword 3**: Electrophysiology
**Keyword 4**: Behavior

**INTERLEUKIN 1 SIGNALING IN THE DENTATE GYRUS OF THE HIPPOCAMPUS: A DOUBLE-EDGED SWORD FOR ADULT HIPPOCAMPAL NEUROGENESIS**

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Adult hippocampal neurogenesis (AHN) is a dynamic process by which neural stem cells (NSCs) in the dentate gyrus (DG) of the hippocampus proliferate and differentiate into newly born granule neurons. AHN is essential for learning and memory; however, the process is regulated by various factors at each phase. One such factor is interleukin-1 (IL-1), a proinflammatory cytokine, that signals via interleukin 1 receptor type 1 (IL-1R1). By using transgenic mouse models with cell type-specific expression of IL-1R1, this study examines how IL-1 signaling impacts the NSCs in the DG. We established a minimum dose of IL-1, delivered stereotaxically via an adeno viral vector, to induce NSC proliferation. Intriguingly, we discovered that low-dose IL-1 promotes NSC proliferation. We found that high doses suppress neurogenesis and induce neuroinflammation. Interestingly, selective expression of IL-1R1 on astrocytes replicates the low-dose IL-1-induced proliferation of NSCs, highlighting astrocytes' critical role in mediating IL-1's pro-neurogenic effects. Hence, IL-1 functions as a double-edged sword in AHN depending on its concentration and cellular target. To further investigate the mechanisms behind these effects, we use retroviral tagging of proliferating NSCs to track their differentiation into mature neurons. We will determine whether the increase in the quantity of newly born neurons will improve learning and memory. By identifying the specific cell types targeted by IL-1 and the pathways involved, this research aims to uncover the dose-dependent and cell type-specific regulation of AHN. Ultimately, this work could lead to new therapeutic strategies to manage neuroinflammation and enhance neurogenesis in neurodegenerative diseases.

**Keyword 1**: adult hippocampal neurogenesis
**Keyword 2**: neuronal cell fate
**Keyword 3**: neurodegeneration
**Keyword 4**: neuroimmunology
**Keyword 5**: interleukin 1

**CHARACTERIZATION OF THE CELLULAR AND MOLECULAR DIVERSITY DURING THE PRIMATE EVOLUTION**

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The brain comprises diverse cell types that are interconnected to form specific neural circuits underlying cognition and behavior. To systematically elucidate the cellular diversity and underlying regulatory mechanisms during primate evolution, we performed large-scale spatial transcriptome, single-nucleus RNA-seq and chromatin accessibility analysis of the primate brain. By developing deep learning models for integrating multi-omics data, we derived a comprehensive cell-type taxonomy and found their laminar and regional preferences as well as neighborhood complexity. Comparative analysis revealed glutamatergic and GABAergic neuron types in the cortex of primates with layer- and subregion-specific distribution and high expression of functionally important genes. Furthermore, our analysis revealed primate-specific CREs for genes related to neurodevelopment and psychiatric diseases. Taken together, these results provide spatial underpinning for molecular understanding of organizing principles of the primate brain.

**Keyword 1**: primate brain
**Keyword 2**: cell atlas
**Keyword 3**: evolution
**Keyword 4**: multi-omics data integration

**THE MICROCEPHALY-ASSOCIATED TRANSCRIPTIONAL REGULATOR AUTS2 COOPERATES WITH POLYCOMB COMPLEX PRC2 TO PRODUCE UPPER-LAYER NEURONS**

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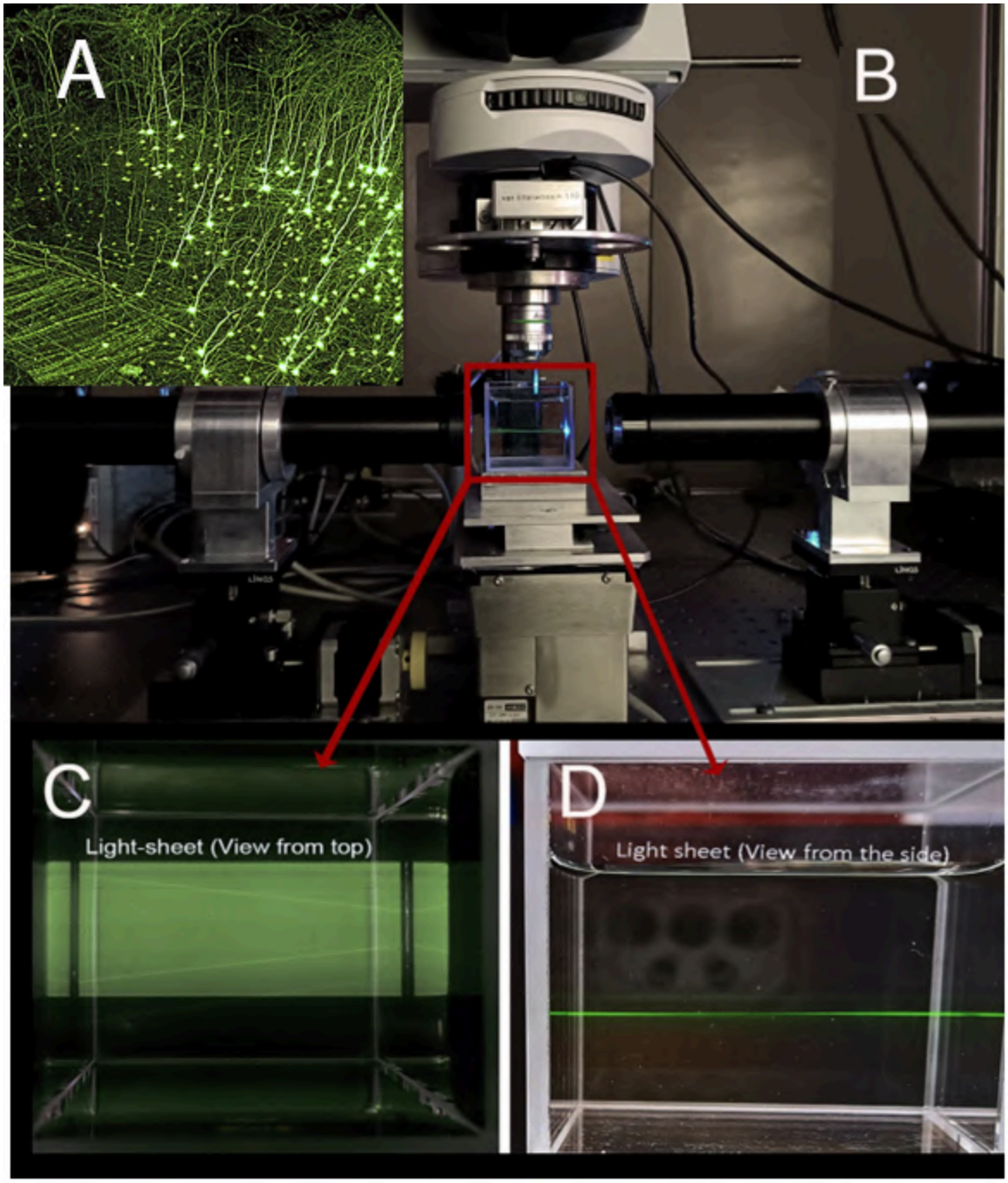
AUTS2 syndrome is characterized by intellectual disability and microcephaly, and is often associated with autism spectrum disorder, but the underlying mechanisms, particularly concerning microcephaly, remain incompletely understood. Here, we analyze mice mutated for the transcriptional regulator AUTS2, which recapitulate microcephaly. Their brains exhibit reduced division of intermediate progenitor cells (IPCs), leading to fewer neurons and decreased thickness in the upper-layer cortex. Increased expression of the AUTS2 transcriptional target Robo1 in the mutant animals suppresses IPC division, and transcriptomic and chromatin profiling shows that AUTS2 primarily represses transcription of genes like Robo1 in IPCs. Regions around the transcriptional start sites of AUTS2 target genes are enriched for the repressive histone modification H3K27me3, which is reduced in Auts2 mutants. Furthermore, we find that AUTS2 interacts with Polycomb complex PRC2, with which it cooperates to promote IPC division. These findings shed light on the microcephaly phenotype observed in the AUTS2 syndrome.

**Keyword 1**: microcephaly
**Keyword 2**: AUTS2
**Keyword 3**: PRC2

**MESO-ASPHERIC BASED TUNABLE LIGHT-SHEET MICROSCOPY: RELIABLE TECHNIQUE FOR PRECISION IMAGING OF NEURAL AND VASCULAR NETWORK**

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Meso-aspheric optics provide complex optical elements that enhance imaging techniques, particularly in high-resolution microscopy. In this work, we present the latest advancements in our tunable light-sheet microscope. By incorporating refractive optics, such as achromatic-aspheric and meso-aspheric lenses, alongside diffractive optics, we have enhanced optical performance, enabling high-resolution 2D and 3D imaging of the neural and vascular networks in biological tissues of varying sizes, as shown in Fig. 1-A.
To prepare the specimens, we use an optimized clearing technique involving an organic clearing agent (DBE), specific wavelength light sources, and ultrasonic waves. The specimens are then fixed and secured in a clamp within a container filled with a clearing medium that matches the refractive index.
The newly developed tunable light-sheet system generates an ultra-thin, uniform-intensity light sheet with an extended Rayleigh range, as shown in Fig. 1B–D. The system is equipped with three continuous Gaussian beam lasers (488 nm, 532 nm, and 660 nm) for fluorescence excitation. Additional components include optical filters, two custom-made light sheet generators for dual-sided illumination, computer-controlled vertical and horizontal stages, and specialized objectives that compensate for refractive index mismatches between air and the immersion medium.
Image acquisition is carried out using an Andor Neo CMOS camera (2560 × 2160 pixels), followed by post-processing with NeuroDeblur deconvolution software and 3D reconstruction using AMIRA 2020-2.
This tunable light-sheet microscope represents a significant advancement in 3D imaging, offering high resolution across all three axes for enhanced biological visualization.




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**Keyword 1**: Light Sheet Fluorescence Microscopy
**Keyword 2**: High Resolution Imaging
**Keyword 3**: Neural and Vascular Network

**EXPLORING THE ROLE OF CEREBROSPINAL FLUID FLOW IN REGULATING NEUROGENESIS AND NEURONAL FUNCTION IN ZEBRAFISH LARVAE**

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Cerebrospinal fluid (CSF) is a clear, colorless fluid essential for brain homeostasis, bathing the central nervous system. CSF flows through interconnected ventricles lined by ependymal cells, which generate directional flow via their motile cilia. Besides motile cilia, factors like heartbeat and body movement also influence CSF movement in the brain. To investigate the role of CSF movement in brain development and physiology, we analyzed two zebrafish models: one mutant with paralyzed cilia and another lacking pulsatile flow mediated by heartbeat. We found that loss of ciliary motility did not change progenitor proliferation, overall brain morphology and spontaneous neural activity, but lead to enlarged ventricles and altered neuronal and glial responses to photic stimulations. In contrast, the mutant lacking pulsatile flow mediated by heartbeat exhibited severe developmental problems, including altered neurogenesis, smaller brains and brain ventricles, and highly reduced neuronal activity. Our findings underscore the crucial and distinct contributions of motile cilia and heartbeat-driven flow to brain development and function, offering new insights into the function of CSF circulation in neural health and disease.

**Keyword 1**: motile cilia
**Keyword 2**: heartbeat
**Keyword 3**: cerebrospinal fluid
**Keyword 4**: zebrafish
**Keyword 5**: development

**THE CALCIUM SENSING RECEPTOR CASR REGULATES MYELINATION IN THE CENTRAL NERVOUS SYSTEM**

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Myelination by oligodendrocytes in the central nervous system can be divided into intrinsic and adaptive myelination. While the former is thought to be coordinated by a gene regulatory network of important oligodendroglial transcription factors such as Sox10, Olig2, and Nkx2.2, adaptive myelination is dependent on the environment surrounding the oligodendrocyte, such as calcium ions derived from neuronal activity. However, how the mechanisms guiding intrinsic and adaptive myelination are linked or converge is poorly understood. While studying interactions of GPCRs and the oligodendroglial gene regulatory network through publicly available ChIP-seq and RNA-seq data, our attention was drawn to the calcium sensing receptor Casr. Casr is specifically and transiently expressed during the onset of oligodendroglial differentiation both *in vivo* and *in vitro* but is not expressed in oligodendrocyte precursor cells. Reporter gene assays and data from Sox10 deleted mouse spinal cord suggest that Casr expression is regulated by the key lineage determinant Sox10. Furthermore, positive modulation of Casr with the calcimimetic drug cinacalcet increases myelination of primary rat oligodendroglia *in vitro*. These results uncover Casr as a target of the oligodendroglial gene regulatory network that may be important for the process of adaptive myelination through its connection to calcium. This may also uncover new potential future therapies for myelin-associated diseases.

**Keyword 1**: Myelination
**Keyword 2**: Oligodendrocytes
**Keyword 3**: Calcium
**Keyword 4**: Transcription

**IMPROVING MATURATION OF HUMAN IPSCS-DERIVED CORTICAL ORGANOIDS**

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Self-assembled three-dimensional cortical organoids made from human stem cells offer a complex and physiologically relevant model of the brain that resembles the human cortex, and uniquely model neurodevelopment. However, due to lack of blood vessels and microglia, the latter being particularly important for synaptic pruning and neural network maturation, brain organoids do not mature past a stage that resembles the human foetal brain at the end of the second trimester in gestation. Given the crucial role of external inputs and activity for neural development, we hypothesize that their absence limits the maturation of organoids and affects their structure. Another challenge with organoid modelling is the substantial variation between batches and interindividual variation within the same batch. This makes analysis challenging and requires a large number of organoids in each group.
Here we have tested several approaches to promote maturation and reduce variability of our cortical organoids. We have used different electrical and optogenetic stimulation regimes to mimic external inputs. In addition, we have also tested the effect of introducing microglia to the organoids through migration. The efficacy of these different experimental approaches was tested on their ability to improve expression of mature neurons and glia cells, synaptic density and neuronal circuits in the organoids.

**CHARACTERISATION OF THE TEMPORAL STEROLOMIC PROFILE OF A STEM CELL MODEL OF HUMAN CORTICAL DEVELOPMENT**

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Altered cholesterol metabolism has been linked to neural developmental defects, including those associated with intellectual disability (ID) and autism spectrum disorders (ASDs). Studies have identified oxidised metabolites of cholesterol, known as oxysterols, to interact with developmental signalling pathways involved in human cortical development, including Hedgehog and Liver X receptor signalling. However, there is a lack of evidence for the endogenous presence of these molecules in human cortical cells, with much evidence being obtained from rodent models or whole brain tissue samples. To address this, we aimed to characterise the temporal profile of the sterols present in human stem cells undergoing cortical development *in vitro.* Using liquid chromatography-tandem mass spectrometry (LC-$MS^n$) techniques, we have been able to quantify the sterols, including "neuro-oxysterols" 24S-hydroxycholesterol and 24S,25-epoxycholesterol, which are present in these human stem cell-derived neuronal models at each stage of cortical development *in vitro*, from neural progenitor stage to cortical neurons. We have further been able to quantify these molecules in cortical astrocytes, a cell type that has been evidenced to have a role in supporting neuronal cholesterol biosynthesis in the postnatal brain. Furthermore, analysis of protein levels of key enzymes involved in cholesterol biosynthetic and metabolic pathways has revealed changes in their activity at different developmental stages. Overall, this temporal characterisation of *in vitro* human cortical development provides us with a clearer idea of what sterols are present during cortical neurogenesis and information on which of these molecules may contribute to human neural development.

**Keyword 1**: Neurodevelopment
**Keyword 2**: Cholesterol metabolism
**Keyword 3**: Oxysterols
**Keyword 4**: Cortical neurons
**Keyword 5**: Human Pluripotent Stem Cells (hPSCs)

**TOP-DOWN REGULATION OF VISUAL PROCESSING AND INNATE BEHAVIORS IN THE NEONATAL BRAIN**

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Innate behaviors are essential for survival, yet the developmental mechanisms underlying their emergence remain poorly understood. In mammals, the orbitofrontal cortex (OFC), a higher-order brain area, is thought to mature slowly and, thus, of minimally, if at all, contributing to behavior early in life. Here, we show that the OFC controls light aversion, a neonatal survival-critical behavior, even before eye opening. Combining optogenetics, electrophysiology, and anatomical tracing, we identify functional top-down projections from the OFC to the primary visual cortex shortly after birth. These projections target somatostatin-positive interneurons, inhibit visual responses, and are solely activated by salient visual stimuli. This data challenges traditional views of cortical development by demonstrating the early behavioral relevance of the OFC in providing top-down sensory control.

**LAYER I OF THE ADULT HUMAN NEOCORTEX: NEURONAL ARCHITECTURE AND MOLECULAR PROFILING**

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Neocortical layer I is a 200-micron-wide zone located beneath the pia mater of the adult human brain, considered as a cell sparse cortical layer. Using immunohistochemical and RNAscope methods on postmortem tissue from five adult individuals, we analyzed the proportion, distribution, and molecular characteristics of layer I neurons in the associative cortex of the frontal, occipital, and temporal lobes. Opposite to prevailing views, our findings demonstrate that layer I contains a significant number of NeuN-positive cells, primarily distributed in its deeper part. These neurons express mRNA for vesicular transporters of either GABA or glutamate. Approximately 20-50% of NeuN-positive neurons in layer I also express calretinin, whereas calbindin-, parvalbumin-, and somatostatin-positive neurons were found sporadically. At the subpial position, intensely stained calretinin-reactive Cajal-Retzius like neurons were identified. Quantitative analysis indicates that at least 5% of all cortical neurons reside in layer I. Moreover, the density of NeuN-positive neurons in its deeper half is comparable to that of other cortical layers, such as the lower portion of layer III. These findings suggest that human neocortical layer I contains a substantial proportion of cortical neurons, prompting a reevaluation of its classification as a hyponeuronal layer.

**Keyword 1**: layer I
**Keyword 2**: GABA
**Keyword 3**: interneurons
**Keyword 4**: cerebral cortex
**Keyword 5**: human

**LIPRIN-A PROTEINS ARE CRITICAL MEDIATORS OF HUMAN PRESYNAPSE ASSEMBLY**

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Formation of a synapses entails the precise alignment of presynaptic release sites with postsynaptic receptors, but the steps whereby nascent axo-dendritic contacts lead to the assembly of a functional synapse remains unclear. Using human gene-edited neurons as a model system to deconstruct synapse assembly on a clinically relevant genetic background, we find that the Liprin-α family of proteins serve as critical mediators of synapse formation by linking trans-synaptic cell adhesion receptor interactions to downstream assembly of presynaptic specializations. In neurons lacking all four Liprin-α isoforms, nascent synaptic contacts are formed but recruitment of active zone components and accumulation of synaptic vesicles is blocked, resulting in ‘empty’ boutons and loss of synaptic transmission. Interactions with presynaptic cell adhesion molecules (CAMs) of either the LAR-RPTP family or Neurexins via CASK are required to localize Liprin-α to nascent synaptic sites. Assembly of human presynaptic terminals is thus governed by a hierarchical sequence of events in which the recruitment of Liprin-α proteins by presynaptic CAMs is a critical initial step.

**NEURONAL TIMESCALES ACROSS DEVELOPMENT AND BRAIN AREAS**

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The brain's ability to perform complex cognitive tasks relies on integrating and storing information across different temporal scales. This ability is potentially facilitated by the heterogeneity of intrinsic timescales (ITs), estimated from the neuronal signal autocorrelation function. ITs form a brain-wide gradient, with sensory regions exhibiting fast ITs supporting rapid processing and associative areas showing slower ITs supporting prolonged integration. This gradient is observed in different recording modalities and conserved across species. However, it remains unclear how ITs develop and whether the ITs gradient is preconfigured or experience-dependent. We investigate ITs development using a large-scale in vivo electrophysiological dataset recorded in several brain regions in mice (postnatal days 4–12) in combination with neural network modeling. We infer ITs from single unit activity (SUA) and local field potentials (LFP). We show that the ITs gradient, similar to that of the adult brain, is already present in the first postnatal weeks. The relationship between SUA- and LFP-derived ITs varies across brain regions, with LFP-derived ITs decreasing over time while SUA-derived ITs remain stable or increase. Moreover, SUA-derived ITs exhibit substantial within-region variability following a right-skewed, heavy-tailed distribution. To gain mechanistic insights into the structural underpinnings of ITs heterogeneity, we used a biophysically constrained spiking neural network model. We found that heavy-tailed ITs distributions arise in networks with skewed synaptic properties, influenced by excitation-inhibition balance and cell-type proportions. Our findings indicate that ITs form a brain-wide gradient from an early age, thereby supporting the theory of a preconfigured brain.

**Keyword 1**: intrinsic timescales
**Keyword 2**: neural network modeling
**Keyword 3**: brain-wide gradients
**Keyword 4**: cortical hierarchy

**LABEL-FREE FUNCTIONAL ANALYSIS FOR THE CHARACTERIZATION OF IPSC-DERIVED NEURAL ORGANOID DEVELOPMENT AND MATURATION**

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Neural organoids generated from iPSCs have emerged as a model of the human brain to study the complex neural network activity observed during early brain formation. The objective of this work is to develop and validate a live-cell analysis workflow for the characterization of neural organoids in vitro. First, whole-vessel imaging with the Omni was used to monitor iPSC colony coverage, count, area, and diameter to ensure consistent iPSC passaging. Following neural induction, organoids were imaged to monitor size and count, illustrating the progression of differentiation. At day 50+, neural organoids were transferred and attached to a multiwell microelectrode array (MEA) plate, and activity was measured on a Maestro Pro. Organoid attachment was quantified by impedance as a measure of cell viability and electrode coverage. The emergence and maturation of electrophysiological activity was tracked via measurements of spiking activity and low frequency oscillations. Next, iPSC-derived midbrain organoids were plated onto MEA plates and dosed with neuroactive compounds (4-aminopyrodine (4-AP) and rotenone). 4-AP increased the mean firing rate and network burst frequency, while rotenone led to a decrease in both metrics. Lastly, a customized 48-well microplate (SpheroGuide) was designed, featuring a funnel design that targets organoids over the MEA. No attachment versus surface coated-mediated attachment of organoids was compared. PEI-coated wells exhibited the best performance with 100% organoid attachment and the development of synchronous network activity within two weeks. This new workflow combines live-cell imaging and MEA measurements to support the continued development of in vitro 3D models of neural function.

**ROLE OF PROTEOGLYCANS IN CORTICAL MICROCIRCUITRY DEVELOPMENT AND SPECIFICATION**

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The specific brain proteoglycans (PG) and their spatial-temporal reorganization during development were researched at the level of protein and transcriptomics using immunohistochemical and RNA-seq analysis in 30 postmortem fetal and perinatal human and rut brains. The results show that the PG expression patterns are highly dynamic in humans between the mid-fetal period and the end of the second postnatal year and between P15-P30 in rats. The chondroitin-sulfate proteoglycans diffuse expression in the intermediate zone shifts to the cortical plate around 33 gestation weeks, defining the sub-laminas of future cortical layers. The versican and keratan-sulfate expression specifies the limbic supra-callosal region and marginal zone at the mid-fetal period. They down-regulate around 35 gestation weeks, concordant with versican up-regulation around some neuron populations forming human perineuronal nets. The glycosylation type of the PGs revealed by a set of different lectins shows the region and lamina-specific expression patterns, confirming its contribution to the future cerebral cortex circuit specification. PG are similarly distributed but less pronounced and have distinct expression patterns during perineuronal net formation in rats. However, the limited time-space resolution of the rat brain confirms the PG comparison between human and rat brains as a superior research model. A significant down-regulation of all diffusely expressed PGs in the third trimester of the fetal period qualitatively and quantitatively affects the imaging signal intensity in MR scans of the fetal and early postnatal brain, placing the knowledge on spatial-temporal PG developmental expression essential for diagnostic, prognostic, and therapeutic approaches in perinatal medicine.

**Keyword 1**: perinatal brain
**Keyword 2**: versican
**Keyword 3**: perineuronal net
**Keyword 4**: synapse
**Keyword 5**: human

**BRAIN-WIDE DISTRIBUTION OF PARVALBUMIN-POSITIVE NEURONS AND PERINEURONAL NETS ACROSS THE DEVELOPING MOUSE BRAIN**

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Perineuronal nets (PNNs) are a condensed form of extracellular matrix that surround neuronal cell bodies and dendrites, playing an important role in regulation of neural plasticity. PNNs are widely expressed in the brain, often in association with parvalbumin (PV)-positive interneurons. During infancy and adolescence, neural plasticity is known to peak during critical periods when the brain undergoes major development and reorganization. This period is also associated with sexually dimorphic increased risk for disturbances leading to disease. In primary sensory cortices PNN's develop after the critical period and limit plasticity, thereby stabilizing functional circuits. It is well known that different brain regions mature at different times, but more specific information about the spatial and temporal distribution of PNNs during development is missing. To address this, we have created a collection of high-resolution, multi-channel fluorescence microscopy images showing PNNs and PV-positive interneurons across mouse brains at different developmental stages. The collection contains coronal sections showing parvalbumin-positive (PV) neurons and perineuronal nets (PNN) in male and female mouse brains harvested at postnatal days (P) 9, 14, 17, 21, 35 and adult. These images are spatially registered to age-matching mouse brain reference atlases, providing a standardized anatomical reference to interpret the images. The image collection will be openly accessible via the EBRAINS Knowledge Graph (https://search.kg.ebrains.eu/), offering a valuable resource for further investigations of the role of PNNs in neural plasticity and brain development.

**Keyword 1**: Perineuronal nets
**Keyword 2**: developmental neuroscience
**Keyword 3**: brain atlas
**Keyword 4**: synaptic plasticity
**Keyword 5**: parvalbumin-positive interneurons

**IMPACT OF XKR8 SCRAMBLASE DEFICIENCY ON SYNAPTIC ACTIVITY OF CA1 PYRAMIDAL NEURONS IN THE MOUSE HIPPOCAMPUS DURING POSTNATAL DEVELOPMENT**

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During early postnatal development, hippocampal pyramidal neurons experience synaptic pruning, which is critical for effective signal processing and functional neural network formation. However, impaired synaptic pruning can lead to neurological disorders like schizophrenia or autism spectrum disorders. Phosphatidylserine (PtdSer), a neuronal "eat me" signal translocated by the Xkr8 scramblase, is one of the signals that regulate synaptic pruning. The Xkr8 scramblase is highly expressed in mouse brains after birth, and its deficiency leads to excessive excitatory nerve terminals, altered neuronal bouton morphology, and increased cortico-cortical and cortico-spinal projections. Moreover, Xkr8 deficiency affects mice hippocampal synaptic activity by postnatal day 40, but the exact timing of these changes remains unclear. This study aimed to investigate how Xkr8 scramblase deficiency affects synaptic activity in the mouse hippocampal CA1 pyramidal neurons during early postnatal development. Whole-cell patch-clamp technique was used to record spontaneous excitatory postsynaptic currents (sEPSCs) in male and female *Xkr8* knockout and wild-type mice. The strength of synaptic connectivity was evaluated measuring the amplitudes of sEPSCs and synaptic activity was estimated from the value of synaptic interevent intervals. Preliminary results indicated that the intensity of synaptic activity increased after the second postnatal week of development in all tested groups. Additionally, Xkr8-deficient pyramidal neurons showed higher synaptic activity during the first two postnatal weeks compared to wild-type mice in females. In contrast, the amplitude of sEPSCs remained unchanged across all groups during early postnatal development. Overall, Xkr8 deficiency increases the intensity of early synaptic activity in female CA1 hippocampal pyramidal neurons.

**Keyword 1**: Synaptic activity
**Keyword 2**: Xkr8 deficiency
**Keyword 3**: Postnatal hippocampal development

**EVOLUTION OF SLEEP ARCHITECTURE DYNAMICS THROUGHOUT DEVELOPMENT**

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Sleep patterns in both humans and rodents undergo significant changes throughout the lifespan. While sleep plays a crucial role in development, our understanding of sleep architecture, the maturation of brain oscillations, and the coordinated communication between brain regions remains limited. Existing methods for studying developmental sleep fall short of elucidating the underlying neuronal mechanisms that drive these changes.

To address this gap, we developed a lightweight, chronic microelectronic device that records muscle activity (EMG) and field potentials from three brain regions—the prefrontal cortex (PFC), parietal cortex (PAR), and hippocampal CA1—in freely behaving rats, from postnatal day 9. By applying principal component analysis to the EMG and electrocorticogram (ECoG) signals, and integrating video-tracking data from DeepLabCut, we were able to efficiently characterize brain states during long-term sleep recordings (>24 hours). This approach provides an in-depth analysis of sleep microstructure and enables mapping of global brain communication throughout development.

Our results reveal dramatic changes in sleep architecture across development, with early stages predominantly consisting of REM-like sleep. We also confirmed that REM sleep is heterogeneous, with distinct tonic and phasic components emerging from juvenile stages onward. Furthermore, we mapped the maturation of sleep-related oscillations, such as theta, gamma, and ripple waves, which reflect ongoing pyramidal cell "wiring." Additionally, we characterized global brain cross-communication during sleep and mapped developmental changes in hippocampal multiunit activity.

In conclusion, our findings offer a comprehensive perspective on the evolution of sleep brain states, highlighting specific stages that dominate distinct developmental windows.

**Keyword 1**: Development
**Keyword 2**: Sleep
**Keyword 3**: Brain oscillation

**DEVELOPMENTAL DYNAMICS OF THE EXPRESSION OF CALCIUM-BINDING PROTEINS IN THE HUMAN FETAL SUBTHALAMIC NUCLEUS**

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Calcium-binding proteins (CaBPs, e.g. parvalbumin, calretinin) have been used for a long time to investigate neuronal subclasses and parcellation of subthalamic nucleus (STN). In the adult STN, these CaBPs have widespread expression throughout the STN, with an opposing gradient of expression. Moreover, the expression of CaBPs could be linked to electrophysiological properties of specific neuronal subclasses. Therefore, studying the spatio-temporal expression patterns of these CaBPs in the human fetal STN could indirectly provide us with data on the maturation of functional circuitry. To address this issue, we performed indirect immunohistochemistry using anti-parvalbumin (PV) and anti-calretinin (CR) antibodies on samples of human fetal STN ranging from 11 PCW (post-conceptional weeks) to 3 months of age. Our results indicate that calretinin is expressed already at the early fetal period, whereas parvalbumin starts to be expressed in the STN during the late fetal period. The timing of parvalbumin expression could indicate the start of electrophysiological neuronal activity and maturation of functional circuitry. Additionally, these PV-immunoreactive neurons could be clinically relevant since they are sensitive to bilirubin toxicity, providing the pathophysiological mechanism of neuronal damage in kernicterus.

**Keyword 1**: Calcium-binding proteins
**Keyword 2**: Human Fetal Brain
**Keyword 3**: Immunohistochemistry
**Keyword 4**: Subthalamic nucleus

# GLS1-MEDIATED GLUTAMINOLYSIS IS REQUIRED FOR POSTNATAL GROWTH OF THE CEREBRAL CORTEX

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The rapid expansion of the cerebral cortex at late development stages is metabolically expensive, but whether the growth burst requires glutaminolysis remains unknown. Here we show that glutaminase (*Gls1*; a key glutaminolysis enzyme) is increasingly expressed in cortical neurons in mouse and man during postnatal development. We generated a mouse model (*Gls1*-cKO) lacking *Gls1* in cortical excitatory neurons and found a way to successfully rescue them from the premature lethality. These mice developed smaller brains mostly due to reduced volume of the cerebral cortex. The smaller volume was neither due to neurodegeneration nor impaired neurogenesis, but due to reduced postnatal growth of neuropil and of white matter resulting in adult microcephaly. The mice exhibited behavioural abnormalities including decreased locomotive activities, motor deficits and impaired responses to new environment. The symptoms largely phenocopied those observed in humans. These findings demonstrate that *Gls1*-dependent glutaminolysis is indispensable for the late development of the cerebral cortex.

**Keyword 1**: glutaminase
**Keyword 2**: microcephaly
**Keyword 3**: glutaminolysis
**Keyword 4**: glutamate metabolism
**Keyword 5**: brain development

**THE EFFECT OF SHANK3 DEFICIENCY ON NEURONAL CELL MORPHOLOGY AND SYNAPTIC PROTEINS IN DOPAMINERGIC BRAIN REGIONS**

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The aim of present study was to 1) examine the impact of *Shank3* deficiency on the morphology of GABAergic neurons isolated from the tegmentum and striatum; 2) assess the effects of transient *Shank3* gene silencing on the neuronal morphology and specific synaptic proteins 3) evaluate the influence of oxytocin on the morphology and synaptic proteins. This study found a significantly reduced number of short neurites in GABAergic neurons isolated from the ventral tegmentum of *Shank3*-deficient mice. A similar trend was observed in neurite arborization following transient *Shank3* gene silencing in primary striatal and tegmental neurons. Incubation of neuronal cells in the presence of oxytocin did not significantly affect neurite arborization in primary neurons isolated from the striatum and tegmentum. Increased levels of the postsynaptic protein Neuroligin 3 were observed in neurons isolated from the striatum of *Shank3*-deficient mice, with no differences found after transient *Shank3* gene silencing. No change in D2 receptor immunofluorescence was found in primary striatal neurons from *Shank3*-deficient mice. In contrast, a significant decrease in D2 receptor and Neuroligin3 immunofluorescence was found following transient *Shank3* gene silencing in primary striatal neurons, independent of oxytocin effects. The morphology of GABAergic neurons in dopaminergic brain regions may contribute to autism-like behavior. Changes in neurite outgrowth are accompanied with alterations in postsynaptic adhesion molecules and dopamine receptors, without additional effects from oxytocin. Altered neuron morphology and connections in dopaminergic regions might contribute to autism development at specific stages. This work was supported by grants APVV-21-0189, VEGA 2/0057/23, and 2/0132/25.

**Keyword 1**: autism
**Keyword 2**: dopamine
**Keyword 3**: neurite
**Keyword 4**: Shank3
**Keyword 5**: oxytocin

**RELN FUNCTIONS IN CORTICAL DEVELOPMENT AND NEURODEVELOPMENTAL DISORDERS**

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Reelin is a large glycoprtotien involved in critical steps of cortical development, including neuronal migration, dendritic architecture and synaptic fuctioning, and is altered across the whole spectrum of neurodevelopmental disorders. Several Reln secreting (Reln+) neuronal populations contribute to RELN levels at various stages of cortical development. First, the Cajal-Retzius cells (CRs) located in the marginal zone (future layer 1) of the developing pallium, represent the only cortical source of RELN from early embryonic day 11 (E11) to E14, before undergoing massive death during the first postnatal weeks. Starting from E14, some Reln+ IN originating from the subpallial ganglionic eminences progressively invade the developing cortical plate and start expressing Reln, to become the main source of Reln by postnatal day 8 (P8). Using genetic models of *reln* conditional ablation, we demonstrate that while single-population *reln* ablations have very restricted effects on lamination, their concomitant ablation has a profound impact reminiscent of *reeler-/-* animals, occuring in a dose-dependent manner. Looking at adult stages in single-population ablation models, we also observe a differential implication of Reln+ populations in emotional and cognitive behaviors, although the contribution of developmental versus contemporary Reln-sources remains to be elucidated. Finally, we also identified differential alterations of *Reln* levels in Reln+ populations upon pre- versus post-natal exposure to serotonin (5-HT) transporter blockade by SSRIs. Altogether, these results support a differential vulnerability of Reln+ cortical populations to environmental stressors as a potential landmark for the etiology of a spectrum neurodevelopmental.

**THE EFFECT OF MEROPENEM ON BEHAVIOR, COGNITION, AND HIPPOCAMPAL NEUROGENESIS IN ADULT RATS**

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Neurogenesis is the process where neural stem cells (NSCs) develop into functional neurons. It is a process affected by multiple factors including antibiotic administration. Meropenem is a broad-spectrum carbapenem that treats various infections. It can penetrate most body fluids and tissues, cross the blood–brain barrier, and obtain a concentration within cerebrospinal fluid close to that of blood plasma. This project aims to investigate the effect of meropenem on rodents' motricity, cognitive function, and hippocampal NSC proliferation. Adult male Sprague-Dawley rats received two intraperitoneal injections of meropenem every 8 hours for 1 week. The experimental group was divided into two: low-dose (177 mg/kg) and high-dose (354 mg/kg) group. Sham group received sterile saline and naïve group received nothing. Abdominal thermal sensitivity test assessed for hyperalgesia, rota-rod test assessed for motricity, and behavioral tests including Y-maze and novel object recognition test assessed for spatial memory and short-term memory, respectively. Bromodeoxyuridine (BrdU) was injected 24 hours prior euthanasia to assess for hippocampal NSC proliferation. Results show no significant effect on cognitive function, pain sensitivity, and motricity. However, further analysis is required to support this conclusion by studying NSC proliferation via confocal microscopy and expression levels of associated genes via quantitative real-time PCR. This will be the first study assessing the neurotoxic effects of meropenem on neuroproliferation and behavior. Clinically, this might provide insight on the appropriate use of this drug and its possible negative effects on the central nervous system.

**DIFFERENTIAL EFFECTS OF ACTIN MANIPULATING DRUGS SUGGEST A GROWTH PROTECTIVE ROLE OF STABILIZED ACTIN FILAMENTS IN LATER STAGES OF NEURONAL DEVELOPMENT**

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Neurons of the central nervous system lose their growth capacity during development and maturation. The cellular mechanisms that cause this decline in growth potential are not fully understood. In early stages of neuronal development, before polarization, the actin cytoskeleton restricts the outgrowth of minor neurites, keeping the cell in an unpolarized state. Destabilization of the actin cytoskeleton with pharmacological drugs, like Cytochalasins, allows neurites to grow out and to become an axon. Further, the tip of the neurite that will become the future axon has a more dynamic actin cytoskeleton. A dynamic actin cytoskeleton is associated with increased growth potential in early developmental stages and during regeneration. However, in maturing neurons, the actin cytoskeleton was described to become more stable and less dynamic. We asked whether are more static and stable actin cytoskeleton contributes to the reduction of growth potential in maturing neurons. Using *in vitro* cell culture approaches combined with pharmacological treatments and immunocytochemistry, we found that the actin cytoskeleton in axonal growth cones becomes less dynamic in the course of development. The onset of actin stabilization already takes place when the axon shows its highest growth rates. Also, additional stabilization and polymerization of the actin cytoskeleton with Jasplakinolide has no growth reducing effect after neurons have polarized. Thus, after polarization, a stabilized actin cytoskeleton is not growth perturbing. We propose that the stabilization of the actin cytoskeleton during later developmental stages is protective as it prevents growth reducing effects that can be observed in early axon stages.

**Keyword 1**: Neurite growth
**Keyword 2**: Axon growth
**Keyword 3**: Actin cytoskeleton

**CHANGES IN NEUROGENESIS AND BEHAVIOUR IN ADULT MICE AFTER EXPOSURE TO FIPRONIL**

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Pesticides are widely used in agricultural and household environments to kill disease vectors, such as pests in agriculture. Fipronil (FPN) is a broad-spectrum insecticide that belongs to the phenylpyrazole chemical family. It is effective in controlling the insects that are resistant to other insecticides by blocking gamma-aminobutyric acid (GABA) chloride channels in insect's central nervous system. FPN also elicits neurotoxicity in mammals by binding strongly to GABA chloride channels, causing hyperexcitability. The neurotoxic effect of this pesticide was tested in the present study with a special emphasis on the proliferation of cells in the mice olfactory neurogenic region involving the subventricular zone (SVZ) and the rostral migratory stream (RMS). Additionally, changes in depressive-like behavior, locomotor activity and anxiety-related behavior after fipronil exposure were evaluated. FPN was administered to experimental mice during the preimplantation period (three oral applications) using a syringe. To explore cell proliferation in the SVZ/RMS neurogenic region, Ki-67 immunohistochemistry was employed. Changes in depressive-like behavior were evaluated by monitoring performance in the forced swimming test. The open field test was used to assess changes in the locomotor activity and the anxiety-related behavior in mice after fipronil exposure. Our findings demonstrate that maternal administration of FPN during the preimplantation period of pregnancy affects cell proliferation in the olfactory neurogenic region SVZ/RMS of the offspring. In addition, maternal exposure to FPN enhances depressive-like behavior, reduces locomotor activity and increases anxiety level in adult mice.
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**Keyword 1**: neurogenesis
**Keyword 2**: fipronil
**Keyword 3**: proliferation
**Keyword 4**: behaviour

**MATURE NEURONS IN THE RAT SUBVENTRICULAR ZONE AND ROSTRAL MIGRATORY STREAM OF INTACT AND MATERNALLY SEPARATED RATS**

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Recent evidence indicates that neurogenesis in the subventricular zone (SVZ) and rostral migratory stream (RMS) is regulated by neurons within these neurogenic regions. Two distinct populations of cells with morphological and biochemical characteristics of mature neurons have been identified in the SVZ/RMS of adult rats: nitric oxide (NO)-producing neurons and neurons expressing secretagogin (SCGN). Our goal was to obtain new morphological data on these neuronal populations in intact rats and in rats after maternal separation (MS) during the first three neonatal weeks (3 hours/day). We examined the distribution and the number of these neurons in the SVZ and in individual anatomical parts of the RMS. Double immunofluorescence labeling for neuronal nitric oxide synthase (nNOS) and SCGN confirmed the presence of nNOS+ and SCGN+ neurons in the SVZ/RMS, each with distinct morphological features and distribution patterns. The response to MS was different for each cell type. Quantitative analysis revealed an increase in the number of nNOS+ cells in the SVZ/RMS of MS rats, which was significant only in the SVZ. Regarding SCGN+ cell distribution, caudorostral decrease in SCGN+ cell number, with the lowest count in the rostral part of the RMS, typical for intact rats, was not observed in MS rats. In these animals, SCGN+ neuron numbers were significantly decreased in the caudal part of the RMS and significantly increased in the rostral part of the migratory pathway. Our results suggest a different functional significance of nitrergic and SCGN-producing neurons in the olfactory neurogenic region.
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**ENHANCEMENT OF BACTERIAL CELLULOSE GRAFTS WITH GLIAL CELL LINE-DERIVED NEUROTROPHIC FACTOR AND GOLD NANOPARTICLES IN PERIPHERAL NERVE INJURY**

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Peripheral nerve injury is a widespread health problem. Bio-cellulose materials are suggested as an alternative graft material. Glial Cell-Derived Neurotrophic Factor (GDNF) is known to significantly support peripheral nerve healing. The aim of this study is to investigate the effects of GDNF-loaded bio-cellulose grafts on sciatic nerve damage.
Bacterial cellulose (BS) produced by the *Gluconacetobacter xylinus* bacterium were functionalized with GDNF and gold nanoparticles to prepare a nerve guidance conduit. Wistar rats were divided into the autologous (n=6), the bio-cellulose (n=6), and the GDNF-loaded bio-cellulose (n=6) groups. Functional evaluations were performed with walking analyses and sciatic functional indices (SFI). At the end of the 8th week, electromyography (EMG) and compound muscle action potential (CMAP) values were recorded. For histology, semi-thin epoxy sections stained with toluidine blue and examined with light microscopy. GraphPad Prism software and the Kruskal-Wallis test (non-parametric ANOVA) were used to compare the data. According to the functional evaluation analyses, the SFI and %CMAP calculations showed that the GDNF graft group exhibited a recovery similar to the autologous graft group. The number of myelinated axons was significantly higher in the autograft group compared to the bio-cellulose graft group ($p<0.001$). In the GDNF graft group, the number of myelinated axons showed an increase compared to the bio-cellulose graft group ($p<0.01$), and the morphology was similar to the autograft group.
The functional and morphological analysis results of this study suggest that the application of GDNF on bio-cellulose grafts could be a potential alternative treatment for peripheral nerve damage.

