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ABSTRACT BOOK

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Symposia

Novel targets in Alzheimer's drug design: restoration of brain cell homeostasis

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Alzheimer's disease (AD) is a multifactorial disease associated with aging and several pathogenic mechanisms. AD is a continuum spanning from normal cognition to dementia with clinical and pathological heterogenity. Chronic inflammation is the main driving factor of AD progression. The primary event is neuroinflammation with activated microglia and the astrocytes, and release of pro-inflammatory cytokines, leading to overproduction of Aβ and hyperphosphorylation of tau protein, resulting in accumulation of toxic Aβ and p-tau aggregates. There is no singular variant gene or mechanism behind sporadic AD, although 50% of AD patients has ApoE4 gene. We have identified three drug targets for breaking the vicious cycle of pathological events by blocking chronic neuroinflammation, protecting synaptic stability and restoring protein homeostasis.

Sigma-1 receptor (Sig-1R) is an intracellular receptor widely expressed in the CNS that modulates signaling pathways, remodels membrane microdomains, and is considered as a drug target in several pathologies. Sig-1R agonists are cytoprotective, reparative, and anti-inflammatory. We used a new ensemble docking-based virtual screening protocol to search for suitable Sig-1R binding compounds using an in-house compound library. Sig-1R binding affinities were measured with a competitive radioligand binding assay. Five novel high-affinity Sig-1R ligands were identified. One of them exhibited anti-inflammatory effect in vivo and good Sig-1R/Sig-2R selectivity.

Synaptic stability requires interconnection of several structural proteins. Some of them have at least one WW domain that binds short peptides with a PXP motif. A library of 65 peptides with PXP motif was synthesized and their neuroprotective effect was measured in vitro (SH-SY5Y cell line, MTT viability assay). The best compound (an all-D amino acid containing pentapeptide: apape) showed good neuroprotective affinity in vivo based on dendritic spine density, learning ability and memory function measurements.

Heat shock proteins (Hsps) have chaperone activity and play a pivotal role in protein homeostasis. Several active Hsp co-inducers were selected from a library of 1,4-dihydropyridine derivatives. One of them, LA 1011 was effective in the APPxPS1 tg mouse model of AD: preserved neurons, increased dendritic spine density, reduced tau pathology, and improved learning and memory indicating its potential as a drug candidate for neuroprotective therapy.

Hyperexcitability as a modifiable risk factor of Alzheimer's disease

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Over the past decade, experimental and clinical findings have consistently shown that cortical hyperexcitability is a common feature in Alzheimer's disease (AD) and plays a significant role in its pathophysiology. Studies using mouse models of AD indicate that recurrent epileptiform activity can promote the deposition of amyloid-beta (Aβ) and phosphorylated tau (p-tau), while also disrupting the balance between inhibitory and excitatory neural networks. This exacerbation of neuronal hyperexcitability and Aβ/p-tau accumulation contributes to cognitive decline in AD.
More than half of individuals with late-onset epilepsy of unknown etiology (LOEU) present with mild cognitive impairment (MCI) at the time of their first seizure, suggesting epilepsy could serve as a non-cognitive prodromal marker of AD. These findings emphasize the need for early cognitive screening in individuals with LOEU to enable timely disease-modifying interventions.
Recent evidence highlights the high prevalence of subclinical epileptiform activity (SEA) in late-onset AD (LOAD), which is strongly linked to accelerated cognitive decline. A higher frequency of epileptic spikes correlates with poorer cognitive performance, indicating a direct association between pathological hyperexcitability and memory impairment. Diagnosing seizures in AD can be challenging, as focal-onset seizures with impaired awareness often mimic memory lapses and other behavioral symptoms of the disease. Overnight electroencephalography (EEG) has proven highly sensitive in detecting epileptiform activity, and its use early in the diagnostic process is recommended to guide timely initiation of antiseizure medications (ASMs) when needed.
The strong association between LOE and faster disease progression in AD, coupled with the 70% likelihood of recurrent seizures following the first episode, underscores the importance of early therapeutic interventions. Levetiracetam has emerged as a promising treatment for managing epileptic seizures in AD.
AD pathogenesis involves a complex interplay of predisposing and exacerbating factors, including epilepsy. To better understand the causal links between cortical hyperexcitability and neurodegeneration, large prospective studies are needed incorporating multimodal diagnostic tools—such as cerebrospinal fluid (CSF) and imaging biomarkers—and long-term EEG recordings.

The work was founded by the HAS NAP III program (2022-I-9/2022), the Lendület (2023_94) program, the János Bolyai Research Scholarship of HAS (bo-78-20-2020) and the EU JPND project (2019-2-1-7-ERA-NET-2020-00006).

Modulation of neurovascular responses by microglia

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Microglia represent the main immune cell population in the brain parenchyma and are key modulators of inflammatory processes. Microglial function is altered in common brain diseases, but how microglial mechanisms impact on neurovascular processes under physiological conditions and in diverse brain pathologies, is not well understood. We have identified novel forms of microglia-neuron interactions, through which microglia sense neuronal activity and injury, while also modulate neuronal function. These purinergic interactions occur at specified areas of neuronal somata and are maintained in accordance with changes in neuronal metabolic states. Microglia also shape vascular responses via purinergic, compartment-specific actions through which microglia modulate cerebral blood flow, neurovascular coupling and cerebral hypoperfusion. In the inflamed brain, altered microglia-vascular interactions are associated with perfusion changes and modulation of central leukocyte recruitment. Thus, understanding the mechanisms of microglia-neurovascular interactions is likely to help the identification of novel therapeutic targets in common neurological disorders.

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Heat shock proteins and Alzheimer's disease

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Heat shock proteins (HSPs) are evolutionarily conserved chaperones that play an important role in the cellular stress response. During ageing, there is a decline in the stress response capacity of cells promoting the development of age-related diseases. As one of the most important functions of HSPs is to maintain cellular protein homeostasis, they may be promising therapeutic targets in protein misfolding disorders, such as Alzheimer's disease (AD). Indeed, a number of studies have confirmed the neuroprotective role of HSPs in different animal models. In the APP/PS1 transgenic mouse model of AD, we found that overexpression of the small heat shock protein, HSP27 ameliorated certain symptoms of the disease, such as amyloid plaque formation and memory impairment. Although the chaperone function of HSP27 may be essential for its neuroprotective effects, there is increasing evidence that HSPs also have several moonlighting functions. For example, in our previous experiment, HSP27 overexpression was found to normalize the increased synaptic excitability in APP/PS1 mice. This hyperexcitability is probably related to the enhanced susceptibility of APP/PS1 mice to epileptic-like seizures, leading to higher mortality. However, according to our latest results, overexpression of HSP27 in APP/PS1 females remarkably reduced the mortality rate. Moreover, emerging evidence suggests a regulatory role for HSPs in inflammation, which is an important factor in the pathogenesis of AD. However, under different conditions, HSPs can induce both pro- and anti-inflammatory cytokines, suggesting their complex role in fine-tuning inflammatory processes. Previously, we found that in response to acute brain injury, HSP27 enhanced certain neuroinflammatory processes without increasing the level of cell death. On the other hand, here we show that HSP27 does not further exacerbate AD-related chronic inflammation, but instead we detected a milder cytokine expression and a slight increase in markers of M2 microglial activation, suggesting a shift towards anti-inflammatory actions and tissue repair. As neurodegenerative diseases, including AD, are a growing problem for our ageing society, the development of new strategies for prevention and treatment is particularly important, and we hope, that our research can contribute to this in the future.

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Alzheimer-stroke continuum: the role of spreading depolarization

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Cerebral amyloid angiopathy leads to capillary obstruction in Alzheimer's disease (AD). AD is a neurodegenerative disorder, however, the altered microvascular function shares similarities with the pathology of capillary dysfunction after acute ischemic stroke (AIS). In accordance, we have limited information about the co-morbidity of AD and AIS. Although the pathologies behind capillary dysfunction remained elusive, here we report the novel, elevated frequency of spreading depolarization (SD) in AD mice after AIS. We hypothesize that SD, that is known to induce capillary constriction in AIS, might worsen the progression of AD.