**Keyword 1**: peripheral nerve injury
**Keyword 2**: bacterial cellulose graft
**Keyword 3**: glial cell line-derived neurotrophic factor

**MODULATION OF GAP43 EXPRESSION THROUGH NDR2 KINASE IN THE AGEING BRAIN**

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Nuclear Dbf2-related protein 2 (Ndr2) is a serine/threonine kinase within the AGC family and a key component of Hippo signaling. Ndr2 is the predominant Ndr kinase in the mouse brain and plays crucial roles in neuronal differentiation and maturation, influencing dendritic and axonal growth as well as synapse formation and synaptic plasticity via integrin receptors. Our research furthermore has shown that deletion of Ndr2 kinase ameliorates the decline of spatial memory formation in aged mice (see presentation by Miguel del Angel). Proteomic analysis of the hippocampus revealed, among others, an altered GAP43 (also called neuromodulin) expression in aged Ndr2 knockout (KO) mice compared to wild type (WT) mice, as Ndr2 KO mice exhibit a much more moderate decrease in expression levels than WT mice. Intriguingly, the GAP43 gene has been identified as a direct target of YAP, a key transcription factor in the Hippo pathway. GAP43 serves as a membrane protein to support neuronal outgrowth, neurogenesis, and plasticity and has been related to ageing-related cognitive decline. Our immunohistochemical analysis revealed that the age-related reduction of GAP43 varies across subregions of the dorsal hippocampus of WT and Ndr2 KO mice. We are currently investigating the impact of Ndr2 kinase-mediated alterations in GAP43 on aging-related hippocampal functional decline, including its role in regulating hippocampal neurogenesis and network physiology.

**HYPOXIA-INDUCIBLE FACTOR 2 PROMOTES PROCESSES THAT INCREASE THE REGENERATIVE CAPACITY OF NEURONAL CELLS VIA PRIMARY CILIA UNDER LOW OXYGEN CONDITIONS**

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Primary cilia are responsible for receiving extracellular signals and transmitting them to the cell interior. These organelles facilitate cell-to-cell communication and enable critical processes such as proliferation and migration. Disruption of primary cilia signaling has been linked to the development of a range of human diseases collectively termed ciliopathies. Often, ciliopathies are linked to malformations of the central nervous system.
Abnormalities in cilia length are a hallmark of inadequate cilia function. In circumstances where the oxygen supply is low, such as during an ischemic stroke, it is imperative to swiftly provide the relevant signals for counter-regulations.
Key factors in the hypoxic response are hypoxia-inducible factors (HIFs). Of particular significance is the promotion of neurogenesis and the protective effect on neuronal stem cells by HIF-2. Through its influence on the formation of new nerve cells, HIF-2 is a relevant factor in the regeneration of cerebral pathologies.
In this study, we have utilised immunofluorescence to localise hypoxia-inducible factor-2alpha (HIF-2α) within the axoneme of murine neuronal primary cilia under hypoxic conditions. This process involves the elongation of the ciliary axoneme, a process facilitated by the direct interaction of HIF-2α with the intraflagellar transport protein 88 homolog (IFT88). Quantitative PCR analyses confirmed the induction of Mek1/2, Erk1/2 and associated target genes only in the presence of primary cilia and HIF-2α. It is therefore suggested that, by increasing MEK/ERK signaling via primary cilia, HIF-2 promotes processes that increase the regenerative abilities of the cell and consequently may improve the outcome of diseases such as stroke.

**Keyword 1**: primary cilia
**Keyword 2**: hypoxia
**Keyword 3**: MEK/ERK signaling

**PERSISTENT CORTICAL DIFFERENCES IN ADULTS WITH PRENATAL POLYDRUG AND OPIOID EXPOSURE: A LONGITUDINAL STUDY FROM CHILDHOOD TO ADULTHOOD**

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Prenatal opioid exposure (POE) is widely associated with low birth weight, neonatal abstinence syndrome, and long-term neurodevelopmental impairments. Although cortical differences have been documented from infancy through adolescence, it remains unclear whether these structural changes persist into adulthood beyond the influence of birth weight. In a 30-year, prospective longitudinal study, we followed up participants with prenatal polydrug exposure (mostly including POE) alongside controls, collecting repeated MRI scans (initiated in childhood), as well as cognitive and clinical measures. Earlier follow-ups showed lower neuroanatomical volumes during childhood and adolescence among substance-exposed individuals. Here, we acquired MRI data from 16 substance-exposed adults (5 male, aged 28–33) and 31 controls (20 male, aged 27–29). Anatomical imaging (T1w, T2w) enabled estimation of cortical volume (CV), thickness (CT), and area (CA), while a 3D-QALAS sequence—which enables the estimation of T1 and T2 relaxation times in a single 3D scan—provided parametric (relaxometry) maps. Significantly lower CA emerged in the substance-exposed group ($p < 0.05$), with more modest differences in CV and CT, even after adjusting for sex and birth weight. By contrast, intracranial volume (ICV) did not differ between groups once sex was controlled for. T1 and T2 values did not differ significantly between groups. These findings suggest that cortical differences associated with polydrug and opioid exposure persist into adulthood independent of birth-weight effects. Implications and limitations of this study are discussed, highlighting the need for further research into the long-term impact of prenatal substance exposure.

**Keyword 1**: Prenatal opioid exposure
**Keyword 2**: Longitudinal MRI
**Keyword 3**: Cortical morphometry
**Keyword 4**: Adult neurodevelopment
**Keyword 5**: T1/T2 relaxometry

**TENEURIN-4 DICTATES NEURONAL MIGRATION VIA STRUCTURALLY EXCLUSIVE INTERACTIONS**

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Teneurins (Ten1-4) are large cell-surface receptors that drive hippocampal wiring and neural migration. In the latter, they signal contact-repulsion by interacting with Latrophilins. Whether homophilic Teneurin interactions also direct neuronal migration is presently unknown; and if yes how these integrate with heterophilic interactions. Our unpublished single-particle cryo-electron microscopy data shows that the Ten2 YD- shell domain homodimerises via a mechanism that sterically clashes with canonical Latrophilin Lec-binding. Based on these results, we produced structure-based Ten mutants that specifically abolish homophilic or Latrophilin binding. We combined these tools with in-utero-electroporation (overexpression/loss-of-function experiments), in- vivo gene editing, nanobody-based tools, and fluorescence microscopy at different resolutions to discover that both Ten4 interactions regulate neuronal migration at different stages. Removal of Ten4- Latrophilin binding results in reduced attachment of neurons to radial glia cell (RGC) fibres, leading to an accumulation of neurons in the intermediate zone. Conversely, ablation of Ten4 homophilic interactions increased RGC association, impeding migration through the cortical plate. Our results showcase how a single receptor can switch between mutually-exclusive structural mechanisms to regulate the attachment of neurons to RGC fibres and direct their migration through diQerent cortical layers in a step-dependent manner.

**Keyword 1**: Cortical development
**Keyword 2**: Neuronal migration
**Keyword 3**: Structural biology
**Keyword 4**: Structure-based protein engineering
**Keyword 5**: Advanced fluorescence microscopy

**NEURONAL IL-1R1 MEDIATES EARLY LIFE STRESS EFFECTS ON SOCIAL AND ANXIETY-RELATED BEHAVIORS**

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Early life stress (ELS) has profound and enduring effects on neurodevelopment and social functioning, contributing to the risk of neurodevelopmental disorders. The interleukin-1 receptor (IL-1R1) in the hippocampus plays a pivotal role in mediating the impact of stress on social behavior. However, the involvement of neuronal IL-1R1 (nIL-1R1) in ELS-induced social deficits remains poorly understood. This study aims to address this gap by utilizing a maternal separation (MS) paradigm and cell-type-specific IL-1R1-expressing mouse lines to elucidate the role of nIL-1R1 in MS-induced long-lasting social interaction deficits. Our investigations revealed dynamic nIL-1R1 expression in the dentate gyrus (DG) during neurodevelopment, particularly within the first two outer granule cell layers from P7 to P14. Repeated MS heightens nIL-1R1 and Δ-FosB expression in the DG and induces social interaction abnormalities in adulthood. Further, we found that glutamatergic nIL-1R1 plays a pivotal role in MS-induced neuronal and behavioral alterations. To evaluate whether nIL-1R1 is necessary or sufficient for MS-induced social deficits, we assessed social interaction alterations in control and MS-exposed nIL-1R1 expressing mice. Additionally, open-field Test assessments revealed that IL-1R1 KO mice spent more time in the center compared to WT mice, suggesting reduced anxiety-like behavior. This finding indicates that nIL-1R1 may contribute not only to social behavior deficits but also to stress-induced anxiety-related behaviors. We seek to gain insights into the molecular mechanisms underlying ELS-induced social behavior difficulties. Unraveling nIL-1R1's role in social behavior deficits holds promise for therapeutic interventions, ultimately improving mental health outcomes for individuals affected by ELS and neurodevelopmental disorders.

**Keyword 1**: Interleukin-1 receptor (IL-1R1)
**Keyword 2**: Early life stress (ELS)
**Keyword 3**: Social and anxiety-related behaviors

**22Q11.2DS IPSCS AS MODEL SYSTEM FOR INVESTIGATION OF MOLECULAR MECHANISM AND NOVEL THERAPEUTIC TARGETS IN NDDS**

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Neurodevelopmental disorders (NDDs) represent considerable public health challenge. One of the syndromes associated with heightened risk of NDDs is 22q11.2 Deletion Syndrome (22q11.2DS). To be able to investigate the molecular signature of NDDs we have generated induced pluripotent stem cells (iPSCs) from 22q11.2DS patients and healthy controls, differentiated them into neurons and examined the expression of miR-185, which have been shown to be downregulated in the whole blood of 22q11.2DS patients. Using CytoTune™-iPS 2.0 Sendai Reprogramming Kit, iPSC lines from 22q11.2DS patients and healthy controls were generated, and characterized by the expression of stemness markers, and differentiation into neurons was confirmed by the expression of neuronal markers using RT-PCR. The expression of miR-185 was assessed by Taqman qRT-PCR specific for miR-185, and miRNet platform, and online available tools GeneTrack, miRNet and Metascape were used to elucidate miR-185 function in NDDs. Generated iPSC lines from seven 22q11.2DS patients and three healthy controls express markers of pluripotency and have the ability to differentiate into all three germ layers. Selected 1.5Mb 22q11.2DS iPSC lines and healthy control were able to generate neurons. Decreased expression of miR-185 was detected in iPSCs derived from patients with 22q11.2DS, as well as in the 22q11.2DS neurons compared to healthy control. Using online available tools, we were able to select three potential target genes of this miRNA related to NDDs – *CSMD2*, *DLG2* and *HIC2*. iPSC lines from 22q11.2DS patients are powerful model system for studying molecular mechanisms and investigation of novel therapeutic targets related to NDDs.

**DELINEATING THE NEUROBIOLOGICAL MECHANISMS INVOLVED IN *SETBP1*-HAPLOINSUFFICIENCY DISORDER USING HUMAN NEURAL ORGANOIDS AND TRANSCRIPTOMICS**

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Haploinsufficiency of the *SETBP1* gene causes a highly heterogeneous neurodevelopmental syndrome (*SETBP1*-haploinsufficiency disorder) with the main phenotypic features including moderate-to-severe speech and language impairments, and wide variability in intellectual functioning. The precise functions of *SETBP1*, encoding the SET-binding protein, are yet to be discovered. Therefore, the neurobiological pathways by which rare loss-of function *SETBP1* variants cause a neurodevelopmental disorder remain largely unknown. By employing induced pluripotent stem cell (iPSC)-derived neural organoids and transcriptomic approaches, we aim to dissect the underlying aetiological pathways. We have generated iPSCs from three patients carrying heterozygous *de novo* truncating variants and sex-matched parents as controls, and *SETBP1*-knockout iPSCs with CRISPR/Cas9 gene-editing. These iPSC lines were differentiated into un-patterned neural organoids and their transcriptomic profiles were analysed at whole organoid and single-cell levels. Morphological examination, cell-type specific differential gene expression analysis and cell lineage tracing were performed at two selected developmental timepoints. Both patient-derived and *SETBP1*-knockout organoids consistently showed gross morphological differences, rosette and transcriptomic anomalies during early organoid development, suggesting aberrations in cell fate commitment during embryonic brain development. Gene ontology analyses demonstrated that dysregulated pathways were related to brain morphogenesis, cilia organisation, axon projection and synaptic function. Together, our work is, to our knowledge, the first mechanistic investigation using iPSCs and organoids derived from *SETBP1*-haploinsufficiency disorder patients. This work promises to offer valuable insights into the fundamental understanding of the currently unknown neurodevelopmental roles of SETBP1 and aetiological mechanisms that go awry in *SETBP1*-haploinsufficiency disorder.

**Keyword 1**: Human neural organoids
**Keyword 2**: Transcriptomics
**Keyword 3**: Monogenic neurodevelopmental disorder

**INTER-STRAIN MOUSE CHIMERAS TO INVESTIGATE NEURODEVELOPMENTAL MECHANISMS IN AUTISM**

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Understanding the genetic and cellular mechanisms of neuronal development is crucial for deciphering how intrinsic and environmental factors shape brain structure and functions, particularly in neurodevelopmental disorders like autism. The BTBR *T*$^{+}$ *Itpr3*$^{tf}$/J (BTBR) mouse model exhibits autism-related behaviors and unique neuroanatomy, including corpus callosum agenesis and other axonal defects, making it an excellent model for studying the etiopathogenesis of neurodevelopmental disorders.
In this study, embryonic stem cells (ESCs) derived from BTBR and control C57BL/6J (B6) mice were established, validated for pluripotency, and engineered to express tau-GFP for tracing neuronal structures. Both lines were validated for pluripotency markers, clonogenicity, and neuronal differentiation potential. Then, *in vitro*-derived neuronal progenitors (NPs) or ESCs were injected into blastocysts, which were transferred to pseudopregnant females to obtain BTBR-B6 chimeras. We found that ESCs and NPs of either strain engraft into the host embryo of the other strain and contribute stochastically to multiple brain areas, neuronal circuits, and non-neuronal brain tissues. Investigating cell fate in chimeras will be useful to neural lineage commitment, axonal growth, and synaptic network formation in vivo. Moreover, addressing strain-specific features of the ESC neural developmental potential and subsequent axonal formation in vitro may help understand the mechanism underlying the unique neuroanatomy of the BTBR mouse model to study autism etiopathogenesis.
This research was funded by the National Science Centre, Poland (grant nr. 2020/39/B/NZ4/02105).

**Keyword 1**: chimera
**Keyword 2**: BTBR mouse
**Keyword 3**: autism
**Keyword 4**: neurodevelopment
**Keyword 5**: stem cells

**DEVELOPMENT OF IPSC-BASED MODEL SYSTEM FROM PATIENTS WITH 22Q11.2 DUPLICATION SYNDROME FOR STUDYING NEURODEVELOPMENTAL DISORDERS**

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22q11.2 Duplication Syndrome (22q11.2DupS) is associated with an elevated risk of developing neurodevelopmental disorders (NDDs), such as autism spectrum disorders (ASD) and attention-deficit/hyperactivity disorder. ASD is detected in 14-25% of patients, making 22q11.2DupS one of the genetic syndromes with the highest prevalence of ASD. To better understand the molecular mechanisms underlying the pathophysiology of NDDs, we have generated induced pluripotent stem cells (iPSCs) from patients with 22q11.2DupS. Mononuclear cells from 22q11.2DupS patients and healthy individuals were reprogrammed into iPSC lines using the CytoTune™-iPS 2.0 Sendai Reprogramming Kit. The established iPSC lines were characterized by examining the expression of stem cell markers through RT-PCR and their ability to differentiate into cells of three germ layers using the STEMdiff™ Trilineage Differentiation Kit. We differentiated the iPSCs into neurons using the dual SMAD inhibition method together with retinoic acid. The expression of neural and neuronal-specific markers in neural progenitors and mature neurons was analyzed using RT-PCR and immunocytochemistry. The established iPSC lines from three patients with an inherited form of 22q11.2DupS, their mothers who carry the microduplication, and three healthy individuals demonstrated the expression of stem cell markers and the ability to differentiate into the cells of all three germ layers. Both iPSCs from carriers of the 22q11.2 microduplication and healthy controls successfully differentiated into neurons, which was confirmed by the expression of neuronal markers. The generated 22q11.2DupS iPSC lines provide a valuable resource for understanding the molecular mechanisms underlying NDDs associated with 22q11.2 microduplication.

**Keyword 1**: 22q11.2 Duplication Syndrome
**Keyword 2**: induced pluripotent stem cells
**Keyword 3**: neural differentiation

**GENE EXPRESSION PROFILING OF ASTROCYTES DERIVED FROM IPSCS OF PATIENTS WITH 22Q11.2 DELETION SYNDROME**

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22q11.2 Deletion Syndrome (22q11.2DS), caused by microdeletion 22q11.2, is the most common microdeletion syndrome in humans. t is closely linked to an increased risk of neurodevelopmental disorders and provides a valuable model for investigating the molecular mechanisms underlying these disorders, which are still not fully understood. Here we analyzed transcriptomic profiling of 22q11.2DS astrocytes derived from iPSCs of mother and child with 22q11.2DS and one healthy control. RNA-seq was carried out on Illumina NovaSeq 6000 sequencer. Bioinformatic processing of raw data was conducted via NVIDIA platform, while differential gene expression analysis was performed in RStudio using DESeq2 R package. The obtained list of DEGs was used for pathway enrichment analysis by employing EnrichR and WikiPathways. 125 DEGs with lower expression and 287 DEGs with higher expression in 22q11.2DS astrocytes compared to the control were obtained. For genes with lower expression in 22q11.2DS astrocytes, 22q11.2 Copy Number Variation Syndrome and Axon Guidance were the top enriched pathways, while for genes with higher expression in 22q11.2DS astrocytes we did not identify biological pathways that are enriched in DEG lists more than would be expected by chance. We found 178 DEGs with lower expression and 205 DEGs with higher expression in astrocytes of symptomatic child with 22q11.2DS compared to non-symptomatic mother with 22q11.2DS. Employing EnrichR and WikiPathways we did not identify biological pathways that are enriched in DEG lists more than would be expected by chance. Our findings offer preliminary evidence of an altered transcriptomic landscape of 22q11.2DS astrocytes.

**Keyword 1**: astrocytes
**Keyword 2**: 22q11.2 Deletion Syndrome
**Keyword 3**: neurodevelopmental disorders
**Keyword 4**: transcriptome

**GENE EXPRESSION PROFILING OF IPSC-DERIVED NEURAL PROGENITORS FROM PATIENTS WITH 22Q11.2 DUPLICATION SYNDROME**

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The molecular mechanisms underlying neurodevelopmental disorders (NDDs) are understudied. 22q11.2 Duplication Syndrome (22q11.2DupS) is associated with an increased risk of NDDs, including autism spectrum disorders, developmental delay, and intellectual disability, while potentially having protective effect against schizophrenia. Here we performed transcriptomic profiling of 22q11.2DupS neural progenitors (NPCs). Total RNA was isolated from iPSCs-derived NPCs of a family trio consisting of a child with 22q11.2DupS, a carrier unaffected mother, and a healthy control father. Paired-end RNA-sequencing was carried out by commercial services. FASTQ files were processed on an NVIDIA platform and differential gene expression analysis was carried out in RStudio using the DESeq2 R package. The resulting lists of differentially expressed genes (DEGs) was utilized for pathway and gene ontology enrichment analysis using clusterProfiler in RStudio. 60 DEGs with lower expression and 58 DEGs with higher expression were obtained in 22q11.2DupS NPCs compared to both carrier and control NPCs. For genes with lower expression in 22q11.2DupS NPCs, "miRNA targets in ECM and membrane receptors" was the top enriched pathway. On the other hand, for genes with higher expression in 22q11.2DupS NPCs, no biological pathways were identified as being significantly enriched in the DEG list beyond what would be expected by chance. However, for genes with higher expression in 22q11.2DupS NPCs, we obtained statistically significant enrichment of molecular functions such as Histone demethylase activity. 22q11.2DupS NPCs exhibit altered signaling pathways and molecular functions, providing preliminary insights into the impact of this microduplication on neural differentiation.

**Keyword 1**: 22q11.2 Duplication Syndrome
**Keyword 2**: iPSCs
**Keyword 3**: transcriptomic analysis

**CANDIDATE PATHWAYS AND BIOMARKERS IN OGT-CDG – A NOVEL INTELLECTUAL DISABILITY SYNDROME**

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O-GlcNAc Transferase Congenital Disorder of Glycosylation (OGT-CDG) is a recently discovered intellectual disability (ID) syndrome caused by missense mutations in the OGT gene. OGT catalyses O-GlcNAcylation, a dynamic post-translational modification affecting thousands of proteins. OGT-CDG patients present with extensive phenotypic heterogeneity, including ID, developmental delay, hypotonia, and dysmorphic features. Despite some overlap between patients, the clinical presentation is highly variable, complicating diagnosis and impeding the identification of underlying mechanisms.
To address this, we enrol a large cohort of OGT-CDG patients and family members to systematically map the heterogeneity of OGT-CDG. Standardised clinical data collection and genetic screening of OGT variants will be used to cluster and stratify patients based on shared and distinct symptom profiles. Blood samples from patients, healthy family members, and OGT-CDG mouse models carrying patient mutations will be analysed for biomarker discovery and validation.
Preliminary data from three different OGT-CDG mouse models show upregulated Ogt mRNA levels and downregulated Oga mRNA levels compared to wild type mice in whole brain samples, in leukocytes, and in whole blood, respectively, suggesting that altered O-GlcNAc homeostasis may contribute to disease pathophysiology.
By integrating clinical, genetic, and biomarker data, this study aims to provide a comprehensive characterisation of OGT-CDG. Defining clinical profiles and associated biological signatures will offer novel insights into the aetiological processes underlying this syndrome, paving the way for improved diagnostic tools and potential therapeutic interventions for affected patients and their families.

**Keyword 1**: Intellectual Disability
**Keyword 2**: Neurodevelopmental Disorder

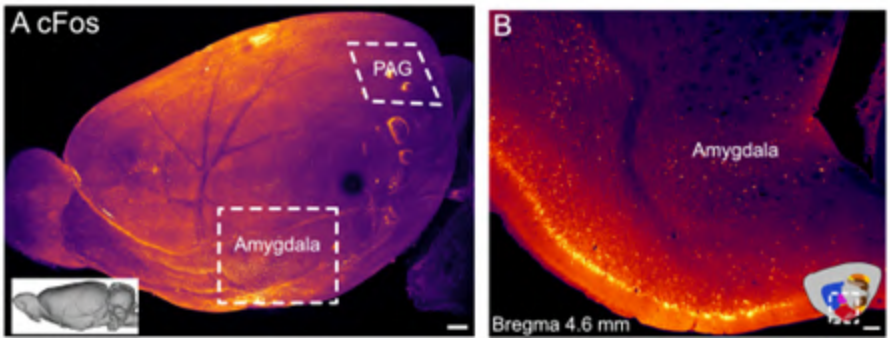
**RATDISCO, A TISSUE CLEARING PIPELINE FOR LARGE RAT TISSUE CAPABLE OF DETECTING STIMULUS-ACTIVATED NEURONS IN MODELS OF DEVELOPMENTAL DISORDERS**

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Social impairments, hypersensitivity to sensory stimuli, and epilepsy characterise neurodevelopmental disorders such as autism spectrum disorders (ASD). Hyper- or hypo-activation in key brain regions involved in social and emotional processing, such as the amygdala, has been assessed in ASD patients and rodent models using neuroimaging and traditional histological methods. While those studies provide valuable information, they are constrained by resolution and slicing artefacts. Currently, no methods are customised to optically clear and immunolabel whole rat brains.

To overcome this, we developed RatDISCO, a novel whole-brain immunolabelling and clearing pipeline that detects proteins of interest throughout large rats' and mice's brains and organs without slicing or compromising transparency or tissue integrity. RatDISCO achieves immunolabelling of deep brain structures by a simple antigen retrieval step before antibody labelling, followed by staged delipidation steps to achieve optically transparent samples ideal for light-sheet microscopy.

We used RatDISCO to identify differentially activated neurons during behaviour, using the immediate-early gene cFos as a proxy for neuronal activation. We created a 3D atlas of the rat amygdala to map and quantify the cell density of cFos-positive neurons across wild-type and Fragile-X knockout ($Fmr1^{-/y}$) rats, a model of ASD. We identified hypoactivation in the basal and basolateral amygdala in $Fmr1^{-/y}$ rats in response to fear during a classical fear paradigm. These results correlate with the observed amygdala-dependent emotional processing alterations in individuals with Fragile-X syndrome.




**Keyword 1**: Light sheet microscopy
**Keyword 2**: Tissue clearing
**Keyword 3**: 3D imaging
**Keyword 4**: Autism spectrum disorders
**Keyword 5**: cFOS

**UNRAVELING THE GENETIC FACTORS OF SECONDARY LISSENCEPHALY IN MARMOSET USING BULK AND SINGLE-CELL TRANSCRIPTOMICS OF BRAIN ORGANOIDS**

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The common marmoset (*Callithrix jacchus*) is a widely used primate model in biomedical research due to its small size and shared physiological mechanisms with humans. One key feature of interest is its abundance of basal radial glial cells (bRG) despite having a lissencephalic brain, making it a valuable model for studying the evolution of this trait. Due to ethical constraints on primate research, brain organoids have become essential models in neurodevelopmental and evolutionary studies. This study compares histology and transcriptomes of marmoset and human brain organoids to identify factors underlying bRG prevalence in the marmoset brain. Bulk RNA-seq of early-stage organoids (embryoid bodies, EBs) indicated an earlier neural commitment in marmoset EBs compared to human EBs. Single-cell RNA-seq analysis further revealed species-specific differences in neural progenitor subpopulation composition. Despite similar overall apical (APs) and basal progenitor proportions, neuronal-committed APs and all identified bRG subtypes showed higher proliferation rates in human than their marmoset counterparts. This may underlie the shortened neurogenic period in marmoset brain development. Functional analysis implicated fatty acid metabolism, apoptosis, and cytoskeleton assembly, as potential drivers of these differences. These findings provide insight into the molecular and evolutionary basis of the secondary lissencephaly in common marmoset.

**Keyword 1**: Brain evolution
**Keyword 2**: Neural Progenitor Cells
**Keyword 3**: Common marmoset
**Keyword 4**: Brain organoids
**Keyword 5**: Transcriptomics

**EVOLUTIONARY MECHANISMS UNDERLYING SECONDARY LISSENCEPHALY MODELED IN COMMON MARMOSET CEREBRAL ORGANOIDS**

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Throughout primate evolution, the neocortex, responsible for complex behaviors and intelligence, has expanded in both size and folding degree (gyrification). However, some species have undergone a secondary loss of cortical folds, becoming secondarily lissencephalic. One such example is the common marmoset (*Callithrix jacchus*), a New World primate whose ancestors are predicted to have had a gyrencephalic cortex. This raises a key question: what evolutionary mechanisms drove the transition to a lissencephalic cortex in common marmoset, and what selective advantages might this change confer? Consistent with previous findings in fetal brain tissue, we found that human and marmoset cerebral organoids at an equivalent developmental stage exhibit a similar composition of neural progenitor cells (NPCs). However, scRNA-seq and histological analyses revealed that NPC populations in marmosets were generally less proliferative, with marmoset apical progenitors (APs) displaying a notably longer cell cycle. Additionally, marmoset APs underwent extensive delamination during the course of development, suggesting that a similar mechanism operates *in vivo* to effectively shorten the neurogenic window. In conclusion, our data suggest that during the evolution of the common marmoset, a combination of mechanisms facilitated the reversal to a lissencephalic cortex. However, a key aspect of this transition remains unresolved: identifying the genetic factors underlying this evolutionary shift.

**Keyword 1**: Cortical evolution
**Keyword 2**: Neural progenitors
**Keyword 3**: Secondary lissencephaly
**Keyword 4**: Common marmoset
**Keyword 5**: Genetic regulation

**INTEGRATING CROSS-SECTIONAL AND LONGITUDINAL DATA: LINKING BRAIN MORPHOLOGY TO COGNITION, MENTAL HEALTH, AND SOCIAL FACTORS IN AN OSLO-BASED ADOLESCENT COHORT**

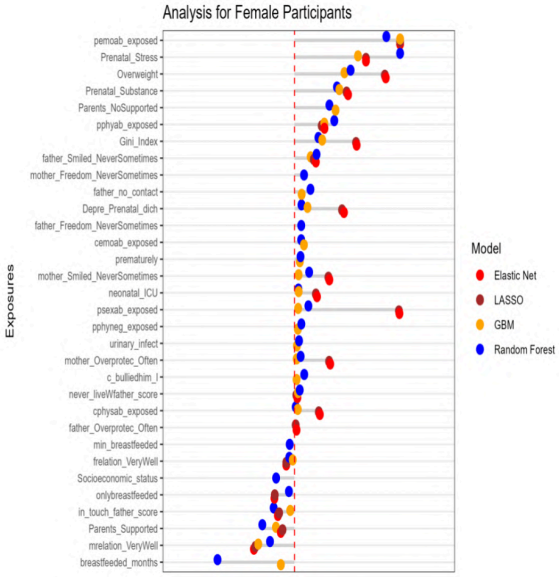
E.S. Blix[1,2], L.C.F. Holthe[2], A. Dahl[1,2], D. Alnæs[2], L.T. Westlye[1,2]
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Adolescence is characterized by increased neuroplasticity, making the brain highly sensitive to environmental factors such as social relationships and socioeconomic conditions. For some individuals this period marks the onset of mental illness. Advancements in the scale of brain imaging studies have shifted the focus from evaluating mental phenotypes on a case/diagnosis level to viewing them as a continuum of function. Here we aim to expand on this work by linking MRI-derived phenotypes to a comprehensive set of social, clinical and cognitive continuous traits in a combined cross-sectional (n ~ 1000) and longitudinal (n ~ 200) adolescent sample (ages 15 to 25). Our combined approach allows us to explore the possibility that certain traits possess morphological signatures consistent across the adolescent age span, while other traits may be more closely associated with individual developmental trajectories. This study enhances our understanding of adolescent brain development at both the group and individual levels and provides a foundation for further investigations into individual developmental trajectories, aiming to unravel the complexity of the relationships between neuroplasticity and adolescent behavior.

**SEX-SPECIFIC EXPOSOME ANALYSIS: TRACING PRENATAL TO POSTNATAL ENVIRONMENTAL INFLUENCES ON PSYCHOPATHOLOGY**

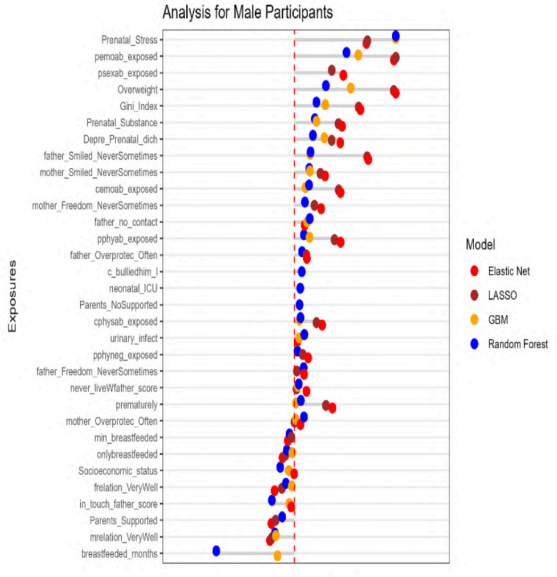
L. Melo Rothmann[1], A. Martins de Oliveira[2], B. Kluwe-Schiavon[3], M. Scopel Hoffmann[4], E. Constantino Miguel[5], G. Abrahão Salum[6], P. Mario Pan[2], L.A. Rohde[7], R. Affonseca Bressan[2], S. Iole Belangero[2], R. Grassi-Oliveira[1]
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The exposome approach considers environmental factors' cumulative and interactive effects on health. Childhood and adolescent exposures, particularly to environmental violence, significantly influence mental health, often with sex-specific differences. This study examined the sex-specific impact of environmental exposure on the severity of the *Child Behavior Checklist (CBCL)* Total Problems Score. The sample included 2,009 children (894 females [age = 10.2 ± 1.9], 1,115 males [age = 10.2 ± 1.8]) from the Brazilian High-Risk Cohort Study, with machine learning models trained separately for males, females, and the whole sample. We employed Elastic Net, LASSO, Gradient Boosting Machines, and Random Forest to evaluate 36 predictors after data curation. Results indicated better predictive accuracy in males ($R^2$ = 0.35, Elastic Net, 28 predictors) compared to females ($R^2$ = 0.27, Elastic Net, 20 predictors) and the combined model using male and females ($R^2$ = 0.28, Elastic Net, 25 predictors). Emotional abuse, overweight, prenatal stress, and socioeconomic inequality were key predictors across the sexes, but their effects differed. Males were more affected by paternal absence and maternal substance use during pregnancy, whereas females showed stronger associations with sexual violence and prenatal maternal depression. These findings underscore the importance of sex-specific analyses in understanding differential susceptibility to adversity. Moreover, the superior performance of linear models over machine learning approaches suggests that the relationships between predictors and CBCL Total Score are primarily linear. Future research should integrate biological and neurodevelopmental mechanisms to refine predictive models and develop sex-sensitive interventions.




| Model | MAE | MSE | RMSE | $r^2$ | n of Features |
|---|---|---|---|---|---|
| Elastic Net | 0.644 | 0.700 | 0.837 | 0.277 | 20 |
| LASSO | 0.645 | 0.703 | 0.838 | 0.276 | 20 |
| Ridge | 0.641 | 0.694 | 0.833 | 0.284 | 32 |
| Random Forest | 15.276 | 407.786 | 20.194 | 0.270 | 31 |
| GBM | 15.276 | 376.269 | 19.398 | 0.326 | 26 |

[1] MSE, Mean Squared Error;
[2] MAE, Mean Absolute Error;
[3] RMSE, Root Mean Squared Error;
[4] $r^2$, R-squared.




| Model | MAE | MSE | RMSE | $r^2$ | n of Features |
|---|---|---|---|---|---|
| Elastic Net | 0.636 | 0.627 | 0.792 | 0.350 | 28 |
| LASSO | 0.636 | 0.629 | 0.793 | 0.348 | 24 |
| Ridge | 0.638 | 0.629 | 0.793 | 0.346 | 32 |
| Random Forest | 16.409 | 476.117 | 21.820 | 0.206 | 31 |
| GBM | 16.409 | 449.268 | 21.196 | 0.251 | 22 |

[1] MSE, Mean Squared Error;
[2] MAE, Mean Absolute Error;
[3] RMSE, Root Mean Squared Error;
[4] $r^2$, R-squared.

**Keyword 1**: Exposome Approach
**Keyword 2**: Sex-Based Vulnerability
**Keyword 3**: Mental Health Prediction
**Keyword 4**: Sex-Specific Effects

**OFFSPRING OUTCOMES FOLLOWING PRENATAL THC AND CBD EXPOSURE: BEHAVIORAL IMPACTS IN ADOLESCENCE**

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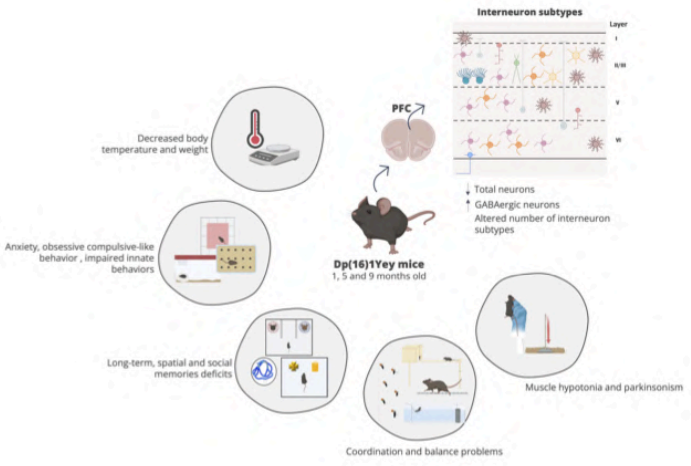
The rising prevalence of cannabis use during pregnancy has raised concerns regarding its potential long-term effects on offspring behavior. Δ9-tetrahydrocannabinol (THC) and cannabidiol (CBD) can cross the placental barrier, yet their distinct behavioral consequences remain poorly understood. While prenatal THC exposure has been linked to neurodevelopmental disruptions, studies investigating CBD exposure within the same framework are scarce. This study aimed to characterize the long-term behavioral alterations following prenatal exposure to THC and CBD. Pregnant mice received THC (5 mg/kg, i.p.), CBD (5 mg/kg, i.p.), or vehicle (VEH) throughout gestation. Male and female offspring underwent behavioral assessments during adolescence to evaluate locomotor activity, working memory, and social behavior. Prenatal THC exposure led to hyperlocomotion in the open field test ($F(2,51) = 7.073$, $p = 0.0019$) and impaired spatial recognition memory in the Y-maze task ($F(2,43) = 7.872$, $p = 0.0012$), indicating long-term cognitive alterations. No significant behavioral differences were observed in the CBD and VEH groups. These findings reinforce concerns about the lasting behavioral consequences of prenatal THC exposure and provide novel insights into the effects of CBD within the same neurodevelopmental context. Further research is warranted to elucidate the underlying molecular mechanisms and their implications for offspring health.

**Keyword 1**: Cannabis
**Keyword 2**: Pregnancy
**Keyword 3**: Behavioral outcomes
**Keyword 4**: Neurodevelopment

**BEHAVIORAL AND NEUROPATHOLOGICAL CHARACTERIZATION OF THE DP(16)1YEY MOUSE MODEL OF DOWN SYNDROME**

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Down Syndrome (DS), caused by triplication of the human chromosome 21 (HSA21), is the most common neurodevelopmental disorder worldwide, associated with altered brain structure and function, physical abnormalities, and intellectual disability. An excitatory/inhibitory (E/I) circuitry imbalance was described in DS patients' brains, but how specific cortical inhibitory circuits mediate DS pathophysiology remains unclear. Thus, we aimed at characterizing the DS mouse model Dp(16)1Yey, throughout development, and comprehend how cortical interneurons are affected in DS. Using a battery of behavioral tests in male and female Dp(16)1Yey and control mice we observed that, starting at 1 month of age, Dp(16)1Yey mice showed anxiety- and obsessive compulsive-like behaviors, impairment of long-term, spatial and social memories and absence of sociability deficits. This was accompanied by coordination and balance difficulties and hindlimb hypotonia, that worsened with age. At the neuronal level, at 5 months of age, we observed reduced number of neurons in some layers of the anterior cingulate and prelimbic areas of the prefrontal cortex along with a tendency to an increased number of total GABAergic interneurons in Dp(16)1Yey mice. Specifically, the number of different interneuron subtypes (parvalbumin-, somatostatin-, neuropeptide Y (NPY)- and nitric oxide synthase (nNOS)-positive neurons) was altered in this DS model, pointing towards an imbalance in the E/I circuitry. Overall, our results confirm that the Dp(16)1Yey model mimics the clinical manifestations observed in humans, being a good model to study the different cellular and molecular mechanisms of the disease, pointing to interneuron subtype imbalances as an important component of the disease.



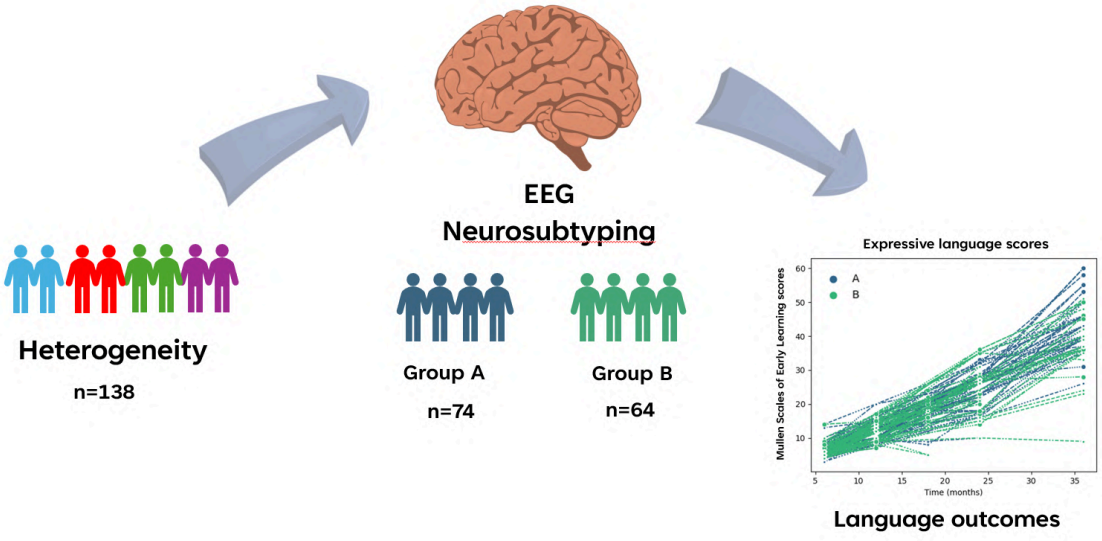

**Keyword 1**: Down syndrome
**Keyword 2**: Cortical interneurons
**Keyword 3**: Cognition
**Keyword 4**: Social
**Keyword 5**: Behavior

**NEUROSUBTYPING IN AUTISM: A CLUSTERING APPROACH TO PREDICT LANGUAGE OUTCOMES IN INFANTS**

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Autism spectrum disorder (ASD) is a neurodevelopmental condition characterized by a wide range of symptoms, causes, and endotypes. This significant heterogeneity remains a major obstacle in research, as traditional case-control paradigms often fail to identify biomarkers and predictors of behaviour. One strategy to combat heterogeneity is "neuro-subtyping", a technique where individuals are characterized based on their brain phenotypes rather than broad diagnostic labels. The current study aims to assess whether this transdiagnostic approach can be applied to infants at elevated risk for autism and predict later language outcomes.

In this study, we analyzed EEG recordings from 138 infants (68 at elevated likelihood for ASD, 70 typical likelihood) aged 6-7 months. Latent profile analysis and hierarchical clustering methods were used to identify subgroups based on two language-associated EEG measures: left frontal connectivity and lateralization of spectral power. Our analyses revealed two distinct subgroups: Group A, defined by high connectivity and leftward lateralization and Group B, defined by lower connectivity and rightward lateralization. Linear mixed-effects models revealed significant group differences in language growth trajectories ($p = 0.018$), with Group A displaying significantly higher expressive language scores by 36 months based on the Mullen Scales of Early Learning (Figure). Notably, EEG-derived subgroups did not predict a later ASD diagnosis, suggesting a lack of evidence for an ASD-specific phenotype during the first year of life. In conclusion, this project contributes to a large body of research that supports using stratification approaches to decode heterogeneity in autism and its role in predicting behavioural outcomes.