Aged (19-23 months) female and male APP/PS1 (AD, n=7) and control (WT, n=8) mice were used. AIS was induced under isoflurane anesthesia (0.8-1%) by the transient (60 min) microfilament occlusion of the middle cerebral artery. At the end of AIS, reperfusion was induced by removal of the microfilament. The AIS caused neurological deficit during the 72 hours of survival period was tested daily using the Composite Garcia Neuroscore Scale (GNS) system. After 3 days, T2 and DWI MRI sequences were recorded to assess infarction, and functional ultrasound imaging was used to characterize neurovascular coupling (NVC) during mechanical whisker stimulation (2-5 Hz).

We have observed no difference in the neurological deficit of mice after AIS (GNS: 13±2 vs. 12±4 points; AD vs. WT). AD mice developed diffuse AIS morphology and frequent SD evolution in response to whisker stimulation (SD number: 13 vs. 3; AD vs. WT). The appearance of SDs confirms an enhanced neuronal excitability to somatosensory activation in AD mice after AIS. Furthermore, the amplitude of the whisker stimulation triggered functional hyperemia of NVC was also reduced in the contralateral (intact) cortex of the AD group (NVC: 9.3±5 vs. 15.5±3.1%; AD vs. WT), suggesting the impaired neurovascular function of AD mice.

Both experimental and clinical data have suggested that protein aggregation in AD fosters cortical and hippocampal hyperexcitability. Indeed, a considerable number of late onset AD patients present with epilepsy. While seizure activity is thought to be the antechamber of SD, our results are novel, as SD evolution has never been observed in AD. The objective of our future experiments is to determine whether the inhibition of SD could represent a novel approach to AD therapy.

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SII.1 Symposium

Secretagogin, a calcium-sensor protein shapes neuronal migration and regeneration

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Neurons of the adult central nervous system reside within an established extracellular scaffold which principally hampers regeneration. New-born cells must migrate from their locus of origin through this framework to reach their destination. We identified a latent neuronal cohort in the brain which is activated on-demand and can focally demolish the extracellular matrix to help neuroblast migration. These neurons form chains and reside in the rostral migratory stream of rats and mice to execute a coordinated release of an endogenous matrix-lytic enzyme, matrix metalloprotease-2 (MMP2), to dynamically restructure the extracellular matrix. Enzyme externalization is annexin-V dependent and regulated by secretagogin, a calcium-sensor protein, the expression of which blueprints this specific neuronal ensemble. Prefrontal cortex projects onto RMS shell cells and inhibition of prefrontal cortex GABA interneurons increases neuroblast migration in the RMS. Upon injury, corridors lined by secretagogin neurons appear between the ventricular wall and the cortex in intimate contact which migrating neuroblasts. Of note, secretagogin neurons are present in the human brain in the olfactory tract as well as in the developing cortex where they form vertical corridors. We argue that secretagogin neurons and their matrix demolishing mechanism might be exploited for therapeutic benefit in rescue strategies.

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SII.2 Symposium

Cell transplantation for the reconstruction of neuronal circuits after brain damage

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The adult mammalian brain lacks the ability to regenerate after an injury, but cell transplantation offers a potential solution for reconstructing neuronal circuits. In recent years, significant progress has been made, with transplantation studies in mouse models of cortical injury yielding promising results in respect to transplant survival, morphological development, and connectivity. These findings provided proof-of-principle evidence that new neuronal cells can successfully integrate into cortical brain circuits. However, it remained uncertain how the cellular and molecular environment of the injury influences the outcome of cell transplantation and whether transplants can accurately and stably reconstruct the cellular makeup and intricate connectivity of the cerebral cortex. By utilizing cell transplantation in the injured mouse neocortex, and leveraging genetic tools, rabies virus tracing, advanced microscopy and quantitative connectomics we investigate these fundamental questions. Our research provides novel insights into the potential of stem cell transplantation for regenerating complex circuitries in the adult mammalian brain.

Brain disease models in human cortical organoids

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Human induced pluripotent stem cells (hiPSC) can be reprogrammed from somatic cells of patients and can differentiate into any type of cells of the human body, offering human-specific models for various diseases. Human iPSC-derived cerebral organoids, three-dimensional models of the forebrain, resembling its cell type diversity and cortical architecture, were shown to model neurodegenerative diseases more accurately than 2-dimensional cultures of hiPSC-derived neurons alone. Air-liquid interface cultures of sliced cerebral organoids (ALI-COs) promote formation of neuronal circuits and allow longer culturing times, leading to the generation of more mature cells as seen in regular cultures of cerebral organoids.

Using the ALI-CO system, we investigated the human-specific disease mechanisms of amyotrophic lateral sclerosis with frontotemporal dementia (ALS/FTD) caused by the mutation of the C9ORF72 gene (C9 ALI-COs). ALS is mainly characterized by the loss of motor neurons, but more recently astrocytes have been shown to be affected too. The C9 ALI-CO model displays distinct neuronal and astrocytic pathology from early on, which turnes into more severe disturbances over the curse of the in vitro culture.

Observation on iPSC-derived astrocyte cultures provided evidence that KIF5A, a microtubule-bound motor protein previously known to be expressed only in neurons is also present in astrocytes. We showed that absence of KIF5A disrupts the structural integrity of astrocyte processes and mitochondrial transport and demonstrated that low KIF5A protein levels underlie cytoskeletal changes in astrocytes derived from ALS patients with SOD1 mutation. Since a disease relevant role of KIF5A was also demonstrated in C9ORF72 mutant ALS/FTD neurons we used the C9 ALI-CO system to provide evidence of shared astrocytic pathology between different genetic forms of ALS.

Chicken embryo as a tool for studying regeneration of human iPSC grafts

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Adult neurogenesis requires the same molecular players and genetic programme described during embryological neurogenesis. Moreover, Species - specific differentiation pace in vitro indicates that some aspects of neural differentiation are governed by cell intrinsic properties. Here we describe a novel in vitro human neural - rosette assay that recapitulates dorsal spinal cord differentiation but proceeds more rapidly than in the human embryo, suggesting that it lacks endogenous signalling dynamics. To test whether in vitro conditions represent an intrinsic differentiation pace, human iPSC - derived neural rosettes were challenged by grafting into the faster differentiating chicken embryonic neural tube iso -chronically, or hetero - chronically into older embryos. In both contexts in vitro differentiation pace was initially unchanged, while long - term analysis revealed iso - chronic slowed and hetero – chronic conditions promoted human neural differentiation. Moreover, hetero - chronic conditions did not alter the human neural differentiation programme, which progressed to neurogenesis, while the host embryo advanced into gliogenesis.

This study demonstrates that intrinsic properties limit human differentiation pace, and that timely extrinsic signals are required for progression through an intrinsic human neural differentiation programme.

Tracing the evolution of the neuroendocrine system behind animal reproduction: functional and evolutionary insights from mollusks

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There is an ongoing debate about the evolution of the endocrine system that mediates animal reproduction, particularly regarding the presence of elements of the HPG axis in invertebrates. Since the 1950s, hundreds of studies have concluded either that mollusks have a functional homolog to vertebrate GnRH, can synthesize sex steroids de novo, appear to contain sex steroid receptors, or respond when exposed to vertebrate steroids. However, starting from 2010, critical reviews began to question the claim that molluscan and vertebrate endocrine systems are similar, underlying the need to revisit the evolution of the endocrine system behind reproduction.

To contribute to this, multilevel experiments were performed on the widely used molluscan model, the great pond snail (Lymnaea stagnalis). In our neural transcriptome data, we identified a homolog sequence to vertebrate GnRH. Our behavioral, immunohistochemical, phylogenetic, and ligand-receptor investigations clearly supported the new concept that previously termed molluscan GnRH peptides are multifunctional and should be classified as corazonins. Our genomic and transcriptomic results demonstrated that FSH, LH, key elements of the vertebrate sex steroid synthesis pathway, and functional nuclear sex steroid receptors are not present in Lymnaea and in mollusks in general. Moreover, we revealed that immunohistochemical studies using antibodies against vertebrate proteins are grossly non-specific and have no value in studying sex steroid synthesis or activity in mollusks. Using radiolabeled sex steroids, we showed that snails can absorb sex steroids from the environment and accumulate them for a long time, confirming that the presence of vertebrate sex steroids in molluscan tissues is not evidence of endogenous origin. We detected sequences homologous to the known vertebrate membrane sex steroid receptors in Lymnaea. However, the signaling assays revealed that none of the candidates interacted with the main vertebrate steroid ligands, providing the first experimental evidence that functional membrane sex steroid receptors are absent in mollusks.