**Keyword 1**: Neurodevelopmental Disorders
**Keyword 2**: Language
**Keyword 3**: Autism
**Keyword 4**: EEG

**SOCIOECONOMIC STATUS MODERATES THE RELATIONSHIP BETWEEN PRETERM BIRTH AND GREY MATTER VOLUME IN ADULTHOOD**

T. Barda[1,2,3], M. Thalhammer[2,3], B. Schmitz-Koep[2,3], C. Zimmer[2,3], P. Bartmann[4], D. Wolke[5], C. Sorg[2,3,6], D.M Hedderich[2,3]
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Very preterm (VPT) birth (≤32 weeks gestation and/or birth weight <1500g) is associated with altered brain development, including reduced grey matter volume (GMV). Socioeconomic status (SES) influences brain structure in full-term (FT) populations, but its role in moderating the relationship between prematurity and GMV remains unclear. This study examines whether SES at birth moderates the association between VPT birth and GMV in adulthood.

Structural T1w MRI scans (3T) were acquired at age 26 from 101 VPT and 111 FT adults in the prospective Bavarian Longitudinal Study, and matched for SES at birth (high, middle, or low). GMV residuals, adjusted for intracranial volume, were extracted using FreeSurfer (v. 7.3.2), and scanner effects were harmonized with NeuroCombat.
Moderation analyses revealed a significant interaction between prematurity and SES ($p = 0.042$, $f^2 = 0.021$), suggesting a small but meaningful effect of SES on GMV residuals. Lower SES was linked to reduced GMV residuals in preterm individuals, while higher SES was associated with greater GMV residuals. A similar moderation effect was observed when considering gestational age as a continuous predictor ($p = 0.037$, $f^2 = 0.022$). Additionally, in the VPT group, GMV residuals significantly predicted IQ ($p = 0.037$, $f^2 = 0.047$), underscoring the functional relevance of these structural differences.
These findings suggest that SES plays an important role in shaping neurodevelopmental outcomes following preterm birth. Future research needs to assess SES and should examine regional brain differences and their cognitive implications to better understand these effects and inform potential intervention strategies.

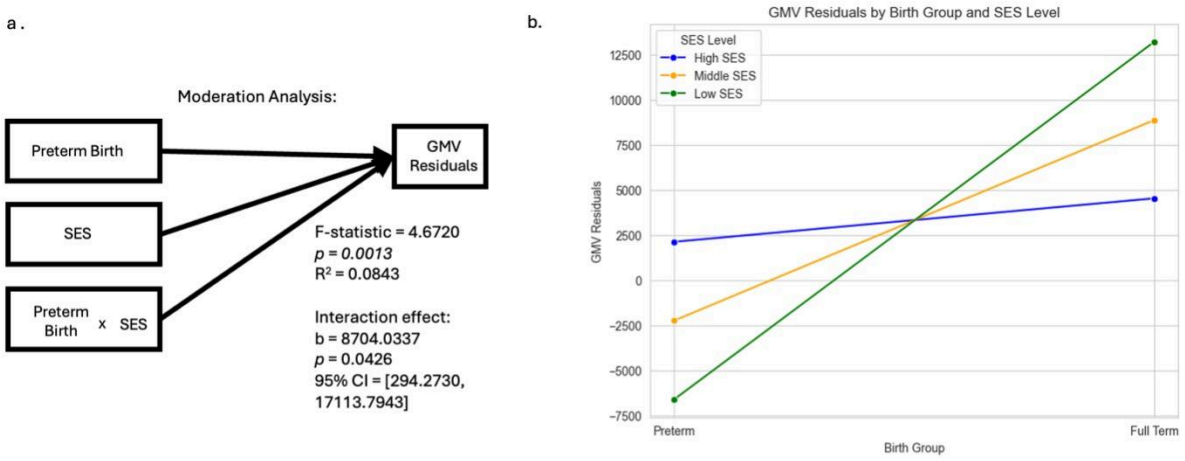



**Figure 1.** Moderation analysis of birth group and GMV residuals by SES.

**(a)** Statistical moderation model showing SES as a significant moderator of the relationship between birth group (preterm vs. full-term) and GMV residuals in adulthood ($b = 8704.03$, $p = 0.0426$, 95% CI [294.27, 17113.79]).

**(b)** Interaction plot illustrating SES-specific trajectories of GMV residuals across birth groups. High SES individuals show relatively stable GMV residuals regardless of birth status, whereas those with middle and low SES exhibit increasingly pronounced reductions in GMV residuals if born preterm.

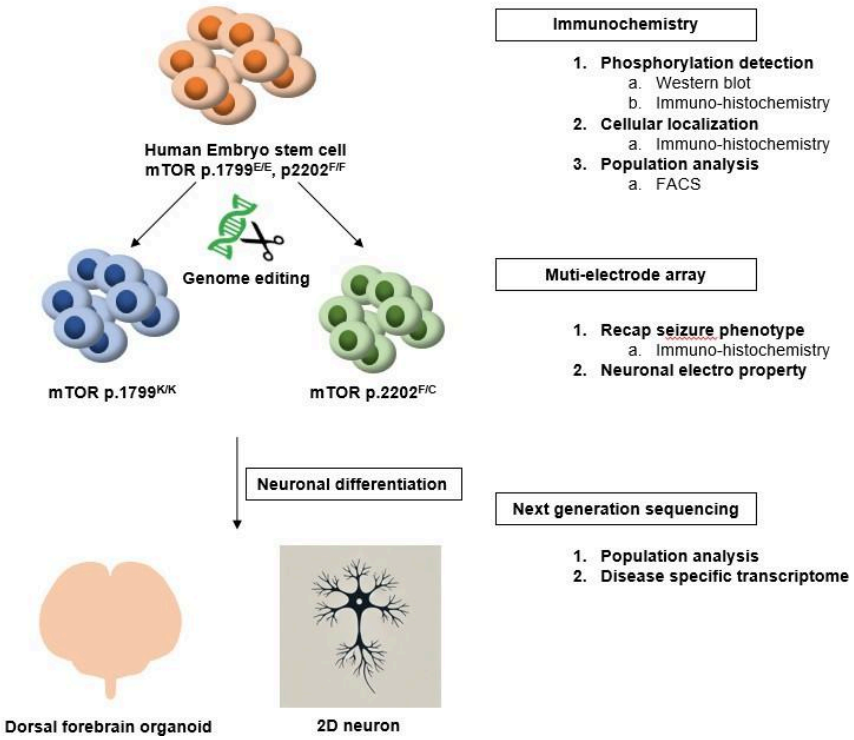
GMV, grey matter volume; SES, socioeconomic status

**Keyword 1**: preterm birth
**Keyword 2**: socioeconomic status
**Keyword 3**: grey matter volume

**MODELING AND MECHANISM ANALYSIS OF SMITH-KINGSMORE SYNDROME USING HUMAN EMBRYO STEM CELLS WITH PATIENT'S GENOTYPE**

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Smith-Kingsmore syndrome (SKS) is a representative mTORopathy characterized by activating mutations in the mTOR gene, leading to hyperactivation of the mTOR pathway and neurodevelopmental abnormalities such as seizures and intellectual disability. Although the correlation between mTOR hyperactivation and neuronal developmental disorders is well established, the downstream molecular mechanisms driving these pathologies remain poorly understood. To elucidate the direct impact of mTOR hyperactivity on neuronal development, we generated human embryonic stem cells (hESCs) carrying the SKS genotype through CRISPR-Cas9 gene editing. We differentiated these hESCs into dorsal forebrain organoids and maintained them in culture for over 10 weeks. SKS organoids exhibited an enlarged size compared to control organoids, consistent with the brain overgrowth phenotype observed in SKS patients. Morphologically, neural rosette-like structures were retained in SKS organoids over prolonged culture periods. Immunostaining analysis revealed increased phosphorylation levels of S6 and S6K1, indicating sustained mTOR pathway activation in the SKS organoids. These findings demonstrate that dorsal forebrain organoids derived from hESCs with the SKS genotype recapitulate key patient-associated phenotypes, including tissue overgrowth and mTOR hyperactivation. This human stem cell-based model provides a robust platform for studying the neurodevelopmental consequences of mTOR dysregulation. Further studies using single-cell RNA sequencing with SKS organoids are expected to identify downstream targets mediating mTOR-driven neurodevelopmental abnormalities, offering new insights into the pathophysiology of mTORopathies and potential therapeutic strategies.




**Keyword 1**: Brain developmental disease
**Keyword 2**: Smith-Kingsmore syndrome
**Keyword 3**: mTOR pathway

**ALTERED RESTING-STATE IN DEVELOPMENTAL DYSLEXIA: A SYSTEMATIC REVIEW AND META-ANALYSIS**

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This systematic review aimed to elucidate the neurobiological foundations of developmental dyslexia (DD) by exploring intrinsic brain functions, assessed through resting-state neural activations (rsNA) and functional connectivity (rsFC). The review encompassed 29 studies, categorized into sensor-based and anatomical-region-based results. Findings from the 13 sensor-based studies indicated higher rsNA in DD across delta, theta, and alpha bands, particularly over left frontal sites, alongside stronger rsFC in the right hemisphere across delta, theta, beta, and alpha bands. Increased whole-brain rsFC in the alpha band further supported these observations, despite consistent reductions in whole-brain rsFC in delta, theta, and beta bands in DD. Data from 16 anatomical-region-based studies consistently showed decreased rsNA in the bilateral cuneus and increased rsNA in the right parahippocampal gyrus, right superior frontal gyrus, and right insula in DD. Additionally, DD was associated with weaker rsFC in the left fusiform gyrus but stronger rsFC in the paracentral lobule and precuneus. These analyses illustrate multifaceted changes in resting-state activity spanning various brain regions. This complexity potentially reflects the dual nature of neural adaptations in dyslexia, encompassing both inherent deficits and compensatory mechanisms. These insights further our understanding of the neural basis of reading difficulties in dyslexia and contribute to identifying potential neural biomarkers of deficits and compensation.

**Keyword 1**: Developmental dyslexia
**Keyword 2**: Resting-state function
**Keyword 3**: Compensatory mechanisms
**Keyword 4**: Systematic review
**Keyword 5**: Meta-analysis

# AI, Neurotechnology, and Computational Neuroscience

**INVESTIGATING THE PHARMACOLOGY OF SEROTONIN (5-HT) RECEPTORS USING COMPUTATIONAL MODELLING**

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Serotonin receptors(5-HTR) regulate neurotransmission and impact various neuropsychiatric disorders. Despite their importance in several neurological processes, much remains unknown about their mechanisms and downstream activity.

There are multiple families/subtypes of 5-HTR. Using computational modelling, and molecular dynamic (MD) simulations-based methods, we aim to assess various functional features of three of these subtypes: 5-HT2a, 5-HT2c and 5-HT7.

Our project has several specific aims. Firstly, we will use MD to assess the structural basis of previously characterised residue mutations that decrease the ability of 5-HT2a to be activated by psychedelics such as LSD; this includes both mutations in the human system as well as the native inability of rat 5-HT2a to be activated by LSD. Further, we will use modelling and coarse-grained (CG) MD to assess the dynamics of 5-HT2c in different membrane environments whilst quantifying key lipid interactions for activation. Finally, we will run a comparative structural analysis of 5-HT7r to understand the role of ligand binding on receptor activation, with a particular focus on screening prospective antagonists against the inactive state of the receptor. Using Isambard 3 HPC, we will employ Well-Tempered Metadynamics (WTMetaD) on the 5-HTR to map activation/inactivation pathways and assess the potential allosteric role of lipid binding.
All findings will be validated using wet lab experimental methods including mutagenesis with functional cell-based BRET assays and imaging using FRET-FILM and FRAP.

Our data will reveal structural details about the underlying mechanism of several 5HTRs. We hope this will provide insights into 5HTR signalling, informing future therapeutic strategies.

**MATHEMATICAL MODELLING OF NEURAL CIRCUITS EVOLUTION**

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Neural circuits of evolutionary distant animals display certain similarity in structure and functions despite differences in properties of individual neurons and their developmental origin. Therefore, the neural circuits' structure and dynamics are more pivotal for the functioning than specific receptors and channels. We are aiming to implement computational modelling to study the evolution of neural circuits on an abstract level, without usage of detailed physiological models of individual cells. This will allow better understanding of relations between the structure and function of the circuits.
We present a modelling pipeline for the neural circuit evolution study *in silico*. The core element of the pipeline is the neuronal class-based inheritance and mutability, which enables generation-to-generation preservation/change of the circuit's properties without assigning them to individual cells. The circuits generated from the subclass-based set of parameters undergo an activity simulation. The external input is being applied in form of an activity of subpopulation of input neurons, and the resulting activity of the output neurons is used for the selection process.
Under the selection pressure, the circuits display rapid changes in structure and dynamics, adjusting the output activity. This leads to fast and reliable quantitative alternations, when the circuit adapts to minor changes in the anticipated input-output relationships. It also allows occasional qualitative shifts, for example beginning of recurrent pattern generation by the circuit that did not display this kind of activity.
The proposed pipeline allows tracking and studying reorganization of the model circuits' structure and dynamics that enables obtaining of new functions.

**Keyword 1**: Neural circuit
**Keyword 2**: Evolution
**Keyword 3**: Mathematical modelling

**3DEEPVOG: A ROBUST DEEP LEARNING-BASED EYE MOVEMENT TRACKING SYSTEM FOR NEURODIAGNOSTIC APPLICATIONS**

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Accurate and efficient tracking of eye movements is crucial for the diagnosis and understanding of neuro-otologic and neurologic disorders, as abnormal eye movements often serve as important diagnostic markers. However, current clinical video-oculography (VOG) systems are expensive and require high-quality data, limiting their accessibility for widespread clinical and limited clinical research use, e.g. in the context of VOG-fMRI experiments. To overcome these limitations, we present 3DeepVOG, an open-source deep learning (DL)--based eye-tracking system that enables robust, low-cost, and high-precision tracking of horizontal, vertical, and torsional eye movements. Our system uses a Convolutional Neural Network (CNN) architecture with an attention-based U-Net for pupil segmentation, achieving the highest accuracy. Trained and evaluated on a large dataset of 163,999 frames and an additional internal dataset of 5,506 frames using the Clinical Open Research Engine (CORE) provided by LMU Klinikum, 3DeepVOG achieves median errors of 0.34° (horizontal gaze), 0.76° (vertical gaze) and 0.16° (torsional gaze) compared to gold standard VOG systems. The results show the potential of DL-based eye-tracking solutions to improve diagnostic capabilities in clinical and research settings.

**Keyword 1**: Eye-tracking
**Keyword 2**: Deep learning
**Keyword 3**: Video-oculography (VOG)
**Keyword 4**: Neurodiagnostics

**MULTISCALE MODELLING USING ”SNUDDA”: FROM SUBCELLULAR BIOCHEMISTRY TO NEURONAL NETWORKS**

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Neural computation is both multiscale and multimodal. The dynamic behavior of synapses and membrane electrophysiology is continuously modulated by a complex chemical machinery composed of a range of neurotransmitters and second messenger systems. Understanding requires cohesive integration of different formalisms, including reaction-diffusion for biochemistry, multicompartmental Hodgkin-Huxley formalism for electrophysiology, and touch detection for network anatomy.
We present an integrated framework bridging these scales by integrating the Reaction-Diffusion (RxD) module of the NEURON simulator into our framework for detailed network topography, ”Snudda”. This framework enables a natural coupling at the mechanistic level between different models: various neurotransmitters in the synapse or extracellular space that bind to their receptors and activate signal transduction cascades, whose downstream second messengers continuously modulate synaptic and ion channel parameters. Models implemented in our framework can easily be extended to integrate and combine existing biochemical models specified in SBML, SBtab, or a JSON format, in detailed neural networks. Using this, it is possible to also study the interaction of several neurotransmitter systems and their effect on the network level.
We apply this framework to study the dopamine system of the basal ganglia by combining earlier biochemical models of the D1R and D2R cascades (Nair et al 2015, 2016, Yapo et al 2017) into our detailed model of the striatal network (Hjorth et al 2020), and the modulation of multiple ion channels, to study their effects in health and disease.

**EVALUATING THE LINK BETWEEN SEX, POLYGENIC RISK FOR SEX-BIASED DISORDERS, AND BRAIN AGING**

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Sex plays a key role in the prevalence and progression of many brain-related disorders, but its interaction with genetic risk to impact brain health remains unclear. Notably, sex-biased conditions such as schizophrenia and Alzheimer's disease have been associated with older brain age compared to controls. In the current study, we evaluated the link between sex and polygenic risk scores (PRS) for Alzheimer's disease, Parkinson's disease, depression, and schizophrenia with brain age gap (BAG) in 16 390 females and 14 639 males from the UK Biobank (aged 40–70). First, we assessed the interaction between sex and PRS with BAG using PRS derived from sex-combined genome-wide association studies (GWAS). However, it is common practice when performing a GWAS to adjust for the effects of sex, which in turn, produce PRS adjusted for sex. Therefore, we additionally assessed the impact of sex-specific PRS derived from sex-stratified GWAS on BAG to determine the role of sex-specific genetic predisposition to the disorders on BAG. Next, we used paired-samples Z-tests to compare sex-combined and sex-specific models. We found that males presented with older brain age compared to females across all sex-combined models. Further, PRS for Alzheimer's disease was linked to older brain age in both the combined-sex and female-only samples. No differences between sex-combined and sex-specific PRS were observed in their association with BAG based on Z-tests. Overall, these findings highlight that minor but potentially clinically relevant sex differences exist in the association between genetics, sex-biased disorders, and brain age.

**Keyword 1**: Sex differences
**Keyword 2**: Polygenic risk score
**Keyword 3**: Brain age gap
**Keyword 4**: Neurodegenerative disorders
**Keyword 5**: Mental disorders

**CAUSAL RISK FACTORS AND POLYGENIC OVERLAP BETWEEN PREGNANCY-RELATED DISORDERS AND BRAIN DISORDERS**

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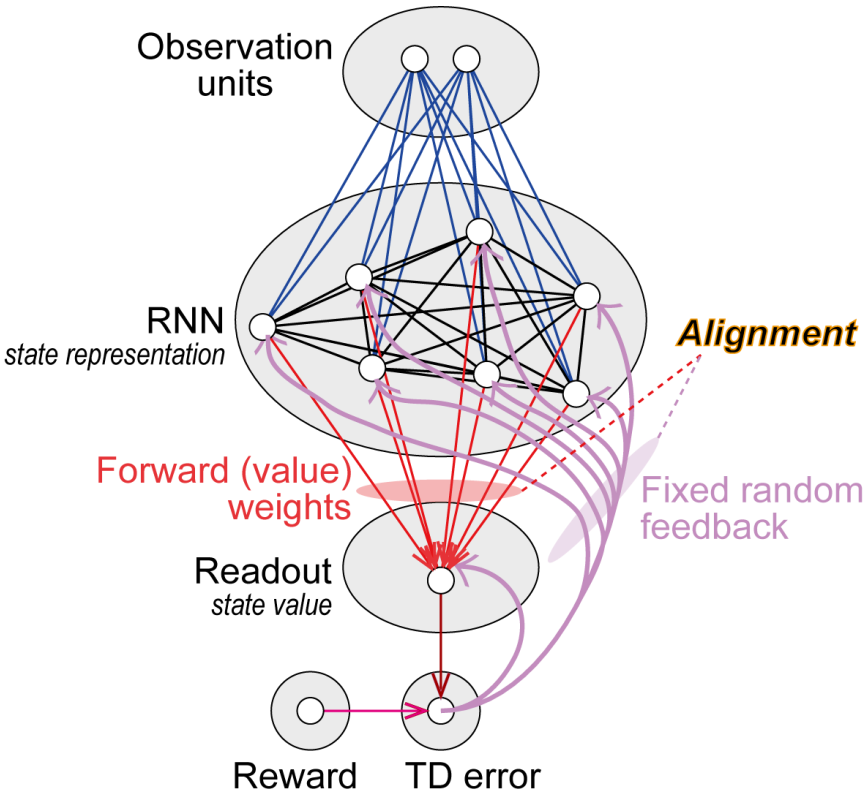
Pregnancy-related disorders, such as hypertensive disorders and postpartum depression, have consequences for maternal brain health. Studies show highly intertwined pathophysiologies between disorders, with shared risk factors such as blood lipids. However, the causality of risk factors and the genetic overlap between disorders has not been established. Here, we used female-specific summary statistics from genome-wide association studies (GWAS) to estimate genetic correlations using linkage disequilibrium score (LDSC) regression and causal associations using Mendelian randomization between blood lipids and inflammatory markers (c-reactive protein (CRP), HDL-cholesterol, LDL-cholesterol, and triglycerides), pregnancy-related disorders (hypertensive disorders of pregnancy and postpartum depression), and brain disorders (Alzheimer's disease and major depressive disorder). We used bivariate causal mixture models (MiXeR) to explore polygenic overlap between disorders. We found widespread genetic correlations between blood lipids, BMI, and pregnancy-related disorders. We further found causal associations between triglycerides and HDL-cholesterol as exposures and hypertensive disorders of pregnancy as an outcome, and CRP, LDL-cholesterol, and triglycerides as exposures and Alzheimer's disease as an outcome. However, multivariable analyses suggested that associations were likely driven by BMI. Postpartum depression was positively correlated and causally associated with major depressive disorder. MiXeR estimated substantial polygenic overlap between disorders, with 29.3K variants influencing major depressive disorder and 7.7K variants influencing postpartum depression, of which 7K variants were shared. The results suggest causal effects of cardiovascular mechanisms, with BMI as an important modifiable risk factor. Further, the findings contribute to discussions on major depressive disorder and postpartum depression as disorders with both overlapping and distinct genetic features.

**Keyword 1**: Pregnancy-related disorders
**Keyword 2**: Brain disorders
**Keyword 3**: Genetic approaches

**NON-NEGATIVE VALUE-RNN ENABLES LEARNING OF REPRESENTATION AND VALUE THROUGH LOOSE ALIGNMENT**

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Recent work (Hennig et al. 2023 PLoSComputBiol) showed that state representation and value can be learnt in a recurrent-neural-network(RNN) and its readout (named "value-RNN"), but it used the biologically implausible "backpropagation(backprop)" algorithm. We propose a biologically plausible alternative. Our model, like the original value-RNN, consists of observation-units, RNN, a readout, and a Temporal-Difference(TD)-error-unit, which trains both recurrent/observation and readout connections. Different from the original value-RNN, we assume fixed random (rather than backprop) feedback from the TD-error-unit to the RNN, and we presume that the model's components are implemented in sensory cortex, association/prefrontal cortex, striatum, and dopamine neurons. Our model can learn appropriate representations and values of states that precede temporally separate reward, even in the presence of probabilistic task structures or reward-non-associated distractors. This learnability comes from alignment of the forward readout (value) weights to the random feedback, as in the "feedback alignment" (Lillicrap etal. 2016 NatCommun), and we show how it mechanistically occurs. Moreover, imposing further biological constraint (non-negative weights, plus monotonic dependence of plasticity on post-synaptic-activity) does not ruin but rather promote learning because the constraint enables loose alignment of the readout weights and the feedback from the beginning. Our model does not assume eligibility-trace nor memorable RNN unit assumed previously (Bellec etal. 2020 NatCommun; Hennig etal.), but can still solve the temporal credit assignment problem to a certain extent presumably because TD learning itself (even without eligibility-trace) is a solution for it. This work appeared as an unrevised reviewed preprint in eLife, and is now in revision.




**Keyword 1**: reinforcement learning
**Keyword 2**: dopamine
**Keyword 3**: recurrent network
**Keyword 4**: Hebbian
**Keyword 5**: state representation

**TEACHING COMPUTATIONAL SKILLS IN NEUROSCIENCE**

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A push for open science in recent years has led to more studies sharing code and data upon publication. However, recent systematic analyses found that a majority of those studies are not computationally reproducible.[1,2] Reasons for this vary, but are likely to at least in part be due to a lack of training in computational skills for researchers. A large corpus of resources for learning the requisite programming and data analysis skills are available, but many are not tailored to neuroscientific applications. This may raise the threshold for researchers to apply those skills in their own projects. We have developed courses teaching both essential computer science skills for making computationally reproducible projects, such as version control, compute environment control, persistent data sharing, and documentation, and data analysis methods for various measurement modalities in neuroscience. Our courses emphasize application to real neuroscience data and realistic problems faced by neuroscience researchers, which ensures relevance for participants and easier translation of skills and knowledge acquired to their own work. Furthermore, the courses implement teaching philosophies adapted to adult learners, where only a short (5-15 minutes) introductory lecture is given at the beginning of each session and the majority of the participants' time is spent solving problems in small groups of 2-3 students. Our preliminary analysis shows that these courses achieve retention rates of up to 90 %, consistent positive feedback, and participants regularly returning for new courses.

1 Samuel and Mietchen, 2024, https://doi.org/10.1093/gigascience/giad113
2 Ziemann, Poulain, and Bora, 2023, doi.org/10.1093/bib/bbad375

**Keyword 1**: Teaching
**Keyword 2**: Programming
**Keyword 3**: Replicability

**DATA-DRIVEN COMPUTATIONAL MODELLING TRACKS EXCITATION/INHIBITION CHANGES IN EPILEPTIC BRAIN TISSUE AND DIFFERENTIATES SEIZURE TYPES**

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**Aims:** Computational modelling is a powerful tool for exploring brain mechanisms underlying neuroimaging data. Nowadays, it is increasingly used to design therapeutic interventions. In this contribution, the computational approach is used to identify the synaptic mechanisms involved in epileptic seizure transitions from EEG data. **Methods:** Building on the modelling work of Wendling et al., we replicate rat slice (n=4, high potassium model of epilepsy) and human intracranial EEG data (4 patients from Motol hospital, 15 patients from the European Epilepsy Database). In a data-driven manner, we fit five-second segments of simulated and real data, providing us with a) an automatized classification of the observed type of epileptic brain activity, and b) estimates of the underlying levels of excitatory and inhibitory synaptic gains across time. We study 205 seizure transitions, across seven different types of seizure onset patterns, in brain locations inside and outside of the seizure onset zone (SOZ). **Results:** The modelling environment could correctly classify epilepsy brain states (interictal, preonset, onset, ictal) with a mean sensitivity of 0.99 on model, 0.77 on rat, and 0.56 on human data (4-way classification task). The transition to seizure was linked to significant model-predicted increases in excitation and inhibition. Seizure types clearly differed in their associated changes (classification of seizure type: $p < .01$), already in the interictal interval and also in non-onset channels. **Conclusions:** Different seizure types are linked to distinct synaptic mechanisms that are pre-determined at least tens of seconds before seizure onset and not (only) localized in SOZ.

**Keyword 1**: Computational Modelling
**Keyword 2**: EEG
**Keyword 3**: Epilepsy
**Keyword 4**: Excitation/Inhibition

**PATHOLOGICAL BRAIN DYNAMICS USING EXPLAINABLE GRAPH GEURAL GETWORKS**

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With the objective of developing novel artificial intelligence methods that can be used for early detection of Dementia, Our methods research aims to understand the brain dynamics that are responsible for cognitive impairments. My PhD hope to develop a system for early detection of Dementia which, as a result, would help to take preventive measures at the early course of the disease. We introduce a novel method for solving the problem of classification of electroencephalography (EEG) signals based on dynamic Graph Neural Networks. Our focus essentially is on using Graph neural Networks (GNN) to study the temporal dynamics of these functional connectivity to classify the EEG signals. We used the model to predict Alzheimer's disease and dementia. Our model is capable of achieving competitive performance on unseen test data subjects. In this study, we use the explanation of those model to understand the dynamics responsible for dementia and Alzheimer's disease. I aim to interpret the model decision and how it is related to the data features which can be used as a biomarker for pathologies such as MCI or dementia. Additionally, we validate the interpretation of models with the well established concepts in neuroscience literature. We quantify the brain regions, functional connectivity networks and different brain waves over frequency bands that are responsible for the decision of the Graph neural Networks model.

**UNDERSTANDING AND IDENTIFYING NOVEL SUBGROUPS IN ALZHEIMER'S DISEASE THROUGH GENE REGULATORY NETWORKS**

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Alzheimer's Disease (AD) is a complex neurodegenerative disorder with poorly understood cellular dysfunction. To explore the molecular dysfunction of AD at the genome-scale level, we propose a biologically informed neural network using single-cell RNA-Seq data from the ROSMAP aging study to classify cells from AD versus Non-Cognitive Impaired (NCI) participants. Unlike traditional models, our approach combines person-specific data with biological knowledge bases to create a disease-specific, interpretable sparse neural network classifier. Our model uses an ensemble of neural networks with varying hidden layers and pathways, optimized through a voting mechanism and threshold-based pruning to enhance precision and accuracy. Shapley values were applied to identify key genes, while activation values of neural network layers were analyzed to find disrupted pathways in AD. The model achieved balanced accuracy of 75% to 85% in classifying excitatory neurons. Key genes identified through Shapley values included mitochondrial genes MT-ND1 and MT-ND4, alongside PDE10A, which inhibits phosphodiesterase and SYT7, affecting protein-protein interactions at synapses. Activation value rankings revealed alterations in several pathways associated with AD, including
RAC1 GTPase cycle, Neddylation, MAP2K and MAPK. These findings align with previous research, supporting the robustness of our method in elucidating AD's molecular mechanisms. Future research will incorporate epigenetic data and extend the analysis to other cell types for a more comprehensive understanding of AD

**Keyword 1**: Biologically informed AI
**Keyword 2**: Alzheimer´s Disease
**Keyword 3**: Deep Learning
**Keyword 4**: Single Cell analyses

**MAPPING TRANSCRIPTIONAL DYSREGULATION IN ALZHEIMER'S DISEASE USING SINGLE-CELL NETWORK INFERENCE**

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The involvement of different brain cell types in Alzheimer's disease (AD) is well established, yet the contribution of cell type-specific transcriptional regulation to AD pathophysiology remains largely unexplored. This study thus aimed to investigate transcriptional dysregulation in AD on a single-cell level.

We reconstructed gene regulatory networks (GRNs) from single-cell RNA sequencing data from the Religious Orders Study and Memory and Aging Project (ROSMAP). Using SCENIC (Single-Cell rEgulatory Network Inference and Clustering), we identified key transcription factors (TFs) driving cell type-specific transcriptional changes. GRNs were reconstructed for study participants with clinical and histological AD diagnosis, as well as participants with no signs of cognitive impairment (NCI). GRNs were inferred using GRNBoost2, a Gradient Boosting Machine method, and regulon activity was assessed with AUCell, which ranks gene expression to score regulon activity and compared between the groups.

Our analysis revealed distinct regulon activity across astrocytes, microglia, oligodendrocyte precursor cells (OPCs), inhibitory neurons, and excitatory neurons. In AD, astrocytes exhibited increased lipid metabolism-associated TF activity, suggesting metabolic dysregulation. Microglia displayed upregulation of inflammatory regulators, including genes linked to pro-inflammatory signaling. OPCs demonstrated altered oxidative stress-related regulatory activity. Inhibitory neurons showed synaptic dysfunction, while excitatory neurons exhibited heightened stress responses through glucocorticoid signaling.
These findings provide new insights into the transcriptional regulatory landscape of AD, highlighting how cell type-specific transcriptional programs contribute to disease pathology. By leveraging single-cell regulatory network inference, this study establishes a framework for identifying potential therapeutic targets that modulate transcriptional activity in distinct brain cell types in AD.

**Keyword 1**: Alzheimers Disease
**Keyword 2**: Gene regulatory networks
**Keyword 3**: Transcriptomics
**Keyword 4**: Single-cell
**Keyword 5**: Stastistical learning

**UNCOVERING THE DYNAMICS OF WHOLE-BRAIN NEURAL CIRCUITS USING TEMPORALLY STRUCTURED RECURRENT BOLTZMANN MACHINES**

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Brain computation emerges from the dynamic orchestration of whole-brain neural activity, characterized by an interplay between stochastic and goal-oriented behavior. To effectively capture these neural properties in model-based analyses, it is necessary to account for this stochasticity and the temporal dependencies while keeping model inference tractable on large-scale datasets. Previous work showed that the compositional Restricted Boltzmann Machine (cRBM) is successfully able to decompose larval zebrafish whole-brain activity (~100k neurons) into a much lower number of neural assemblies (~100-200 assemblies); however, assembly interactions in this model are static, thereby failing to capture temporal dynamics underlying network activity. To address this shortcoming, we show that the Recurrent Temporal Restricted Boltzmann Machine (RTRBM), a probabilistic recurrent model, can effectively be used as an extension of the cRBM to account for the time domain. By initializing RTRBMs with cRBM-derived assemblies, we can infer the temporal evolution of these assemblies, revealing novel insights into the interactions underlying whole-brain dynamics. Our results show that the RTRBM estimates significantly improve prediction accuracy as they outperform the cRBM models in temporal predictivity on simulated and whole-brain recordings in zebrafish. Furthermore, results demonstrate that the RTRBM model can accurately identify the timescales of the dynamics of identified neural assemblies. Overall, our work shows that the RTRBM is an effective model for capturing neural dynamics in large-scale neural recordings, with potential for broader application to large-scale neural datasets, including human fMRI.

**Keyword 1**: Probablistic Modeling
**Keyword 2**: Systems Neuroscience
**Keyword 3**: Timeseries Analysis
**Keyword 4**: Whole-Brain Activity
**Keyword 5**: Neural Assemblies

**POLYHEDRAL DECOMPOSITIONS AND TOPOLOGICAL TRANSFORMATIONS IN POPULATIONS OF NEURONS**

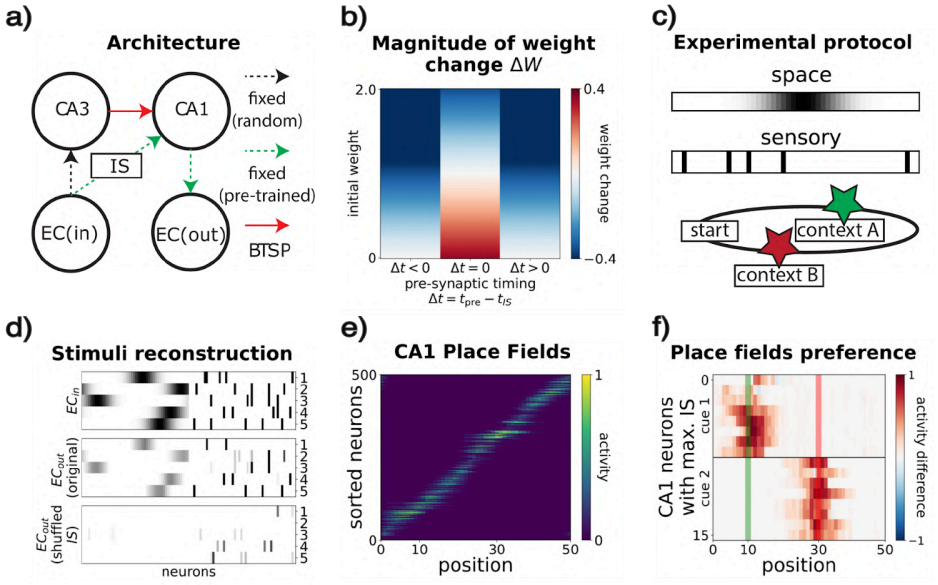
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The idea of neural representations living on smooth manifolds has become a prevalent metaphor in neuroscience. One way to characterize the structure of such manifolds is to study their topological invariants, often done with the help of persistent homology. While this approach has proven to be fruitful, it is questionable whether it is applicable to the study of higher dimensional manifolds that require an immense amount of sampling. In this work we train recurrent ReLU neural networks and use the framework of polyhedral decompositions to split the input (stimulus) space into regions on which a network operates linearly. Given such a decomposition we further define another decomposition (the overlap decomposition), that tells us which pieces of an input space are being glued together as the input space moves through the layers of a network. Given this knowledge, the homology groups of a neural representation can be calculated through a relative homology sequence which only requires knowledge of model parameters and therefore does not necessitate applying persistent homology on the final neural representation. This approach avoids any problems related to sampling or the highly non-linear nature of neural representations. To illustrate its power, we perform several modelling experiments in which we vary the topology of a stimulus space and study its homology groups of recurrent neural networks. Finally, we fit ReLU networks to real data from visual cortex and hippocampus and use our approach to describe the expected topology in response to unseen manifolds of stimuli.

**MAINTAINING DECODABILITY OF MEMORIES FORMED VIA BEHAVIOURAL TIMESCALE SYNAPTIC PLASTICITY (BTSP)**

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Behavioural timescale synaptic plasticity (BTSP) enables rapid synaptic changes based on neuronal activity at behaviourally relevant timescales. In CA1 neurons, BTSP requires both plateau potentials and presynaptic input from CA3. These plateau potentials, dependent on the Entorhinal Cortex (EC), are thought to act as instructive signals (IS) guiding synaptic changes. BTSP allows for one-shot learning, influencing episodic memory formation.
A critical challenge is understanding how downstream regions like the output from EC(out) maintain stable memory decoding despite changing CA1 representations. While additional plasticity mechanisms could link CA1 activity to downstream targets, this would demand flexible, context-dependent adaptation. Here, we explore the alternative hypothesis that the IS carries memory-related information, ensuring that future reactivations of CA1 neurons align with the original memory content. We propose a model in which the IS approximates the latent layer of an autoencoder loop EC(in)→CA1(IS)→EC(out). When presented with an input pattern, BTSP links the activity induced in CA3 with CA1 neurons selected by the IS. Because this signal targets neurons that lead to input reconstruction in EC(out), reactivation of a similar pattern in CA3 (to memory encoding) promotes recovering the original memory in EC(out) via CA1 activity. Simulating a circular track paradigm, we show that EC(out) reliably reconstructs original EC(in) patterns across multiple BTSP learning episodes. As a control, randomizing the IS disrupts this reconstruction, highlighting its role in maintaining decodability of CA1. The model also generates context-dependent place fields, consistent with experimental findings in episodic memory tasks.




**Keyword 1**: episodic memory
**Keyword 2**: synaptic plasticity
**Keyword 3**: hippocampus

**COMPUTATIONAL MODELING OF NEURON-ASTROCYTE INTERACTIONS: TRIPARTITE CONNECTIVITY AND SYNAPTIC MODULATION**

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Astrocytes are integral components of neural circuits, actively participating in synaptic transmission and plasticity as well as in network function. This study advances computational modeling of neuron-astrocyte interactions, focusing on astrocyte-induced synaptic currents (SICs) and their modulatory influence on neuronal activity. We developed a biologically grounded model incorporating tripartite connectivity, where astrocytes selectively modulate the postsynaptic terminal of a neuron, extending beyond traditional binary synaptic connections. Implemented within the NEST simulation framework, our model integrates astrocytic calcium signaling and its downstream effects on postsynaptic neuronal excitability through SICs, offering a mechanistic representation of astrocyte-mediated modulation of synaptic activities.

Simulations reveal that astrocytes modulate local neuronal network dynamics, with the extent of astrocyte-postsynaptic interactions significantly shaping overall network activity. By fine-tuning astrocytic parameters, the model exhibits transitions between asynchronous and synchronized neuronal states, demonstrating the role of astrocytes in coordinating neural oscillations and network states. Our results suggest that astrocytes influence neuronal excitability by regulating their glutamate release and modulating synaptic currents at the postsynaptic terminal.

The NEST implementation of tripartite connectivity provides a generic framework for combining arbitrary rule-based primary and third-factor connectivity. Using our model, we show that our approach scales well up to at least 1 million cells. Our framework thus facilitates realistic and reproducible modeling of neuron-glia interactions, providing insights into the role of astrocytes in both physiological and pathological conditions. By offering an accessible and computationally efficient tool for simulating neuron-astrocyte dynamics, this study supports further investigation into glial contributions to neural function and disease.

**Keyword 1**: Glia
**Keyword 2**: Astrocyte
**Keyword 3**: Simulation
**Keyword 4**: High-performance computing

**LEARNING PLACE CELL REPRESENTATIONS AND CONTEXT-DEPENDENT REMAPPING THROUGH A SIMILARITY-BASED OBJECTIVE**

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Hippocampal place cells are fundamental to spatial navigation and memory, exhibiting spatially selective firing patterns that encode an animal's location. These cells also respond to contextual cues and demonstrate remapping—a dramatic reorganization of firing fields in response to environmental changes. How these place-like representations emerge and how they remap remains poorly understood. Here, we propose a minimal similarity-based objective function that translates proximity in physical space to proximity in neural representation space. We demonstrate that neural networks trained to minimize this objective learn place-like representations similar to biological place cells. Networks develop key characteristics including spatially localized firing fields, coverage of the entire environment, and variable firing rates across the population. When extended to encode contextual information in the same way as space , our networks exhibit global remapping between distinct contexts, with firing fields that become uncorrelated when comparing across different contexts—mirroring observations in hippocampal recordings. Furthermore, we show that our learned representations are invariant to orthogonal transformations, allowing new spatial representations to be generated from existing ones without explicit relearning. This suggests a novel mechanism for understanding how place cells may form new maps in novel environments. Our recurrent network implementation also reveals the emergence of band cell-like representations alongside place fields, suggesting multiple cell types may cooperate during path integration. These findings offer insight into the computational principles underlying spatial representations in the hippocampus and demonstrate how a simple similarity-preserving objective function can account for complex neurobiological phenomena like context-dependent remapping.

**Keyword 1**: Cognitive map
**Keyword 2**: Remapping

**EXPONENTIAL MAP MODELS AS AN INTERPRETABLE FRAMEWORK FOR GENERATING SPATIAL REPRESENTATIONS**

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In mammals, the fundamental ability to navigate within an environment relies on specialized neurons in the hippocampal formation, such as place, grid, and boundary cells. Although the distinct firing patterns of hippocampal neurons are well studied, the mechanisms underlying how these patterns form remain unclear. Recent computational approaches have demonstrated that artificial neural networks trained on navigation tasks can develop spatial representations resembling biological cells. However, these models encompass a wide range of disparate neural networks and tasks, raising questions about whether their emergent patterns result from a choice of loss function, network architecture, or both.
In this work, we introduce a two-parameter expanded exponential map model that generates diverse spatial representations without neural networks or gradient based training, enabling a systematic exploration of spatial representation formation. We demonstrate that our mathematically transparent model produces interpretable spatial tuning modes, such as place and grid cell-like representations, that align with observed neuronal activity. Moreover, we demonstrate that constraints suggested in recent literature, such as equinorm-states and path invariance, can be directly achieved through simple constraints applied to the generator matrices within our model.
Our findings provide a theoretically grounded and interpretable mechanism for the formation of spatial representations, offering insights into the computational principles that may underlie navigation in the brain. This work bridges the gap between abstract mathematical models and biological observations, potentially advancing our understanding of the neural basis of spatial cognition and navigation behavior.

**Keyword 1**: Cognitive map
**Keyword 2**: Remapping
**Keyword 3**: Theory

**A MINIMAL BIOPHYSICAL MODEL OF ASTROCYTE–NEURON INTERACTIONS IN POTASSIUM HOMEOSTASIS AND EPILEPSY**

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Astrocytes critically regulate extracellular potassium ($K^+$) levels, a function that, when disrupted, contributes to pathological states such as epilepsy. Here, we present a reduced yet mechanistically grounded model that integrates both short- and long-range astrocytic $K^+$ buffering processes. By focusing on a “syncytial astrocyte” design—incorporating gap junction connectivity and high $K^+$ permeability—the model captures essential astrocyte properties while remaining sufficiently compact for interpretability.We validate the model against in vitro experimental data, confirming that it reproduces key features of astrocytic membrane dynamics, including the ability to buffer extracellular $K^+$ during heightened neuronal activity. Notably, a parameter-space exploration reveals degeneracy in the system: multiple distinct parameter sets lead to the same functional outcome, underscoring astrocytes’ robust adaptability. Furthermore, extending the model to include a reduced Hodgkin–Huxley neuronal component shows how local and spatial buffering mechanisms can influence seizure onset and evolution, consistently aligning with established seizure taxonomies.Because of its minimal and biophysically guided structure, the model can be translated into a mean-field or population-level formulation. This scalability opens opportunities to interface with large-scale connectome-based simulations, supporting personalized whole-brain modeling platforms for epilepsy. Thus, our model offers a tractable yet comprehensive framework for dissecting neuron–glia interactions, especially in the context of diseased states. By shedding light on astrocytic mechanisms that govern normal and pathological $K^+$ regulation, this work paves the way for more refined therapeutic strategies targeting glial function in epilepsy and beyond.