Our results have demonstrated at multiple levels that the endocrine systems of mollusks and vertebrates are fundamentally different. The findings contributed to the functional and evolutionary understanding of the neuroendocrine and reproductive systems of mollusks and provided a basis for a more precise understanding of certain general laws of neuroendocrine regulation.

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A dopaminergic circuit mechanism links past and future learning through shifts in perception+

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Learning and long-term memory formation are important but energetically costly processes. Neural strategies that could help identify the most relevant or fruitful associations are therefore likely to be beneficial for an animal's survival. Neuronal mechanisms to support such guided learning, however, are not well established. One candidate to help instruct new memory formation is a shift in perception induced by an animal's prior experience. Here, we used an established molluscan system (Lymnaea stagnalis) to directly probe the relationship between past memory, perception, and new learning. We elucidate a circuit mechanism in Lymnaea, which enables past memory to shape new memory formation through changes in perception. Specifically, strong classical conditioning drives a positive shift in perception that facilitates the robust learning of a subsequent and otherwise ineffective weak association. Circuit dissection approaches reveal the neural control network responsible, characterized by a mutual inhibition motif. This both sets perceptual state and acts as the master controller for gating new learning. Pharmacological circuit manipulation in vivo fully substitutes for strong paradigm learning, shifting the network into a more receptive state to enable subsequent weak paradigm learning. Our study reveals a key mechanism for coupling past and future learning through changes in perception. We hypothesise that this serves to signal to the animal a potentially learning-rich environment, allowing new positive associations to form to cues that would otherwise be ignored.

+Dedicated to the memory of Professor Paul Benjamin, who passed away in October 2024.

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A translational and multidisciplinary approach to studying the Garcia effect, a higher form of learning with deep evolutionary roots

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Most of us have tried a new food and later felt sick. While factors like improper preparation might be the actual cause, we instinctively 'blame' the new food, avoiding it in the future as even the thought evokes nausea. What makes this link between a novel taste and illness so strong that it overrides other experiences? The first clues emerged during World War II when ecologist C.S. Elton observed that rodents, after consuming poisoned bait, avoided it for weeks, even after recovering. This phenomenon, known as "bait-shyness," was soon used in pest control. In 1951, Dr. John Garcia revealed its broader implications, coining the term "the Garcia effect." His experiments showed that rodents rapidly develop a long-lasting, taste-specific aversion to a new food causing illness, even with a significant delay between ingestion and symptoms. By the 1970s, clinical studies extended these findings to humans, highlighting its role in conditions like chemotherapy-induced nausea. Despite its significance, research into the Garcia effect has declined over the last two decades. Long thought to be exclusive to mammals, we recently demonstrated its existence in an invertebrate, the pond snail (Lymnaea stagnalis). Sickness was induced by injecting snails with the bacterial endotoxin lipopolysaccharide (LPS), leading to a long-lasting, taste-specific Garcia effect. Molecular analyses revealed that LPS significantly upregulated immune-related genes such as Toll-like Receptor 4 (TLR4), consistent with mammalian studies. While LPS alone did not alter neuroplasticity-related gene expression, pairing it with taste conditioning upregulated key genes involved in learning and memory, including glutamate receptor subunits (LymGRIN1, LymGRIN2A, LymGRIN2B) and the transcription factor LymCREB1. Strain-specific differences in long-term memory (LTM) were observed: laboratory-inbred snails exhibited a 24-hour LTM, whereas freshly collected snails and their first-generation offspring formed a 48-hour LTM. Moreover, pre-treatment with aspirin (a non-steroidal anti-inflammatory drug) before LPS injection blocked both the sickness state and the Garcia-like effect at behavioral and molecular levels. These findings pave the way for translational and ecological studies aimed at characterizing the conserved mechanisms underlying this form of learning with deep evolutionary roots, which can be used to address a range of different biological questions.

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Large-scale deorphanisation of GPCRs in placozoans, reveals the ancestral origin of neurotransmitter signalling systems.

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Placozoans, simple marine animals without nervous systems or organs, offer valuable insights into early animal evolution. Despite lacking neurons, they display complex behaviours, regulated by neurotransmitters. Neurotransmitters are critical for modulating processes like movement, feeding, and environmental responses in the majority of animals. Studying these molecules in placozoans reveals how early forms of communication might have evolved before the development of synaptic nervous systems, shedding light on the origins of complex cellular signalling and nervous system evolution. During this talk I will mostly focus in the results obtained by the large-scale deorphanisation of receptors on Trichoplax adhaerens. Testing more than 50 against more than 20 different small amine molecules has allowed us to identify that some of these signalling systems are more ancestral than previously thought.

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Neural dependence and genes of the CNS regeneration

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Reparative regeneration (the ability of renewing lost body parts) is a distinctive feature that is mastered by earthworms. The earthworm's superior ability to regenerate body parts is exemplified by the fact that they can regenerate an extirpated cerebral ganglion ('brain') within weeks. The nervous system is thought to drive this capacity for regeneration, but to date no direct experimental evidence has emerged to support this hypothesis. Standardized extirpation experiments revealed that the majority of the neuropile reorganized from neural processes, originated from the circumpharyngeal connectives and prostomial nerves. These neural processes formed an anatomical scaffold to which stem cells attached and differentiated to neurons, glial, muscle and connective tissue cells. This was then followed by the appearance of anchoring and communicating cell junctions, and axonogenesis, then vasculo-and angiogenic features were observed. The size, form, and histological characteristics of the regenerated brain resembled the extirpated brain by the 6th postoperative week, but marked differences were observed in terms of the transmitter specific neural networks. Whilst the GABAergic network reorganized within the 3rd postoperative week, others (e.g. peptidergic and serotonergic systems) required more time, respectively. Global transcriptomic expression profiling of the truncated central nervous system as well as the newly regenerated brain revealed characteristic differences between the original and regenerated structures even at the 10th postoperative week. Detailed transcriptomic analyses confirmed that distinct gene sets are expressed as regeneration progresses. During the early stage of regeneration genes were modulated that contribute to the reorganization of damaged extracellular matrix and determine the formation of an anatomical scaffold to which stem cells attach. Thereafter genes were overexpressed that govern neural tissue development, which in turn influence cell proliferation and differentiation, axonogenesis and synapse formation, development of cell junctions etc. Changes in the distribution pattern and concentrations of biometals (Ca, Fe, Zn) in the regenerating brain revealed that especially iron plays a crucial role in neural tissue regeneration, suggesting that iron cofactors are key players of regeneration at the cellular level. Taken together we define the histological and molecular fingerprints of complex neuronal regeneration in earthworms.

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POTENTIAL USE OF SIGMA-1 RECEPTOR LIGANDS AND CANNABINOID ALLOSTERIC MODULATORS IN BRAIN DISORDERS

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Recent studies have exhibited therapeutic potential for sigma-1 receptor (Sig-1R) agonists and cannabinoid receptor 1 (CB1R) allosteric modulators in the treatment of several disorders such as cerebral ischemia and alcohol use disorder (AUD). Developing novel pharmacological tools to investigate agonist and allosteric modulator strategies could aid in the mechanistic understanding of functional selectivity in Sig-1R and CB1R which could ultimately help develop effective and safer therapies. Accordingly, we designed, synthesized, and evaluated a series of novel endogenous and synthetic Sig-1R and CB1R compounds using in silico, in vitro and in vivo assays.

Selective and potent compounds targeting Sig-1R and CB1R with agonist and allosteric potential were screened. Novel compounds with high affinity and selectivity for Sig-1R and CB1R in the sub- and low nanomolar range were tested in functional assays using [35S]-GTPγS binding. The tested CB1R compounds retained high potency as CB1R antagonists. Six of these compounds exhibited non-competitive antagonism at CB1R in the GTPγS binding assay, with Schild plot analysis indicating negative allosterism. In pharmacokinetic (PK) studies, the tested non-competitive antagonists demonstrated good systemic exposures in 3 mg/kg intraperitoneal (i.p.) injections. Acute treatments with enantiomerically pure compounds at 3 mg/kg dose showed maximal in vivo efficacy for CB1R antagonism, fully attenuating the effect of CB1R agonist in upper gastrointestinal (GI) motility assay. CB1R ligand-1 was peripherally restricted with 8% brain/plasma ratio, while CB1R ligand-2 displayed moderate brain penetration, with 34% brain/plasma ratio. Unlike rimonabant (10 mg/kg), neither of the two tested compounds (10 mg/kg) induced hyperambulatory activity. Additionally, both compounds dose-dependently (1, 3, 10 mg/kg) reduced alcohol consumption in drinking in the dark (DID) experimental paradigm.

We identified five novel, high-affinity, and selective Sig-1R ligands with in vivo activity. Additionally, we developed moderately brain-penetrant or peripherally restricted allosteric CB1R modulators that exhibit favorable pharmacokinetics and potent in vivo efficacy, without inducing anxiogenic effects. Future studies are warranted to further characterize these compounds in different experimental models and behavioral paradigms to demonstrate in vivo functional selectivity and improved CNS safety.

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Does N,N-dimethyltryptamine modulate microglial activity?

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Microglia exhibit elevated intracellular Ca2+ concentrations in response to acute brain injury. In turn, intracellular Ca2+ transients have been postulated to regulate microglial activation, which initiates neuroinflammation and pro-inflammatory cytokine production. Microglia express sigma-1 receptors (Sig-1R) on the mitochondria-associated membranes (MAM) of the endoplasmic reticulum (ER), which have been implicated in the cellular stress response. Here, we tested whether pharmacological activation of Sig-1R with N,N-dimethyltryptamine (DMT) suppresses the shift of microglia from a quiescent to an activated phenotype and how it modulates microglial proteomics.

Primary microglial cultures were prepared from the cortex of neonatal Sprague-Dawley rats. On day 6, the plated cells were treated with lipopolysaccharide (LPS; 20 ng/ml) to activate microglia and with DMT (Sig-1R agonist) alone (5-10-20-50μM) or in combination with LPS for 24 h. Microglial activation was evaluated by Iba1 immunolabeling. The degree of arborization was expressed by a transformation index (TI) calculated from cell perimeter and surface area. Iba1 protein levels were quantified by Western blot analysis, and phagocytic activity was visualized with fluorescent microbeads. Protein was isolated from harvested cells and processed for ptoteomics analysis.

Microglia acquired an amoeboid, activated morphological phenotype after LPS treatment. When the LPS-treated cell cultures were treated with DMT, significantly more ramified cells were observed. Increased Iba1 signal intensity in Western blot analysis confirmed microglial activation by LPS treatment, which was particularly reduced by DMT. Control microglia engulfed a few microbeads. In contrast, LPS challenge increased microglial phagocytic activity, which was significantly attenuated by DMT. The proteomics data identified a total of 2793 proteins, of which 244 were altered in abundance. The combined administration of DMT and LPS resulted in the upregulation of 21 proteins and the downregulation of 84 proteins.

DMT effectively reduced microglial activation. We hypothesize that DMT exerts its protective effect by modifying the ER stress response and microglial Ca2+ homeostasis. This hypothesis is supported by previous findings that microglial activation is associated with increased intracellular Ca2+ concentration and that Sig-1R in the MAM regulates Ca2+ transport between the ER and mitochondria.

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Effects of natural and synthetic sigma-1 receptor agonists in experimental cerebral ischemia

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Intracellular sigma-1 receptors (Sig-1Rs) regulate Ca2+ turnover between the endoplasmic reticulum and mitochondria, inhibit free radical formation and support the survival of stressed cells. Recently, it has been suggested that the use of Sig-1R agonists as adjunctive neuroprotective therapy may contribute to the treatment of ischemic stroke. Our experiments investigated the effects of two Sig-1R agonists, the endogenous dimethyltryptamine (DMT), known for its psychedelic effects, and the newly synthesized (S)-L1, in a rodent model of ischemic stroke.

Global forebrain ischemia was induced in isoflurane-anesthetized adult male Sprague-Dawley rats (n=81) by bilateral common carotid artery ligation. Ischemia was exacerbated by repeated induction of spreading depolarizations (SDs) and transient anoxia. After one hour, the common carotid arteries were released to induce reperfusion. In separate groups of animals, DMT, (S)-L1, the selective Sig-1R agonist PRE-084, the Sig-1R antagonist NE-100, and the broad-spectrum serotonin receptor antagonist azenapine (1 mg/kg/h) were continuously administered intravenously from the onset of ischemia. Cortical local field potential was recorded by a microelectrode, and changes in cerebral blood flow by laser Doppler flowmetry. The extent of neuroprotection and cellular localization of Sig-1Rs were characterized by immunocytochemistry. The affinity of DMT and (S)-L1 for Sig-1R was confirmed by competition receptor binding assay.

The presence of Sig-1Rs was confirmed by epifluorescence and confocal microscopy in the perinuclear soma and in the processes of astrocytes and microglia. The binding affinity of (S)-L1 in brain tissue homogenates far exceeded that of DMT (Ki: 14.8 ± 1.2 μM vs. 21 ± 1.8 nM). DMT, (S)-L1 and PRE-084 reduced the amplitude of SD, which was compensated by the addition of NE-100. The azenapine-induced increase in SD amplitude was suppressed by the addition of DMT. The treatments did not cause significant changes in baseline cerebral blood flow, or the blood flow response to SD. Importantly, DMT and (S)-L1 reduced neuronal apoptosis and ferroptosis and supported astrocyte survival.

Overall, our results suggest that the natural DMT and the synthetic (S)-L1 most likely achieve their neuroprotective effect via Sig-1R activation. Thus, the timely administration of Sig-1R agonists may be considered as adjuvant pharmacological therapies in the treatment of acute cerebral ischemic injury.

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Posters

Postpartum enhancement of spatial learning and cognitive flexibility: an IntelliCage study

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The postpartum period involves significant physiological, emotional, and neurobiological changes to support maternal care and offspring survival. Key adaptations include improved cognitive flexibility and spatial learning, enabling mothers to navigate environments, access resources, and meet their offspring's needs. However, the mechanisms behind these cognitive enhancements are not yet fully understood.

The IntelliCage system is a cutting-edge tool for studying cognitive changes in rodents under semi-natural, socially enriched conditions. It uses RFID technology to monitor multiple animals simultaneously, minimizing stress by eliminating human intervention and enabling the analysis of spontaneous behavior. IntelliCage supports advanced behavioral tests, assessing aspects like spatial learning and cognitive flexibility.

Our study used a series of behavioral tasks to evaluate cognition across pregnancy, postpartum, and non-pregnant states. Animals were acclimated to the IntelliCage and trained in nosepoke behaviors for water access. Spatial learning and cognitive flexibility were assessed via place and reversal learning tasks, requiring corner associations and adaptation to changing rewards. However, fixed schedule drinking tasks tested anticipatory and persistent behaviors under restricted access.

The results revealed significant enhancements in spatial learning and cognitive flexibility in postpartum females compared to pregnant and control animals. During place learning, postpartum females demonstrated faster acquisition of the correct location associated with water and a steeper improvement in performance over time. Similarly, in the reversal learning phase, postpartum females showed greater adaptability to the new reward location, indicating superior cognitive flexibility. Fixed schedule drinking tasks highlighted increased anticipatory and persistent behaviors in postpartum females, reflecting heightened motivation.

In conclusion, the present study is the first to determine that motherhood improves spatial learning performance and cognitive flexibility using IntelliCage.

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Cortico-thalamic and cortico-preoptic projections from the medial prefrontal cortex differently affect the social behaviour in rats

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The medial prefrontal cortex (mPFC) plays a role in social behaviour, and a lesion of this area can be the cause of many neuropsychiatric disorders, such as autism spectrum disorder or schizophrenia. mPFC neurons project to different subcortical areas, exerting influence from the mPFC. The objective of this study was to determine the projection pattern of two types of projection neurons and examine their role in social behaviour using chemogenetics.