**Keyword 1**: Astrocyte
**Keyword 2**: K+ buffering mechanisms
**Keyword 3**: minimal biophysical model

**A GEOMETRIC MODEL ILLUSTRATES THE KEY ROLE OF WIRING COSTS AND SPATIAL DISTRIBUTIONS IN NETWORK DEVELOPMENT**

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Biological neural networks are characterized by short average path lengths, high clustering, and modular and hierarchical architectures. These complex network topologies strike a balance between local specialization and global synchronization via long-range connections, resulting in highly efficient neural communication. However, studies have shown that the structural components enabling these features are disrupted in neurodegenerative diseases such as ALS, demonstrating defects in neurite outgrowth and connectivity. Here, we use a geometric network model to capture the intricacies of the interplay between spatial conformations of neurons and varying wiring cost principles on the resulting network structures. This is done by combining connection probabilities favoring either intermediate or long-range connections with neurons organized into increasing spatial clusters embedded on a plane. We find that neither the long-range nor the intermediate connection probability conforms to small-world architectures for neurons in random distributions, with only larger spatial clusters of nodes producing small-world networks. For larger degrees of spatial clustering, local wiring cost is reduced, resulting in a larger number of connections between neurons in close vicinity. Here, we find the average path length is reduced, and modularity and clustering increased. Our findings suggest that both wiring costs and spatial distributions play a key role in neural network development and modelling. Furthermore, such simplified models may improve and help guide our understanding of compensatory mechanisms in the face of adaptive or maladaptive responses to neurodegenerative disease.

**INVESTIGATING DEGENERACY IN BIOPHYSICALLY DETAILED MODELS OF JUVENILE CA1 PYRAMIDAL NEURONS**

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Neurons exhibit a phenomenon known as degeneracy, where different combinations of ion channels result in similar electrophysiological behaviour, allowing stable function despite variability in ion channel expression. The study of degeneracy is essential for understanding neuronal regulation under biological variability. Most recent models have used data from adult animals; however, the presence of degeneracy in young animals remains unclear. In this study, we prepared acute hippocampal slices from young Wistar rats (postnatal days 11-13) and performed whole-cell patch-clamp recordings in 47 CA1 pyramidal neurons, extracting 171 electrophysiological features. We used 10 published morphologies of CA1 pyramidal neurons that matched our conditions for computational modelling. Using the BluePyEModel pipeline, we optimised maximal ion channel conductances and selected passive parameters. An evolutionary algorithm minimised a cost function representing the total Z-score across features, defining valid models as those with Z-scores below 5. Over 10 independent optimisations per morphology yielded 15,000 models, of which 9,915 were valid. Preliminary analysis indicates strong correlations between certain ion channels, supporting the theory of degeneracy. Principal component analysis shows that models cluster by morphology, with overlap suggesting that morphology influences parameter distribution. Future work will explore degeneracy in terms of neuronal energy efficiency and functional properties. Funded by the EU NextGenerationEU through the Recovery and Resilience Plan for Slovakia under the project No. 09I03-03-V04-00388.

**Keyword 1**: compartmental model
**Keyword 2**: degeneracy
**Keyword 3**: CA1 pyramidal neuron
**Keyword 4**: morphology

**SYNAPTIC POTENTIATION STABILIZES STRUCTURAL AND FUNCTIONAL NETWORK ABNORMALITIES IN ALS MOTOR NEURONS**

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Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease causing progressive degeneration of upper and lower motor neurons. Growing evidence suggests that early pathological hallmarks include synaptic impairment and associated circuit-level disruptions such as cortical hyperexcitability and increased functional connectivity. Importantly, these synaptic alterations precede overt neurodegeneration and symptom onset, making the synapse a relevant target for early therapeutic intervention.

To strengthen and stabilize ALS synapses, we treated ALS-patient derived motor neuron networks with the long-term potentiation (LTP)-inducing potassium channel blocker tetraethylammonium (TEA). Compared to healthy networks, untreated ALS networks were more functionally interconnected, less specialized, and displayed a higher firing rate and more bursting than the healthy control networks. The ALS networks also showed upregulated synaptic energy metabolism and protein synthesis, aberrant neurite growth and branching, and a time-dependent loss of dendritic spines.

Blocking of potassium channels in ALS networks upregulated proteins involved in synaptic organization, maturation, and synaptic vesicle cycle, and stabilized the density of dendritic spines, consistent with synaptic potentiation. Furthermore, synaptic stabilization was accompanied by restoration of aberrant branching, transient suppression of hyperexcitability and excessive synchrony, and stabilization of dysregulated synaptic pathways.

Together, these findings demonstrate that mobilizing inherent synaptic plasticity mechanisms can temporarily restore structural and functional network abnormalities in ALS networks. This underscores the contribution of synaptic dysfunction to ALS pathology, and highlights synaptic plasticity as a viable avenue for modulating disease-related network dynamics.

**Keyword 1**: Network neuroscience
**Keyword 2**: Neurodegenerative disease

**BEYOND DIAGNOSTICS: A DIMENSIONAL APPROACH TO MENTAL PROFILING**

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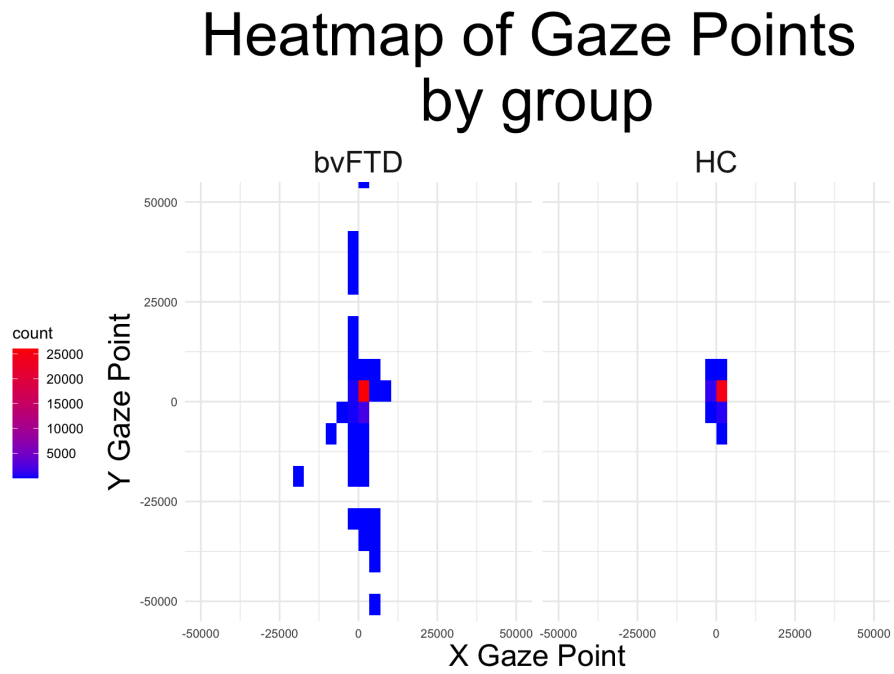
Mental health extends beyond the absence of mental illness (MDx). It arises from a complex interplay between hereditary factors and exposures, encompassing emotional, psychological, and social dimensions. However, ambiguity between pathological and non-pathological states challenges traditional diagnostic classifications, necessitating a dimensional approach for precision psychiatry. This study aimed to explore data-driven methods for stratifying mental health risk based on psychometric and biological markers. Here we analyze data from a large, young, representative and deep-phenotyped European sample. Data included psychometric assessments (e.g., personality, cognition, emotional regulation), brain-imaging, circulatory OMICs markers, and polygenic scores (PGSs) for a wide range of psychiatry-related traits. Archetypal (soft-clustering) analyses was applied to psychometrics and PGSs to display participants on the phenotype spectrum as convex combinations of extreme observations. Associations between archetype scores, OMICs and imaging data were assessed. Deep psychometric archetypal profiling effectively stratified participants into risk clusters defined by personal and familial MDx history. Highlighting their biological basis, clusters exhibited distinct biological features, with individual PGSs (e.g., well-being, MDx, and brain MRI measures) predicting liability to psychometry-based archetypes. Intriguingly, psychometric and PGS-based archetypes significantly overlapped, identifying high-risk subsets with multiple MDx diagnoses and elevated genetic risk. Our findings demonstrate the feasibility of data-driven risk stratification of the general population the relevance of multimodal archetypal analyses for uncovering latent psychopathology structures. While replication in larger, longitudinal cohorts is warranted, our study advances the understanding of mental health by embracing a dimensional perspective, laying the foundation for personalized mental health care strategies.

**Keyword 1**: Precision Psychiatry
**Keyword 2**: Personalised Medicine

**EXPLORING THE OCULAR DYNAMICS OF PEOPLE WITH BVFTD AND APATHY IN ECOLOGICAL SETTINGS**

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Apathy reduces motivation and goal-directed behavior (Rascovsky et al., 2011; Levy & Dubois, 2006) and is a core symptom within behavioral variant frontotemporal dementia (bvFTD) which disrupts social, emotional, cognitive, and behavioral functioning. The ICM's ECOCAPTURE initiative investigates apathy in bvFTD and identifies digital biomarkers within ecological settings. Previous research (ECOCAPTURE@LAB, Clinicaltrials.gov: NCT03272230) showed that apathy in bvFTD impairs both exploratory and goal-directed behaviors, as evidenced by reduced environmental exploration in a waiting room (Batrancourt et al., 2019). Building on these findings, we incorporated eye-tracking to refine the behavioral signature of apathy in bvFTD. By evaluating visual exploration in a replicated waiting room setting (ECOCAPTURE@Lab), we hypothesized that apathy in bvFTD would be linked to reduced visual efficiency and engagement. Our results confirmed that individuals with bvFTD exhibited a significantly lower overall saccade frequency during free exploration than controls. Additionally, greater variability in saccade frequency, which was positively correlated with apathy scores, was observed. This suggests inefficient exploration may appear as increased visual scanning but lacks goal-directed intent. Heatmap analyses during the 7-minute free exploration and a structured task (puzzle-solving) confirmed the link between increased visual exploration and ineffective engagement, through dispersed gaze patterns. This study identifies two impairments in exploration -initiation and efficiency- and demonstrates eye-tracking as a powerful tool for assessing apathy and its behavioral mechanisms. As a next step, we will use eye-tracking to study visual exploration efficiency in real-world settings by observing participants with bvFTD and apathy preparing a tartine in their kitchens (ECOCAPTURE@HOME).




**Keyword 1**: Eye-tracking
**Keyword 2**: Apathy
**Keyword 3**: Natural environment
**Keyword 4**: bvFTD
**Keyword 5**: Exploration

**THE ROLE OF OSCILLATIONS IN GRID CELLS' TOROIDAL TOPOLOGY**

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Persistent homology applied to the activity of grid cells in the Medial Entorhinal Cortex suggests that this activity lies on a toroidal manifold. By analyzing data and a model, we show that neural oscillations play a key role in the appearance of this topology. To quantitatively monitor how changes in spike trains influence the topology of the data, we first define a robust measure for the degree of toroidality of a dataset. Using this measure, we find that small perturbations of spike times have little influence on both the toroidality and the hexagonality of the ratemaps. Jittering spikes by 100-500 ms, however, destroys the toroidal topology, while still having little impact on grid scores. These critical jittering time scales fall in the range of oscillations periods between the theta and eta bands. We hypothesized that these oscillatory modulations play a key role in the appearance and robustness of toroidal topology and the hexagonal spatial selectivity is not sufficient. We confirmed this hypothesis using a model for grid cells activity, consisting of an ensemble of independent rate-modulated Poisson processes. When these rates were modulated by oscillations, the network behaved similarly to the real data. In the absence of oscillations, this similarity was substantially lower. Furthermore, we find that experimentally recorded spike trains exhibit temporal modulations at the eta and theta bands, and that the ratio of the power in the eta band to that of the theta band correlates with the critical jittering time at which the toroidal topology disappears.

**NETWORK DYNAMICS AND PLASTICITY IN NEURONAL CULTURES WITH ENGINEERED CIRCUITS**

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When neurons are cultured in the lab, synaptic formation and, by extension, the neuronal network architecture, is in most cases an almost entirely random process. Neurons tend to form synapses indiscriminately across the entire population, resulting in a network where feedback circuits are the predominant feature. We've recently developed a microchip with the capacity to guide neurite growth, which imposes a bias on network architecture, allowing experimenters to engineer neuronal network architectures effectively. Here, we report on the morphological and functional aspects of neurons cultured on such chips. We integrate these chips with micro-electrode arrays and examine network behavior and functional connection characteristics compared to neurons cultured using more traditional methods. Through stimulation, we examine these neurons' plasticity and ability to differentiate multiple stimulus patterns.

**NEURODEBLUR: A NOVEL SOFTWARE FOR FAST AND ACCURATE DECONVOLUTION OF LARGE LIGHT-SHEET, CONFOCAL, OR BRIGHT-FIELD MICROSCOPY STACKS**

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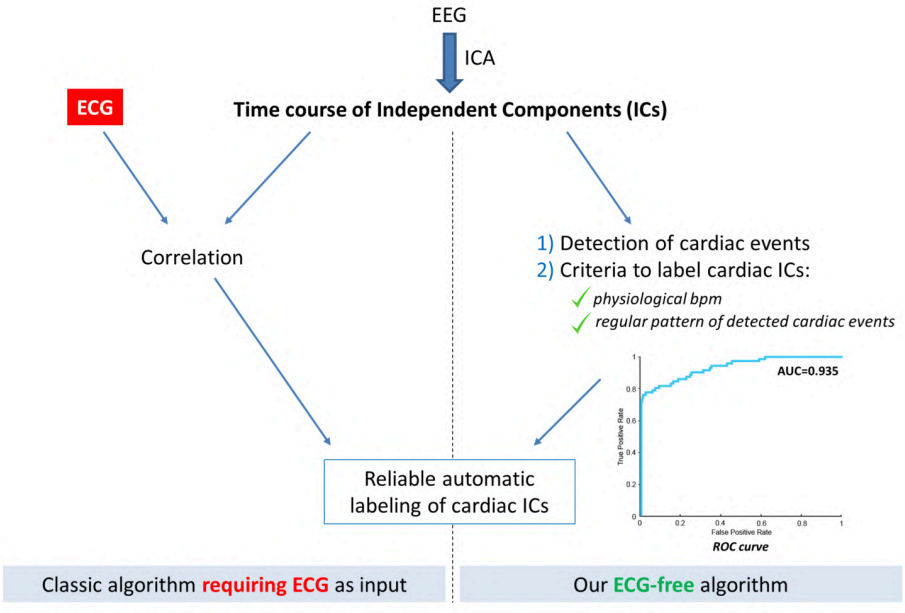
We developed NeuroDeblur, a novel software solution for deconvolving 3D microscopy data that significantly enhances the visibility of fine details—such as neurons or nerve fibers—in large image stacks captured using light-sheet, confocal, or bright-field microscopy. NeuroDeblur employs synthetic point spread functions (PSFs) tailored to each imaging modality by leveraging a sophisticated optical model of the imaging process. Additionally, experimental PSFs derived from recordings of fluorescent beads can be utilized. A newly developed module automatically extracts and averages the PSF. The software also features an advanced adaptive background removal algorithm and automatic block-wise processing of large datasets, enabling the handling of very large image stacks (>100 GB). Through extensive parallelization and GPU acceleration, NeuroDeblur achieves high-speed performance; for example, on a PC equipped with a state-of-the-art NVidia graphics board, a 3D stack comprising approximately 1 billion voxels can be deconvolved in less than 5 minutes.

**Keyword 1**: Neuroimaging
**Keyword 2**: deconvolution
**Keyword 3**: light sheet microscopy
**Keyword 4**: confocal microscopy
**Keyword 5**: image processing

**NEW AUTOMATED TOOL FOR IDENTIFYING CARDIAC INDEPENDENT COMPONENTS IN EEG WITHOUT ECG INPUT**

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Independent Component Analysis (ICA) is widely used to separate EEG signals into Independent Components (ICs). Efficient automatic labeling of cardiac ICs is essential for artifact removal, but most existing methods require ECG recordings, which are not always available. To address this limitation, we developed a novel tool that identifies cardiac ICs using only the IC time course.
We applied an existing function (github repository: https://github.com/dnacombo/heart_functions) designed to detect cardiac events (PQRST) in ECG signals to each IC time course. Analysis of the output enabled the differentiation of cardiac ICs (whose timecourse are very similar to an ECG) from non-cardiac ICs (where unrelated signal variations are incorrectly identified as cardiac events). ICs were labeled as cardiac based on two criteria: (1) a physiological average RR interval and (2) a regular pattern of detected cardiac events, assessed by the standard deviation-to-mean ratio of RR intervals, PQ intervals, and R amplitudes.
In our preliminary analyses, we tested our algorithm on 107 resting-state EEG recordings from the INSIGHT-preAD cohort (2min, 256 electrodes). A total of 3,808 ICs were classified both automatically and through visual inspection. ROC analysis yielded an AUC of 0.935 (specificity=1, sensitivity=0.69). Next, we plan to test the algorithm on a larger open-access dataset with ECG (see Wakeman & Henson 2015, https://openneuro.org/datasets/ds000117/versions/1.1.0) to compare its performance with existing methods.
We present a reliable ECG-free algorithm for detecting cardiac ICs in EEG, offering a practical solution when ECG is unavailable. The tool is currently implemented in MATLAB, with plans for a Python version.




**Keyword 1**: Methodological advances
**Keyword 2**: EEG preprocessing tool

**CUTTING-EDGE VIRAL VECTOR TOOLS AND THEIR APPLICATIONS IN NEUROSCIENCE**

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Recent advancements in molecular and genetic approaches are revolutionizing experimental research, enabling a deeper understanding of complex biological mechanisms in health and disease. However, the optimal use of these tools relies on the ability to deliver them precisely and efficiently to target cells within complex organs like the mammalian brain, in living organisms. Achieving anatomical specificity in transgene delivery, matching the precision at which neuronal circuits operate, not only enhances our understanding of normal and pathological brain function but also holds promise for therapeutic applications. Genetically engineered viral vectors are at the forefront of biomedical research, providing powerful tools for delivering foreign genes to precise cellular targets at specific times and locations. In neuroscience, where studies often involve intricate neuronal circuits, viral vector approaches have brought about a true revolution. These tools facilitate gene expression and modulation, anatomical/functional circuit mapping, and neuronal activity monitoring and manipulation, enabling unprecedented insights into brain function.
Among the widely used viral vectors, Adeno-Associated Virus, Lentivirus, and Rabies Virus each offer distinct advantages, making them suitable for different neuroscience research applications. Additionally, specific scientific questions may require the development of novel viral tools with tailored properties, necessitating innovative vector designs. At the Viral Vector Core of the Kavli Institute, NTNU, we have the expertise and infrastructure to produce high-quality viral vectors. As part of the NORBRAIN, a national research infrastructure program, the VVC operates as a non-profit entity. We offer consultation, vector design, and viral vector production services, enhancing research capabilities across the Nordic region.

**Keyword 1**: Adeno-Associated Virus (AAV)
**Keyword 2**: Lentivirus
**Keyword 3**: Rabies Virus
**Keyword 4**: Viral vector
**Keyword 5**: Gene therapy

**INTEGRATION OF AUDITORY AND VISUAL STIMULI IN THE AUDITORY CORTEX**

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Understanding speech in noisy environments can be challenging. Seeing the speaker provides additional cues that aid comprehension. However, it remains unclear which exact aspects of the visual signal support speech comprehension, and how auditory and visual information integrate in the auditory cortex. Here we investigate different visual signals, from simplified outline faces to AI-generated avatars and natural videos. We determine to which degree they support speech-in-noise comprehension and how they influence the neural tracking of speech rhythms in the auditory cortex.
Therefore, we recorded MEG data from 18 normal-hearing participants while they watched videos of a speaker articulating grammatically correct but semantically unpredictable German sentences. After each sentence, participants repeated the words they understood. Sentences were presented in seven conditions: three video types (original, avatar, outline) with and without audio, plus an audio-only condition.
We first analyzed the behavioural data and found that all visual signals aided speech-in-noise comprehension. We analyzed the data using temporal response functions (TRFs) for source-reconstructed auditory cortex activity, focusing on delta, theta, and broadband neural responses linked to speech processing. Significant neural activity was observed across all audio-only and audio-visual conditions. To assess audio-visual integration, we subtracted the audio-only response from the audio-visual conditions, revealing differences, particularly in later peaks (~600ms). In addition, we examined the relation between the neural measures and the behavioural results.
These findings suggest that AI-generated avatars can enhance speech processing by modulating neural entrainment, offering insights into audio-visual integration and potential applications for improving communication in challenging listening environments.

**Keyword 1**: Auditory Processing
**Keyword 2**: Auditory Cortex
**Keyword 3**: Audiovisual Integration

**EFFECTS OF ACTIVE DENDRITIC PROPERTIES ON NETWORK DYNAMICS IN THE STRIATAL-PALLIDAL LOOP**

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There is an emerging view that the external globus pallidus (GPe) of the basal ganglia (BG) acts as an integrative hub owing to its interconnectivity with the cortex, thalamus, and other basal ganglia structures. Earlier experimental and theoretical studies have suggested that pallidal neurons depend on dendritic action potentials to integrate synaptic inputs, but the role of this phenomenon for shaping at the network level activity is not understood. We study this by reconstructing the GPe *in silico*, using biophysically detailed multicompartment neurons, and couple these with an existing model of the dorsolateral striatum based on their morphology and network anatomy. Emulated cortical and subthalamic synaptic inputs are used to study the dynamics of this network. The resulting model predicts that single neuron excitability, as well as detailed electrophysiological features, under current injection can be modelled equally well regardless of dendritic properties. Active dendrites, however, confer a qualitatively different integrative mode in response to synaptic stimulation, where spatiotemporally correlated inputs on the dendrite are preferentially amplified. The increased activity of GPe neurons resulting from spatiotemporally correlated inputs is reflected by inhibition among their afferent striatal targets, suggesting that the effects of correlation-based mechanisms propagate to the network level with effects on striatal activity.

**Keyword 1**: Globus Pallidus
**Keyword 2**: Basal Ganglia
**Keyword 3**: Multicompartment
**Keyword 4**: Dendrites

**THE MOLECULAR CORRELATES OF TOPOGRAPHICAL MAPS IN THE VERTEBRATE OLFACTORY BULB**

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The vertebrate olfactory bulb exhibits a topographical organization, where distinct odor classes are encoded in spatially distinct regions. This chemotopic (or odotopic) map plays a crucial role in olfactory computations. However, the molecular correlates of this organization in projection neurons and interneurons are not fully understood. By generating and utilizing single-cell and spatial transcriptomic atlases for both zebrafish and mouse, we investigated the molecular correlates of olfactory bulb topography. We identified and matched basic cell types across species, revealing a continuum of gene expression diversity in classes of glutamatergic projection neurons and GABAergic interneurons. This molecular diversity correlates with topographical organization of the olfactory bulb, revealing higher transcriptomic similarity among nearby neurons. Both zebrafish and mouse projection neurons in spatially distinct olfactory bulb zones exhibit a high degree of molecular similarity, clearly different from spatially distant olfactory bulb zones. These molecularly identifiable groups of projection neurons are organized into non-overlapping regions across the dorsoventral and mediolateral axes of the olfactory bulbs. In zebrafish, these regions overlap with zones encoding alarm, food, and social odors. However, interneurons exhibit a different logic of molecular topography. In both species, the major axis of molecular diversity is the depth from the surface of the olfactory bulb, where interneurons at various depths exhibit prominent transcriptomic differences. While we observed no obvious zonal organization of interneuron molecular topography along the dorsoventral and mediolateral axes of the mouse olfactory bulb, the zebrafish olfactory bulb exhibits a prominent separation of molecularly different interneuron groups along these dimensions.

**ASSESSING MULTI-ECHO T2*-WEIGHTED FMRI RELIABILITY ACROSS SESSIONS AND SITES IN A MULTI-CENTER STUDY**

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Ensuring the reliability of fMRI data across sessions and sites is crucial for reproducible multi-center studies. Multi-echo (ME) fMRI, which captures data at multiple echo times, enhances signal quality by improving the signal-to-noise ratio and reducing physiological and scanner noise. However, the reliability of ME fMRI - particularly ME T2*-weighted signal components - remains underexplored in multi-center settings. This pilot study evaluates session-to-session and site-to-site reliability of ME T2*-weighted fMRI compared to conventional ME data. Twenty-four healthy participants (8 women, 16 men, aged 21–58) underwent scanning in Brno (Siemens Prisma 3T) and Olomouc (Siemens Vida 3T), each completing three task-based ME runs: Visual Oddball (VOB), Motor Task (SFO), Verbal Fluency Task (VFT). Acquisition parameters included three echo times (14, 34, 54 ms), 3×3×3 mm voxel size, 700 ms repetition time, and a multi-band factor of 6. Data was processed using a composite ME model and T2*-weighted estimation, with reliability assessed via cosine, Dice, and Pearson similarity across the whole brain and task-specific regions of interest (ROIs) from AAL atlas. While contrast maps showed slightly higher reliability for ME model, t-statistics maps revealed comparable reliability for both approaches. Pearson and cosine similarity indicated almost equal performance between ME and T2*-weighted models for SFO and VFT tasks globally. These findings demonstrate that ME fMRI achieves good reliability across sites and sessions and also T2*-weighted data has shown potential for supporting reliability and task-relevant signal integrity, however, our hypothesis of achieving better results than ME fMRI has not been confirmed.

| | Cosine sim. | | Pearson sim. | | Dice sim. | |
|---|---|---|---|---|---|---|
| | mean | SD | mean | SD | mean | SD |
| ME SFO | 0,5192 | 0,1407 | 0,5782 | 0,0988 | 0,4800 | 0,1674 |
| ME VFT | 0,6128 | 0,2205 | 0,6655 | 0,1510 | 0,3924 | 0,2097 |
| ME VOB | 0,5303 | 0,2380 | 0,3895 | 0,2018 | 0,1336 | 0,1609 |
| ME SFO T2* | 0,4944 | 0,1134 | 0,5096 | 0,0996 | 0,4447 | 0,1143 |
| ME VFT T2* | 0,5728 | 0,1795 | 0,5815 | 0,1620 | 0,4400 | 0,1383 |
| ME VOB T2* | 0,2912 | 0,1770 | 0,2029 | 0,1188 | 0,0311 | 0,0437 |

**Keyword 1**: fMRI
**Keyword 2**: multi-echo
**Keyword 3**: data reliability
**Keyword 4**: T2*

**INTRACORTICAL MECHANISMS OF AFTER-DISCHARGES ELICITED BY INTRACRANIAL 50 HZ STIMULATION OF EPILEPTIC PATIENTS**

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Rhythmic stimulation-induced discharges, known as after-discharges (AD), have long been correlated with epileptogenic processes. Nevertheless, the latent neuronal processes are still poorly understood.
Our goal was to delineate cortical domain-specific characteristics of polyspike burst type ADs derived from intracranial macro- and microelectrode recordings. Our study examines the data of 7 drug-resistant epileptic patients undergoing presurgical evaluation with subdural grid electrodes, presenting ADs after 50 Hz stimulation. Simultaneously, laminar multielectrode arrays (LME) were implanted in the hypothesized epileptogenic zone.
Recordings were evaluated during stimulation (n = 6) and ADs (n = 5). We examined 989 stimulation events along with 50 AD-series with overall 797 AD events. Stimulation elicited either increased (81/989), decreased (430/989) or no change (189/989) in multi-unit activity (MUA), depending on the localization of stimulated site relative to the LME. More pronounced change in MUA predicted AD appearance. Additionally, non AD-generating events showed higher MUA when stimulating an otherwise AD-producing site compared to regions where no ADs emerged.
ADs proved to be very localized, detectable changes were found on LMEs in 17/50 series. The initial spikes of individual ADs, associated with infragranular sinks and prominent MUA, were followed by an upper-middle layer wave gradually extending to deep layers.
In sum, stimulation induces excitation in cortical neurons, accompanied by pronounced surround inhibition. The magnitude of this effect is related to the cortical susceptibility to generate ADs. ADs engage cortical layers in a specific sequence. A better understanding of stimulation-dependent neural dynamics may shed light to epleptogenic process within the cortex.

**Keyword 1**: epilepsy
**Keyword 2**: direct cortical stimulation
**Keyword 3**: after-discharge
**Keyword 4**: laminar microelectrode
**Keyword 5**: multi-unit activity

**OPENMINDS SANDS: MAKING BRAIN ATLASES FINDABLE AND INTEROPERABLE USING LINKED DATA**

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Brain atlases are used in all stages of neuroscientific research to visually explore or analyse anatomical data in context of a common spatial framework. Consequently, brain atlases are now increasingly implemented as spatial reference frameworks in digital applications such as atlas viewers, image registration software, and atlas-based analysis tools. However, due to significant variation in the organization and representation of atlas data and associated metadata, digital applications typically employ customized atlas representations. This customization results in a lack of interoperability and comparability among digital applications, and in turn corresponding problems with the data that are semantically or spatially registered to the atlases. To address these challenges, we developed openMINDS SANDS, a metadata model for describing brain atlases and anatomical location data as Linked Data. The SANDS extension is the practical implementation of the Atlas Ontology Model (AtOM; Kleven et al., SciData 10:486, 2023) in the broader openMINDS metadata framework (https://openminds-documentation.readthedocs.io). Here we present the needed adaptations to adopt AtOM and highlight the applicability of openMINDS SANDS for selected commonly used brain atlases. We further showcase the impact of increased machine-actionability of brain atlases for digital application, such as visualization and comparison of brain atlases across distributed systems, and atlas driven analysis of scientific data.

**Keyword 1**: reference atlas
**Keyword 2**: FAIR
**Keyword 3**: knowledge integration
**Keyword 4**: graph database
**Keyword 5**: automatization of computational processes

**THE SHAREBRAIN WORKFLOW: AUTOMATED EXTRACTION, STANDARDIZATION AND OPEN SHARING OF RE-USEABLE METADATA FROM CALCIUM IMAGING AND MULTI-ELECTRODE RECORDING STUDIES**

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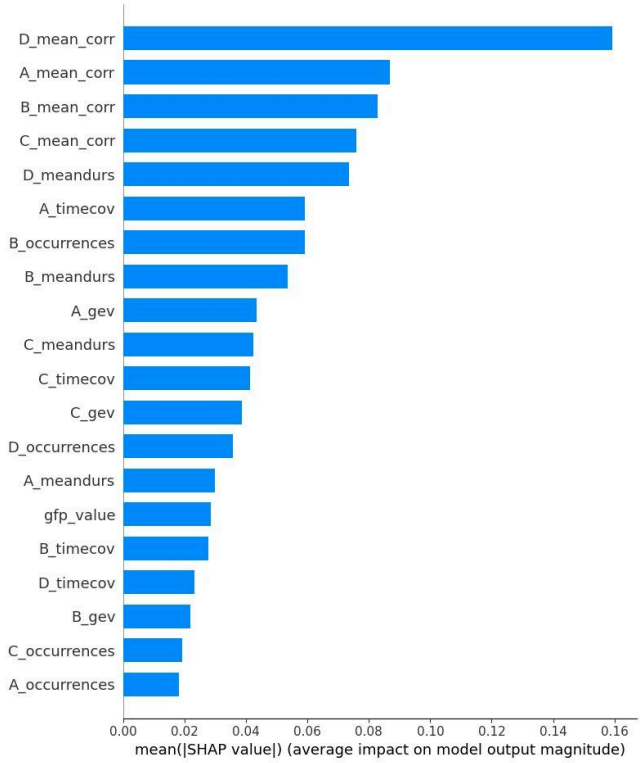
Despite increasing emphasis on open data, approaches to organizing advanced experimental electrophysiology data vary considerably across laboratories, hampering re-use of data. As variability is intrinsic to heterogeneous technical setups and laboratory practices, efficient tools for extracting and organizing metadata in standards suitable for automated retrieval, integration and analysis of data are needed. The SHAREbrain workflow is a user-friendly approach bridging the gap between specialized laboratory practices and community-endorsed standards such as Neurodata Without Borders (NWB) and openMINDS. The workflow builds on the interactive data management platform NANSEN and is designed to eliminate boilerplate handling of diverse file types and metadata. Researchers map existing folder structures and lab-specific file formats through a stepwise graphical user interface, generating standardized representations of subjects, sessions, and variables. Custom file adapters are readily specified for previously unsupported file types. Data can be converted to NWB and metadata to openMINDS using dropdown menus, form dialogs, and modular “converter functions”, rather than intricate code. We validated the workflow by generating consistently organized and rigorously annotated calcium imaging- and multi-electrode recording data integrated into the EBRAINS Research Infrastructure for heightened discoverability and citation. The SHAREbrain workflow allows laboratories to maintain existing data acquisition routines, while offering intuitive, GUI-driven standardization tools lowering the barrier to sharing interoperable neurophysiological data. Looking ahead, we envision expanded support for data analyses within NANSEN, two-way dataset import, and advanced features such as automated metadata extraction aided by large language models, together accelerating the transition to robust, shareable, and reproducible neuroscience data practices.

**Keyword 1**: FAIR
**Keyword 2**: Data standardization
**Keyword 3**: Data Reuse

**AN EXPLAINABLE EEG MICROSTATE ANALYSIS FRAMEWORK FOR DEMENTIA AND MCI CLASSIFICATION**

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Dementia is a growing global health challenge, emphasizing the need for early and accurate diagnostic methods. Electroencephalography (EEG) offers a promising non-invasive approach to detecting subtle neurological changes, yet conventional methods often fail to capture brain activity's transient and complex nature. To this end, we introduce an end-to-end framework that leverages EEG microstates-discrete, quasi-stable topographical patterns to identify dementia-related biomarkers, and feature ranking to identify key neural biomarkers distinguishing dementia, mild cognitive impairment (MCI), and healthy controls. Our approach encompasses three key stages: (1) automated extraction of microstate features, (2) classification using machine learning algorithms to distinguish between dementia, MCI, and healthy controls, and (3) feature ranking via Shapley Additive Explanations (SHAP) to identify the most relevant microstate features contributing to disease differentiation. Experimental evaluations on the publicly available Chung-Ang University Hospital EEG dataset demonstrate that our framework not only achieves high classification accuracy but also enhances interpretability by revealing specific microstate features associated with cognitive decline. In particular, our microstate-driven approach attains a classification accuracy of 92%, outperforming state-of-the-art baselines. Shapely-based feature importance ranks D_mean_corr, A_mean_corr, B_mean_corr, and C_mean_corr as the top predictors, underscoring the critical role of per-time-point correlation values assigned to each microstate in detecting dementia. Our findings highlight the efficacy of microstate-based features in dementia detection, particularly when integrated with advanced machine-learning classifiers. By harnessing EEG's high temporal resolution and capturing transient brain dynamics, this framework paves the way for scalable, cost-effective diagnostic tools for early dementia detection.

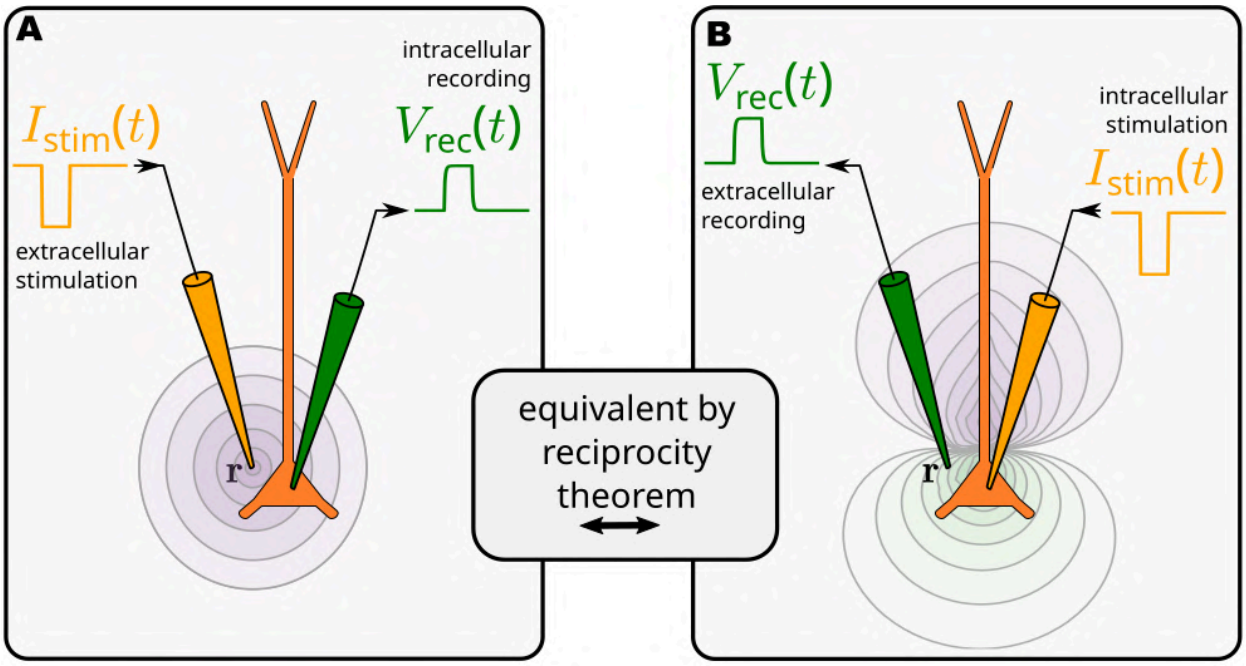



**Keyword 1**: Dementia
**Keyword 2**: EEG
**Keyword 3**: AI
**Keyword 4**: Microstates

**PREDICTING NEURAL RESPONSES TO INTRA- AND EXTRA-CRANIAL ELECTRIC BRAIN STIMULATION BY MEANS OF THE RECIPROCITY THEOREM**

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Electrical stimulation of nervous tissue is grounded in well-established biophysical principles, yet understanding its precise effect on neural activity remains challenging. This represents a major obstacle to both scientific and clinical applications of electrical brain stimulations. We here show that the reciprocity theorem of electromagnetism can be applied more broadly than previously acknowledged, providing a framework for elucidating the effects of electrical brain stimulation on neurons. Through this perspective, we account for the observed weak frequency-dependence of extracellular stimulation–induced membrane potential changes, and the $1/r$ falloff for nearby and $1/r^2$ falloff for distant neurons. We also show that transcranial electrical stimulation at established safety limits (<4 mA) induces somatic membrane potential changes less than 0.1 mV in pyramidal cells, exhibiting weak spatial but strong orientation dependency across human neocortex.




**Keyword 1**: electric brain stimulation
**Keyword 2**: transcranial electric stimulation
**Keyword 3**: extracellular potentials
**Keyword 4**: modelling

**AN INNOVATIVE APPROACH FOR CONDUCTING 3D ELECTROPHYSIOLOGICAL RECORDINGS WITHIN INTACT ORGANOIDS**

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Organoid technology is a pivotal tool for exploring human physiology and diseases. Despite its potential, current readout capabilities constrain organoid electrophysiological research. Classical microelectrode arrays (MEA) fall short in capturing data from intact organoids, which may flatten in the 2D-MEA surface, jeopardizing physiological responses and data validity. To overcome this, we pioneered a mesh MEA, reducing morphological deformations, fostering 3D growth, and facilitating electrical activity recording within intact organoids over an extended period. Electrophysiological recordings of human brain organoids were performed in an MEA-2100 headstage from MultiChannel Systems, accommodating classical MEA and mesh MEA chips. Extracellular neural activity, sampled at 25 kHz and filtered at 400 Hz for spike detection, accurately reflected action potential events on the membrane. Neuronal migration around the mesh was monitored using light microscopy. The mesh MEA integrates 60 titanium nitride electrodes (30 µm diameter) at the nodes of a 2D polymer mesh with a pitch of 200 µm and filament width of ~20 µm and thickness of ~10 µm. The mesh scaffold is suspended 2 mm from the bottom of the well. From preliminary measurements, spike time analysis revealed heightened activity after seven days on the mesh MEA (mean firing rate 34 Hz) compared to acute recordings on a classical MEA (5 Hz). Microscopy images illustrated neuronal migration, dendritic growth, and axon development around the mesh structure and electrodes. These findings suggest that the mesh MEA holds great promise for comprehensive, long-term organoid electrophysiological studies, providing deeper insights into human functions and disorders.

**Keyword 1**: Organoids
**Keyword 2**: neuronal activity
**Keyword 3**: synaptic plasticity

**SIGNAL INTEGRATION AND COMPETITION IN A BIOPHYSICAL MODEL OF THE SUBSTANTIA NIGRA PARS RETICULATA**

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The substantia nigra pars reticulata (SNr) is a primary output for basal ganglia signalling. It plays an important role in motor control, integrating inputs from upstream structures in the basal ganglia, before sending organised projections to a wide range of targets in the midbrain, brainstem and thalamus. Here we present a detailed *in silico* model of the mouse SNr, including its major afferent inputs. The electrophysiological and morphological properties of SNr neurons are characterised in acute brain slices via whole cell patch-clamp and morphological reconstruction. Using reconstructed morphologies, multicompartmental models of single neurons are instantiated within the NEURON simulation environment and populated with relevant modelled ion channels. Model parameters are optimised via an evolutionary algorithm, such that simulated neurons faithfully reproduce recorded electrophysiological behaviour. Using the simulation infrastructure software *Snudda*, single neuron models are incorporated into a circuit-level model, where the sparse lateral connectivity within the SNr is recreated. We simulate the mouse SNr at scale, featuring realistic volumes and neuronal density. The unique synaptic properties of different afferent sources are captured *in silico*, and several experimental observations are reproduced by simulating afferent activation. Our model suggests that paradoxical activity increases in response to striatal stimulation can be explained by lateral connectivity. Furthermore, we employ this model to predict SNr responses to afferent inactivation, temporally coincident competing inputs, and to predict the functional impact of altered glutamatergic signalling.

**Keyword 1**: Basal Ganglia
**Keyword 2**: substantia nigra pars reticulata
**Keyword 3**: simulation

**3D RANDOM-ACCESS RECORDING OF MEMBRANE VOLTAGE OF CORTICAL NEURONS FOR *IN-VIVO*SPIKE DETECTION**

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Optical sampling of spiking activity of neurons in 3D-distributed cortical circuits during animal behaviour permits analysis of cortical circuit function. However, continuous volumetric scanning schemes in two-photon microscopy are inefficient when sampling from 3D-distributed cells at high temporal resolution. Custom-access serial holography (CASH) is a more efficient 3D random-access scan mode that uses holographic light patterning for two-photon excitation of activity indicators to maximize their single-shot fluorescence yields, speed of acquisition and robustness against *in-vivo* cell target motion. Here, we demonstrate CASH action potential recordings at 4 to 10 kHz of *in-vivo* spontaneous activity in up to 10 neurons in layer2/3 of visual cortex expressing FORCE1f, a novel ultrafast membrane voltage indicator optimized for supra-threshold voltage signals, in a head-fixed mice on a treadmill. We compare the experimental data to synthetic data obtained from a dynamic model of the FORCE1f indicator to predict spike detection yields as function of sampling rate, signal-to-noise, applied denoising and level of fluorescence background.