To examine the neurons projecting to the medial preoptic area (MPOA), a retrogradely spreading Cre-expressing adeno-associated virus (AAV) was injected into the MPOA and a Cre-dependent second virus expressing stimulatory and inhibitory designer receptors into the mPFC. The mPFC neurons projecting to the MPOA gave rise to collaterals to several subcortical areas, including the accumbens nucleus, ventral pallidum, lateral septum, paraventricular hypothalamic nucleus, ventromedial hypothalamic nucleus, and medial amygdala but not to the thalamus. In a further experiment, AAV expressing DREADDs driven by the calcium/calmodulin-dependent protein kinase II (CaMKII) promoter was injected into mPFC using viral gene transfer. The CaMKII neurons of the mPFC projected only to the paratenial, mediodorsal (MD), submedius, and reticular thalamic nuclei but not to any cortical or subcortical region. Double retrograde tracer injections into the MD and the MPOA revealed that the majority of MPOA-projecting neurons are located in layer V, while MD-projecting neurons in layer VI.

The stimulation of the mPFC neurons projecting to the MPOA resulted in a reduction in the sociability of the animals in the three-chamber test. However, the duration of direct social interactions between freely moving animals remained unchanged. In contrast, the stimulation of CaMKII-containing cells in the mPFC resulted in a decrease in the time spent with conspecifics in the three-chamber test, as well as a reduction in the duration of several elements of social interactions between freely moving rats. Conversely, inhibition of these neurons had the opposite effect.

In conclusion, the mPFC exerts an inhibitory influence on sociability and social behaviours. The differential effects of manipulation of the two types of mPFC projection neurons indicate that they may have distinct roles in this inhibitory action.

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Thalamic control of aggression

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Lack of social interaction has been demonstrated to induce aggressive behaviour in rodents. In our previous research, we demonstrated that projections from the posterior intralaminar thalamus (PIL) to the medial preoptic area (MPOA) enhance the duration of positive valence social contacts. The present study aimed to elucidate the role of the PIL-MPOA pathway and the VMH to PIL projection in the induction of aggressive behaviour in male rats subjected to social isolation.To chemogenetically manipulate PIL neurons that are activated by social interaction, we emplyed the vGATE protocol. This methodology permitted the expression of excitatory or inhibitory DREADDs in PIL neurons that had previously been c-Fos activated in response to social interactions. Following a period of recovery, behavioral tests were conducted on the animals. On the first day of the experiment, a resident-intruder test was initiated 1.5 hours after a control, vehicle injection. On the subsequent day, the experiment was repeated with the administration of CNO. The stimulation of vGATE-labeled PIL neurons reduced aggressive behavior in the animals, whereas the inhibition of these neurons was found to increase aggression. Furthermore, the use of c-Fos immunolabeling revealed that stimulation of vGATE-labeled PIL neurons resulted in the stimulation of neurons in one of its target areas, the MPOA. In turn, the inhibition of PIL cells reduced c-Fos expression during aggressive behavior in the MPOA region. To investigate the PIL-MPOA neural pathway, an anterogradely spreading virus expressing DREADDs was injected into the PIL and intracerebral cannulas were implanted above the MPOA. This protocol permitted the selective manipulation of terminal fibers originating from the PIL in the MPOA region through local CNO administration. Selective stimulation of the pathway also reduced aggression, while its inhibition increased it Additionally, the role of VMH-PIL projections in aggressive behavior was examined, and it was found that the activation of this pathway increases aggressive behaviour. In conclusion, the activation of PIL neurons by social interaction leads to the inhibition of aggressive behaviour through their projections to MPOA neurons. This may contribute to the prevention of aggressive behavior through social interactions. Meanwhile, the projections of VMH neurons that trigger aggression can counteract the prosocial effects of the PIL-MPOA pathway

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Intermale aggression is regulated by oxytocin receptor expressing neurons in the medial preoptic area

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The medial preoptic area (MPOA) has been demonstrated to receive input from the paraventricular hypothalamic nucleus (PVN) as well as the posterior intralaminar thalamic nucleus (PIL). The former contains oxytocin, a prosocial neuropeptide known to exert its function via the oxytocin receptor (OTR). PIL neurons, in turn, contain the neuropeptide parathyroid hormone 2 (PTH2), which we showed previously to increase social behaviours with positive valence. Furthermore, our findings have also established that PIL neurons suppress aggressive behavior in rats through their projections to the MPOA. This study aims to elucidate the influence of MPOA OTR neurons on social interaction in male rats and to identify their projections and whether they are targeted by PTH2 terminals.

To study the function and projections of OTR-positive neurons, we used OTR-Cre transgenic rats to specifically target these cells with an AAV viral agent containing an inhibitory DREADD and mCherry fluorescent protein, which was also used to visualize the projections of the MPOA OTR neurons. To evaluate the aggressive behaviours of the animals behavioural tests were conducted. On the initial control day of the experiment, the animals had been injected with a vehicle and underwent a male intruder test. On the second day, clozapine-N-oxide (CNO) was administered to activate the DREADDs, and the test was repeated. Our results demonstrated that the inhibition of OTR neurons led to an increase in aggressive behaviour and a reduction in the duration of positive valence contacts. The visualization of the MPOA projections revealed that the OTR neurons exhibited the highest intensity of projection to the ventromedial (VMH) and the paraventricular hypothalamic nuclei, the infralimbic cortex (ILC), and the medial amygdaloid nucleus (MeA). Furthermore, double immunolabelling demonstrated that a high density of fiber terminals containing PTH2 closely apposed OTR neurons in the MPOA.

In conclusion, the findings reveal that OTR-positive neurons in the MPOA play a pivotal role in reducing aggressive behaviour and promoting positive valence social interactions, possibly by distinct projections to multiple brain regions, including the VMH. This result suggests that oxytocin may exert some of its prosocial functions via the stimulation of MPOA OTR neurons. In turn, PTH2-containing neurons projecting from the PIL to the MPOA may target the same neuronal population to exert their prosocial actions.

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Oxytocin receptor-expressing neurons in the medial preoptic area control social behavior in rats

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The neuropeptide oxytocin is released during social interactions, acts through oxytocin receptors (OTRs) to regulate social behavior. The medial preoptic area (MPOA) of the hypothalamus, exhibiting a high density of neurons expressing OTRs. While the role of the MPOA in reproductive behavior has been extensively studied, recent evidence suggests that the MPOA is also involved in the control of affiliative social behavior.
The objective of this study was to investigate the functional role of OTR-expressing neurons in the MPOA in social behavior. MPOA OTR+ neurons were selectively stimulated by chemogenetics in OTR-Cre female rats. Adeno-associated virus (AAV) expressing excitatory designer receptors (DREADDs) was injected into the MPOA in a Cre-dependent manner. The treatment day was compared to the previous and subsequent control days, during which the animals received only vehicle injections.
Following the stimulation of OTR+ neurons through the injection of the DREADD's ligand clozapine-N-oxide (CNO), a significant increase was observed in the number and duration of the following behavioral elements: allogrooming, body sniffing, mounting, and chasing. A significant reduction in the frequency and the duration of non-social behaviors was also observed. A control group of rats was also established, in which the subject animals were injected with AAV without the DREADDs. In this group, the behaviors were not affected by CNO treatment, they exhibited comparable behaviors to those observed in the rats in the excitatory group during the vehicle control days. Furthermore, the stimulation of the excitatory DREADDs did not alter the animals' sociability, social preference, depression-, and anxiety-like behavior. Using fiber photometry, we found that MPOA OTR+ neurons are activated during anogenital sniffing. Finally, the projections of MPOA OTR+ neurons were mapped by anterograde viral tract tracing demonstrating projections to multiple brain regions, including the periaqueductal grey matter (PAG) and the lateral septum (LS). Both regions are implicated in the control of social behavior. We also showed that OTR+ MPOA neurons are innervated by parathyroid hormone 2 containing terminals ascending from the thalamus, which are known to relay touch-related signals during direct social interactions. These observations suggest that MPOA OTR+ neurons may facilitate social interactions between adult female rats via projections to the PAG and the LS.

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Caskin scaffold proteins regulate repetitive and anxiety-like behaviour in an isoform-specific manner

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Synaptic scaffold proteins are essential for organizing and maintaining neurotransmitter receptor localisation and neuronal signalling pathways. Caskin proteins form a new family of scaffold proteins, with an already established indication to regulate synaptic functions. Caskin1 is known to be enriched in postsynaptic regions, where it interacts with proteins involved in synaptic plasticity, like AMPA receptors. Caskin2 isoform is structurally similar, but its potential role remains poorly understood. Preliminary results suggest that Caskin1 is capable of liquid-liquid phase separation, facilitating the formation of membraneless compartments.

According to our previous observations, Caskins modulates synaptic architecture, and the deletion of both Caskin isoforms impairs spatial learning and memory without a profound effect on general mobility in the open field test. Others have shown recently that Caskin1 KO mice show mildly increased anxiety, however the exact molecular mechanisms regulated by Caskins are not yet clarified.

To investigate the age-dependent behavioral consequences of lacking Caskin1 and/or Caskin2 isoforms, we assessed transgenic mouse strains of Caskin1 KO, Caskin2 KO, or double knockouts (dKOs) alongside wild-type (C57Bl6/J) and double heterozygous (dHz) controls at 2, 4, and 6 months of age. Self-grooming and marble burying assays revealed that activity of the heterozygous animals differs significantly from wild-type controls, indicating dose-dependent effects of Caskin genes. Absence of Caskin 1 or deletion of both isoforms reduced the duration and average time of self-grooming bouts in an age-dependent manner. In the marble burying test, Caskin1 KO and dKO mice exhibited an age-dependent increase of their interest towards the marbles. Mice lacking only Caskin2, on the other hand, showed similar activity to dHZ, further emphasising that complex behaviour are regulated predominantly by Caskin1. Immunohistochemistry results also indicate that deletion of both Caskin isoforms lead to increased expression of GluA1 subunits within the prefrontal cortex and the basolateral amygdala, with a more pronounced localisation within the perinuclear region instead of the expected postsynaptic localisation of AMPA type receptors.

These findings underscore the importance of Caskin1 in regulating repetitive and anxiety-related behaviours, further emphasizing its importance in behaviour, also affected in neuropsychiatric and neurodevelopmental disorders

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The influence of antibiotic cocktails on posttraumatic stress disorder like behaviour in male mice

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Posttraumatic stress disorder (PTSD) is a debilitating condition that affects 8%-13% of the general population and 20%-30% of military personnel. The heterogeneity of PTSD, including its comorbidity and partial symptom overlap with other mental disorders, further complicates this attempt. Most available treatments rely on psychotherapy and pharmacotherapy, but alternatives are required due to high number of treatment resistance. The gut-brain axis may play an important role in modulating the stress response and emotional regulation thus, gut might be a new therapeutic target.

We aimed to test the hypothesis that drinking antibiotic (AB) cocktail can affect PTSD-like symptoms.

Male CD1 mice was used and AB cocktail was given to the drinking water for 28 days before trauma. We used electric foot shock as trauma and in a conditioned fear paradigm concentrated on freezing as major outcome. As a first step, in an open field test we tested whether the AB treatment influence locomotion as well as anxiety. Traumatized animals were compared to controls, who were put into shock chamber without trauma and in both groups AB treated and control mice were also compared. Twenty-four hours as well as fourteen days after trauma we put the animals back to trauma environment to study acute stress disorder-like as well as PTSD-like behaviour.

Initially AB cocktail was unpleasant, the animals drop weight, but than they used to it and their condition normalized. There was no major impact of the chronic AB treatment on locomotion and anxiety, thus, the results of the PTSD-like behaviour are reliable. The traumatized mice spent significantly more time freezing and jumped also more than non-traumatized animals. Previous AB treatment influenced both the acute stress reaction measured 24h after trauma as well as PTSD-like behaviour measured 14 days after electric foot shock.

Our findings could offer new insights on how ABs influence trauma- and anxiety-related behaviours in neuropsychiatric conditions. We added further knowledge to the brain-gut axes confirming the role of microbiome in our behaviour.

Investigation of behavioural changes after acute dehydroepiandrosterone treatment for the therapy of Alzheimer's disease in mice

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Alzheimer's disease (AD) is a neurodegenerative disorder, and the most typical cause of dementia. Anxiety and depression are fairly common and well-modellable symptoms in AD, severity of which is depending on the progression of the disease. Dehydroepiandrosterone and its water-soluble form, dehydroepiandrosterone-sulphate (DHEAS) are endogenous hormones, which might have a positive effect on depressive behaviour, anxiety, and cognitive impairment. Thus, our goal was to evaluate the behavioural changes of acute treatment in a model of AD.

Seven months old male 3xTg-AD (B6;129-Tg(APPSwe,tauP301L)1Lfa Psen1tm1Mpm/Mmjax) and +/+ mice were treated intraperitoneally with DHEAS or saline (10mg\10mL/kg). Previously we observed that acute DHEAS treatment, given 30 minutes before tests had no behavioural effect but diminished morphological alteration 48h later. Thus, this time a later timepoint (24h) was chosen for behavioural examination. To test learning and memory Y-maze (working memory) and conditioned fear tests (CFT) were used. In the later fear extinction was also followed with conditioned stimuli (80dB sound).

In Y-maze test the typical genotype difference was observable (3xTg-AD mice moving less, $p<0.001$) as a possible sign of anxiety). Additionally, CFT also confirmed the increased anxiety of 3xTg-AD mice, with more time spent in immobile posture than the +/+ counter pairs. The extinction was observable in all four studied groups. However, no treatment-induced change was found. The morphological examination showed genotypical alterations.

All in all, we could not confirm that 3xTg-AD mice are more anxious, as a possible early sign. So far, no significant behavioural effect was found with the DHEAS treatment. We can assume that other dose or timing would be required for such changes.

THE INVOLVEMENT OF CHOLINERGIC LATERAL SEPTUM NEURONS IN ANXIETY AND THEIR ROLE IN PROCESSING OLFACTORY CUES IN MALE AND FEMALE MICE

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The lateral septum (LS) is known to play a crucial role in regulating emotional states like anxiety and aggression, as well as influencing social behaviors, potentially in a sex-specific manner. Despite ample experimental evidence supporting these functions, the precise mechanisms through which the LS exerts these effects remain unclear due to conflicting findings. Although traditionally considered to consist solely of GABAergic cells, our research has identified a subset of LS cells expressing markers characteristic of cholinergic neurons.

In this study, we aim to study the function of LS cholinergic cells (LSCNs) by selectively manipulating their activity using optogenetic tools and assessing behavioral responses in various anxiety-related tasks, including the Open Field Test, Elevated Plus Maze, Place Preference Test. Additionally, we explore neuronal activation patterns in response to olfactory stimuli using the fox odour test coupled with c-Fos staining, alongside the male pheromone darcin-containing urine samples, then quantifying the cellular overlap.

Our findings support that optogenetic activation of LSCNs induces anxiety-like behavior in males but not in females. We confirmed that a large proportion of LSCNs receives input from somatostatin (SOM) terminals. Predator odour exposure elicited stronger activation of LSCNs in females, whereas males had more SOM input-receiving neurons activated as a result to the olfactory cue. However, males showed a higher rate of LSCN activation than females after exposure to darcin.

We plan to employ a diverse array of techniques, including chemogenetic inhibition and stimulation, optogenetic inhibition, fiber photometry, electrophysiology, and anatomical and cellular composition studies, to further unravel the functional role of LSCNs within the LS and their broader implications for emotional regulation, social behavior and cognitive function in physiological and dementia mice models.