**Keyword 1**: Holography
**Keyword 2**: Random-access
**Keyword 3**: Voltage imaging
**Keyword 4**: Visual cortex

**ACOUSTIC AND LINGUISTIC MARKERS IN SPEECH FOR SCREENING AND MONITORING OF SUICIDE RISK IN PATIENTS WITH MOOD DISORDERS**

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Suicide risk assessment in psychiatric patients often relies on self-disclosure and interviewer expertise. This study investigated acoustic and linguistic features of speech to improve risk assessment. We collected voice recordings from 104 patients diagnosed with major depressive disorder or bipolar disorder during clinical interviews at baseline and follow-ups over a year. Suicidal ideation and behavior were assessed using Beck's Scale for Suicidal Ideation and the Columbia Suicide Severity Rating Scale. Using automatic speech recognition, 217 speech features were extracted, and speech patterns were compared between suicidal and non-suicidal patients, adjusting for covariates including age and sex. Gradient boosting models were trained to classify patients at high risk of suicide and to detect increases in suicide risk over time. Model performance was evaluated using fivefold cross-validation and external validation. We identified robust differences in speech patterns in suicidal patients, including lower mean sentiment scores, higher use of predicative endings, and death-related words. Acoustic and linguistic markers of suicidality varied by sex. The between-person classifier identified high-risk individuals with an area under the curve (AUC) of 0.734 (95% CI: 0.732–0.737), while the within-person model, which detected worsening suicidality over time, achieved an AUC of 0.669 (95% CI: 0.665–0.673), compared to a null model AUC of 0.611 (95% CI: 0.607–0.616). Our findings highlight the potential of automated voice analysis as an objective and scalable tool for suicide risk assessment. Implementing speech-based screening in clinical and telemedicine settings may enhance early detection and intervention for individuals at risk.

**Keyword 1**: Speech analysis
**Keyword 2**: Suicide
**Keyword 3**: Mood disorder
**Keyword 4**: Automatic speech recognition
**Keyword 5**: Machine learning

**VALIDATION STUDY OF THE COGNISCAN COMPUTERIZED TEST FOR MEMORY AND PROCESSING SPEED IN A BRAZILIAN SAMPLE**

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Objective: To evaluate the psychometric properties of the Cogniscan screening test, a self-administered software for assessing episodic and working memory, focusing on validating its accuracy in a Brazilian clinical sample. Participants and Methods: This study involves 300 volunteers aged 18 years or older with at least four years of schooling. Participants will answer a sociodemographic and quality of life questionnaire, in addition to taking the G-38 intelligence test, the Recognition Memory Test (TEM-R-2), attention tests (AOL) and the computerized Cogniscan, which assesses episodic and working memory and processing speed. The results of the Cogniscan are expected to be equivalent to those of the TEM-R-2, showing impressive reliability and construct validity indices. Results: Preliminary data from 54 participants show a mean age of 36.7 years (SD = 13.4), with a predominance of women (66.7%, n = 33) over men (33.3%, n = 18). Regarding education, 42.6% of the participants had higher education (n = 23), and 37% (n = 20) had postgraduate degrees. The results of the cognitive tests were expressed as means and standard deviations, demonstrating the variation in the use of Cogniscan as a screening tool. Conclusions: Initial results suggest that the Cogniscan test has potential as an effective tool for cognitive monitoring and early detection of declines. Validation of this software may significantly contribute to preventive and therapeutic interventions, promoting a better quality of life in the different stages of aging.

**Keyword 1**: Computerized neuropsycholopgical testing
**Keyword 2**: Memory complains
**Keyword 3**: Neurophsychological assessement

**PREVALENCE OF DRUG-INDUCED MOVEMENT DISORDERS IN MENTAL DISORDERS USING AI-POWERED DATA EXTRACTION**

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This meta-analysis systematically examines the global prevalence of extrapyramidal symptoms (EPS) among patients with mental disorders taking antipsychotic medication. A comprehensive search of databases such as PubMed, MEDLINE, EMBASE, PsycInfo, and Google Scholar was conducted for literature from 2018 to 2024 using predefined keywords. Observational studies reporting antipsychotic-induced EPS were included. Data extraction was performed using an artificial intelligence (AI) based retrieval-augmented generation (RAG) tool. Our proposed tool does not require any data-specific fine-tuning to extract data for meta-analysis studies. Meta-analysis was conducted using SPSS and R software. Subgroup analysis was performed based on geographical location. Nine studies, with a total of 15,332 patients, met the specified criteria for inclusion. The pooled prevalence of EPS was 26.70%, with drug-induced parkinsonism being the most common (16.51%). Subgroup analysis revealed higher effect sizes in Asia (0.28) and Africa (0.27) compared to Europe and America (0.13).
Our study shows that antipsychotic-induced EPS are prevalent globally, with regional population-specific variations. Drug-induced parkinsonism was the most frequent EPS. Further research on demographic and clinical factors influencing EPS is needed to optimize patient care.

**Keyword 1**: mental health
**Keyword 2**: Extrapyramidal Symptoms
**Keyword 3**: Antipsychotics

**A NOVEL SCIENTIFIC KNOWLEDGE GRAPH TO INTEGRATE PUBLIC METADATA AND ANALYZE TRENDS IN NEUROSCIENCE RESEARCH**

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As open research data grows, the heterogeneous and complex nature of neuroscience research outputs makes it difficult to collect, organize, and analyze data, hindering data integration, retrieval, and impact monitoring. Scientific Knowledge Graphs (SKGs) are increasingly used to represent and link research objects, but face overwhelming challenges related to the huge amount and variability of data representations. To pave the way for more efficient representation, retrieval and impact monitoring of neuroscience data, the Neuroscience pilot of the EU funded SciLake project has established a novel neuroscience SKG by integrating scholarly metadata automatically harvested from journal publications in the OpenAIRE Graph (https://www.openaire.eu/) with open and curated neuroscience datasets from the EBRAINS research infrastructure (https://search.kg.ebrains.eu/). We connected metadata for neuroscience-related publications in OpenAIRE to corresponding openMINDS metadata (https://github.com/openMetadataInitiative) used in the EBRAINS Knowledge Graph to create a new SKG that was loaded into an AvantGraph (https://avantgraph.io/) instance to facilitate for advanced citation analyses. An entity recognition tool from SIRIS (https://www.sirisacademic.com/r-d) was used to establish connections between datasets and publications via domain-specific entities. The SKG can be accessed programmatically or through a user interface that highlights linked datasets and publications along with citation metrics. We here demonstrate how this SKG accommodates innovative queries and yields novel information about emerging trends such as the uptake and relevance of relevant datasets or publications. The neuroscience SKG provides researchers and services with a powerful tool for exploring research uptake, impact and trends in the neuroscientific research landscape, optimising research and data sharing efforts.

**Keyword 1**: Scientific Knowledge Graph
**Keyword 2**: Impact monitoring
**Keyword 3**: Research landscape analysis
**Keyword 4**: Multimodal data

**BIDS2OPENMINDS: FACILITATING AUTOMATED CONVERSIONS BETWEEN NEUROSCIENCE DATA STANDARDS**

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BIDS and openMINDS are expert- and community-driven standards that facilitate FAIR data management in the field of neuroscience. BIDS is a commonly adopted data model that standardizes the file organization and metadata annotations per dataset within a data repository, such as openNeuro.org or publicneuro.eu. The openMINDS metadata framework provides integrated metadata models for Linked Data, libraries of ontology-based metadata instances and supports rich search capabilities in graph database systems, such as the EBRAINS Knowledge Graph. To facilitate ingestion of metadata from BIDS datasets into such graph database systems, we developed the bids2openminds converter. bids2openminds is an open-source project, provided as a python package with a command line interface (https://github.com/openMetadataInitiative/bids2openminds). It builds on the functionalities of, pyBIDS and openMINDS_Python. However semantic mapping between BIDS and openMINDS terminologies had to be hard coded. Currently, the converter supports the BIDS modalities structural and functional Magnetic Resonance Imaging (MRI), Electroencephalography (EEG), and Intracranial Electroencephalography (iEEG). We demonstrate the metadata conversion using bids2openminds on BIDS compliant, open access datasets. Converted to openMINDS and registered in the EBRAINS Knowledge Graph, data and metadata have an increased findability via the search user interface and advanced query options. Through the semantic integration data are linked to other datasets within and beyond the EBRAINS database, increasing their interoperability and reusability. The bids2openminds converter facilitates automatic registration of BIDS compliant datasets in openMINDS-based graph databases, fostering interoperability and alliances across different data management systems (e.g., publicneuro.eu and EBRAINS).

**Keyword 1**: automated conversion
**Keyword 2**: graph database
**Keyword 3**: Linked Data
**Keyword 4**: FAIR

# Sensory and Motor Processing and Perception

**OSCILLATORY DYNAMICS IN DYSLEXIA: INVESTIGATING NEURAL AND BEHAVIORAL CORRELATES OF TEMPORAL SAMPLING DEFICITS IN CHILDREN**

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Disruptions in neural oscillations are increasingly recognized as key contributors to neurodevelopmental disorders, including dyslexia that affects up to 7% of schoolchildren and is characterized by persistent reading and phonological processing difficulties. Recent studies suggest a sampling deficit in low-gamma band in dyslexic adults leading to the inability to properly track phonemes in a speech flow and thus, impaired grapho-phonological correspondence deteriorating reading acquisition capacities. Emerging evidence suggests broader uncompensated disruptions across multiple temporal scales in children with dyslexia, including slower oscillations critical for prosodic segmentation. This study investigates neural deficits in auditory sampling in children with dyslexia and their relationship to reading and phonemic processing abilities. Using auditory steady-state responses (ASSR) to amplitude-modulated white noise at specific frequencies, we examine phase synchronization and oscillatory power in dyslexic (N = 26) and control (N = 26) children, aiming to characterize how oscillatory dysfunctions across different temporal scales contribute to reading difficulties. Preliminary findings indicate that dyslexic children perform significantly worse than controls on linguistic tests (phonemic awareness and reading tasks). Neural data reveal a sampling deficit in the auditory cortex at two key temporal scales: slow rhythms (2 Hz) and fast rhythms (30 Hz). Across all tests we could not find the correlation of 2 Hz activity with behavioural scores, while the 30-Hz deficit was associated specifically with phonological performance. These findings support the hypothesis that dyslexia involves a multi-scale disruption in neural speech tracking, affecting phonemic processing, therefore, providing potential neurophysiological targets for intervention.

**Keyword 1**: dyslexia
**Keyword 2**: reeducation
**Keyword 3**: rhythm
**Keyword 4**: speech
**Keyword 5**: reading

**VISUAL ENCODING BY RETINAL GANGLION CELLS IN OPTOGENETIC MODELS FOR VISION RESTORATION**

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Retinal degeneration is one of the leading causes of blindness and optogenetics as a potential therapy has garnered much attention. Naturally light-sensitive molecules like Channelrhodopsin (ChR2) and other engineered ion channels are inserted into the retina to play the role of light-sensing elements after the loss of photoreceptors. Previous studies have shown responses of retinal ganglion cells (RGCs) in blind animal models with optogenetically modified retinas based on simple light stimuli. Our study aims to directly compare encoding by RGCs under photoreceptor and optogenetic stimulation in response to spatiotemporally complex and natural stimuli. Furthermore, we would like to estimate an optimal stimulation of such modified retinas to elicit responses like that in a normal retina. Our experiments using multielectrode array recordings with RGCs expressing ChR2 showed light-dependent optogenetically driven responses in the RGCs. Temporal filtering was faster under ChR2 activation and the estimated receptive fields of the RGCs with photoreceptor-evoked and ChR2-evoked responses had a significant difference in size. The responses of RGCs to natural images under photoreceptor and optogenetic stimulation were characteristically different with distinct shapes of response curves. Based on this, the natural images were modified by thresholding, scaling and blurring and presented to the RGCs under ChR2 activation. We saw that certain types of modifications elicited stronger and more similar responses to the photoreceptor-evoked responses than unmodified images under ChR2 activation in a cell-type dependent manner. These findings will help in generating better optogenetic therapies by optimizing stimulation of optogenetically modified retinas to achieve more natural vision.

**Keyword 1**: Optogenetics
**Keyword 2**: Vision restoration
**Keyword 3**: Retina

**CORTICAL AUDITORY EVOKED POTENTIALS IN SUBJECTS WITH MILD COGNITIVE IMPAIRMENT AND ALZHEIMER'S DISEASE: A CROSS-SECTIONAL STUDY TAKING HEARING LOSS INTO ACCOUNT**

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Alzheimer's disease (AD) is the most common cause of dementia, accounting for 60-70% of dementia cases. Early diagnosis and follow-up of disease progression are important for timely interventions. Cortical Auditory Evoked Potentials (CAEPs) have shown promising results as an early prognostic biomarker for cognitive decline and disease progression. However, it is important to note that hearing loss alters CAEP morphology similarly to cognitive impairment, i.e. prolonged peak latencies and reduced peak amplitudes. Hearing loss is common among older adults and a known risk factor for the development of dementia. Nevertheless, studies investigating the effect of both cognitive impairment and hearing loss on CAEP morphology are lacking. This study aimed to evaluate the effect of cognitive impairment on CAEP components while correcting for hearing loss. This cross-sectional study included 54 subjects with mild cognitive impairment (MCI) or AD and 54 cognitively healthy control subjects matched for sex, age, and hearing levels. CAEPs were evoked using a classic auditory oddball paradigm and CAEP waveforms were compared between cognitively impaired and cognitively healthy subjects. Furthermore, correlations between CAEP signals and Repeatable Battery for the Assessment of Neuropsychological Status for Hearing Impaired Individuals (RBANS-H) total score and subdomain scores were assessed. This study observed no significant alterations in CAEP morphology in subjects with MCI or AD when the effect of hearing loss was considered. Additional CAEP studies investigating the effect of both cognitive impairment and hearing loss are needed to confirm these findings and to investigate the effect of both on CAEP morphology.

**Keyword 1**: Evoked response potentials
**Keyword 2**: Cognition
**Keyword 3**: Hearing loss

**THE INFLUENCE OF STIMULUS DURATION ON THE ACOUSTIC CHANGE COMPLEX IN NORMAL-HEARING ADULTS**

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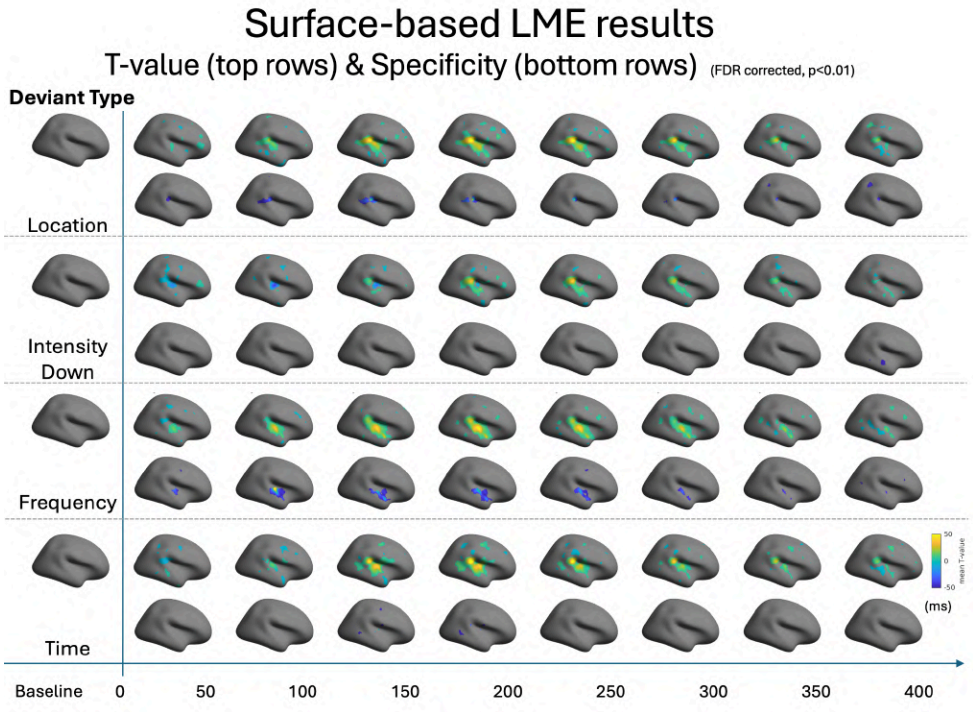
**Aims**: The acoustic change complex (ACC), a cortical auditory evoked potential, reflects the brain's ability to detect changes in ongoing sounds and can be clinically applied for auditory evaluation. This study investigates the influence of stimulus duration on the ACC in normal-hearing adults, highlighting that shorter durations are beneficial for clinical applications, while longer durations enhance amplitude size of the ACC.
**Methods**: Twenty normal-hearing adults participated. ACC N1-P2 responses were recorded using stimuli of varying durations. The stimuli consisted of a 1000 Hz base frequency that increased by 12%, with base frequency durations varying from 1000 to 5000 ms. Data were analyzed using linear mixed models exploring the effects of stimulus durations and sex on ACC amplitude and latency.
**Results**: ACC N1-P2 amplitudes significantly increased with increasing base stimulus duration. Stimulus durations of 5000 ms resulted in 2.3 times larger amplitudes compared to those evoked by a duration of 1000 ms. A duration of 3000 ms was the shortest duration that yielded clear responses (> 3 μV) in all participants. Female participants exhibited significantly larger ACC N1-P2 amplitudes and shorter N1 latencies compared to males across stimulus durations. At every stimulus duration, the ACC N1-P2 amplitude is 1.5-2 times larger in female compared to male participants.
**Conclusions**: Longer base stimuli enhance the ACC response, improving signal-to-noise ratios, while also highlighting consistent sex differences in auditory processing. Clinically, using a 3000 ms stimulus duration is recommended for short, yet reliable ACC measurements, balancing test efficiency and patient comfort.

**Keyword 1**: acoustic change complex
**Keyword 2**: frequency change
**Keyword 3**: stimulus duration
**Keyword 4**: normal hearing

**SPATIOTEMPORAL DYNAMICS OF AUDITORY PREDICTION ERROR PROPAGATION VIA INTRACRANIAL EEG IN HUMANS**

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The neural network underlying human auditory deviance detection is not fully understood. To address this, we recorded SEEG from 31 adult patients with drug-resistant epilepsy who had depth electrodes implanted in all brain lobes. Patients passively heard a stream of bilaterally presented tones while reading. We used the Optimum-1 paradigm [1], which consisted of 300 standard tones interleaved with 300 randomly presented deviant tones per block (3 to 10 blocks per patient). Deviant tones differed from standards in: 1)intensity, 2) frequency, 3) sound source location, 4) shorter duration, or 5) silent gap in the middle (the last two types condensed as Time deviants). Electrode coordinates were obtained from MRI and CT images using the iElectrodes toolbox and projected to a standardized brain surface space. Non-epileptic channels were bipolar referenced (N=2050), and high-frequency band activity (HFA, 64-256 Hz) was extracted. We used linear mixed-effects models to analyze the effect of the different deviant types on the HFA at all cortical vertices with a 10ms temporal resolution. The figure below shows the spatiotemporal dynamics of prediction error propagation. The top rows show the main effects per deviant type, and the bottom rows show region-specific larger prediction errors against other deviant types.
Overall, the spatiotemporal dynamics of prediction errors of “What” (Frequency/Intensity) tend to follow the ventral auditory pathway, whereas the “When and where” (Location/Time) tend to follow the dorsal pathway.
[1] Näätänen et al., 2004, DOI:10.1016/j.clinph.2003.04.001

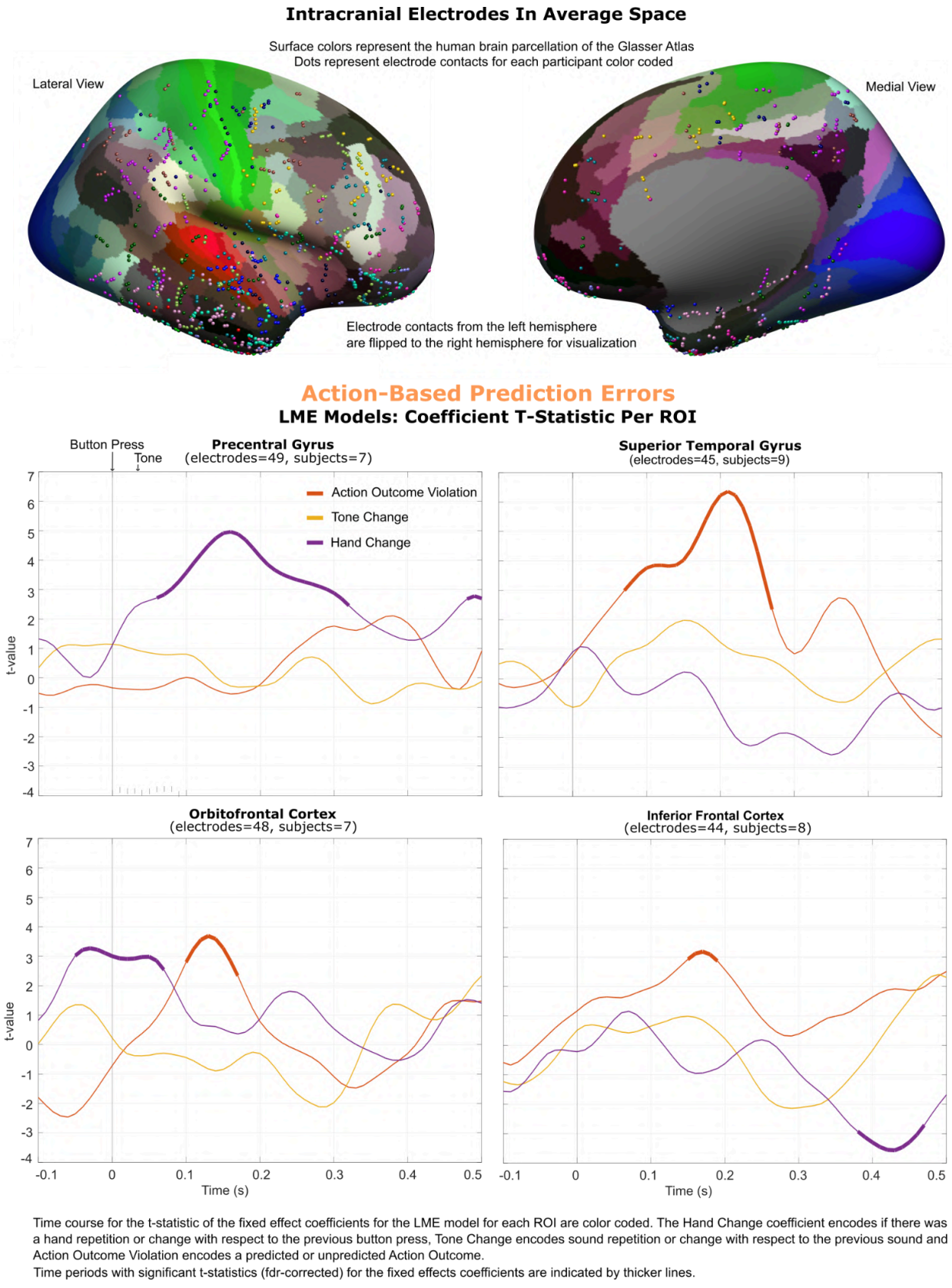


**Keyword 1**: predictive processing
**Keyword 2**: deviance detection
**Keyword 3**: mismatch
**Keyword 4**: auditory ventral pathway
**Keyword 5**: auditory dorsal pathway

**INTRACRANIAL CORRELATES OF ACTION-BASED AUDITORY PREDICTION ERRORS IN HUMANS**

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The brain predicts sensory consequences of actions to prepare sensory cortical areas and reduce their processing activity. This capacity is thought to rely on action-related forward prediction by an internal motor-based forward model. Relatedly, the predictive coding framework suggests that perception relies on internal models making predictions about upcoming sensory events based on statistical probabilities.
The goal of the study was to delineate the brain network involved in action-based prediction error processing caused by environment-related action outcomes (i.e., action-sound associations learned in the experimental setting), by analysing intracranial high-frequency activity (75-145 Hz), which is a proxy for local neural firing. A self-paced, two-choice random generation task, infrequently inducing deviant action outcomes was used. Participants repeatedly pressed a right and a left button associated with a 70ms long 1kHz and 2kHz tone, respectively. Occasional deviants inverted the learned button-tone association. Participants were instructed that button presses should be random, at a regular but self-paced tempo of one per 1-2s, and that they should press both buttons with equal probability.
We analyzed intracranial EEG data from 17 adults with drug-resistant epilepsy undergoing presurgica




l monitoring via implanted stereotactic electrodes. Electrode coordinates and anatomical labels

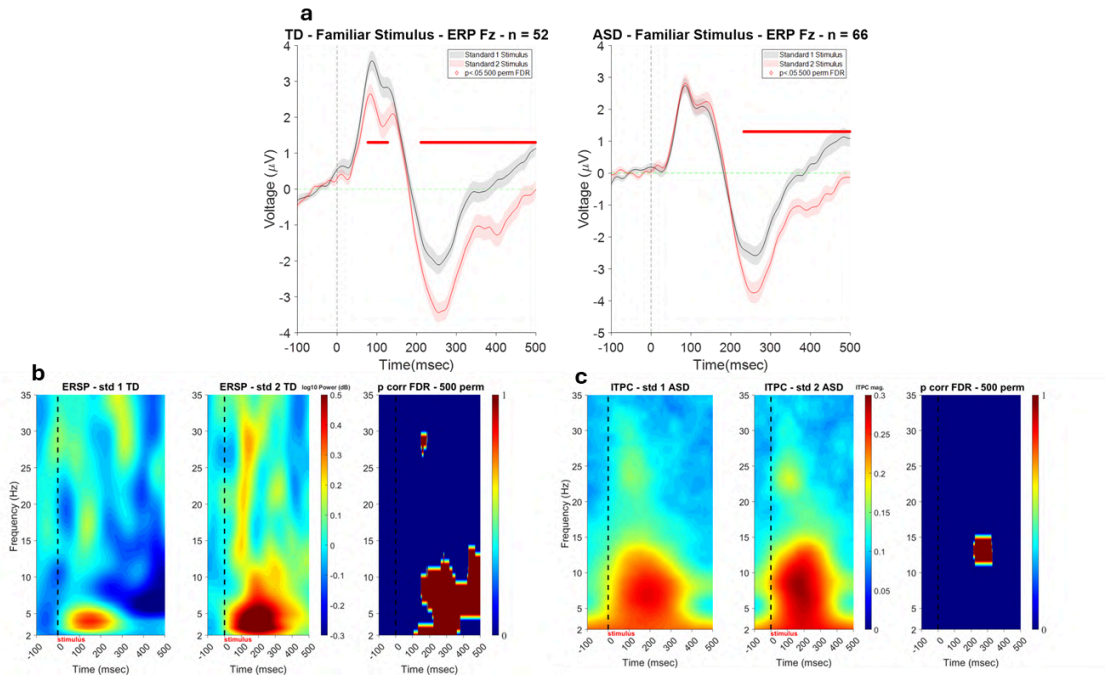
were obtained from coregistered MRI and CT images using iElectrodes toolbox. The results indicate that violations of the sensory effects of actions modulated high-frequency activity in widely distributed brain regions including the insula, temporal and prefrontal cortices, but not motor areas, resembling brain networks reported for sensory prediction errors based on statistical probabilities of the stimuli.

**Keyword 1**: Intracranial EEG
**Keyword 2**: Action Based Sensory Prediction
**Keyword 3**: Prediction Error
**Keyword 4**: High Frequency Activity
**Keyword 5**: Auditory Perception

**CORTICAL RESPONSE TO REPEATED SOUNDS IN CHILDREN, ADOLESCENTS AND ADULTS WITH AND WITHOUT AUTISM**

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In the auditory domain, neurophysiological responses reduce to repeated sounds (repetition suppression/ RS). This process is fundamental to learning as it enables filtering out of irrelevant information, facilitating novelty detection. Differences in the processing of auditory inputs, as reflected by changes in RS, have been observed in autism.
We examined RS in an auditory oddball paradigm on time series from 173 typically developing (TD) and 203 autistic individuals aged 6-31 years. EEG was pre-processed using an automated pipeline. Event-related changes in temporal and spectral domains were determined for whole scalp and Fz channel.
In children, there was significant RS for all components only in TD group and this was accompanied by an increased amplitude in delta, theta and alpha waves. In autistic group RS was observed only for the N2 component and this was accompanied by an increase in inter-trial phase coherence in alpha waves (Figure 1). Both TD and autistic adolescents displayed RS. While RS in TD adolescents was accompanied by an increase in amplitude across ~ 4-14 Hz frequency range, this spectral change was present in a narrower range (~ 5-12 Hz) in the autistic group.
We showed differences in both temporal and spectral domain in response to repeated auditory stimulus in autistic relative TD group. Changes in excitation – inhibition (E/I) balance and dysfunction in GABA signalling are important mechanisms underlying differential cortical response to auditory stimuli. Currently we are examining E/I alterations on resting state time series from same individuals to further understand cortical response in RS.



**Keyword 1**: autism
**Keyword 2**: ERP
**Keyword 3**: ERSP
**Keyword 4**: ITPC
**Keyword 5**: auditory

**THALAMOCORTICAL-LIKE CIRCUITS SHOW HIERARCHICAL SENSORY REPRESENTATIONS IN THE ANCESTRAL VERTEBRATE FOREBRAIN**

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Sensory information is first processed by various thalamic nuclei before reaching the pallium in mammals, where it is first processed by sensory selective cortices before reaching the associative cortices. This feature has been shown to be highly important for separating different sensory information streams and later combining them for higher-ordered computations such as object-recognition and spatial navigation. In zebrafish, the dorsal telencephalon is considered analogous to the vertebrate pallium and supports complex behaviors such as social interactions, learning and spatial navigation. However, it remains unclear how sensory information is transmitted to and processed in this cortical-like structure. Using a combination of anatomical tracing, electrophysiological circuit mapping, and in vivo Ca2+ imaging, we characterized a thalamocortical-like pathway in juvenile zebrafish. We found that the zebrafish dorsal telencephalon receives the majority of its incoming sensory information (visual and mechano-sensation) from the preglomerular nucleus (PG). By imaging the PG neurons and their axonal projections in the dorsal telencephalon, we revealed that they transmit topographically organized and sensory-selective responses to distinct subregions in the dorsal telencephalon. In contrast, topographically organized dorsal telencephalic neurons displayed diverse responses following sensory stimulation ranging from sensory-specific responses to mixed selectivity and nonlinear responses. Similarly to the thalamocortical systems in mammals, our results revealed that the zebrafish dorsal telencephalon integrates sensory information in a hierarchical manner from the thalamic-like PG to distinct zones in the dorsal telencephalon. This indicates that pallial hierarchical sensory processing is a highly conserved and important feature found across vertebrates.

**Keyword 1**: Thalamocortical
**Keyword 2**: Zebrafish
**Keyword 3**: Multisensory integration
**Keyword 4**: Hierarchical processing
**Keyword 5**: Population code

**THE MINIMAL EXPOSURE DURATIONS REQUIRED FOR PERCEIVING AND EMBODYING EMOTION**

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Human faces contain multiple attributes, including information about configuration, emotion, and intention. While the visual system is specially attuned to detect and recognise human faces, the order in which it extracts meaningful attributes—and the extent to which this extraction occurs in the absence of awareness—is largely unknown. Here, we used a novel LCD tachistoscope that enables visual presentations as brief as 0.002 ms to determine the minimal exposures required for the visual system to extract information about facial configuration and emotional expression by combining psychophysical, electroencephalographic, and electromyographic markers of emotional processing, as well as markers of conscious awareness. Importantly, we combined EEG measures of perceptual emotion processing (i.e., markers that distinguish between emotional and non-emotional stimuli) with EMG measures of embodied emotion processing (i.e., facial mimicry of emotional expressions). We found that both perceptual and embodied markers of emotional processing emerged with 4 ms of exposure—a duration that was sufficiently long to also elicit psychophysical and electroencephalographic markers of conscious awareness. Our findings suggest that the processing of facial emotion does not occur in the absence of awareness but rather arises alongside it. Furthermore, our findings challenge the hypothesis that facial mimicry—a phenomenon believed to be critical for empathy and social communication—occurs unconsciously by showing that facial mimicry unfolds as emotional information enters awareness.

**Keyword 1**: Consciousness
**Keyword 2**: Face perception
**Keyword 3**: Emotion
**Keyword 4**: EEG
**Keyword 5**: Psychophysics

**NON-VERIDICAL TACTILE EXPERIENCES IN VIRTUAL REALITY: THE ROLE OF MULTISENSORY INTEGRATION AND BODY OWNERSHIP IN SHAPING TACTILE PERCEPTION**

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Vision and touch interplay can shape tactile perception and body ownership (BO), as vision can guide and deceive touch[1,2] and BO illusions may alter tactile perception[3]. Using a Virtual Reality (VR) setup, we studied the role of multisensory integration and BO in generating non-veridical tactile experiences. Fifty participants viewed either a virtual hand (Hand-condition; HANDc) or cube (Cube-condition; CUBEc) on a VR table. A visual stimulus targeted the virtual object at one location, while tactile stimulation was applied to the same (Same-stimulation; SAMEs) or different (Different-stimulation; DIFFs) location on the participant's hand. Participants localized the perceived touch on their hand. They also completed an ownership task, reporting ownership over the virtual object after 60 seconds of SAMEs or DIFFs. Tactile localization errors and ownership were analyzed. Errors included vision-biased-errors (VBE; touch perception shifted toward the visually-stimulated location) and mislocalization-errors (MISE; where reported location matches neither the real hand nor visually-stimulated location). In HANDc, VBE in DIFFs were greater than MISE in SAMEs and DIFFs [$p<.001$]. In CUBEc, VBE in DIFFs were greater than MISE in SAMEs [$p<.01$] but not DIFFs. VBE in DIFFs were higher in HANDc than CUBEc [$p<.01$]. Ownership was greater in HANDc than CUBEc [$p<.001$]. We found VBE in both HANDc and CUBEc, showing that multisensory integration can generate non-veridical tactile experiences even in the presence of non-body-like objects. However, the higher VBE in HANDc, where higher ownership was found, indicates that the feeling of BO over a virtual hand amplifies the altered feeling of touch.

**Keyword 1**: Body Ownership
**Keyword 2**: Multisensory Integration
**Keyword 3**: Virtual Reality
**Keyword 4**: Non-veridical tactile experience

**ATTENTIONAL MODULATION OF OTOACOUSTIC EMISSIONS EVOKED BY SPEECH-DERIVED STIMULI: EXAMINING THE ROLE OF RESOLVED AND UNRESOLVED HARMONICS**

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The human auditory system excels at selectively focusing on target sounds such as speech while suppressing background noise. This may be aided by efferent feedback from the brain to the inner ear, or cochlea. The efferent feedback may be able to regulate the amount of mechanical amplification in the cochlea in specific frequency bands to aid selection of a target voice.
Here we employ otoacoustic emissions (OAEs), a byproduct of the cochlear amplification, to study this issue. We previously developed a novel mathematical framework to measure speech-OAEs that arise as distortion product otoacoustic emissions (DPOAEs) from the harmonics of the fundamental frequency of a voice [1]. Here we extend this methodology to include multiple pairs of harmonics instead of a single pair only. This allows, in particular, to assess both resolved and unresolved harmonics in the same recording.
In total, 40 participants aged 18 to 31 took part in the study. First results indicate that attentional focus indeed modulates the morphology of the DPOAEs. Further analysis will give insight into the differences between resolved and unresolved harmonics and their degree of modulation due to attentional effects.
Our study highlights the potential of speech-OAEs to study the role of top-down feedback to the inner ear for speech processing. It also demonstrates the importance to differentiate between resolved and unresolved harmonics.

[1] Saiz-Alía, M., Miller, P., & Reichenbach, T. (2021). Otoacoustic emissions evoked by the time-varying harmonic structure of speech. Eneuro, 8(2).

**Keyword 1**: Otoacoustic emissions
**Keyword 2**: Auditory attention decoding

**DIFFERENCES IN NEURAL TRACKING OF THE SPEECH ENVELOPE BETWEEN HEARING-AID AND COCHLEAR-IMPLANT USERS**

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Despite advances in hearing-aid (HA) and cochlear implants (CI) technology, speech perception in cocktail party situations remains a major challenge for their users. Neurofeedback, using EEG to identify the attended speaker, could optimize HA and CI processing, but a deeper understanding of neural speech tracking in hearing impaired individuals is required.
To this end, we collected EEG data from 25 bilateral CI users, 29 bilateral HA users and 29 age-matched controls in a selective auditory attention paradigm, where competing audiobooks were presented through loudspeakers. We assessed the participants' speech comprehension and their subjective listening effort.
A linear backward model was used to reconstruct the attended speech envelope and decode the attended speaker. One-way ANOVA was used to test for group differences. If applicable, post-hoc comparisons were carried out using unpaired t-tests. All p-values were FDR-corrected.
Behaviorally, we found that subjective listening effort ratings increased successively from controls to HA users to CI users (all $p<0.01$). Comprehension scores were significantly reduced in the CI group only ($t=6.91$, $p<0.001$). The decoding accuracy of the attended speaker was lower for the CI group ($t=6.31$, $p<0.001$), which could be explained by higher reconstruction scores of the distractor envelope ($t=4.84$, $p<0.001$). Also, the HA group showed increased reconstruction scores of the distractor speaker compared to the control group ($t=2.01$, $p=0.048$), while no group differences in reconstructing the attended envelope emerged.
Our findings provide evidence that speech perception difficulties in cocktail party environments among hearing-impaired listeners are linked to impaired cortical suppression of the distractor speaker.

**Keyword 1**: EEG
**Keyword 2**: Neural envelope tracking
**Keyword 3**: Hearing Aids
**Keyword 4**: Cochlear Implants

**CORTICAL BRODMANN AREA 7 MODULATES THE DEFENSIVE BLINK REFLEX RESPONSE**

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The blink reflex is a prototypical defensive behaviour, mediated by a medullary brainstem circuit. Despite being a reflex response, the BR size reflects the nervous system's implicit estimation of the potential to harm of the eliciting stimulus. When elicited by electrical stimulation of the human median nerve (hand-blink reflex, HBR), the HBR is strongly modulated by a number of experimental factors related to the value of blinking. For example, the HBR doubles in magnitude when the stimulated hand is close to the face . Although such modulation of the HBR medullary circuit relies on top-down cortical efference, it is unclear which *cortical* areas are involved. Here, in healthy human participants, we assessed the proximity-dependent HBR enhancement following manipulation of Brodmann area 7 (BA7), the human homologue of the ventral intraparietal area (VIP) in monkeys, which encodes the value of actions related to interacting with objects near the body. We used transcranial direct current stimulation (tDCS) to either enhance or inhibit BA7 activity, as well as transcranial alternating current stimulation (tACS) to entrain BA7 activity at the frequency of beta-band oscillations (i.e. 20 Hz). We found that enhancing BA7 activity significantly increased both the overall HBR magnitude as well as the proximity-dependent HBR enhancement compared to a sham condition. Moreover, 20-Hz entrainment significantly inhibited the blink reflex response. These results indicate that BA7 modulates the excitability of the brainstem circuitry subserving the HBR, and hence is part of a cortical network involved in estimating the value of defensive blinking.

**Keyword 1**: Hand Blink reflex
**Keyword 2**: Defensive Behaviour
**Keyword 3**: Brain Stimulation and Electrophysiology
**Keyword 4**: Brainstem Circuit
**Keyword 5**: Sensorimotor Processing

***CHARACTERIZATION OF PUPILLOMETRY FLUCTUATIONS IN FREELY MOVING RATS DURING PERCEPTUAL MISMATCH ERRORS***

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The brain constructs a coherent representation of the environment based on sensory and proprioceptive information. This critical ability to interpret dynamic signals relies not only on multisensory integration, but also on less understood body-brain coordination. Together, these processes form the neural basis of stable perceptual experience. Traditionally, investigations of neural mechanisms underlying perception have focused on visual processing in head-fixed subjects performing passive perceptual tasks. Therefore, how pure visual information is integrated with dynamic body posture or internal states remains unclear. To better understand how the brain processes visual, postural and motion signals to maintain perceptual stability during natural movements, we study neuronal activity in the primary visual cortex of freely moving rats during rapid behavioural and sensory transitions. Our experimental design combines high-density electrophysiological recordings (Neuropixels) and behaviour monitoring (3D motion capture and facial tracking), with tightly synchronized presentation of 360° visual projections in an enclosed dome. Rats experience either carefully calibrated visual stimuli (eg. distinct visual textures, prediction error signals) or passive conditions (eg. global luminosity changes). In preliminary experiments, we extracted pupil dynamics from facial tracking videos to identify psychometric responses at transitions between distinct visual stimuli. These responses are aligned to discrete postural features (eg. rearing during active visual sampling) and serve as proxy for novelty or mismatch detection. By studying neural activity coupled with body kinematics and pupillometry during specific visual events, we aim to uncover context-dependent sensory processing and gain insight on how predictions in primary visual cortex are flexibly implemented during naturalistic behaviours.

**Keyword 1**: sensory processing
**Keyword 2**: visual perception
**Keyword 3**: facial tracking
**Keyword 4**: posture
**Keyword 5**: error prediction

**AUDITORY PREDICTION RESPONSE IN THE FRONTAL POLE REVEALED USING A NOVEL CASCADE ROVING PARADIGM: A MACAQUE ECOG STUDY**

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Auditory prediction is essential for understanding the structure of the world and living safely. In recent years, mismatch negativity (MMN) has attracted attention as a predictive error signal and is well investigated in psychiatric disorders. However, prediction signals are more difficult to detect than prediction error signals. To elucidate both auditory prediction and prediction error signals, we recorded electrocorticogram (ECoG) during a sequence of auditory stimuli in two macaque monkeys. High-density, custom-made flexible electrodes were used to comprehensively search for prediction signals in higher auditory networks (i.e., auditory and prefrontal cortex). The complex cortical structures of non-human primates, which closely resemble humans, is well suited for translational research. We applied an original auditory paradigm “cascade roving”, a variation of the typical oddball paradigm, to dissociate prediction of novel sounds from adaptation to repetitive sounds. A previous human ECoG study showed broader distribution of prediction error signals in the inferior frontal gyrus for this paradigm (Fujitani et al., 2024). In this study, we found prediction signals in the form of high-beta and gamma oscillations in auditory cortex and prefrontal cortex, particularly in the anterior auditory cortex and frontal pole. Prediction error signals were observed in the orbitofrontal cortex (OFC). In summary, the novel “cascade roving” paradigm made it possible to separate prediction signals from prediction error signals and is expected to provide a new understanding of the hierarchical relationship between them.

**Keyword 1**: auditory prediction
**Keyword 2**: ECoG

**THE ROLE OF INFORMATION ENTROPY IN THE NEURAL PROCESSING OF UNCERTAINTY IN THE AUDITORY SYSTEM**

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Perception relies on precision weighting, which describes the impact of prediction errors (PE) on model updating. Precision weighting is thought to depend on the entropy (i.e., predictability) of sensory inputs: lower-entropy environments enable more precise predictions, whereas higher-entropy environments lead to less precise, more uncertain predictions. The mismatch negativity (MMN) component of the event-related potential is considered a neural marker of PE signaling. In this study, we investigate how information entropy at different hierarchical levels of auditory processing affects MMN and P3 responses in a modified local-global paradigm. Three conditions are presented: (a) low-entropy condition with sequences of harmonic complex tones where deviants differ only in fundamental frequency (f0), (b) moderate-entropy condition with sequences of inharmonic complex tones with jittered and fixed-order sounds, (c) high-entropy condition with sequences of inharmonic complex tones with jittered, randomly ordered sounds. Local and global deviants are introduced by omitting or altering the final element of the sequence. By varying sequence-level and stimulus-level entropy, we aim to dissociate stimulus-based uncertainty from sequence-based uncertainty and examine their effects on MMN and P3 complex. We hypothesize that MMN amplitude will be modulated by local and global rule predictability, with larger PEs in low entropy conditions due to higher precision weighting, and attenuated PEs in high-entropy conditions due to increased uncertainty. The data collection is under way and the results will be presented at the FENS meeting in Oslo.