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Thalamic input of the medial preoptic area promotes maternal care in rats

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Mothers receive a set of different sensory cues from the pups, which are then processed by a complex integrative neural circuit. The medial preoptic area (MPOA) as the regulatory center of maternal behavior is a key component of this network by receiving diverse inputs from brain regions involved in sensory processing. Although, preoptic neuronal cell types promoting maternal care have been extensively studied, the specific afferent neural pathways participating in the integration of pup-related cues remain unknown. MPOA receives input from a thalamic region, the posterior intralaminar thalamic nucleus (PIL) which came into focus in recent years. PIL neurons expressing the maternally induced neuropeptide, parathyroid hormone 2 (PTH2), are supposed to transmit suckling stimuli to higher-order brain areas. In light of these data, our objective was to ascertain whether pup-related cues are transmitted from PIL neurons to the MPOA in mother rats. First, we examined the involvement of PIL neurons in the regulation of pup-directed behaviors. Therefore, chemogenetic stimulation of PIL neurons was performed by injecting a virus expressing an excitatory designer receptor into the PIL. Following ligand administration, the time spent with nest building and pup-related behaviors such as licking and grooming of the pups was increased. Furthermore, the latency of pup-retrieval was reduced upon stimulation. In order to examine the pup-induced activation of the PIL-MPOA pathway, we applied retrograde tracer injection into the MPOA and performed c-Fos immunolabelling. It was found that almost all MPOA-projecting PIL neurons are activated following pup exposure. Furthermore, a considerable proportion of these neurons are part of the PTH2-expressing neuronal cluster and were also labelled with calbindin (Cb). We also investigated whether Cb+ PTH2-expressing neurons of the PIL are activated specifically due to the exposure of touch-related pup stimuli. We established that these cells were prominently activated in mothers suckling their pups compared to mothers being separated from their pups. The MPOA is composed of various cell clusters involved in stimuli integration, and our findings demonstrate that one of these receives somatosensory inputs from the pups via Cb+ PTH2-expressing PIL neurons. In conclusion, our data indicate the role of the PIL-MPOA pathway in the formation of the maternal phenotype based on the processing of pup-associated direct contact-related cues.

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Functional characterisation of the lateral septal calbindin neurons

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The ventral subdivision of the lateral septum (LSv) is a forebrain region that has been linked to maternal care, but the specific cellular networks that are responsible remain unidentified. A high density of calbindin-containing (Cb+) neurons is present in the LSv, yet the properties, neuronal connections and functions of these cells remain unknown. In the present study, patch-clamp electrophysiological recordings were made of calbindin-containing neurons in coronal slices containing the lateral septum. Our findings revealed that these neurons exhibit regular firing patterns and possess a notably high membrane resistance. The mean amplitude and rise slope of spikes were found to be significantly lower in these neurons than in Cb- LSv neurons. To establish the projections of Cb+ LSv neurons, we applied viral-based, cell-type-specific anterograde tracing, and revealed that they send extensive projections to the medial preoptic area (MPOA), a region crucial for the regulation of maternal behaviour regulation. This projection was subsequently validated through retrograde tracing. To confirm whether information relevant to maternal behaviour is transmitted from the LSv Cb+ neurons to the MPOA, the pup-induced activation of these neurons was described using the c-Fos technique. To elucidate the pathway through which maternal signals reach the Cb+ LSv neurons, a retrograde tracer was injected into the LSv. This revealed input from the posterior intralaminar thalamus, which expresses the maternally activated parathyroid hormone 2 (PTH2) neuropeptide. Using double labelling, we identified PTH2 receptors on Cb+ LSv neurons. These receptors are likely activated by PTH2 released from nearby PTH2 terminals, which we also identified around the Cb+ neurons in the LSv. Using electron microscopy, we confirmed a synaptic connection between PTH2+ fibres and maternally activated inhibitory neurons in the LSv. Given that LSv Cb+ neurons have been demonstrated to be GABAergic, it seems plausible that they may be innervated by the PTH2+ terminals. Finally, functional studies using Cb-Cre mice revealed that the inhibition of LSv Cb+ neurons reduced pup-licking behaviour without affecting other maternal behaviours. In conclusion, our findings collectively suggest that activation of inhibitory Cb+ neurons in the LSv is essential for pup-licking behaviour, likely mediated through their projections to MPOA neurons.

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AI-based analysis of direct social interactions in rodents: development of a new software tool, Emerenka, to identify behavioural elements from the output matrix of DeepLabCut

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There is now a growing interest in elucidating the underlying mechanisms of the neurological basis of social interactions and neuropsychiatric disorders characterized by impaired social behavior. A significant proportion of this research employs rodent models, the applicability of which is contingent upon the accuracy with which rodent behavior can be interpreted. A frequently employed behavioral test for the measurement of social behavior is the social interaction test, in which two subjects are placed in a shared environment where direct contact between them is feasible. However, this makes it challenging to evaluate the data, as the animals block each other's view of the camera. The DeepLabCut (DLC) software tool, which is based on artificial intelligence, is capable of determining the positions of two labelled animals during a social interaction. The output matrix of DLC contains the positions of the body parts of the two animals, but does not provide the information on behavioral elements they engage in. Therefore, we have developed a web-based software solution for the purpose of extracting behavioral elements from the output matrix of DLC. The data analysis software has been developed with a user-friendly and flexible graphical interface that allows settings to be changed. The software determines the behavioral event based on the relative positions and distances between the body parts of the two animals. For example, in the context of anogenital sniffing, it is postulated that the tip of the nose of one animal is in close proximity to the base of the tail of the other, while the remaining body parts are situated at a greater distance. A total of nine body parts were considered, and 11 behavioral elements were identified for both animals. If the requisite conditions for a specific behavioral event are not met, the software will proceed to the next behavioral element, continuing until all have been evaluated. The software has two outputs. The first assigns a behavioral event to each frame defined by DLC, thus enabling verification of the results. The second provides a summary of the number of frames in which each behavioral event occurs. The software we have developed allows the evaluation of direct social interactions rapidly and accurately. The results of the software analysis were verified by comparison with the results of a manual analysis. Thanks to its high flexibility, the software can also be used to analyze additional tests evaluated by DLC.

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A distinct population of neurons in the mouse pretectum projects to subcortical motor centers to shape behavior

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The diencephalic-mesencephalic border harbors ambiguous neuron populations whose function and hodology are largely unknown. We here report about a group of neurons in the pretectal region of the mouse brain with a common genetic blueprint. The cranio-caudal chain of these secretagogin-neurons extended from the rostral pretectum until the periaqueductal region of the midbrain and showed a largely identical projection pattern. Cre-dependent viral tracing demonstrated that these neurons did not project to the cortex but sent extensive efferentation to the ventral pallidum, motor thalamic nuclei, zona incerta as well as to the pontine gigantocellular nuclei and the locus coeruleus. Pretectal secretagogin neurons received massive afferentation from the cingulate cortex. Behavioral testing using chemogenetics showed that neuronal activation by AAV particles carrying Cre-dependent DREADD expression system decreased the time spent in the open arm during elevated plus maze test but left motor abilities intact. Conclusively, we described a neuronal ensemble at the mouse diencephalic-mesencephalic border which broadly project onto subcortical motor regions. Since they receive massive prefrontal input, we suggest that these secretagogin-neurons are ideally positioned to hub top-down control onto behavior-driven motor activity.

Positive valence regulated by pontine inhibitory cells: fiber photometry evidence

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The lateral habenula (LHb) is an evolutionarily well-preserved structure responsible for motivational and cognitive functions. Using viral gene delivery methods in transgenic mice, we found a novel, inhibitory, pontine cell population projecting to the LHb. We analyzed the activity of this GABAergic (gamma-aminobutyric acid) pontine nucleus using head-fixed fiber photometry measurements. In water-deprived mice, we found that the consumption of water droplets led to an increase in its activity, whereas aversive airpuffs also activated this nucleus. We also found that the nucleus was significantly activated by an otherwise neutral tone if it was previously associated with a positive or a negative experience. Furthermore, when we presented multiple airpuffs with a relatively short interval between airpuffs, we observed a short-term accommodation represented by a decrease in its activation to subsequent airpuffs. Our results suggest that this novel, inhibitory nucleus plays a role in the processing of aversive and rewarding experiences and may prevent overactivation of the lateral habenular negative processing circuitry. Therefore, these cells may play a role in mood-related pathologies.

Exploring the time course of visual letter processing: an RSA approach

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Letter recognition is one of the most fundamental subcomponents of reading, and even the leading computational models of word recognition take it for granted. Not much is known, however, about the exact timeline of visual letter processing. We present findings from a representational similarity analysis (RSA) investigation, where the electrophysiological data was correlated with three main variables of the stimulus set: letter identity, letter case and font.