**Keyword 1**: Predictive processing
**Keyword 2**: Mismatch negativity
**Keyword 3**: Local-global
**Keyword 4**: Entropy
**Keyword 5**: Audition

**CENTRE-SURROUND ORGANISATION OF VISUAL RECEPTIVE FIELD MAY REPRESENT A MODERN ADAPTATION FOR TERRESTRIAL VISION**

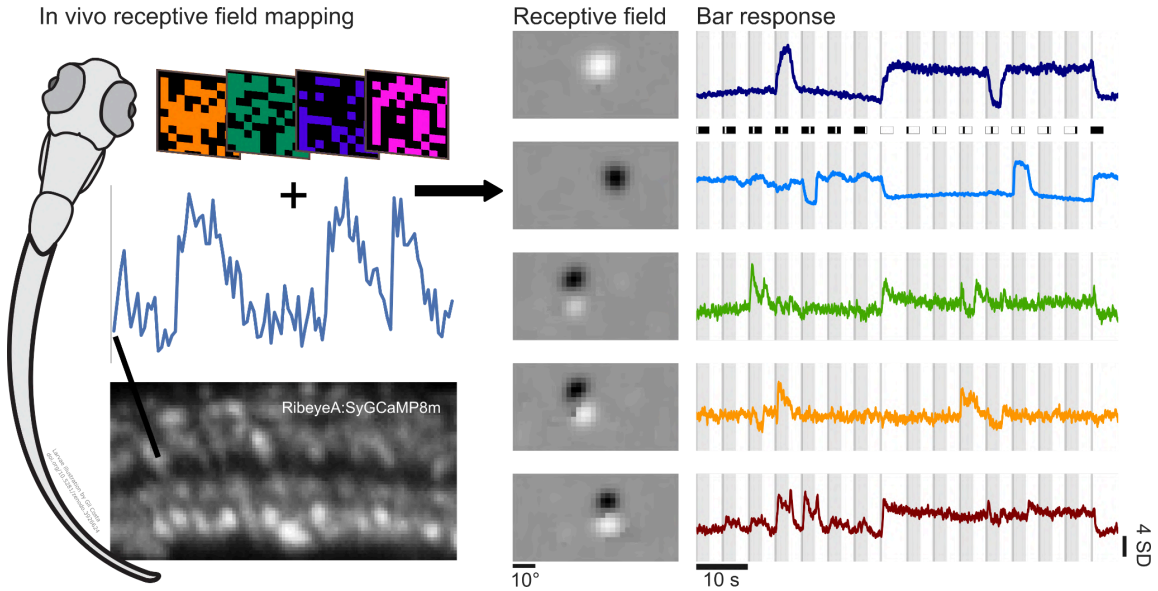
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The centre-surround organisation of receptive fields is thought to represent a fundamental principle of visual circuit organisation: Visual circuits decorrelate spatial contrasts and enhance edge detection via a small receptive field centre and a large antagonistic surround that are approximately balanced.

However, this notion is overwhelmingly based on studies of land-dwelling animals. Terrestrial scenes feature high complexity of high spatial contrasts. Yet, vision originally evolved under water, where spatial contrasts are generally weak, associated with the visual foreground, and systematically varies in spectral content. Accordingly, receptive field organisation should differ versus terrestrial species.

We used 2P in-vivo imaging to record spatio-temporal and spectral receptive fields of retinal bipolar cells in larval zebrafish. We find receptive fields generally invested into spectral rather than spatial decorrelation: Antagonistic surround fields were mostly weak or altogether absent and associated with the Off channel. On bipolar cells instead exhibited notable spectral diversity. Approximately 20% of receptive fields were strongly centre opponent but again lacked an obvious surround.

Next, pharmacological removal of inhibitory inputs increased receptive field sizes in the Off-channel, indicating removal of weak antagonistic surround. By contrast, On-receptive fields remained the same size. This suggests that in the On-channel, zebrafish inner retinal inhibitory circuits do not serve spatial decorrelation. Instead, On and Off bipolar cells systematically retuned their wavelength selectivity following the removal of inhibition.
Together, our results suggest that the antagonistically balanced and ‘spectrally neutral’ centre-surround organisation of visual receptive fields in terrestrial species may represent a



modern adaptation for vision on land.

**Keyword 1**: Vision
**Keyword 2**: Zebrafish
**Keyword 3**: Bipolar cells
**Keyword 4**: Circuits
**Keyword 5**: Receptive fields

**NOVEL COMBINATION OF PI3KDELTA AND CHONDROITINASE ABC GENE THERAPY IN TREATMENT OF SPINAL FOR INJURY**

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Spinal cord injury (SCI) is an untreatable condition characterized by loss of motor function due to axon damage and the inability of CNS neurons to regenerate them. Endogenous factors blocking regeneration are represented mainly by the switch of adult neuron transcriptome from growth promoting programmes to synapse supporting ones. Exogenous factors include the remodelled extracellular matrix (ECM) after SCI which prevents regeneration through chondroitin sulphate proteoglycans (CSPGs). We have shown that PI3Kd overexpression leads to long distance axon regrowth, albeit after longer time periods, and results in significant improvements in fine and gross motor function. Chondroitinase ABC (ChABC) is an enzyme that degrades CSPGs confirmed in many studies of its efficacy in SCI treatment. Here, we used a model of C4 SCI and gene therapy to deliver a novel combination of PI3Kd and ChABC to the brain and spinal cord respectively. Right motor cortex of adult Lister Hooded rat was injected at 4 sites at 1.5 mm depth with AAV1-hSYN-PIK3CD (2.17 x $10^{12}$ gc/ml) + hSYN-GFP (1 x $10^{12}$ gc/ml), concurrent with 2 intraspinal injections near the SCI border (0.5ml/site) with Lenti-PGK ChABC/Lenti-PGK-empty. Control rats received AAV1-hSYN-GFP and Lenti-PGK-ChABC/PGK empty. Weekly testing for 16 weeks to assess grip strength, horizontal ladder crossing and pellet retrieval revealed that PI3Kδ or ChABC administered alone significantly improve upper limb motor function. However, their combination is not synergistic. Further analysis of tissues will assess the combinations effect on regeneration of corticospinal tract neurons and CS-56 analysis to confirm CSPGs degradation.

**Keyword 1**: spinal cord injury
**Keyword 2**: PI3Kdelta
**Keyword 3**: ChABC
**Keyword 4**: gene therapy

**UNRAVELLING THE ROLE OF HEMOKININ-1 IN AGE-RELATED DETERIORATION OF MOTOR COORDINATION AND MUSCLE STRENGTH**

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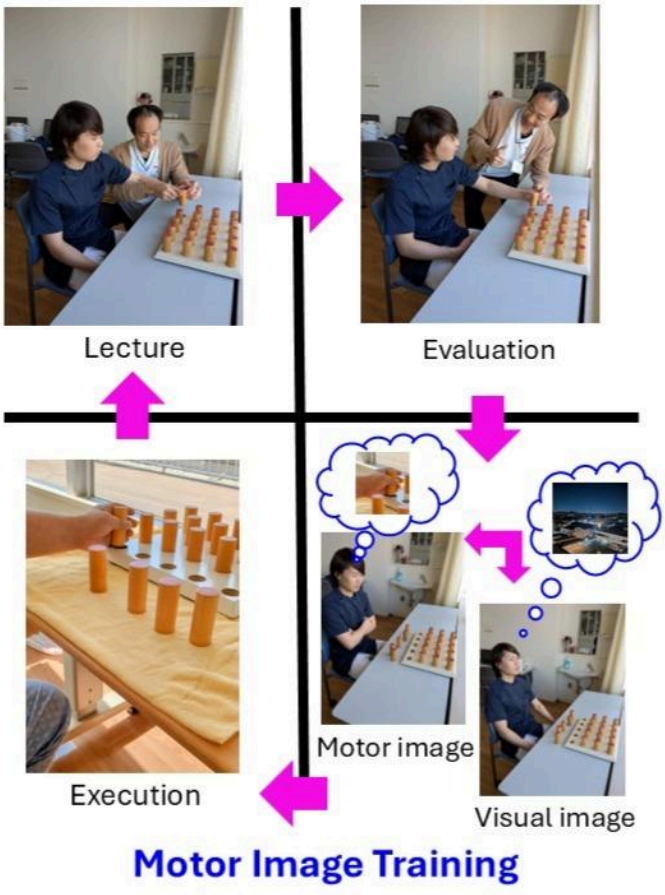
Musculoskeletal problems and consequent fractures significantly reduce the quality of life in old age. Hemokinin-1 (HK-1), the newest member of the tachykinin family, is present in high concentrations in important motor coordination centers, reproductive organs as well as in bones and muscles. Therefore, we investigated its role in age-related motor coordination and muscle function decline in 3-4-, 12- and 18-month-old C57BL/6 wildtype (WT) and HK-1-deficient (Tac4 KO) male and female mice (n=12-20).
We assessed motor coordination in the rotarod and the static rod tests, and muscle strength in the grid and the horizontal bar tests.
No difference was found between the WT and gene-deficient groups in the rotarod test. In aged animals (12 and 18 months), both males and females, a significant deterioration in locomotor coordination was observed in the static bar test, which was significantly more severe in 12-month-old male Tac4 gene-deficient animals compared to the WTs ($p<0.01$), but gene deficiency improved this ability in 18-month-old females ($p<0.01$). In both tests, a significant decline in muscle strength was also detected in older WT animals. In the grid test, the loss of muscle strength was significantly smaller in females compared to males, a phenomenon also observed in the horizontal bar test.
Our results suggest that HK-1 may play a complex regulatory role in motor coordination in old age, also sex differences can be observed. Therefore, elucidating the mechanism of action of HK-1 and its interactions with sex hormones may be important for drug developmental purposes.

**Keyword 1**: Hemokinin-1
**Keyword 2**: motor coordination
**Keyword 3**: muscle strength
**Keyword 4**: tachykinin

**EFFECT OF MOTOR IMAGERY TRAINING ON ACUTE PHASE REHABILITATION IN A STROKE PATIENT WITH PREMOTOR AND PRIMARY MOTOR CORTEX DAMAGE**

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This study was to investigate the effect of motor imagery training on a 52-year-old right-handed male patient with cerebral infarction affecting the right premotor cortex (PMC) and primary motor cortex (M1). Previous academic studies have reported that motor imagery activates PMC function and its connection with M1. However, evidence supporting its efficacy in acute-phase rehabilitation remains limited in clinical settings. The patient presented with motor impairments on the left upper extremity without any higher brain dysfunction. He had no history of psychiatric or neurological disorders.
The motor imagery training method consisted of four stages: (1) Lecture: The patient practiced reaching and gripping a peg with kinesthetic assistance to enhance understanding; (2) Evaluation: The vividness of the patient's motor imagery was assessed through verbal explanation; (3) Motor Imagery: The patient alternated between 20 seconds of visual imagery for a landscape and 30 seconds of motor imagery for reaching and gripping a peg, repeated eight times per session; (4) Execution: The patient physically practiced reaching and gripping various objects. The training was conducted over approximately two weeks during acute phase rehabilitation.




Main outcome measures included timed reaching and gripping actions with five pegboards, five marbles, and ten clothespins. Pre-intervention times were 60.43, 60.15, and 53.11 seconds, respectively. After two weeks of hospitalization, post-intervention times improved significantly to 15.28, 11.93, and 32.03 seconds, respectively.
In conclusion, these findings suggest that motor imagery training positively may impact motor recovery in stroke patients with PMC and M1 damage during the acute phase clinical setting.

**Keyword 1**: motor image
**Keyword 2**: premotor cortex
**Keyword 3**: reaching and gripping action
**Keyword 4**: acute phase rehabilitation
**Keyword 5**: primary motor cortex

**FUNCTIONAL REINNERVATION OF DEGENERATED FORELIMB MUSCLES FOLLOWING CERVICAL VENTRAL ROOT AVULSION INJURY: THE USE OF A DETAILED GAIT ANALYSIS SYSTEM**

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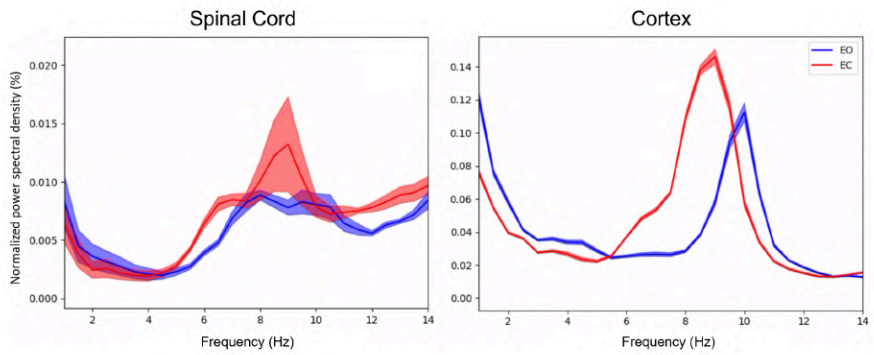
Avulsion injury of the brachial plexus provokes functional disability in the affected limb. Without therapeutic intervention the majority of the affected motoneurons face rapid degeneration leading to paralysis of muscles innervated by the affected segment. With the immediate reimplantation of the affected ventral roots a number of motoneurons can be saved resulting in reinnervation of the affected muscles. In a prior research involving avulsion injuries of the lumbar spinal segments we successfully established our detailed locomotor pattern analysis system. This system has proven to be sensitive and cost-effective tool for detecting alterations in movement patterns of the impaired hind limb. In this current study we explored the diagnostic opportunities of our system in the forelimb following cervical avulsion injuries and reimplantation. To investigate the impact of cervical avulsion injuries, C7 avulsion and immediate reimplantation on Sprague-Dawley rats was performed. We recorded the gait patterns of the animals biweekly. A custom-made plexiglas runway with a mirror system was used to record the steps of the rats with a high-resolution and high-speed digital camera. In total, we measured nine parameters including joint angles, different distances among the participating bones and the footprint area. Twelve weeks after the reimplanation surgery, Fast Blue, a fluorescent retrograde tracer was applied to label the reinnervating motoneurons. Morphological analysis of the spinal cord was carried out with the use of fluorescent microscopy. Our results confirmed the sensitivity of our system and showed a relationship between the kinetic improvement and the morphological reinnervation of the forelimb muscles.

**Keyword 1**: kinematic analysis
**Keyword 2**: spinal cord injury
**Keyword 3**: riluzole
**Keyword 4**: motoneuron death

**RESTING-STATE ACTIVITY IN NON-INVASIVE ELECTROPHYSIOLOGY OF THE SPINAL CORD AND CORTEX IN HUMANS**

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Decades-old invasive electrophysiological recordings in humans have revealed spontaneous background activity at the level of the cervical and lumbo-sacral spinal cord. Invasive spinal electrophysiological recordings alongside surface recordings from the scalp in humans have also revealed similar patterns of resting-state activity in the spinal cord and cortex by contrasting the power of signals in the delta (<4Hz) and alpha (8-12Hz) bands during eyes open and eyes closed recordings. In addition to these electrophysiological approaches, it is furthermore possible to investigate corticospinal resting-state networks in a non-invasive manner via fMRI, but this provides only an indirect measure of neuronal activity.
Here, we are non-invasively investigating resting-state activity using surface electrodes arranged on the scalp (electroencephalography – EEG), as well as the neck and back (electrospinography – ESG). Simultaneous EEG and ESG resting-state recordings were acquired from 24 participants: 2.5 minutes of recording with eyes closed and 2.5 minutes with eyes open. Preliminary analyses revealed differences in power spectral densities at the spinal cord and cortical level during eyes open versus eyes closed recordings at the single participant level (see Figure). Further analyses will now include investigating these potential differences at the group level, examining the coherence of signals across the central nervous system from spinal cord to cortex, as well as determining whether the power in specific frequency bands, such as alpha and delta, hold any predictive value with respect to the participant's state (eyes closed or eyes open).




**Keyword 1**: Human
**Keyword 2**: Electroencephalography
**Keyword 3**: Electrospinography
**Keyword 4**: Resting State
**Keyword 5**: Spinal Cord

**EXPERIENCE-DEPENDENT STRUCTURAL VARIATION IN THE INDIVIDUALLY-MAPPED GENITAL CORTEX OF ADULT WOMEN AFTER CHILDHOOD SEXUAL ABUSE EXPOSURE**

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There is substantial experience-dependent plasticity in the human brain. Previous research identified reduced cortical thickness in the genital field of the primary somatosensory cortex of adult women after childhood sexual abuse (CSA). Functional mapping studies suggest variability in the location of the genital field and demonstrated its plasticity as a function of sexual activity. Notably, the previous study in CSA survivors did not individually map the representation area and did not assess whether its change stems from early developmental programming or lowered sexual frequency in adulthood. To address these gaps, we used an fMRI-compatible sensory-tactile paradigm to individually map the genital cortex in N=128 women with and without CSA exposure. Cortical thickness was extracted from the 10 most activated vertices in this region of interest. We compared the thickness of the individually-mapped field between groups and examined associations with age of abuse onset and sexual frequency over the past year. Preliminary analyses replicate previous findings with higher precision, showing reduced cortical thickness in the individually-mapped genital cortex of CSA survivors. Earlier abuse onset predicted greater cortical thinning. No correlation was found between genital cortex thickness and sexual frequency in the past year. These early results suggest that exposure to CSA has an impact on genital cortex structure, perhaps reflecting an adaptation to developmentally inappropriate and aversive sensory exposure that may be a substrate for sexual dysfunction later in life. Future studies should assess the functional implications of reduced genital field thickness and develop novel interventions to rehabilitate its sensory function.

**MACROPHAGE MIGRATION INHIBITORY FACTOR (MIF) ANTAGONIST ATTENUATES NEUROPATHIC PAIN AFTER NERVE INJURY IN A GENDER DEPENDENT WAY**

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Peripheral neuropathic pain significantly impairs quality of life and remains challenging to treat. Nerve injury triggers complex peripheral and central mechanisms, including interactions between the immune and nervous systems. Macrophage migration inhibitory factor (MIF), a proinflammatory cytokine, plays a crucial role in neuropathic pain development. We investigated the effects of ISO-1, a MIF antagonist, using a chronic constriction injury (CCI) model of the sciatic nerve in adult male and female mice. Behavioral, electrophysiological, immunohistochemical, and molecular analyses were conducted. CCI induced robust mechanical allodynia and thermal hyperalgesia, accompanied by increased excitatory and decreased inhibitory currents recorded in spinal superficial dorsal horn neurons. Additionally, macrophage infiltration was observed in the CCI lesion of the sciatic nerve and dorsal root ganglia (DRG). ISO-1 treatment significantly alleviated these changes in male mice but had no effect in females. MIF mRNA expression in the DRG reflected these sex-specific differences. Our findings suggest that ISO-1 has potential as a neuropathic pain treatment following nerve injury. However, they also reveal sex-dependent differences in neuropathic pain mechanisms, emphasizing the need for targeted therapeutic approaches. Supported by GACR 24-10712S.

**DIVERGENT EEG DYNAMICS FOR FACE ACCESS VS. DISAPPEARANCE IN FLASH SUPPRESSION**

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Breaking and Reverse Continuous Flash Suppression (bCFS and revCFS) are valuable tools for exploring the dynamics of visual awareness. This study investigated the electrophysiological underpinnings of conscious access (bCFS) and disappearance (revCFS) of face stimuli using a time-resolved eXplainable Multivariate Pattern Analysis (xMVPA). We compared EEG activity at each time point against a baseline (the trial's start) to quantify deviations. We employed an explainable AI approach to assess the relevance of each electrode for baseline-versus-time classification.

In a pilot study of 18 subjects, distinct neural patterns emerged when contrasting the dynamics of access and disappearance across tasks. During the first 1000 ms, when the face stimulus ramped from 0 to maximum intensity (suppressed in bCFS, dominant in revCFS), both tasks exhibited increased parieto-occipital activity, with stronger and earlier activation in bCFS. Furthermore, the comparison between access and disappearance revealed that conscious access was characterized by sustained parieto-occipital activity peaking around 400 ms before the response. In contrast, conscious disappearance in revCFS was marked by prominent frontal activity.

Our findings clarify the temporal and electrophysiological dynamics of conscious access and disappearance, indicating that early visual areas primarily modulate access, while frontal regions chiefly regulate disappearance with minimal parieto-occipital involvement.

**Keyword 1**: conscious access
**Keyword 2**: continuous flash supression
**Keyword 3**: unconscious perception

**"CHASING THE LIGHT: THE ROLE OF ARTIFICIAL LIGHT AND SMARTPHONES IN SHAPING SLEEP PATTERNS – A LONGITUDINAL STUDY IN TOBA/QOM COMMUNITIES IN ARGENTINA"**

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Before electricity, human activity was closely tied to daylight, with sleep predominantly confined to the night. The advent of artificial lighting altered this pattern, extending wakefulness. Our research focuses on isolated Toba/Qom native communities in Argentina to understand the evolution of this complex phenomenon. Since 2012, our longitudinal wrist-actimetry studies have shown that individuals in rural areas without electricity exhibit earlier sleep onset and longer sleep duration compared to their urban counterparts (de la Iglesia et al., 2015).
Over time, rural settlements have gained varying access to electricity and mobile devices. With approximately 10,000 nights of recorded sleep data via actigraphy and information via sleep diaries, we monitor shifts in sleep habits considering factors such as season, age, and gender. Our recent findings show a significant delay (~50 min, $p<0.05$) in sleep onset and a reduction (~40 min, $p<0.01$) in total sleep, correlating with increased artificial light exposure. Additionally, we found that smartphone use affects sleep patterns, delaying sleep onset (~25 min, $p<0.05$) and midsleep (~22 min, $p<0.05$). These results highlight the impact of mobile devices on circadian rhythms and sleep, emphasizing the need to continue understanding how technology shapes human behavior.
Our results provide a deeper understanding of how access to electric light and mobile devices influences sleep habits in humans, expanding the knowledge of the complex neurobiology of sleep, which is especially relevant in native communities located in low-middle income countries, where studies on this topic have not been widely conducted.

**Keyword 1**: Sleep
**Keyword 2**: circadian rhythms
**Keyword 3**: native communities

**THE SINGLE-CELL TRANSCRIPTOMIC ATLAS IPAIN IDENTIFIES SENESCENCE OF NOCICEPTORS AS A THERAPEUTIC TARGET FOR CHRONIC PAIN TREATMENT**

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Chronic pain presents a significant clinical challenge, yet its underlying pathophysiology remains poorly understood. Recent studies mapping gene expression in various pain models have revealed distinct patterns. In this study, we constructed unified single-cell atlases of mice dorsal root ganglia (DRG) and trigeminal ganglia (TG) by combining diverse single-cell RNA sequencing datasets from different pain models and time points. Our unified atlases enabled a thorough examination of transcriptome and epigenome transitions from acute to chronic pain and from chronic pain to recovery states. We identified a senescence mechanism underlying chronic pain development and persistence in mice DRG, confirmed in TG and human DRGs in chronic pain conditions. Subsequently, we tested a series of senolytic drugs and observed varying efficacy in reversing the pain phenotype typically present in mice post-injury. Overall, our study suggests a novel therapeutic strategy by targeting cellular senescence in chronic pain conditions.

**Keyword 1**: Pain
**Keyword 2**: Senescence
**Keyword 3**: scRNA-seq
**Keyword 4**: Omics

**THE LATERAL SEPTUM'S ROLE IN PAIN PERCEPTION: UNVEILING MOLECULAR DIVERSITY IN ITS DEVELOPMENT AND ADULTHOOD IN MICE AND HUMANS**

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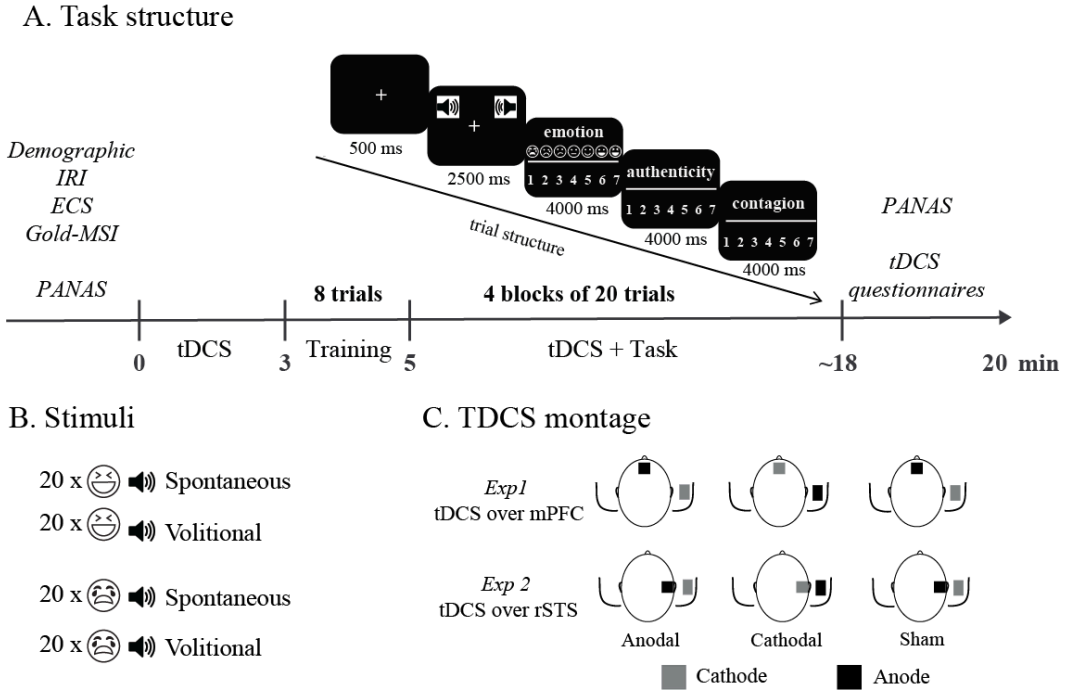
Pain is a crucial mechanism for perceiving and avoiding harm, but its exacerbation in chronic conditions can significantly impact various facets of life. Recent research has identified the lateral septum (LS), a subcortical limbic structure in the brain as a novel component in pain perception in mice. To understand its cross-species conservation, we employed single-nuclei RNA-sequencing (snRNA-seq) along with existing datasets to investigate the cross-species conservation of the lateral septum during development and adult stages in both mice and humans. Our cross-species analysis revealed numerous conserved molecular identities in LS neuronal populations stemming from early developmental timepoints. We then further characterized the gene expression changes of the LS during acute pain stimulation and in chronic pain conditions in mice. Thus, this work lays a foundational framework for further research on the role of LS in pain and therapeutics.

**Keyword 1**: lateral septum
**Keyword 2**: Pain
**Keyword 3**: Development
**Keyword 4**: scRNA-seq

**CAN VOCAL EMOTION PROCESSING BE MODULATED BY TDCS?**

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Inferring and recognising emotions is fundamental for adaptive social functioning. Transcranial direct current stimulation (tDCS) has shown promise in modulating emotional processing, particularly in the visual domain. However, its effects on auditory emotional processing remain underexplored. We aimed to address this gap by investigating tDCS effects over the medial prefrontal cortex (mPFC) and the right superior temporal sulcus (STS), key regions for emotional and social cognition, during emotion discrimination of vocalisations. Participants were divided into two groups (mPFC: n=24; STS: n=25) and underwent sham, anodal, and cathodal stimulation. A 1.5mA current was applied for 5 minutes before and during a task in which participants rated the emotional intensity (on a sad-happy continuum), authenticity, and contagion of spontaneous and volitional cries and laughs. Results for both groups consistently showed that laughs were rated as happier, more authentic, and more contagious than cries, and that spontaneous expressions were perceived as more authentic, positive, and contagious than volitional ones. Stimulation over the mPFC did not modulate these perceptions. In turn, cathodal stimulation over the STS led to an overall sadder perception of all vocalisations compared to sham. These findings highlight the robust capability of humans to recognise emotional vocal expressions and their authenticity, corroborating previous research. The effect of cathodal STS stimulation suggests its potential as a target for modulating auditory emotional processing and underlines the relevance of further examining tDCS effects in vocal emotion perception using alternative protocols, especially in clinical populations with disturbances in this domain.



**Keyword 1**: Emotional vocalisations
**Keyword 2**: Noninvasive brain stimulation
**Keyword 3**: Auditory emotion recognition
**Keyword 4**: Mentalising network

# Brain-Body Interactions: Neuroendocrinology and Metabolism

**THE ROLE OF UNCOUPLING PROTEIN 2 (UCP2) IN STRESS ADAPTATION: INSIGHTS FROM A KNOCK-OUT**

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Stress profoundly impacts both somatic and mental health, and the hypothalamic-pituitary-adrenal (HPA) axis is crucial in stress adaptation. Although uncoupling protein 2 (UCP2), an important mitochondrial protein essential for maintaining energy balance, is expressed in the paraventricular nucleus (PVN) and anterior pituitary, components of the HPA axis, its role in the development of stress-related psychiatric disorders, such as anxiety and depression, remains unclear. To explore this, we utilized a UCP2 knockout (KO) rat model. Behavioural tests showed genotype- and sex-dependent differences. Male UCP2 KO rats displayed increased depressive-like behaviour in the splash test, while female KO rats exhibited increased anxiety-like responses in the contextual fear test compared to wild-type controls. These differences may be due to sex-dependent baseline anxiety and potential ceiling effects. Thirty-six hours after a single foot shock, at the nadir of HPA axis activity, we measured resting corticosterone levels in serum and examined the mRNA expression of stress-regulating neuropeptides in the PVN, pituitary, and adrenal glands. Mineralocorticoid receptor mRNA was significantly altered in KO animals, decreasing in the PVN and increasing in the pituitary, while the expression of other HPA axis components (corticotropin-releasing hormone, proopiomelanocortin, glucocorticoid receptor) remained stable. Given that glucocorticoid levels did not change, mineralocorticoids, such as aldosterone, may contribute to the behavioural differences. Our results highlight the importance of energy regulation in stress adaptation and suggest that UCP2 influences stress-related behaviours, offering new insights into the mechanisms of stress-related disorders. Supported by Project 2024-2.1.1-EKÖP, EKÖP-24-1.

**Keyword 1**: UCP2
**Keyword 2**: stress
**Keyword 3**: HPA axis

**PUTATIVE FENESTRATIONS VISUALIZED IN CAPILLARIES FROM HYPOTHALAMIC TISSUE USING SCANNING ELECTRON MICROSCOPY**

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The blood-brain barrier primarily consists of continuous non-fenestrated endothelial cells (ECs), that regulate brain homeostasis and protect the brain from environmental influences that might damage the brain. However, this also limits the entry of blood borne signaling molecules (e.g., hormones and nutrients). Therefore, circumventricular organs contain continuous fenestrated ECs, which express transcellular pores (fenestrations) allowing for exchange of signaling molecules between blood and brain. One of these regions, the median eminence (ME), is located next to arcuate nucleus, and is critical for sensing blood-derived information. Fenestrated ECs in the ME undergo dynamic remodeling in response to physiological changes, although only a few factors have been identified that trigger these changes. Therefore, we aim to understand the dynamic nature of remodeling of the ultrastructure of ECs in the ME in response to fundamental physiological conditions, such as protein restriction and energy restriction. Visualizing fenestrations is challenging as they are smaller than the light diffraction limit. However, our preliminary data using scanning electron microscopy of rat hypothalamus reveals putative fenestrations in hypothalamic capillaries, which seem to be smaller than fenestrations in liver sinusoidal ECs and are not clustered in sieve plates. Ongoing experiments are extending this work to compare different physiological conditions and see whether characteristics of the putative fenestrations change. In addition, we are using atomic force microscopy to examine fenestrations in liver endothelial cells. Together, this work will further our understanding of the complex remodeling of fenestrated ECs in the brain in different nutritional states.

**Keyword 1**: Fenestration
**Keyword 2**: Median eminence
**Keyword 3**: Electron microscopy
**Keyword 4**: Protein restriction

**IS PROLACTIN A KEY PLAYER IN THE ACUTE STRESS RESPONSE WITH SEX-SPECIFIC DIFFERENCES?**

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Fight or flight survival demands acute stress activation of the hypothalamic-pituitary-adrenal axis (cortisol) and the sympathetic nervous system (adrenaline). Prolactin (PRL), an anterior pituitary lobe peptide hormone, is traditionally known for its function in lactation and reproduction, as well as in chronic stress adaptation and recovery due to its anti-apoptotic and anti-inflammatory protective effects. We found evidence supporting a novel function of PRL as an acute stress modulator. This study focuses on the cellular distribution and vesicle intensity of PRL immunofluorescence (IF) in adult rat lactotrops subjected to acute stressors (restraint versus forced swimming tests) to explore the sex-specific differences. Twenty-six animals were sacrificed to harvest the pituitary glands and embedded in resin. Semi-thin tissue sections were immunostained for PRL and IF analyses showed that PRL distributions and intensities in rat lactotrophs were increased in response to acute stress. The female forced swimming stress group exhibited the highest IF PRL vesicles intensity and manifested more widely scattered abundant PRL distribution patterns, compared to the female control group. In contrast, the male control and restraint stress groups showed higher PRL IF intensity than their female counter-parts. This could mean that PRL *de novo* synthesis and secretion are stimulated by acute stress in a sex-specific manner, attributed to differences in PRL expression and cellular stress intolerance between females versus males. Our findings highly suggest that PRL is not merely involved during lactation but serves as an acute stress modulator, which may be among the functional properties of PRL in males.

**Keyword 1**: Prolactin
**Keyword 2**: Acute-stress
**Keyword 3**: Lactotrophs
**Keyword 4**: Immunofluorescence
**Keyword 5**: Sex-specific

**DUALISTIC NATURE OF MUSCLE-BRAIN CROSSTALK**

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Physical exercise (EX) is the most effective non-pharmacological strategy for promoting health, preventing diseases, and aiding rehabilitation in stroke, depression, or neurodegenerative disorders. Its benefits are largely mediated by brain-derived neurotrophic factor (BDNF), a key regulator of neuroplasticity and neuroprotection. The critical role of BDNF is evident, as anti-BDNF strategies abolish EX-induced cognitive benefits. Recent findings highlight an endocrine dialogue between the brain and contracting muscles, where myokines secreted into the bloodstream stimulate cerebral BDNF synthesis. However, vulnerable populations (e.g., elderly, stroke, COPD or heart failure patients) may have limited capacity to engage in traditional EX. For individuals unable to perform dynamic exercise, electromyostimulation (EMS) - a transcutaneous peripheral electrical stimulation - emerges as a potential alternative to counteract cognitive decline and support brain health. Our team previously demonstrated that acute EMS (two sessions, one week apart) enhances cognition in humans and neuroplasticity in animals via muscle-brain humoral interactions. To amplify these benefits, we applied a chronic EMS protocol (7 days, 30 minute/day; 40 Hz frequency, 400 µs pulse width, 7s ON/14s OFF, progressive intensity: 6-20 mA) on 8-week-old Wistar rats. Unexpectedly, chronic EMS disrupted hippocampal BDNF signaling, reducing neuroplasticity-related proteins. At the muscular level, EMS induced tissue damage and elevated pro-inflammatory cytokines, with increased serum corticosterone. This heightened inflammatory response extended to the hippocampus, where EMS-treated animals exhibited exacerbated neuroinflammation. These findings reveal a dualistic nature in muscle-brain crosstalk and underscore the need to optimize EMS protocols for individuals unable to engage in conventional physical activity.

**Keyword 1**: BDNF
**Keyword 2**: Electromyostimulation (EMS)
**Keyword 3**: Muscle-brain crosstalk
**Keyword 4**: Inflammation
**Keyword 5**: Cognition

**MODERATE-INTENSITY CONTINUOUS TRAINING AND HIGH INTENSITY INTERVAL TRAINING INDUCE GAINS IN BRAIN ENERGETIC METABOLISM THROUGH DIFFERENT PATHWAYS IN AGED RATS**

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Physical exercise is widely recognized as a safe and effective strategy to reverse age-related declines in brain health. Indeed, endurance training can enhance energy metabolism markers associated with brain plasticity. However, the respective effects of two endurance training regimens commonly used in aging population – i.e., the Moderate Intensity Continuous Training (MICT) and the High Intensity Interval Training (HIIT) – in brain energy markers remain unknown in aging. The purpose of this study was to compare the effect of 4 weeks of MICT or HIIT on brain metabolic markers, at both the RNA and protein levels in the cortex and hippocampus of aged rats.
Twenty-two old male rats were randomly assigned to one of the following groups: MICT (n=7), HIIT (n=5) where rats followed MICT or HIIT program respectively, and CONTROL (n=8) in which no training program was performed. To assess endurance performance, incremental exercise tests were performed at PRE and POST. Subsequently, both cortex and hippocampus were removed for Western blot and RNA sequencing analyses.
Both training strategies improved endurance performance, but HIIT induced higher gains. At the molecular level, while both MICT and HIIT influenced specific cerebral metabolic pathways in the cortex and hippocampus, MICT had a broader impact. Furthermore, only MICT improved angiogenesis.
In conclusion, MICT and HIIT are two different effective ways to improve brain metabolic markers. Thus, given their complementary effect, both training strategies should be considered in endurance program for older adults to maximize benefits on brain metabolism-related pathways and endurance performance.

**Keyword 1**: brain metabolism
**Keyword 2**: endurance training
**Keyword 3**: RNA sequencing
**Keyword 4**: aging

**THE SUBJECTIVE AND BEHAVIOURAL EFFECTS OF HEROIN DEPEND ON PERIPHERAL OPIOIDERGIC MECHANISMS**

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The behavioural effects of heroin arising from its subjective and incentive motivational properties have long been considered to be mediated by μ-opioid receptors (μORs) expressed in the brain. However, in humans and relevant preclinical models of substance use disorder (SUD), heroin is self-administered systemically exerting a plethora of pharmacological effects outside the central nervous system. The role of peripheral μORs in the subjective, reinforcing and motivational properties, or the loss of control over the intake, of heroin remains unclear. Capitalising on a novel, highly selective peripherally-acting μOR antagonist, 9g, in a series of experiments in rats, we tested the contribution of peripheral μORs to the discriminative and reinforcing properties of heroin as assessed under a drug discrimination task (DDT) and intravenous self-administration (IVSA), respectively. Pre-treatment with 9g resulted in an increased intake of heroin during acquisition of IVSA, without influencing incentive motivation, but was accompanied by greater acute responding for heroin under an extinction challenge, suggestive of increased incentive properties of IVSA-associated contextual cues. Under extended access, rats pre-treated with 9g exhibited an accelerated loss of control over heroin intake accompanied by higher intake at relapse following 3 days of forced abstinence. Lastly, rats trained to discriminate heroin from saline on a DDT displayed a greater response accuracy over time for heroin when pre-treated with 9g. These findings reveal a hitherto overlooked involvement of peripheral μORs in SUD-relevant behavioural effects of heroin. Additionally, they shed new light on pharmacological mechanisms underlying interoceptive control over the behavioural effects of heroin.

**Keyword 1**: Heroin
**Keyword 2**: Peripheral opioid receptors
**Keyword 3**: Interoception
**Keyword 4**: Substance use disorder
**Keyword 5**: Intravenous self-administration

**HETERODIMERIZATION OF MELANOCORTIN 3 RECEPTOR (MC3R) IN THE PRESENCE OF *A MEMBRANE-ASSOCIATED E3 LIGASE (*RNF11)**

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MC3R-expressing neurons in the hypothalamic arcuate nucleus regulate pathways associated with appetite, energy balance and metabolism, playing crucial role in the body weight control. Additionally, MC3R is linked to growth, with mutations causing late sexual development. MC3R loss-of-functions such as Thr6Lys/Val81Ile MC3R are associated with childhood obesity. In literature, MC3R-GHSR 1A (growth hormone secretagogue receptor) dimerization was found to decrease GHSR 1A intracellular signaling. In this study, we investigated how RFN11 affects the heterodimerization of MC3R with GHSR 1A, as well as how the Thr6Lys/Val81Ile mutations in MC3R influence this dimerization. The interaction efficiency between WT and Thr6Lys/Val81Ile MC3R-GHSR 1A was investigated using spectral FRET in HEK293 cells. The excitation signal was used as 450nm and the emission spectrum was collected with 5nm intervals between 470-650nm. Emission signals were normalized and the Acceptor/Donor ratio was calculated. We found that RNF11 enhances the dimerization of WT MC3R with GHSR 1A, increasing the Acceptor/Donor ratio in FRET experiments. However, RNF11 did not affect the dimerization of Thr6Lys/Val81Ile with GHSR 1A. The efficiency was investigated further by using a specific MC3R ligand, gamma melanocyte-stimulating hormone (γ-MSH) and the RNF11 increased the FRET signal similarly and no significant difference was found for the Thr6Lys/Val81Ile MC3R-GHSR 1A dimer. This suggests that RNF11 promotes the functional interaction of WT MC3R-GHSR 1A heterodimers, potentially influencing anorexigenic signaling, while the Thr6Lys/Val81Ile MC3R variant may disrupt this process. The findings could help guide future strategies for treating MC3R-related obesity.

**Keyword 1**: Hypothalamus
**Keyword 2**: MC3R
**Keyword 3**: Childhood Obesity
**Keyword 4**: Protein Interactions

**UNRAVELLING HOW CEREBROSPINAL FLUID FLOW AND DIFFUSION SHAPE SOLUTE TRANSPORT IN THE BRAIN**

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Cerebrospinal fluid (CSF) is integral to brain architecture, providing mechanical and nutritional support while clearing metabolites. Recent research reveals its potential role in neuromodulation through transport of signaling molecules. Yet, to what extend CSF impacts the direct delivery of such molecules is poorly understood. CSF circulation is tightly regulated by ciliary beating of ependymal cells and pressure gradients driven by cardiac activity and respiration. To investigate the role of motile cilia in CSF flow and transport, we combined computational and experimental approaches. First, we computed flow patterns and simulated solute transport using the geometry of the zebrafish larval brain ventricles, for which we have detailed knowledge of cilia properties and CSF motion velocities and frequencies. Next, we validated our computation model with experimental data by monitoring the dynamics of a photoconvertible fluorescent protein expressed within the ventricles. Our results show that diffusion plays a major role in the transport of small solutes, whereas cilia majorly contribute to the movement of larger particles. We also found that the location of cilia and ventricular geometry impact solute distribution. Next, we investigated the distribution of endogenous metabolites in the adult zebrafish brain by adapting the spatially resolved mass spectrometry technique MALDI-TOF. We identified that large metabolites, such as lipids, are more prone to be spatially organized than smaller molecules, supporting the idea that size matters for transport across the brain. Altogether, our work presents a computational framework together with new concepts of how molecules are transported within the brain and ventricles.

**Keyword 1**: cerebrospinal fluid
**Keyword 2**: modelling
**Keyword 3**: cilia
**Keyword 4**: transport
**Keyword 5**: ventricle

**KEY PREDICTORS OF AGING RATE IN PSYCHOSOCIALLY STRAINED CONDITIONS**

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This study examines physiological and psychological indicators of aging under chronic stress. The sample included 168 adults (mean age = 43.2±5.6 years) in Ukraine during a social and psychological strain period. Twenty-six indicators were analyzed across anthropometric, physiological, and psychological domains. The aging rate was calculated using physiological indicators. Multiple Linear Regression (MLR) and Principal Component Analysis (PCA) identified key predictors and latent factors.
MLR explained 85% of the variance. The strongest predictors of aging rate were waist-to-hip ratio ($\beta = 0.706$, $p < 0.001$), adaptive potential ($\beta = 0.354$, $p < 0.001$), and age ($\beta = -0.524$, $p < 0.001$).
PCA revealed five components with parameters expressing the highest component loadings:

1. Psychological well-being (22.5% variance): subjective well-being (0.943), positive mental health (0.891), personal anxiety (-0.813.
2. Somatic aging component (13.7% variance): waist circumference (0.907), weight (0.813), aging rate (0.790), waist-to-height ratio (0.788).
3. Aging indicators (13.4% variance): chronological age (0.971), biological age (0.953), psychological age (0.921).
4. Cardiovascular function (8.3% variance): systolic blood pressure (0.831), diastolic blood pressure (0.813), adaptive potential (0.732).
5. Respiratory & balance (8.1% variance): Stange test (0.884), Hentsch test (0.862), balance (0,586).

Findings support a multi-component aging model, where somatic dysfunction accelerates aging while psychological resilience mitigates stress-related decline. Anxiety and depression exacerbate aging-related processes. These findings highlight the need for integrated interventions targeting physiological and psychological health to counteract stress-induced aging.

**Keyword 1**: Stress
**Keyword 2**: Well being
**Keyword 3**: Aging

**ASTROCYTIC FOXO1 IN THE HYPOTHALAMUS REGULATES METABOLIC HOMEOSTASIS**

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Astrocytes play important role in the regulation of brain energy metabolism. Forkhead box transcription factor O1 (FoxO1) is a master regulator of cellular metabolism and hypothalamic FoxO1 controls food intake and energy balance. However, role of astrocytic FoxO1 in the regulation of brain energy metabolism and systemic homeostasis is unknown. Here, we report that FoxO1 is critical to maintain the glycolytic nature of astrocytes by regulating the pyruvate dehydrogenase kinase/pyruvate dehydrogenase (PDK/PDH) axis. FoxO1 inhibition shifts the cellular glucose metabolism of astrocytes towards oxidative metabolism, increasing cellular production and release of astrocytic ATP into extracellular environment. Accordingly, astrocytic FoxO1 ablation induces an overactivation of hypothalamic neuronal circuits leading to overfeeding and impaired glucose regulatory mechanism in response to metabolic changes under fasting-refeeding condition and predisposes mice to glucose dyshomeostasis and diet-induced obesity. Targeted deletion of hypothalamic astrocyte FoxO1 replicates such metabolic alterations, suggesting that astrocytic FoxO1 in the hypothalamus plays a key role in the control of brain energy metabolism and whole-body glucose homeostasis.

**Keyword 1**: Astrocyte
**Keyword 2**: FoxO1
**Keyword 3**: energy metabolism
**Keyword 4**: glucose homeostasis
**Keyword 5**: hypothalamus

**METABOLIC FUNCTIONS OF THE HIPPOCAMPUS**

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Survival depends on effective regulation of metabolism. While homeostatic control is mainly attributed to the hypothalamus, recent studies suggest that the neural mechanisms that control metabolism are more complex and distributed beyond the hypothalamus — a notable example being the hippocampus. Traditionally recognized for its role in memory, the hippocampus produces distinct state-dependent neural oscillations, such as sharp wave ripples (SWRs), which support memory consolidation during sleep. Yet, its dense connections to the hypothalamus and expression of receptors for metabolic signals suggest a less recognized metabolic role.
To test this idea, in my previous work, we combined hippocampal recordings with continuous glucose monitoring in freely behaving rodents and discovered that SWRs reduce peripheral glucose levels. Subsequently, I investigated whether SWRs are sensitive to metabolic states and revealed that SWRs are enhanced following meal intake, suggesting a role in postprandial glucose metabolism.
These findings raise critical questions about the role of the hippocampus in metabolic regulation — is it essential for systemic metabolic health? Does it influence the activity of peripheral organs, such as the pancreas? And what would be the broader metabolic consequences of hippocampal dysfunction? In my current work, I aim to address these questions by combining in vivo electrophysiology, neural manipulation tools, and comprehensive metabolic assessment methods. Additionally, I plan to investigate the developmental trajectory of hippocampal metabolic functions, identifying key periods when metabolic regulation emerges. Together, this research will illuminate an overlooked aspect of metabolic control, deepening our understanding of how memory and metabolism are intertwined.

**THE DIFFERENTIAL EFFECTS OF SALVIA SCLAREA L. OIL AND LINALYL ACETATE ON DEPRESSION LEVELS IN DIABETIC FOOT ULCER PATIENTS WITH T2DM**

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Diabetic foot ulcer (DFU) is a significant global health concern due to its substantial burden on individuals and healthcare systems. In addition to its physical impact, DFU has emotional effects on patients. This study analyzed the effects of inhaling Salvia sclarea **L.** (CS; clary sage) or linalyl acetate (LA) on depression and psychological factors in patients with DFU. This study, performed at the Diabetic Wound Center, enrolled DFU patients, categorized as having mild or moderate to severe depression based on visual analog scale (VAS) for depression scores. Patients were randomized to inhalation of 5% CS oil, 5% LA or almond oil (control) by natural breathing. Blood pressure, heart rate, Depression-VAS, Anxiety-VAS, and Stress-VAS were measured before and after inhalation. This study enrolled 72 patients with DFU, including 43 with mild and 29 with moderate to severe depression. Of the 43 patients with mild depression, 14, 14, and 15 were randomized to inhalation of CS, LA, and almond oil, respectively. Of the 29 patients with moderate to severe depression, 9, 11, and 9 were randomized to inhalation of CS, LA, and almond oil, respectively. Compared with inhalation of almond oil, inhalation of CS oil significantly reduced stress and anxiety in patients with mild depression, whereas inhalation of LA significantly reduced anxiety and depression in patients with moderate to severe depression. These findings suggest that adjunct therapy in DFU patients should be individualized according to the degree of depression. This work was supported by NRF (RS-2024-00353184) and NST (CRC22021-300). *Corresponding author.

**Keyword 1**: Depression
**Keyword 2**: Clary sage
**Keyword 3**: Linalyl acetate
**Keyword 4**: Diabetic foot ulcer
**Keyword 5**: Complementary and alternative medicine

**THE COMPUTATIONAL PSYCHIATRY OF INSIGHT: PARAMETERS OF INNER AND OUTER BODY BELIEF UPDATING IN ANOREXIA NERVOSA**

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Background. Patients with anorexia nervosa (AN) typically hold altered beliefs about their body that they struggle to update. These can vary from sensory, local beliefs about how full their stomach feels to global, prospective beliefs about how much they can trust their own body. Questionnaire studies revealed the role of such beliefs in the onset, maintenance, and treatment of AN, yet the parameters that contribute to their formation and maintenance remain unknown. Aims and Methods: I present two studies with independent samples at the acute (total N = 106) and post-acute AN state (N = 113), compared to matched healthy controls (N = 220) that aimed to: (1) assess interoceptive and exteroceptive perception and metacognition using state-of-the-art methods and statistical approaches such as the Heart Rate Discrimination Task (Legrand et al., 2022; M ratio analyses); (2) develop and validate a novel neurocomputational approach using a Bayesian Learning Framework to determine the parameters that may influence explicit belief formation and updating; (4) relate some of these parameters to clinical symptoms such as insight into illness, using measures validated specifically in eating disorders. Results and Conclusions. AN patients showed lower precision-weighted, learning rates than controls in both retrospective and prospective belief updating across domains. However, exteroceptive and interoceptive metacognition, and key, latent parameters of belief updating, predicted different disruptions of clinical insight; exteroceptive, local belief updating difficulties were linked with unrealistic body image beliefs, while difficulties in interoceptive metacognition predicted global insight difficulties.

**Keyword 1**: interoception
**Keyword 2**: body-brain connections
**Keyword 3**: belief updating
**Keyword 4**: interoceptuve metacognition

# Neurochemistry and Neuropharmacology

**THE HCN CHANNEL INHIBITOR ORG 34167 REDUCES EPILEPTIFORM ACTIVITY IN AN *IN UTERO* ELECTROPORATION-BASED MOUSE MODEL OF TSC/FCD2B**

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Tuberous sclerosis complex (TSC) and focal cortical dysplasia type 2B (FCD2B) are developmental disorders caused by pathogenic variation in genes that are part of the PI3K/AKT/mTOR signalling pathway, leading to mTOR hyperactivity. Approximately two thirds of patients with TSC or FCD2B have lifelong refractory epilepsy. These conditions are characterised by the presence of cytomegalic dysmorphic neurons in the cortex, which exhibit ectopic expression of hyperpolarization-activated cyclic nucleotide-gated channel 4 (HCN4). Evidence suggests that this ectopic HCN4 expression can drive mTOR-dependant seizure activity in mice, presenting HCN4 as a promising drug target. We generated a mouse model of TSC/FCD2B utilising *in utero* electroporation with the concomitant injection of a plasmid encoding a constitutively active *Rheb* variant ($Rheb^{CA}$), to increase the activity of mTOR in the developing cortex. This mouse model recapitulates the physiological and morphological characteristics of TSC/FCD2B, including spontaneous seizures and the presence of cytomegalic dysmorphic cortical neurons. We used this mouse model to study and compare the efficacy of antiepileptic drugs, as well as the brain-penetrant broad-spectrum HCN channel inhibitor Org 34167, in TSC/FCD2B. $Rheb^{CA}$ mice display epileptiform spiking on electrocorticography (ECoG), which we used as a quantifiable measure of drug effect. Phenytoin and valproate significantly increased ECoG spike frequency, and there was a strong trend for topiramate to increase spiking. In contrast, both vigabatrin and Org 34167 significantly reduced ECoG spike frequency, with Org 34167 showing the greatest reduction. These data highlight the potential utility of a small molecule HCN channel inhibitor as a precision medicine for TSC/FCD2B.

**Keyword 1**: Epilepsy
**Keyword 2**: Cortical dysplasia
**Keyword 3**: HCN channels
**Keyword 4**: Small molecule
**Keyword 5**: Tuberous sclerosis complex

**ACUTE TREATMENT WITH CURCUMIN ENHANCES HIPPOCAMPAL DELTA OSCILLATIONS SUPPORTING IMPROVED OBJECT MEMORY IN MICE**

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Curcumin, a bioactive compound derived from Curcuma longa, is recognized for its antioxidant and neuroprotective effects. While long-term curcumin administration improves memory, the effects on cognition in the short term remain uncertain. Curcumin affects neurotransmitter systems, increasing levels of monoamines such as dopamine, which suggests a modulatory role in memory through enhanced neural activity. Despite its low bioavailability, which limits its systemic circulation, curcumin's lipophilic nature allows for crossing the blood-brain barrier. We hypothesized that a high oral dose of curcumin would ensure sufficient concentration in the brain to modulate monoamine levels, thereby enhancing cognitive performance. Electrodes were implanted into the hippocampus and medial prefrontal cortex in mice, and curcumin was acutely administered. The curcumin-treated group exhibited better performance in the novel object recognition task. We found stronger delta oscillations in the hippocampus during exploration of novel objects compared to familiar objects. These results suggest that curcumin enhances memory through increased hippocampal delta oscillations. This effect is likely attributable to changes in neural activity within the entorhinal cortex, which sends efferents to the hippocampus. Curcumin may increase dopamine levels and facilitate transitions between Up and Down states in the entorhinal cortex, leading to an increased frequency of delta oscillations. Respiratory rates were shown to increase during object exploration and may synchronize with hippocampal delta oscillations. Considering the established role of delta oscillations in memory retrieval, the curcumin-induced enhancement of these oscillations likely facilitates more effective memory encoding and recall.

**Keyword 1**: curcumin
**Keyword 2**: delta
**Keyword 3**: memory
**Keyword 4**: hippocampus
**Keyword 5**: mouse

**COADMINISTRATION OF SCOPOLAMINE AND MGLU2 NAM VU6001966 AS A NOVEL ANTIDEPRESSANT APPROACH: LOWERING EFFECTIVE DOSE AND REDUCING COGNITIVE SIDE EFFECTS**

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Conventional antidepressants typically require weeks of daily dosing to achieve full response. In contrast, clinical studies have shown that scopolamine, a non-selective muscarinic receptor antagonist, can induce rapid and potent antidepressant effects within a few days of treatment. However, its side effects, particularly memory impairments, remain a concern. Our studies demonstrated the antidepressant-like effects of coadministering scopolamine with the mGlu2 negative allosteric modulator (NAM) VU6001966 in the unpredictable chronic mild stress (UCMS) model of depression in mice. To address scopolamine's adverse effects, this study aimed to investigate the impact of this drug combination on spatial and non-spatial learning in both naïve and stressed mice. Additionally, we examined spontaneous excitatory postsynaptic currents (sEPSCs) frequency and amplitude to assess synaptic transmission properties in layer V medial prefrontal cortex (mPFC) pyramidal neurons. Subchronic coadministration of scopolamine and VU6001966 did not alter object investigation time [%] in the OLT and NORT tests compared to the non-stressed control group. Electrophysiological recordings revealed the main effect of stress on the sEPSC amplitude without altering sEPSC frequency in mPFC neurons. However, the tested drug combination did not modulate the effect of stress on either sEPSC amplitude or frequency. These findings suggest that coadministration of scopolamine with mGlu2 NAM VU6001966 may offer a promising alternative to scopolamine monotherapy for depression, potentially allowing for a lower therapeutically effective dose and reducing adverse cognitive effects.

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**LONG-TERM EFFECTS OF ADOLESCENT 5F-MDMB-PICA INTRAVENOUS SELF-ADMINISTRATION (IVSA): NEUROBEHAVIORAL CONSEQUENCES AND MEDIAL PREFRONTAL CORTEX DYSFUNCTION IN ADULT MICE**

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Synthetic cannabinoids (SC) are the largest group of new psychoactive substances monitored worldwide (EMCDDA, 2022). 5F-MDMB-PICA is a recent SC classified as a potent full agonist at CB1/CB2 receptors able to activate the mesolimbic dopamine (DA) transmission in adolescent but not adult mice (Musa et al, 2020). Here, we have studied its reinforcing effects in adolescent mice and characterized the neurochemical and behavioral effects induced at adulthood. First, by self-administering varying doses (1-5 µg/kg/25µl) in an intravenous self-administration (IVSA) paradigm, we observed an inverted U-shaped trend. The dose of 2.5 µg/kg/25ul elicited behavior aimed at obtaining the substance, as measured by different response ratios. Adult mice exposed to 5F-MDMB-PICA during adolescence showed behavioral and neurochemical differences compare to controls, including a propensity for aggression and reduced social interaction, an anhedonic state, and delayed mPFC DA response to olfactory stress as estimated by in vivo brain microdialysis. In addition, the analysis of the excitatory neuronal activity in the mPFC, by using the fiber photometry technique, revealed diminished responses to aversive stimuli in 5F-MDMB-PICA-exposed mice, compared to controls. These results correlate with the neurochemical analyses indicating disrupted DA signaling. This study provides the first evidence that 5F-MDMB-PICA IVSA is acquired and sustained by adolescent mice at lower doses than that of the prototypical SC JWH-018 (Margiani et al., 2023), confirming a higher abuse liability of this newer SC. Moreover, 5F-MDMB-PICA IVSA during adolescence induced long-term behavioral and neurochemical changes confirming the detrimental consequences related to the use of SC during adolescence.

**THE PROTECTIVE EFFECTS OF ICARIIN IN A RAT MODEL OF CISPLATIN-INDUCED PERIPHERAL NEUROPATHY**

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Effective treatment for cisplatin-induced peripheral neuropathy (CIPN) is still unresolved problem and ongoing research continues to explore potential therapeutic options for this dose-limiting side effect. This study aims to investigate the potential regenerative and restorative effects of icariin in a rat model of CIPN using behavioral (neuropathic pain tests), morphological (immunohistochemistry), and electrophysiological (EMG) assessments. Thirty-two male Sprague Dawley rats (250-300 g, n=8/group) were used in the study. CIPN was induced by ip administrations of cisplatin (3 mg/kg/week for 5 weeks). To prevent nephrotoxicity via hyperhydration, ip saline was concurrently administered to cisplatin-treated rats. ICA (50, 100 mg/kg) was also administered alongside cisplatin. Body weight, mechanical allodynia, thermal hyperalgesia, and cold plate assessments were conducted at baseline (before drug administration) and on the 6th day following each drug administration. Rota rod, footprint and activitymeter tests were performed at baseline and at the end of drug administration period. EMG recordings were obtained using PowerLab. NF200, P-III-tubulin, MBP and S100 levels were evaluated by immunohistochemistry in sciatic nerves. DRG and sciatic nerves were also stained with toluidine blue and examined histopathologically. Cisplatin was found to induce mechanical allodynia, impair locomotor activity and motor balance. ICA at doses of 50 mg/kg and 100 mg/kg improved cisplatin-induced mechanical allodynia but did not significantly affect the motor activity and balance deficits caused by cisplatin. Additionally, icariin alleviated cisplatin-induced EMG disturbances. Immunohistochemical and histopathological degenerations caused by cisplatin seemed to be partially ameliorated by icariin. *Supported by Eskisehir Osmangazi University “Scientific Research Projects” Commission (TCD-2023-2601).*

**Keyword 1**: cisplatin
**Keyword 2**: icariin
**Keyword 3**: neuroregeneration
**Keyword 4**: peripheral neuropathy

**RGH-397 INHIBITS THE FUNCTION OF RECOMBINANT AND NATIVE A5 $GABA_A$ RECEPTORS**

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Excessive activity of α5 subunit containing $GABA_A$ receptors might play an important role in psychiatric disorders, such as depression. Selective reduction of the function of α5 $GABA_AR$ could restore the normal neuronal activity and alleviate the symptoms. Chemical optimization of novel naphthyridine derivatives resulted in a subtype-selective negative allosteric modulator (NAM) of α5 $GABA_ARs$, named as RGH-397. The biological characterisation of this molecule is presented here. Receptor function was investigated in human α1β3γ2, α2β3γ2 and α5β3γ2 $GABA_AR$-expressing cell lines and membrane preparations with displacement assays and whole-cell patch clamp methods. Voltage clamp experiments were carried out in rat brain slices, as well. Finally, effective doses were determined in behaving rats followed by the evaluation of the brain concentrations of the compound. RGH-397 had high affinity to human α5 $GABA_ARs$ and moderate affinity to α1 and α2 $GABA_ARs$ ($K_i$ values of 4.1, 221 and 161 nM), respectively. The compound inhibited α5 $GABA_AR$-mediated current with an $IC_{50}$ of 330 nM and showed no significant inhibition of α1 and α2 $GABA_AR$ currents up to 30 μM. In hippocampal interneurons RGH-397 partially blocked the α5 $GABA_AR$-mediated tonic currents in rat brain slices at the concentration of 100 nM and above. Brain concentrations (56 -182 nM) measured at the effective doses in rats were close to the effective concentrations found in *in vitro* assays. RGH-397 is a potent and selective NAM of α5 $GABA_ARs$. Altogether, the efficacy of RGH-397 was proven on human recombinant receptors, in rat brain tissue and in rodent models as well.

**Keyword 1**: α5 GABAAR
**Keyword 2**: allosteric
**Keyword 3**: patch clamp
**Keyword 4**: depression
**Keyword 5**: cognition

**RGH-560, A NOVEL SELECTIVE POSITIVE ALLOSTERIC MODULATOR OF ALPHA7 NICOTINIC ACETYLCHOLINE RECEPTOR ENHANCES CHOLINERGIC NEUROTRANSMISSION IMPLYING THERAPEUTIC POTENTIAL IN THE FIELD OF COGNITIVE DISORDERS**

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Homomeric alpha7 nicotinic acetylcholine receptors (nAChRs) are ligand-gated ion channels expressed in the central nervous system in cognition relevant areas including the prefrontal cortex, the hippocampus and other subcortical limbic structures. Numerous studies indicate the potential of nAChR ligands to improve cognitive functions. Despite the extensive efforts in the nAChR field effective treatments for neurocognitive disorders remain an unmet need. Therefore, we have developed novel alpha7 nAChR selective positive modulator compounds. Modulators were selected by their effect on intracellular $Ca^{2+}$ elevation using plate-reader based fluorometry and by their effect on the kinetics of choline-evoked currents using automated patch clamp in recombinant human alpha7 nAChR expressing cells. Selected compounds were characterized by manual patch clamp in recombinant and native cells and by patch clamp and multiphoton $Ca^{2+}$imaging in interneurons from rat hippocampal slices. Our candidate compound (RGH-560) is an example of a new azetidinespirochromone chemotype. At 100 nM RGH-560 shows decelerating effect on current decay of choline-induced current without agonist effect. At 1 μM it evokes inward current and more pronounced effect on current decay. RGH-560 increased both the potency and the efficacy of choline to evoke responses. It also significantly enhanced choline-evoked intracellular $Ca^{2+}$ elevation and spontaneous activity in hippocampal neurons. All these effects can be blocked by the alpha7 nAChR selective antagonist MLA. RGH-560 was shown to be effective in vivo in rodent models of cognition. Our data uncover the unique and promising properties of this novel positive modulator of alpha7 nAChR.

**Keyword 1**: nicotinic acethylcholine receptor
**Keyword 2**: patch clamp
**Keyword 3**: allosteric modulator
**Keyword 4**: cognition

**EVALUATION OF CENOBAMATE'S EFFECTS ON CISPLATIN-INDUCED PERIPHERAL NEUROPATHY IN RATS**

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Peripheral neuropathy is a common and dose limiting side effect of chemotherapy, particularly associated with platinum-based drugs like cisplatin, which can lead to sensory, motor, and autonomic nerve damage. This study investigates the potential effects of cenobamate, a new antiepileptic drug, to prevent or treat cisplatin-induced peripheral neuropathy in a rat model. Male Sprague-Dawley rats (200-300g) were divided into four groups, 8 rats each,: control, cisplatin, and two senobamate-treated groups (10mg/kg and 30mg/kg). Peripheral neuropathy was induced by ip administrations of cisplatin (3mg/kg) once weekly for five weeks. Cenobamate was co-administered with cisplatin. Neuropathic pain was assessed at baseline and every six days after cisplatin injection using mechanical allodynia, cold allodynia and thermal hyperalgesia tests. Motor function (rotarod, locomotor activity and footprint) was evaluated at baseline and at sixth week. Data were analyzed using two-way ANOVA for repeated measures followed by Tukey's test as post hoc in SPSS software. The results showed significant improvements in paw withdrawal latency at week six in both cenobamate-treated groups compared to the cisplatin group across all three neuropathic pain tests. In footprint analysis, cenobamate treatment showed significant improvement in the in-between steps distance compared to the cisplatin group (p=0,002), that may indicate enhanced motor function. These findings suggest that cenobamate may mitigate cisplatin-induced peripheral neuropathy, highlighting its potential as a therapeutic agent for that condition. Further research is needed to elucidate cenobamate's mechanisms and refine therapeutic strategies and dose regimens.
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**Keyword 1**: Peripheral neuropathy
**Keyword 2**: Cenobamate
**Keyword 3**: Cisplatin
**Keyword 4**: Rat model

**EVALUATION OF THE EFFECTS OF A DIABETES TYPE 2 RAT MODEL AND SEMAGLUTIDE TREATMENT ON THE SUCROSE PREFERENCE TEST**

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Diabetes type 2 (T2D) is the most common metabolic disorder worldwide and can be associated with eating disorders and/or depression. Recent evidence suggests that semaglutide, glucagon-like peptide 1 agonist and antidiabetic drug, may affect appetite, food preference, but also mood, although preclinical data are lacking or conflicting. We therefore investigated the effects of T2D model based on high-fat diet and streptozotocin administration in middle-aged rats and semaglutide treatment on anhedonia or binge eating.
Sprague-Dawley rats of both sexes were fed high-fat or control diet and treated with streptozotocin or vehicle. When T2D model or control was developed, rats were treated or not with semaglutide/solvent. These animals were studied in separate cohorts subjected to behavioral battery including sucrose preference test.
The main effects of model, sex and treatment were found to be significant for sucrose preference, with T2D rats, female rats and semaglutide-treated rats, respectively, with the former showing decreased and two latter increased levels compared to respective controls. In addition, the main effects of model and sex showed differences in sucrose intake per 100 g animal body mass (SI), with T2D and female animals, showing decreased and increased levels, respectively, compared to their respective controls.
Our results showed that T2D rats had reduced sucrose intake compared to controls, which could be related to anhedonia, and SI seems to be a more appropriate parameter than sucrose preference for this model. Semaglutide treatment had no effect on this parameter, which emphasizes the importance of dietary changes in T2D and warrants further investigation.

**Keyword 1**: diabetes type 2 rat model
**Keyword 2**: semaglutide
**Keyword 3**: sucrose preference test

**ANTIDEPRESSANTS ESCITALOPRAM AND VENLAFAXINE CAUSE HISTOLOGICAL ABNORMALITIES IN THE DEVELOPING BRAIN AND ALTER NEURITE OUTGROWTH IN THE CHICKEN EMBRYO MODEL**

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Antidepressants are frequently used to treat depression and anxiety problems in individuals, including those who are pregnant. Many elements of antidepressant action are still unknown, particularly concerning how they affect neurodevelopment when taken during pregnancy. To evaluate the cellular and histological effects of antidepressant use, we used a 3R-friendly chicken embryo (*Gallus gallus*) model. We chose the two most widely prescribed antidepressants: venlafaxine, a serotonin and noradrenaline reuptake inhibitor (SNRI), and escitalopram, a selective serotonin reuptake inhibitor (SSRI), both of which are often taken during pregnancy. We injected both drugs into embryos at embryonic day (E) 13 (E13) and E16 through the chorioallantoic membrane. Brains were isolated at different time points, and drug distribution was investigated by LC-MS/MS. Histological analysis was performed in the cerebellum of E17 embryos. Neurite outgrowth was measured using a live-cell analysis system using cerebellar granule neurons. According to our analysis of drug distribution, both drugs remained in the developing chicken embryos' brains for several hours after reaching clinical concentrations. Venlafaxine resulted in histological abnormalities, including a decrease in the cerebellum's cortical and molecular layer thickness and an increase in white matter. Both medications affected this critical brain germinal zone by decreasing the number of mitotic events and the thickness of the external granular layer. Additionally, neurite measurements revealed that venlafaxine significantly decreased neurite outgrowth, although escitalopram did not. These results highlight the key differences between venlafaxine and escitalopram and raise the possibility of adverse neurological effects in newborns exposed to those antidepressants during pregnancy.

**Keyword 1**: antidepressant
**Keyword 2**: chicken embryo model
**Keyword 3**: cerebellum
**Keyword 4**: neuropharmacology
**Keyword 5**: neurodevelopment

**DOI AND DEPRESSION: PSYCHEDELIC PLASTICITY AND PERFORMANCE IN THE FSL MODEL**

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Rapid-acting antidepressant are the future, but how do they work? Recent advances in neurobiology and clinical psychiatry emphasize that synaptic plasticity plays a crucial role in mental disorders in general, and depression in particular. Moreover, many antidepressant drugs have been shown to increase synaptic plasticity, and the recent interest in psychedelic drugs can be seen in the light of their ability to enhance synaptic plasticity. We have investigated the effect of the psychoplastogen 2,5-dimethoxy-4-iodoamphetamine (DOI) in the Flinders Sensitive Line (FSL) rat model of depression. Synaptic plasticity and behavioral outcomes relevant for antidepressant action (the marble burying test (MBT) and forced swim test) after one dose of 1 mg/kg DOI was evaluated at 24 hours and 1-week post-treatment. In the MBT, DOI-treated FSL rats left more marbles unburied and exhibited reduced digging at 1-week post-treatment compared to saline treated FSL rats, independent of sex. This can be interpreted as an anxiolytic effect of DOI. Synaptic plasticity was evaluated in field excitatory postsynaptic potential recordings in acute hippocampal slices at the CA3-CA1 synapse, using theta bursts to induce long term potentiation (LTP). Preliminary results suggests that LTP is enhanced at 24 hours and 1 week after DOI treatment. Our findings reveal that DOI enhances synaptic plasticity and normalizes behavior in the marble burying test in a rat model of depression. Continued research into DOI and related psychoplastogens will focus on elucidating the mechanism behind the enhanced synaptic plasticity, aiming to contribute to the development of more rapid-acting antidepressant alternatives.

**Keyword 1**: Long term potentiation (LTP)
**Keyword 2**: Psychoplastogen
**Keyword 3**: Marble burying test

**INVESTIGATION OF WEIGHT-GAIN ASSOCIATED WITH PAROXETINE USE IN THE LIGHT OF SEROTONIN-2C RECEPTOR**

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Selective serotonin reuptake inhibitors (SSRIs) are most commonly prescribed for psychiatric disorders. Over the last few decades, the use of psychiatric medications has increased considerably and the side effects of these medications have become more of a concern. Although they are successful medications for treating psychiatric problems, they have many side effects, particulary weight gain. However, the molecular mechanism of weight gain caused by SSRI drugs has not been fully elucidated. This study examines the molecular mechanisms of the effects of Paroxetine, a known weight-gaining SSRI, on weight gain and its relationship with the serotonin-2C receptor which is associated with well-studied mechanisms of weight gain. Besides, the well-known association of the serotonin-2C receptor with anxiety makes it a good target for eliminating other side effects of SSRI use on cognitive abilities. Using C57BL/6 mice, we investigated the effects of paroxetine on weight control pathways and neuropeptides in the hypothalamus.Various molecular techniques such as RT-qPCR, Western Blotting and deep sequencing were employed for the investigation. In addition, eliminating paroxetine-induced weight gain and anxiety with a neutral serotonin-2C receptor antagonist treatment was another aim of the project. Our preliminary results demonstrated that hypothalamic neuropeptide gene expression changes caused by weight gain resulting from paroxetine use and revealed that neutral antagonist treatment was successful in restoring the gene expression levels. Behavioral studies also indicated the efficacy of neutral antagonist therapy.

**Keyword 1**: Serotonin
**Keyword 2**: Weight Gain
**Keyword 3**: SSRI
**Keyword 4**: Obesity

**HNK, DESCENDANT OF KETAMINE: SIMILAR YET DIFFERENT?**

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Ketamine has emerged as a fast-acting antidepressant. Its derivative, hydroxynorketamine (HNK), is promising due to its efficacy in animal models and lack of psychotomimetic side effects. A phase 1 clinical trial has been completed. While some studies suggest that HNK interacts with the TrkB receptor, others suggest that it affects pre- or postsynaptic mGluRs. Naive and chronic despair mice were treated with HNK. Behavioural experiments and ex vivo electrophysiological measurements in the hippocampus were performed. Different modulators of the receptors in question were used to decipher HNK's potential targets. HNK showed significant antidepressant effects that were reversed by activation of presynaptic mGluRs and blockade of the TrkB receptor. Electrophysiologically, HNK increased basal transmission and blocked associative long-term depression. The increase in basal transmission was again not observed with TrkB or mGluR modulation, while the aLTD blockade was only reversed by TrkB blockade. Blockade of one of the presynaptic mGluRs did not alter behaviour, whereas a combination of mGluR2 and 7 antagonist mimicked the electrophysiological effects of HNK and the behavioural antidepressant effects. This finding was supported by the observation that when one of the receptors was silenced by siRNA, the other was compensatorily upregulated. Despite structural similarities to ketamine, HNK has distinct mechanisms. It primarily restores LTD and enhances basal transmission, involving the BDNF pathway. These findings highlight the unique potential of HNK as an antidepressant with fewer side effects than ketamine and the potential role of mGluRs in antidepressant treatment.

**Keyword 1**: Depression
**Keyword 2**: HNK

**ANTIMICROBIAL, ANTIPROLIFERATIVE, AND ANTI-SEIZURE PROPERTY ASSESSMENT OF *ARTEMISIA* PLANTS FROM KAZAKHSTAN**

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Epilepsy, characterized by recurrent seizures, remains a global health challenge due to the limited efficacy of current antiepileptic drugs (AEDs) and the issue of drug-resistant epilepsy. Inflammation and oxidative stress are increasingly recognized as key contributors to seizure development. Targeting these pathological processes may enhance seizure control and provide neuroprotection. Medicinal plants, with their anti-inflammatory and antioxidant properties, offer promising alternative therapies. *Artemisia* species, known for their diverse pharmacological effects, including neuroprotection, are of particular interest.
This preliminary study evaluated the antimicrobial, cytotoxic, and antiproliferative activities of ethanol root extracts from *Artemisia vulgaris* (AV) and *Artemisia glauca* (AG), harvested in Kazakhstan, and assessed their potential antiepileptic effects *in vivo*. AV and AG extracts exhibited broad-spectrum antimicrobial activity, particularly against gram-positive bacteria. While AV showed low cytotoxicity and no neurotoxicity, AG displayed both, indicating a narrower therapeutic window.
*In vitro* assays revealed dose-dependent antiproliferative effects on astroglioma (U-87 MG) and primary astroglial cells, suggesting potential modulation of neuroinflammation and gliosis relevant to epilepsy. *In vivo*, adult CD-1 mice received intraperitoneal AV extract (50–500 mg/kg) 30 minutes before pentylenetetrazole (PTZ) administration (6 x 40 mg/kg). Seizure severity (Racine's scale) and behavior (Open Field, Elevated Zero-Maze, Barnes maze tests) were assessed. Neither AV extract nor the positive control (naringin, 80 mg/kg) significantly impacted seizures or behavior.
These findings suggest AV extract is well-tolerated but may require model optimization to reveal antiepileptic benefits. Future studies will explore a chronic seizure model with increased PTZ doses.
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**Keyword 1**: Epilepsy
**Keyword 2**: Artemisia
**Keyword 3**: Medical plant
**Keyword 4**: Antiinflammatory

**NEXT-GENERATION GPCR-BASED INDICATORS FOR IN VIVO IMAGING OF SPATIOTEMPORAL NOREPINEPHRINE DYNAMICS**

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Norepinephrine (NE) is a key monoamine neuromodulator involved in various neurophysiological processes and disorders. Genetically encoded fluorescent indicators based on GPCRs allow real-time, high-resolution imaging of NE release in living animals. These indicators utilize a GPCR scaffold with a circularly permuted fluorescent protein (cpFP) in intracellular loop 3, translating receptor activation into fluorescence changes while retaining ligand specificity. Our lab recently developed a multicolor family of NE indicators based on the Alpha1a adrenergic receptor. Through mutagenesis and screening, we engineered nLightG2 and nLightR2, which exhibit a several-fold improvement in dynamic range over their parental versions. Importantly, NE selectivity over dopamine (DA) is very high for both indicators. These indicators enable enhanced detection of endogenous NE release in acute brain slices via two-photon imaging and in vivo using fiber photometry during optogenetic or behavioral stimuli. Notably, nLightR2 allows in vivo two-photon imaging of NE release in the hippocampus during a reward collection task. We anticipate that these optimized indicators will be valuable to the scientific community, advancing the study of NE physiology and pathophysiology.

**Keyword 1**: GPCRs
**Keyword 2**: neuromodulators
**Keyword 3**: genetically-encoded sensors
**Keyword 4**: imaging
**Keyword 5**: fluorescence

**EFFICACY OF INDIVIDUALIZED ESCITALOPRAM DOSING BASED ON PLASMA LEVEL MONITORING: FINDINGS FROM A CROSS-SECTIONAL STUDY**

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Escitalopram, a widely used antidepressant, exhibits considerable interindividual variability in metabolism and response, which is primarily influenced by CYP2C19 and CYP2D6 polymorphisms. The therapeutic plasma range is 15-80 ng/ml, with optimal exposure at 25-50 ng/ml. With the standard dosage of 10 mg/day, genetic variability is often neglected, leading to suboptimal results. The aim of this prospective cross-sectional study is to investigate the impact of TDM-guided and pharmacogenetically adjusted dosing of escitalopram on patient outcomes in real-life clinical practice. In addition, the study will investigate the impact of CYP2C19 and CYP2D6 genotypes on optimizing drug exposure. The study involved 89 patients (18-65 years) diagnosed with depression and treated at the Institute of Mental Health in Belgrade. After two weeks of taking 10 mg/day of escitalopram, plasma levels were measured and the dose was adjusted based on TDM. In patients with levels <25 ng/ml, the dose was increased, while in patients with levels >50 ng/ml, the dose was reduced. Plasma levels were quantified by HPLC-MS/MS. Genotyping is planned for the next study phase. At baseline, only 19 patients were in the therapeutic range, 28 were slightly underdosed, 41 were significantly underdosed and one was overdosed. After TDM-based adjustments, 37 patients reached the ideal exposure, the proportion of underdosed patients decreased by 3.4-fold, and the proportion of optimally dosed patients almost doubled (1.9-fold). A standard dose of 10 mg/day is insufficient for most patients. TDM-guided dosing significantly improved target exposure and reduced underdosing. Further genetic and biochemical analyzes will improve personalized antidepressant therapy.

**Keyword 1**: Escitalopram
**Keyword 2**: TDM
**Keyword 3**: Personalized Medicine
**Keyword 4**: Pharmacogenetics
**Keyword 5**: Depression

**ENGINEERING CHIMERIC PROTEINS FOR IMMUNOGENIC TARGETING OF $A3$B4* NICOTINIC ACETYLCHOLINE RECEPTORS (NACHRS)**

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The $α3$β4*-nicotinic acetylcholine receptor (nAChR) plays a crucial role in addiction, neurodegeneration, and psychiatric disorders. However, developing specific immunoreagents is challenging due to the receptor's structural complexity and low stability. To overcome this, soluble chimeric proteins were engineered by fusing the immunogenic N-terminal domains of $α3- and $β4-nAChR subunits with acetylcholine-binding protein (AChBP) from *Aplysia californica*, a stable pentameric scaffold that mimics the extracellular domain of nAChRs. These AChBP-based constructs provide a reliable alternative to membrane-bound nAChRs, facilitating structural and functional studies. In this project, chimeric proteins ap$α3-nAChR-AcAChBP and ap$β4-nAChR-AcAChBP were developed, aimed at generating nanobodies against properly folded immunogenic regions of $α3- and $β4-nAChR, streamlining antibody production.
Using overlap-extension PCR, cDNA sequences encoding these chimeras were constructed and transfected into HEK293GnT1- cells, establishing stable cell lines that express these proteins. The purified proteins exhibited proper pentameric assembly, confirmed by size-exclusion chromatography, and displayed high purity verified through SDS-PAGE analysis. This indicates that the chimeric constructs successfully folded into their intended structures for further research applications.
These novel chimeric proteins serve as powerful tools for advancing research on $α3$β4*-nAChRs, paving the way for targeted immunoreagents and potential therapeutic strategies for cholinergic dysfunction in neurological diseases.

**WHEN DOPAMINE MEETS MICROGLIA; MICROGLIAL D1 AND D2 RECEPTORS CONTROL NEUROINFLAMMATORY STATE AND BEHAVIOUR**

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Microglia play prime roles in physiology and pathology of the CNS, due to their capacity to adjust to various signals from their environment. One major signal is dopamine (DA). DA modulates fundamental functions including movements, reward, emotional and cognitive processes. Study of DA functions has traditionally relied on the expression of DA receptors on neurons; however emerging evidence suggests their expression in microglia.
Given microglia's established role in inflammatory processes and the link of inflammation to vulnerability to many psychiatric diseases, we aim to investigate how DA receptors D1 and D2 expressed in microglia affect immunomodulatory responses, behavior, and neuronal function in vivo.
Our findings show that microglia express D1 and D2 in the striatum, and expression increases following chronic psychostimulant administration; microglial D1KO leads to hypo-anxiety and abolishes baseline repetitive, compulsive-like behavior. Surprisingly, microglial D1KO leads to diminished hyperlocomotion in response to acute amphetamine and blunts behavioral sensitization to cocaine only in female mice. Microglial D2KO mainly results in altered striatal cytokines profile.
Conclusion: Microglia sense and respond to fluctuations in striatal DA release. Microglial D1 function is associated with physiologic regulation of compulsive-like behavior and anxiety. In addition, microglial D1 modulates response to psychostimulants in a sexually dimorphic fashion. Microglial D2, on the other hand, plays a major role in the homeostatic striatal cytokine profile. This is an ongoing project and the findings will provide crucial and unprecedented insight into the functional roles of microglial DA receptors along with their association with homeostatic neuronal activity and behavior.

**Keyword 1**: dopamine
**Keyword 2**: microglia
**Keyword 3**: D1R
**Keyword 4**: D2R

**QUANTIFICATION OF BRAIN METABOLITES IN TRAUMATIC BRAIN INJURY USING ADVANCED SPECTROSCOPY**

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Effective neurocritical care requires continuous monitoring of brain extracellular chemistry, crucial for informed decision-making in traumatic brain injury (TBI) treatment. Traditional manual sampling methods disrupt the continuity of data collection and are labour-intensive. Recently we have employed label-free, laser-based mid-infrared (mid-IR) transmission spectroscopy achieving clinically relevant detection limits for key TBI metabolites -glucose, lactate, and pyruvate.
Our study here aimed to refine the limit of detection (LOD) for pyruvate below the previously established 0.07 mM by integrating enhanced mid-IR spectroscopy with cerebral microdialysis techniques. Using a Culpeo (Daylight Solutions, Inc), an external cavity quantum cascade laser (EC-QCL) system operating within the 978-1441 $cm^{-1}$ spectral range (“fingerprint” region of the mid-infrared), we analysed pyruvate concentrations ranging from 0.01 to 1 mM. These analyses utilised microfluidic pathlengths of 51 and 76 micrometres, 400 scan averages with three repetitions per measurement, facilitated by an SPM 100 syringe pump maintaining a steady flow rate of 50 microlitre/min. LODs were calculated using the formula LOD = 3 × (noise) / slope of the calibration curve.
The optimised methodology substantially improved the sensitivity of pyruvate measurements, achieving a new LOD of 0.023 mM at the 51-micrometre pathlength, particularly noted at the 1176 $cm^{-1}$ peak characteristic of pyruvate in the “fingerprint” region. This improvement in EC-QCL mid-IR spectroscopy advances pyruvate monitoring capabilities, promising more accurate clinical assessments. Future efforts will focus on further technological optimisation and broadening the clinical application of this method to improve outcomes by quantifying low concentrations of pyruvate in brain microdialysates.

**Keyword 1**: Traumatic brain injury
**Keyword 2**: Brain metabolism
**Keyword 3**: Spectroscopy
**Keyword 4**: Sensors
**Keyword 5**: Pyruvate

**DOPAMINERGIC CIRCUITRY IN THE JAPANESE QUAIL: FIBER ANALYSIS AND SYNAPTIC TARGETS IN THE NIDOPALLIUM CAUDOLATERALE**

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The nidopallium caudolaterale (NCL), the avian counterpart to the mammalian prefrontal cortex, is characterized by dense dopaminergic input and basket-like synaptic structures across various bird species, including pigeons, chickens, crows, and zebra finches. In Japanese quail (Coturnix japonica), we identified the NCL based on tyrosine hydroxylase (TH)-immunopositive (+) fiber densities and basket-like formations, which were also present in the island fields of the caudal nidopallium (NCIF), suggesting shared features among galliform birds. To assess and quantify dopaminergic fiber distribution, we generated heat maps using a custom fiber density analysis software. The highest fiber densities were observed in the NCL, followed by the NCIF, with lower densities in other pallial regions. Double labeling of TH+ baskets in the NCL revealed putative postsynaptic targets, including calbindin (CB), secretagogin (SCGN), and CaMKIIα-positive cells, while parvalbumin (PV) and calretinin (CR) were not identified as targets. In the ventral tegmental area (VTA), the primary source of dopaminergic input, TH co-localized with CR and SCGN but not with PV. Additional double-labeling for GABA with TH and GABA with calcium-binding proteins were performed in the NCL and VTA to evaluate their co-expression and potential overlap. These findings contribute to our understanding of dopaminergic architecture in avian species, providing insight into the neurochemical composition of the avian nidopallium.

**DECIPHERING SPATIAL CELLULAR HETEROGENEITY IN NEURODEGENERATIVE BRAIN PATHOLOGY WITH IMAGING MASS CYTOMETRY**

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The etiology of neurodegenerative diseases (NDs), such as Parkinson's disease (PD), Alzheimer's disease (AD) and multiple sclerosis (MS), requires understanding the complex spatial biological mechanisms leading to neuronal tissue deterioration, formation of protein aggregates, immune cell infiltration and myelin loss. It is essential to maximize the information acquired from a single sample due to the limited availability of postmortem human brain tissue. Imaging Mass Cytometry™ (IMC™) technology is a spatial biology technique that provides quantitative phenotypic evaluation of 40-plus markers. We report the application of IMC imaging modes with a 41-marker panel for ND research. Tissue Mode was applied to perform a scan of the entire tissue followed by pixel-clustering analysis to uncover spatial distribution of relevant markers.
Preview Mode was applied to sample the tissue at low resolution, improving informed selection of areas for high-resolution Cell Mode imaging on the same slide. Using Tissue Mode with pixel-clustering analysis, we quantified the spatial distribution of immune cells in tissue compartments of ND tissues. Preview Mode and Cell Mode highlighted MS lesions devoid of myelinization and a network of αSynuclein in PD tissue. Single cell analysis identified heterogeneity of amyloid precursor protein aggregates in AD that encompassed total and phosphorylated species of Tau and phosphorylated αSynuclein. Overall, whole slide imaging techniques using IMC technology provide quantitative immunological and structural insights into the spatial biology of ND.

**PLASMA METABOLOMIC PROFILING IN PATIENTS WITH MAJOR DEPRESSIVE DISORDER**

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Major depressive disorder (MDD) is a complex psychiatric disorder affecting millions of people worldwide. A subset of patients exhibits treatment-resistant depression (TRD), characterized by insufficient response to conventional antidepressants. Identifying metabolomic variations between MDD and TRD is crucial for improving treatment strategies. To examine metabolomic changes associated with therapeutic response to duloxetine and combined duloxetine-bright light therapy, blood plasma samples were obtained from individuals of both sexes diagnosed with MDD (N=33) and TRD (N=31), before and eight weeks after the treatment. 36 healthy control (HC) subjects were also included. An untargeted metabolomics analysis of plasma was performed using an Agilent 7890A gas chromatograph coupled with a 5975C quadrupole mass spectrometer. Plasma preparation included deproteinization and derivatization, and metabolites were identified using NIST and Fiehn libraries. Univariate and multivariate statistical analyses were applied to detect significant changes in metabolomic profiles across groups. Comparison between three groups showed significant decrease of arachidonic acid (AA), glycine and phosphoric acid (PA) in the TRD group before the treatment, indicating neuroinflammation, disruptions in neurotransmitter balance and energy metabolism associated with TRD. After duloxetine treatment, AA levels significantly increased, indicating a treatment-specific effect on lipid metabolism, while in patients undergoing combined therapy, AA and PA levels were elevated, suggesting additional, possible restoration of mitochondrial function and improved energy metabolism. Glycine levels remained unchanged, underscoring persistent glutamatergic dysfunction in TRD. These findings highlight distinct metabolic patterns differentiating MDD from TRD and suggest potential indicators for assessing therapeutic response. However, further research is required to confirm these findings.

**Keyword 1**: Major depressive disorder (MDD)
**Keyword 2**: Metabolomics
**Keyword 3**: Duloxetine
**Keyword 4**: Bright light therapy (BLT)
**Keyword 5**: Biomarkers of therapeutic response

**METABOLOMIC ANALYSIS OF 3XTG-AD MICE PLASMA: EXPLORING BIOMARKERS AND EVALUATING EFFECTS OF DEHYDROEPIANDROSTERONE SULFATE TREATMENT**

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Alzheimer's disease (AD) is a progressive neurodegenerative disorder marked by cognitive decline and memory impairment. Since AD is often diagnosed at a late stage, which further reduces the effectiveness of treatment, identifying novel biomarkers is crucial for developing more reliable diagnostic tests and effective therapeutic strategies. Dehydroepiandrosterone sulfate (DHEAS) is a neurosteroid that plays an important role in neuroprotection, synaptic plasticity, oxidative stress, and neuroinflammation. Variations in DHEAS levels have been observed in various neuropsychiatric disorders, including AD. Metabolomics serves as a powerful tool for investigating metabolic changes associated with AD and for discovering potential biomarkers. In this study, we utilized an untargeted metabolomic approach involving liquid and gas chromatography coupled with mass spectrometry (LC-MS and GC-MS) to analyze the metabolomic profile of 3xTg-AD mice plasma. The study included 40 male mice (9-10 months old), divided into three groups: 3xTg (+/+) control mice, untreated 3xTg-AD mice, and those treated with DHEAS. This study aimed to investigate metabolomic alterations associated with AD and the potential effects of DHEAS treatment. The results of this study revealed significant alterations in key metabolic pathways, including amino acid, lipid, and energy metabolism, reflecting systemic metabolic dysregulation in AD. Furthermore, DHEAS treatment appeared to modulate certain metabolic pathways, suggesting a possible mechanism of action. These findings enhance our understanding of the metabolic changes associated with AD. However, further research is needed to identify reliable biomarkers of AD, while also exploring the precise role of DHEAS along with its potential therapeutic relevance.

**Keyword 1**: Alzheimer's disease
**Keyword 2**: DHEAS
**Keyword 3**: Metabolomics
**Keyword 4**: LC-MS
**Keyword 5**: GC-MS

**AT2 RECEPTOR STIMULATION PROMOTES ANGIOGENESIS IN THE SPINAL CORD TISSUE AFTER SEVERE INJURY IN RATS**

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Traumatic spinal cord injury disrupts the blood-brain barrier, contributing to the development of hypoxic environment. Neuronal tissue is highly sensitive to hypoxia, making a continuous oxygen supply crucial for its function. While the hypoxic conditions partially promote neuronal tissue repair, particularly through endogenous revascularization, this process is often insufficient. Insufficient integration of newly formed blood vessels with the existing functional vascular network can be one of the reasons. Therefore, maintaining adequate blood flow is vital to support the survival and functionality of the neuronal tissue. The renin-angiotensin system, predominantly acting through the AT1 receptor, regulates blood pressure and hydro-electrolytic balance. However, the AT2 receptor (AT2) also plays a crucial role, especially in processes related to neuroregeneration. In the present study, the AT2 was stimulated in an experimental model of severe spinal cord compression in adult Wistar rats. Selective stimulation with AT2 agonist led to upregulation of gene expression of key pro-angiogenic growth factors and their receptors in the spinal cord tissue. Moreover, significant increase in protein expression of selected markers, such as vascular endothelial growth factor A (VEGF-A), predominantly in cranial segments, was observed. These changes correlated with functional improvement in both locomotion and micturition in the rats. Such positive effects were not observed in the animals after AT2 blockade. These findings strongly suggest the potential of AT2 stimulation as a therapeutic intervention for spinal cord injuries in promotion of angiogenesis and the regeneration of reversibly damaged neurovasculature. The study was supported by APVV-22-0248 and VEGA 2/0123/23.

**Keyword 1**: spinal cord injury
**Keyword 2**: neurovasculature

**AT1 RECEPTOR BLOCKADE AS A POTENTIAL THERAPEUTIC STRATEGY FOR NEUROGENIC BLADDER DYSFUNCTION FOLLOWING SPINAL CORD INJURY**

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Research on spinal cord injury primarily aims to develop therapies that enhance motor function. However, the loss of mobility is often accompanied by various autonomic systemic disorders. One of the most severe complications leading to repeated hospitalizations in patients is neurogenic bladder dysfunction. The renin-angiotensin system, particularly angiotensin receptors, presents a potential pharmacological target for alleviating these complications. In this study involving adult female Wistar rats, treatment with the angiotensin receptor blocker candesartan during an episode of transient systemic hypertension accelerated bladder emptying, with improved function observed by day 14 compared to day 26 post-trauma. Mechanical stress induces the release of Angiotensin II, which promotes the production of nerve growth factor (NGF) in the bladder, contributing to the pathogenesis of overactive bladder. Four weeks post-injury, rats treated with candesartan exhibited a significant reduction in NGF levels (127.3 ± 28.28 pg/ml compared to 202.9 ± 12.51 pg/ml) and its receptors, as well as a marked decrease in brain-derived neurotrophic factor (BDNF) levels (171.3 ± 8.64 pg/ml compared to 218.0 ± 5.8 pg/ml). Additionally, we observed significant changes in the distribution of vanilloid receptors TRPV1, TRPV2, and TRPV4, which are associated with detrusor hyperexcitability, along with alterations in mechanoreceptor channels ASIC and potassium channels TREK-1. Our findings suggest that blocking AT1 receptors may significantly reduce neurogenic bladder dysfunction following spinal cord injury. This approach shows considerable potential as a clinically relevant therapy, considering the complex changes induced by spinal cord injury.
*This study was supported by PostdokGrant grant No.APD0084.*

**Keyword 1**: Angiotensin II receptors
**Keyword 2**: neurogenic bladder dysfunction
**Keyword 3**: spinal cord injury

**SIGNALING MECHANISMS OF DELTA RECEPTORS, ATYPICAL SYNAPSE ORGANIZERS**

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Among the four classes of ionotropic glutamate receptors (iGluRs), the Delta class (composed of GluD1 and GluD2) is the least understood. GluD receptors exhibit atypical pharmacology and signaling compared to other iGluRs: they do not bind glutamate, but instead bind D-serine, glycine, and GABA for GluD1. Notably, agonist binding does not induce channel opening. Compared to GluD2, the physiological roles of GluD1 remain poorly understood. It has been shown that GluD1 contributes to the formation and maintenance of inhibitory synapses in the cortex and regulates inhibitory synaptic strength in the hippocampus. However, the underlying mechanisms remain elusive, mainly due to the challenge of studying an electronically silent receptor. To overcome this issue, we developed a fluorescence-based technique called VCF to study GluD1's molecular gating mechanisms and pharmacology. We tracked fluorescence signals to observe relative motions of receptor components and how different ligands influence gating states. We observed distinct fluorescence signatures for D-serine and GABA, despite their shared binding site. This suggests that the two agonists induce different conformational changes and thus distinct signaling pathways. We also investigated how pathological mutations in the GRID1 gene affect receptor function and signal transduction. Two variants at the interface between LBD subunits altered the effects of D-serine and GABA, either enhancing or nearly eliminating their efficacy. Additionally, we examined the impact of calcium on these variants. This study provides valuable insights into GluD1 signal transduction, contributing to a better understanding of its physiology, pharmacology, and potential patient phenotypes.

**Keyword 1**: ionotropic glutamate receptors
**Keyword 2**: Delta receptors
**Keyword 3**: molecular conformation
**Keyword 4**: signaling mechanism
**Keyword 5**: synaptic transmission

**GLYT1 INHIBITOR, BITOPERTIN CHANGES THE EXCITATORY AND INHIBITORY NETWORK CONNECTIVITY IN MOUSE MEDIAL PREFRONTAL CORTEX**

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Cognitive deficits related to schizophrenia are often linked to an imbalance in the excitation-inhibition (E/I) transmission within the medial prefrontal cortex (mPFC). This imbalance is believed to be related to the hypofunction of N-methyl-D-aspartate receptors (NMDAR) and the dysfunction of GABAergic neurotransmission. We have previously demonstrated that bitopertin, a glycine transporter 1 (GlyT1) inhibitor, can rectify this E/I imbalance. However, the exact mechanism remains unclear. In this study, we used channel rhodopsin-assisted circuit mapping (CRACM), 2-photon *ex vivo* functional imaging, and slice electrophysiological recordings in mouse mPFC to investigate the effects of bitopertin. Our results show that bitopertin reduces NMDA-mediated excitatory drive onto interneurons expressing vasoactive intestinal peptide (VIP IN) and increases it in somatostatin- (SST IN) and parvalbumin-expressing (PV IN) interneurons. Additionally, bitopertin strengthens the synaptic connections between PV and pyramidal neurons (PNs), increases the excitatory input onto PV neurons, and potentially decreases the activity of pyramidal cells. Our study reveals the role of GlyT1 inhibitor in modulating the E/I balance in the mPFC, potentially contributing to its therapeutic effects in schizophrenia-related cognitive deficits. The strengthened synaptic connections and altered neuronal activity indicated the intricate role of GlyT1 inhibitor, bitopertin in neuronal communication. To sum up, our work provides comprehensive insight into the impact of GlyT1 inhibition on the local network and its potential mechanisms, which could usher in more efficient therapies for conditions marked by E/I imbalance-mediated cognitive disorders.

**Keyword 1**: GlyT1 inhibitor
**Keyword 2**: E/I imbalance
**Keyword 3**: Neuropsychiatric disorders
**Keyword 4**: Mental health
**Keyword 5**: Prefrontal cortex

**GLUTAMATE AND GLUTAMINE CHANGES IN TRANSIENT GLOBAL AMNESIA (TGA) MEASURED WITH 7T MR SPECTROSCOPY**

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Transient global amnesia (TGA) is a neurological syndrome characterized by the sudden onset of hyperacute total anterograde and partial retrograde amnesia, lasting up to 24 hours before resolving spontaneously. Despite decades of research, its exact etiology and pathophysiology remain elusive, making it one of the most intriguing conundrums in clinical neurology.
Magnetic Resonance Imaging (MRI) typically reveals focal diffusion restriction in the hippocampus, appearing 6-8 hours after symptom onset and resolving within 14 days—features reminiscent of ischemic-related insults. Given its abrupt onset, research has largely focused on possible cerebrovascular etiologies; however, conclusive evidence is lacking. An alternative hypothesis is that TGA may be driven by focal glutamate release and excitotoxicity.
In this study, we utilized ultra-high-resolution MR Spectroscopy at 7 Tesla at three time points in 18 patients: (1) the acute phase upon admission, (2) the next day at discharge, and (3) 14 days later. In preliminary analyses, we found elevated relative concentrations of glutamate and glutamine relating to the time since TGA symptom onset. The average signal-to-noise ratio (SNR) and linewidth across the three time points ranged from 9-17 S/N and 14-23 Hz, respectively.
Changes in glutamate and glutamine could support the hypothesis that glutamate-induced excitotoxicity plays a role in the pathophysiology of TGA episodes.

**Keyword 1**: transient global amnesia
**Keyword 2**: spectroscopy
**Keyword 3**: MRI
**Keyword 4**: glutamate-induced excitotoxicity
**Keyword 5**: ultra-high resolution MR

**VASOPRESSIN AT THE CROSSROAD OF DEPRESSION AND SCHIZOPHRENIA: STUDIES IN RATS**

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Arginine vasopressin (AVP) is an antidiuretic and vasopressor hormone involved in stress regulation. Elevated AVP levels are linked to anxiety and depression, whereas spontaneous AVP-deficient Brattleboro rats serve as schizophrenia models, suggesting that balanced AVP is crucial for mental health. Cre knockin mice was confirmed to have – paradoxically- AVP-deficiency. Here, we investigated AVP-Cre/Cre Sprague-Dawley rats for AVP deficiency and associated behavioral and hypothalamic-pituitary-adrenal (HPA) axis alterations.
Thirteen-week-old male and female rats underwent behavioral tests assessing anxiety/depression-like (open-field test [OFT], elevated plus maze [EPM], light-dark box [LD], marble burying, splash test) and schizophrenia-like behaviors (novel object recognition [NOR], social discrimination [SD], acoustic startle). Tissue samples were collected for molecular analysis.
AVP-Cre/Cre rats exhibited increased drinking. In OFT, females were more active, while AVP-Cre/Cre males were less active and avoided the center. No significant differences emerged in EPM, LD, or marble burying. Splash test revealed reduced grooming in AVP-Cre/Cre females. NOR results showed only AVP-Cre/Cre males successfully discriminated objects; in SD, only control males distinguished familiar from unfamiliar rats. AVP-Cre/Cre females displayed reduced prepulse inhibition.
Our findings confirm that homozygous Cre knockin can disrupt (in our case) AVP expression. Despite prenatal AVP deficiency, AVP-Cre/Cre rats differed from Brattleboro rats, showing no clear anxiolytic or antidepressant phenotype but displaying cognitive deficits and schizophrenia-like traits.

**Keyword 1**: vasopressin
**Keyword 2**: schizophrenia
**Keyword 3**: depression
**Keyword 4**: behavior
**Keyword 5**: animal model

**NEURONAL AND BEHAVIORAL ALTERATIONS IN A NITROGLYCERIN-INDUCED MIGRAINE MODEL**

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Migraine is a debilitating neurological disorder associated with sensory and social impairments. Using the nitroglycerin (NTG) model of migraine and transgenic mice, we identified significant neuronal activation in multiple brain regions, including the amygdala and hypothalamus, upon pain induction. To investigate the impact of migraine on social behavior, a three-chambered social interaction test was conducted. NTG-treated mice, regardless of sex, exhibited increased social withdrawal, spending more time in isolation. Additionally, both male and female mice demonstrated heightened photophobia, preferring the dark chamber. Given the critical involvement of the chosen brain regions in migraine pathology, we employed chemogenetic approaches and TRAP2 (Targeted Recombination of Activated Population) mice to investigate their functional role in migraine-like symptoms. Social behavior was assessed for the same, using Crawley's test, while mechanical allodynia and anxiety-like behaviors were examined by Von Frey test and Elevated Plus Maze respectively. These findings enhance our understanding of the neural circuits underlying migraine and its associated behavioral impairments.

**Keyword 1**: Migraine
**Keyword 2**: NTG
**Keyword 3**: Chemogenetics
**Keyword 4**: Transgenic mice

**MITF REGULATES GENE EXPRESSION IN MIDDLE TUFTED NEURONS AND OTHER PROJECTION NEURONS OF THE OLFACTORY BULB**

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Microphthalmia-associated transcription factor (MITF) is a master transcription factor in melanocytes, crucial for their development and function. It also plays a role in other cell types, such as retinal pigment epithelial cells, mast cells, and osteoclasts. In the central nervous system, MITF is expressed in projection neurons (PNs), mitral and tufted cells, of olfactory bulb, the primary site for odor processing in the brain. MITF was reported as a direct regulator of intrinsic homeostatic plasticity through the regulation of a potassium channel subunit *Kcnd3*, which encodes Kv4.3 protein. However, its role and function in neurons have yet to be fully understood. Here, we identified several putative MITF target genes in PNs through global gene expression analysis (RNA-seq), single molecule fluorescent *in situ* hybridization (smFISH), and bioinformatic tools. The focus was placed on genes that showed reduced expression in the absence of *Mitf* due to the anticipation that genes regulated by MITF would show reduced expression, while those inhibited by MITF would demonstrate increased expression. Selected genes were tested by smFISH. Genes were evaluated in two groups: those likely to suppress neuronal activity and genes specifically expressed in middle tufted cells (mTCs), a subcluster of tufted cells. The smFISH confirmed reduced expression of these genes and absence of mTCs marker genes in the OB of *Mitf* mutants (*Mitf$^{mi\text{-}vga9/mi\text{-}vga9}$*). ChIP-seq and CUT&RUN analyses of melanoma cells showed MITF binding peaks in some of these genes. These findings suggest a role for *Mitf* in the generation, survival, and/or function of mTCs.

**Keyword 1**: MITF
**Keyword 2**: Olfactory bulb
**Keyword 3**: Projection neurons
**Keyword 4**: Middle tufted cells

**ISOFORM-SPECIFIC EFFECT OF CYTOCHROME C ON NA,K-ATPASE**

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Na,K-ATPase is a fundamental enzyme that plays a key role in sustaining sodium and potassium ion gradients across the cell membrane, a process essential for various physiological functions across different tissues and organs. Given its pivotal role in cellular homeostasis, any disruption in Na,K-ATPase activity can lead to significant physiological effects. This makes it a valuable target for pharmacological interventions, with drugs designed to modulate its function being widely used in clinical treatments.
Cytochrome c (Cytc) is a multifunctional protein with distinct roles in the cell. Within mitochondria, it is indispensable for ATP production and cellular energy metabolism. However, under apoptotic conditions, Cytc is released into the cytoplasm, where it initiates programmed cell death through the intrinsic apoptotic pathway. Beyond its well-established function in apoptosis, Cytc is also involved in non-apoptotic cellular processes, though these roles are less extensively characterized.
In this in vitro study, we present novel evidence of Cytc's influence on Na,K-ATPase activity. An analysis of Na,K-ATPase isoform, and subunit specificity in response to Cytc demonstrated that activation at a concentration of 6 ng/ml requires the presence of the α1 isoform. In contrast, elevated Cytc levels result in the inhibition of all three isoforms—α1, α2, and α3. Additionally, we demonstrate that Cytc alters the affinity of p-chloromercuribenzoic acid (PCMB) for Na,K-ATPase, confirming that both Cytc and PCMB target the same molecular site—specifically, the cysteine thiol groups within the enzyme.

**Keyword 1**: Na,K-ATPase
**Keyword 2**: Enzyme kinetic
**Keyword 3**: Cytochrome c
**Keyword 4**: Cysteine

**OUABAIN AND NA,K-ATPASE**

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Na,K-ATPase is an electrogenic transporter embedded in the plasma membrane of cells, playing a fundamental role in sustaining life in animals. This enzyme is highly vulnerable to inhibition by cardiotonic steroids (CTS). Recent research has expanded our understanding of Na,K-ATPase beyond its classical ion-pumping role, highlighting its involvement in cellular signaling. Specifically, the ouabain-binding site contributes to multiple physiological processes. At elevated concentrations, ouabain suppresses $Na^+$ and $K^+$ ion transport through Na,K-ATPase, whereas at lower concentrations, it enhances the enzyme's signaling activity.
Interestingly, Na,K-ATPase does not conform to the conventional classifications of G protein-coupled receptors or ligand-gated ion channels. This suggests that it may function as a distinct type of cell surface receptor, mediating interactions with signaling molecules via allosteric mechanisms.
In this study, we have identified novel modulators that influence the enzyme's sensitivity to ouabain and investigated the kinetic effects of physiologically relevant ouabain concentrations on hippocampal Na,K-ATPase. Our findings indicate that ouabain binding to Na,K-ATPase triggers conformational rearrangements, promoting transitions between different enzymatic states.

**Keyword 1**: Na,K-ATPase
**Keyword 2**: ouabain binding site
**Keyword 3**: enzyme kinetic
**Keyword 4**: cysteine

**OPTIMIZATION OF A MULTIPLEXED, CELL-BASED ASSAY OF NEURONAL FUNCTION**

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In vitro neuronal models are valuable tools for studying neural diseases and neurotoxicology. With the use of microelectrode arrays, the Maestro Pro can measure the electrical activity of neural cultures in a high throughput, label-free manner. Here, an optimized in vitro assay is demonstrated to characterize the response of neural cultures to neuroactive compounds. A total of 40,000 cortical rodent neurons per well were plated on CytoView 24-well plates, and neural activity and viability were monitored on the Maestro Pro. To determine culture conditions for compound dosing, neural network activity, synchrony, and oscillation were assessed at DIV14 in three different media systems. BrainPhys + SM1 was the optimal media for this application because it induced a moderate level of baseline activity, allowing for the detection of increases or decreases in each measure following compound dosing. After optimizing neural culture conditions, cultures were dosed and recorded on the Maestro Pro. Potassium channel blocker 4-aminopyrirdine led to increases in activity, synchrony, and oscillation. In contrast, glutamate significantly increased activity but decreased synchrony and oscillations. Picrotoxin increased synchrony but did not affect oscillatory behavior, while carbachol decreased synchrony slightly but significantly increased oscillations. Viability did not significantly change following dosing, indicating that changes in neural activity were not due to cell death. Taken together, this study illustrates that the Maestro Pro is a valuable tool for optimizing in vitro neural models and detecting electrophysiological changes from neuroactive compounds.

**HETEROGENEOUS EXPRESSION OF THE CYSTINE-GLUTAMATE EXCHANGER IN THE MOUSE BRAIN**

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The cystine-glutamate exchanger (xCT) is believed to be a significant contributor to the extracellular glutamate levels, possibly modulating neuronal signaling and, in pathological situations, contributing to excitotoxicity. Questions, however, remain, especially since in vivo measurements of ambient glutamate levels in xCT deficient animals have shown inconsistent results. We hypothesize that this might be explained by regional variance in xCT expression. Quantitative and qualitative mapping of xCT protein in C57BL6 mouse brain revealed a higher expression in cerebrum and diencephalon than in midbrain, cerebellum and brain stem. Here, we aimed to characterize the heterogeneity of xCT expression further, on a regional, subregional and cellular level, using tissue from xCT knockout mice as negative control. Immunohistochemistry and single-cell RNA-seq analysis suggest that xCT is highly expressed in the leptomeninges, in a subset of pericytes, and in a subpopulation of astroglia across brain. In the isocortex, xCT is densely expressed in protoplasmic astroglia, although with varying expression levels among subsets of astrocytes. Lower expression levels are found in fibrous astroglia in subcortical white matter regions including corpus callosum. In the hippocampus, xCT displays a scattered pattern with greater expression in astrocytes of subiculum, hippocampal CA1 and gyrus dentatus, and lower levels in CA3. Within CA1, the highest levels are found in the stratum lacunosum moleculare. In the cerebellum, xCT is expressed in cerebellar white matter astroglia and absent from EAAT1-positive Bergmann glia. In sum, consequences of xCT deficiency for glutamate homeostasis might differ between different (sub)regions and cellular networks.

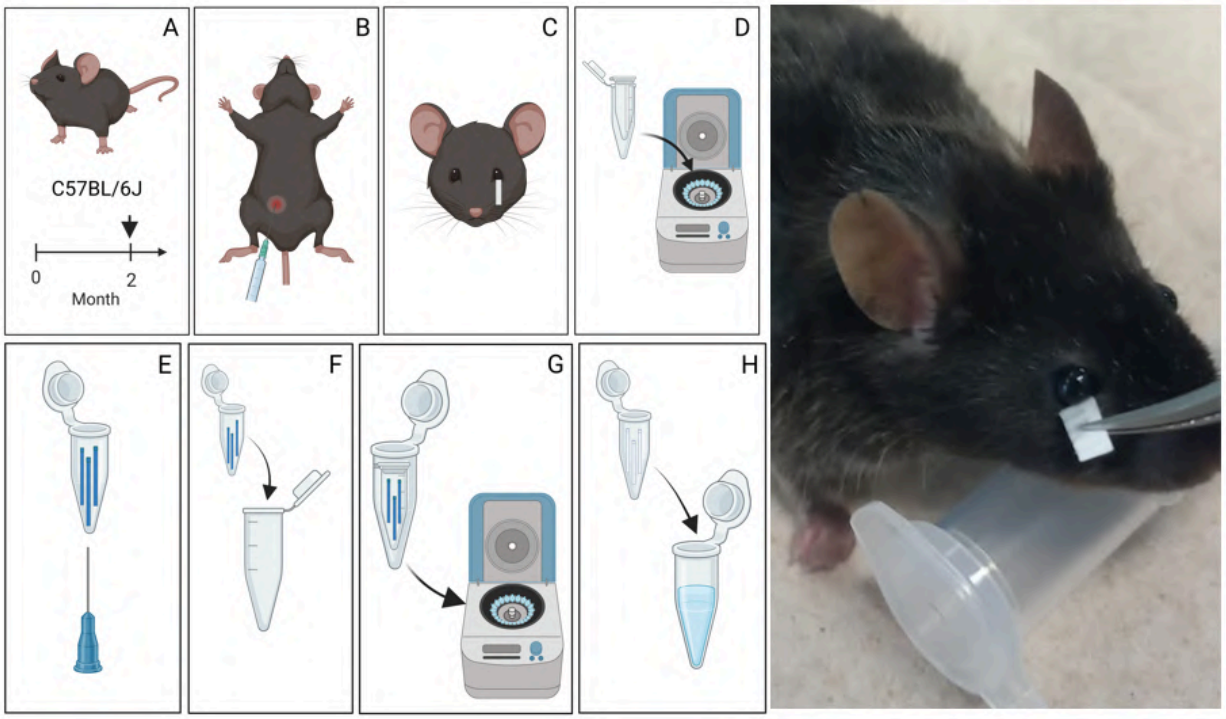
**Keyword 1**: Glia
**Keyword 2**: Astrocyte heterogeneity

**OPTIMIZING TEAR COLLECTION IN MICE FOR MRNA AND PROTEIN ANALYSIS**

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The tear film is a highly dynamic biofluid capable of reflecting pathology-associated molecular changes, not only in the ocular surface but also in other tissues and organs. Molecular analysis of this biofluid offers a non-invasive way to diagnose or monitor diseases, assess medical treatment efficacy, and identify possible biomarkers. Due to the limited sample volume, collecting tear samples requires specific skills and appropriate tools to ensure high quality and maximum efficiency. Various tear sampling methodologies have been described in human studies. We have developed a reliable method for extracting tear-related protein information from experimental animal models, especially mice. This method includes the pharmacological stimulation of tear production in 2-month-old mice, followed by sample collection using Schirmer strips and the evaluation of the efficacy and efficiency of the protocol through standard procedures like SDS-PAGE, qPCR, and digital PCR (dPCR). The aim of this study was to bridge the gap between human and animal research, by establishing an affordable, standardized, and optimized tear sampling protocol for animal models, facilitating translational studies and accelerating advancements in the field of ocular and systemic disease research.

**Keyword 1**: tear fluid isolation
**Keyword 2**: proteins
**Keyword 3**: non-invasive
**Keyword 4**: mice model



**IMPACT OF PSYCHIATRIC MEDICATION ON SUICIDE REATTEMPT RISK IN PATIENTS WITH DEPRESSION: A RETROSPECTIVE COHORT STUDY USING NATIONAL HEALTH INSURANCE DATA IN KOREA**

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Suicide is a critical public health concern globally, with depression recognized as a major risk factor. While psychiatric medications are widely used to mitigate suicide risk in depressed patients, their efficacy remains debated. South Korea, with the highest suicide rate among OECD countries, urgently requires evidence-based interventions. This study aims to assess the impact of psychiatric medication on the risk of suicide reattempts in patients with depressive disorder who have previously attempted suicide. Using data from the Health Insurance Review and Assessment Service (HIRA) in South Korea, we conducted a retrospective cohort study of 4,925 patients diagnosed with depressive disorders who attempted suicide in 2014. Patients were categorized based on psychiatric medication use within six months post-attempt. Inverse-Probability-of-Treatment-Weighting (IPTW) was employed to adjust for baseline differences. Logistic regression and survival analyses were conducted to compare suicide reattempt risks. Without adjustment, patients on psychiatric medication showed a higher suicide reattempt rate than those without medication (21.7% vs. 17.8%, P = 0.0015). However, there were baseline imbalances after IPTW adjustment, logistic regression revealed a 35% reduction in suicide reattempt risk in the medicated group (OR = 0.65, 95% CI: 0.504–0.846, P = 0.001). Survival analysis indicated no significant long-term difference. Subgroup analysis showed significant short-term risk reduction in individuals under 65, but no effect in those 65 and older. Psychiatric medication significantly reduces the short-term risk of suicide reattempts in depressed patients, particularly in younger populations, though long-term effects are limited. Tailored, age-specific interventions are recommended to enhance suicide prevention strategies.

**Keyword 1**: Suicide
**Keyword 2**: Depression
**Keyword 3**: Psychiatry
**Keyword 4**: Medication
**Keyword 5**: Psychotropics

# Neuroethology and Comparative Neuroscience

**UPPSALA UNIVERSITY BEHAVIORAL FACILITY (UUBF), YOUR NEW PARTNER IN BEHAVIORAL STUDIES IN MICE, RATS OR FISH**

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Behavioral core facilities are becoming key features in academic institutes, because they provide expertise and specialized services for researchers within neuroscience and neighboring fields. The Uppsala University Behavioral Facility (UUBF; www.uu.se/uubf) is a non-profit core facility supported by the Faculty of Medicine and Pharmacy, Uppsala University, Sweden. With employed personnel, UUBF's main aims are to provide administrative and organizational services for research groups performing behavioral studies within and beyond Uppsala University. UUBF is a unique infrastructure as it includes support and expertise for three of the most common vertebrate model organisms: mouse, rat and fish (mainly focused on zebrafish), and also involve researchers investigating insect behavior. UUBF provides premises, equipment and protocols for behavioral experiments and assists with research in a wide range of methodologies. Examples include setting up new behavioral tests, behavioral and optogenetic integrations, welfare studies as well as deep learning approaches and statistical methodologies. UUBF also helps with data analysis, interpretation of results, writing of ethical applications, and training and guidance in experimental design. Furthermore, UUBF offers graduate courses on animal behavior, e.g. "How to study behavior in vertebrates with focus on fish and rodents". In summary, UUBF can assist in the whole process of behavioral experimentation, from design, performing, analyzing and publishing your data. You are welcome to contact UUBF (uubf@uu.se) to discuss your future behavioral experiments.

**Keyword 1**: Core facility
**Keyword 2**: Behavioral phenotyping

**EVERY MOUSE COUNTS! THE VALIDATION OF THE *EXPERIMENTAL UNIT* IN THE FOCUS OF ANIMAL WELFARE**

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To justify animal experiments, modern research relies on advanced methodologies, with statistical techniques playing a key role in reducing animal use. Often overlooked, statistics help minimize subject numbers through biometrical predictions and, importantly, correct identification of the experimental unit (EU).
The EU is defined as the smallest unit that can be treated differently within an experiment. Proper EU implementation can increase animal numbers when "treatment" refers to housing conditions, as the cage, regardless of the number of animals, is the EU. Approaches such as ignoring social behavior, testing a "cage representative," or using the mean of the cage data can result in surplus animals. Alternatively, testing all animals within a cage but using only the cage mean could address this.
To evaluate these approaches—single housing, "cage representative," "cage mean," or testing all individuals within a cage—we assessed inbred C57BL/6NRj and outbred SWISS mice of both sexes. We examined animal welfare through behavior and physiology, including activity, anxiety, nociception, sensorimotor skills, exploration, and corticosterone levels. Social hierarchy was evaluated weekly to assess correlations with behavior.
Our results indicate that housing condition did not affect outcomes. Data quality, assessed by coefficient of variation, depended on the parameter measured, not housing condition. Hierarchical ranks remained stable and did not correlate with behavior.
Using the EU as a "mean" or "representative" of the cage produces similar results to individual analysis, still avoiding single-housing and therefore prioritizing social needs and improving animal welfare, with greater respect for the individual lives of the mice.

**Keyword 1**: animal welfare
**Keyword 2**: behavior
**Keyword 3**: statistics
**Keyword 4**: mouse
**Keyword 5**: ethics

**COMPARATIVE ANALYSIS OF THE MOLECULAR, SPATIAL, AND FUNCTIONAL DOMAINS OF VERTEBRATE HABENULA**

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The habenula (Hb) is a conserved diencephalic nucleus implicated in neuropsychiatric disorders and adaptive behaviors. Two main Hb subdivisions, the lateral habenula and medial habenula, have demonstrated distinct roles in a variety of behaviors, such as classical and operant learning, predicting outcomes, addiction, fear, and social interactions. However, our understanding of cytoarchitecture and topography of the vertebrate Hb, as well as how different Hb microcircuits are organized across different species, remains limited. In this study, we utilized a combination of single-cell and spatial transcriptomic approaches across multiple species to investigate how habenular subdivisions are organized and how this organization compares across species in relation to Hb function. First, we identified numerous molecularly and spatially distinct subdomains of habenula across species and revealed that these subdomains show high spatial organization. We also observed that medial Hb has more molecular diversity than lateral Hb. Secondly, we showed that although the zebrafish habenula is typically highly asymmetrical between hemispheres, some of its subdomains are symmetrical, similar to those in the mouse habenula. Likewise, while the mouse habenula is generally viewed as symmetrical across hemispheres, we identified several subregions that displayed significant asymmetries. These findings indicate that asymmetry in the vertebrate habenula varies along a continuum among different species. Lastly, we demonstrated that habenular cell types are relatively conserved across species, with some exceptions. Our ongoing investigation aims to elucidate how the molecular topography of the zebrafish Hb relates to its functional architecture.

**Keyword 1**: Habenula
**Keyword 2**: Single-cell Transcriptomics
**Keyword 3**: Spatial Topography
**Keyword 4**: Spatial Transcriptomics
**Keyword 5**: Zebrafish

**DISTINCT TRAJECTORIES OF SYMPTOMS, COGNITIVE FUNCTIONS AND DAILY ACTIVITIES IN ELDER TWINS DISCORDANT FOR PAST DEPRESSION: AN EXTENDED NETWORK ANALYSIS**

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Depression's direct and indirect health effects compound the disease burden of normal aging. Personalised prevention is limited by existing diagnostic classifications. The network theory conceptualises individual depressive symptoms and associated protective factors (e.g., cognition) as a complex system of interrelated components, allowing the identification of potential personalisation targets. We evaluated the temporal stability and connectivity of extended networks including symptoms, cognitive functions and daily activities in elderly co-twins discordant for past depression, and explored possible genetic contributions to the structure of associations between the networks' elements. Gaussian graphical models estimated the networks of 228 like-sex (104 monozygotic and 124 dizygotic) co-twins aged >70 from the Danish Twin Registry at three timepoints, set two years apart. The networks of both the affected and unaffected by past depression co-twins showed stable global strength and global structure across time. At each timepoint, the affected co-twins showed stronger global network associations and stronger local associations among both depressive symptoms and cognitive functions than the unaffected networks. In affected co-twins, *anhedonia* was the most central network element (i.e., with the strongest independent associations with remaining variables), and *learning* – the most central cognitive function. In unaffected co-twins, memory functions were the most influential elements in the extended networks of depressive symptoms. Frequency of engagement in daily activities were not central in neither networks at any timepoint. No significant differences were observed when comparing monozygotic to dizygotic networks. Depression preventative strategies in the elderly should target anhedonia and stimulating/maintaining memory functions.

**Keyword 1**: network analysis
**Keyword 2**: depression
**Keyword 3**: cognitive functions
**Keyword 4**: twin study

**THE EVOLUTIONARY REORGANIZATION OF THE DENTATE GYRUS AND CA3 IN AN ONTOGENETIC PERSPECTIVE**

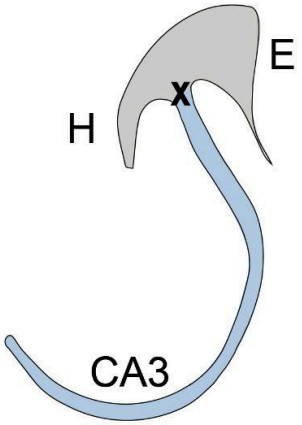
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The hippocampal formation and entorhinal cortex are interconnected brain regions essential for spatial navigation and episodic memory. Axonal projections from entorhinal cortex to the cornu ammonis 3 (CA3) field of the hippocampus are mediated through the dentate gyrus (DG), a unique mammalian feature. While the overall structure of the hippocampal formation is similar across mammals, the anatomical relationship between the DG and CA3 varies considerably. To better understand the possible functional properties of the two regions, we compared their mutual topography across 13 species, including insectivores, rodents, carnivores, lagomorphs, artiodactyls, and primates, using Timm-thionin- and Golgi-stained histological cross-sections, supplemented with public histological images. Observations of selected anatomical features in relation to the site of junction (labelled ‘X’ in the figure) between the CA3 pyramidal layer and the DG polymorphic layer allowed sorting of species into four groups, exhibiting progressive structural differences reflecting the ontogenetic development previously described in rat, rabbit, and human embryos. In less advanced mammals, the DG is situated at the edge of a fully stratified CA3, where palisade arranged bipolar pyramidal cells are supplied from the DG by a compact bundle of granule cell axons with *en-passant* giant boutons. In more advanced species, the DG-near part of the CA3 is progressively transformed into a 'destratified' and medially reflected blade underneath the hidden blade of the DG, through which scattered mossy fibres target dispersed multipolar CA3 pyramidal cells. This change may represent a substrate for increasing capacity for differentiated analysis of impressions.

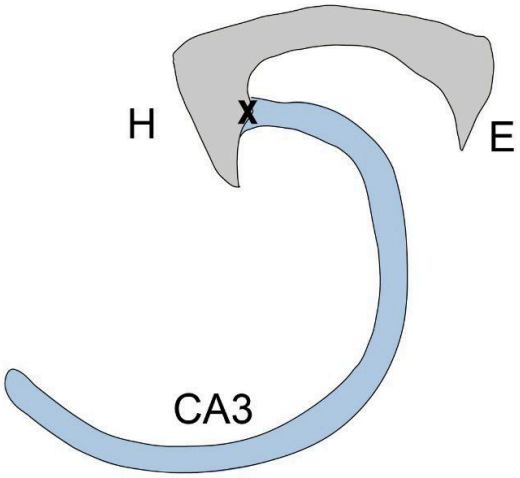
**Keyword 1**: hippocampus
**Keyword 2**: comparative anatomy
**Keyword 3**: dentate gyrus
**Keyword 4**: Timm-staining
**Keyword 5**: Golgi impregnation

# Four groups of DG-CA3 morphology

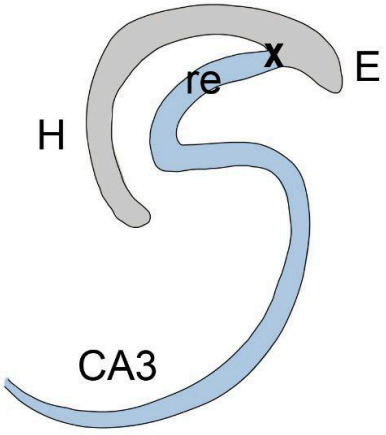
**1. Hedgehog, hamster, mouse, rat**



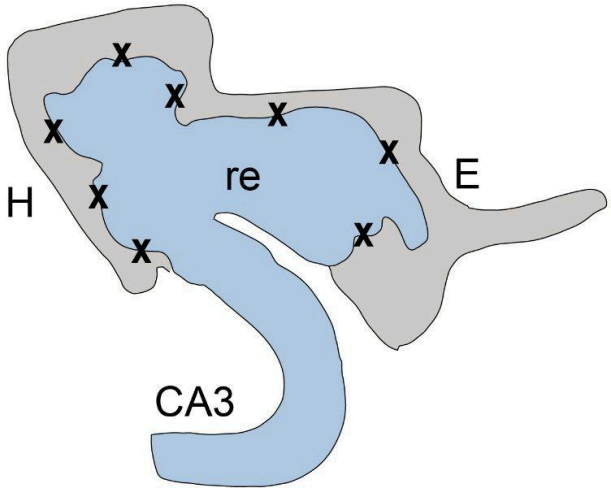

**2. Mole, mink, cat**




**3: rabbit, guinea pig, sheep, pig**




**4: monkey, human**




DG polymorphic layer  CA3 pyramidal cell layer

**Abbreviations:** E, polymorphic layer in exposed blade of DG; H, polymorphic layer in hidden blade of DG; re, pyramidal cell layer in the reflected blade of CA3; X, site of junction of CA3 pyramidal layers with DG polymorphic layer.