Twenty-six university students (21 female, 5 male, mean age: 21.8 years) with no reported reading difficulties participated in an EEG experiment. Letter stimuli were presented on a screen serially, and the participants performed a simple detection task (1600 non-target and 160 target stimuli). Non-target stimuli were of 4 identities (“a”, “e”, “f”, “g”), 2 cases (lower, UPPER) and were printed in one of 5 different fonts (in total, 40 different stimuli, each presented 40 times). Target stimuli were letters of different identities (“h”, “i”). During the stimulus presentation, a 64-channel EEG was recorded.

EEG data were pass-band filtered (0.5-40 Hz) and cleared of blink artifacts, using denoising source separation, based on vEOG peaks. The recording was then epoched relative to stimulus presentation and sorted according to the stimuli. Pairwise comparisons of EEG patterns were performed with an SVM classifier trained on 40-40 epochs of the corresponding individual stimuli. Classification accuracy was measured by LOOCV. This produced the neural representational dissimilarity matrix (RDM), a measure of how distinguishable all pairings of stimuli are, at each timepoint. The neural RDMs were Spearman-correlated with hypothesis-driven predictor RDMs, and time periods of significant correlations on a population level were determined with TFCE.

Correlation with the letter identity predictor was significantly larger than zero from as early as 90 ms after stimulus presentation. Similar correlations were seen with an across-case letter identity predictor matrix, showing that case invariance is detectable with our method, although the significant period started later. Letter case and font predictors were showing significant correlations with the neural data in distinctive time periods. Further RSA studies could shed light on yet unknown factors in letter processing, or even word recognition.

Effects of chronic 5G exposure on well-being and cognitive performance of adolescent rats

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Recently, the newest fifth-generation (5G) mobile telecommunication system has gained much popularity for its faster data transmission rate and higher communication quality. However, the potential health risks of 5G technology remain uncertain, raising widespread societal concerns. Adolescents represent a particularly vulnerable segments of population, as they are in a critical phase of rapid development, potentially increases their sensitivity to 5G.

Here we aimed to investigate the effects of chronic 5G exposure on the well-being and cognitive performance of adolescent (5-8 weeks old) rats. The 5G exposure (1hour¸/day) was used at 3500 MHz frequency with a peak power density of 30 W/m2, 3 W/m2 and 0 W/m2 (Sham-exposed) for 30 days. For neurological screening a battery consisting of 12 assessments was used. General locomotor activity, exploratory drive and anxiety-like behaviour of rats were measured with open field test (OF). Furthermore, cognitive performance of the animals were tested with novel object recognition (NOR)- and Morris-water maze (MWM) paradigms.

Results showed that neither mid- nor long-term neurological effects of 5G exposure were observed in adolescent rats. Regarding general activity in the OF, groups who received low or high intensity of 5G exposure showed less line crossing and rearing, in addition they entered less in the inner circle compared to the Sham-exposed group, which effect persisted until the end of the exposure period. Compared with the Sham-exposed rats, the irradiated groups did not discriminate between the objects in the NOR at the mid-exposure. However, long-term effect of the high-intensity exposure did not persist, as discrimination performance of the irradiated rats achieved the performance of sham-exposed group. Rats who received low-intensity radiation still showed bad memory performance at the end of exposure. Similar results were seen in the MWM: the recall of long-term memory tendentially impaired in the irradiated groups compared to the Sham-exposed group. In the post-exposure period, we did not observe any cognitive or behavioural differences between any of the groups, indicating that cognitive performance of the irradiated animals was restored.

To sum up the present study suggests that chronic 5G exposure may induce signs of transient behavioural and cognitive impairment in adolescent rats. Further investigations will be necessary to reveal the related cellular and molecular mechanisms.

Chronic treatment with estrogen-like compound shows antidepressive and neuroprotective potential in a triple transgenic mouse model of Alzheimer's disorder

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Alzheimer's disease is the most common type of cognitive dementia, affecting elderly women 1.6-3x more than similar aged man, or the younger generations. The advanced progression can be due to decreased hormone synthesis in post-menopause. 17β-estradiol (E2) is well-known for its neuroprotective effects. However, due to the risk of serious side effects, using a modified version of the compound is essential. Activators of non-genomic estrogen-like signalling (ANGELS) could be promising.

Based upon our previous experiments we aimed to examine the neuroprotective effects of a chronic treatment with an ANGELS molecule in a triple-transgenic mouse model of Alzheimer's disease (3xTg-AD).

The experiment was performed on 6-month-old genetically modified female 3xTg-AD mice, and their control strains. Animals received a chronic (2 month) daily s.c. treatment of vehicle and 33ng/g ANGELS compound (A2). During treatment behavioral tests were performed: Y-maze, open field (OF), novel object recognition (NOR), social discrimination (SD), Morris water maze (MWM) and forced swim test (FST). The Aβ1-42 plaques, Tau aggregates, choline acetyltransferase (ChAT) cell number, and acetylcholinesterase (AChE) fiber density in the brain were determined with immunohistochemistry.

AD animals show bad working memory, tend to be more anxious and move less than control strains. The ANGELS treatment did not affect working (Y-maze) and spatial memory (MWM), nonetheless, significantly improved object memory. The tested ANGELS had antidepressive effects in FST. The treatment increased the AChE positive fiber density in the somatosensory cortex, without influencing ChAT cell numbers in the basal forebrain.

The tested ANGELS had antidepressive effects and improved object memory after chronic treatment. Moreover, they proved to be protective in morphological examinations. We believe that these or similar compounds may provide a novel approach in AD therapy.

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Effect of Amblyopia on Visual Prediction Computations

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We aim to establish the role of predictive processing in neurodevelopmental disorders, focusing on amblyopia as a key case study. To achieve this, we developed a novel visuomotor feedback task combined with brain-wide activity readout using EEG to: (1) identify brain regions involved in visuomotor prediction in healthy individuals, (2) evaluate the effects of amblyopia on prediction-related activity patterns, and (3) construct functional network models and apply a longitudinal study design to infer which brain regions and functions are restored or remain impaired following amblyopia treatment.

Our task environment involves participants pedaling on a home-trainer bike within a virtual reality corridor while brain activity, eye movements, and markers of attention are recorded. At random intervals, participants encounter visual mismatches — events where visual motion abruptly halts in a specific region of the screen.

To sustain a continuous flow of visual predictions, the task capitalizes on the brain's intrinsic ability to anticipate changes in the visual scene caused by self-generated motion. We analyze mismatch-related EEG signatures to identify predictive processing abnormalities in amblyopic participants compared to healthy controls.

We also evaluate static visual acuity in participants by incorporating the classical visually-evoked potential (VEP) test into our experimental framework.

Analysis of control recordings revealed a distinctive P200 response across several cortical regions following mismatch events. Additionally, we observed asymmetries in response patterns based on the location of mismatches within the visual field.

Our approach enables the examination of neural responses to controlled disruptions in predictive streams and facilitates comparisons across various factors (e.g., attention, gaze location). We validate our experimental framework using advanced data analysis tools, ensuring its robustness for future investigations.

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Influence of Semantic Content and Verbalizability of Visual Stimuli on Audiovisual Equivalence Learning in Migraine Patients

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In acquired equivalence learning, equivalence forms between different stimuli due to their shared consequences. During the learning phase of the applied tests designed to investigate this phenomenon, the basal ganglia–frontal cortex loop plays a primary role, whereas the test phase relies mainly on the hippocampus–medial temporal lobe. Healthy controls performed significantly worse in an audiovisual test using visual stimuli with low semantic content (polygons, SoundPolygon test) compared to tests with stimuli having higher semantic content (faces, SoundFace test).

In this study, we aimed to investigate whether a similar trend can be observed in migraine patients and whether there are differences in performance between migraine patients and matched controls in the SoundPolygon test.

We analyzed the equivalence learning performance of 43 patients with migraine without aura during the interictal phase using two audiovisual tests (SoundFace and SoundPolygon). These tests included identical auditory stimuli but differed in the semantic content of the visual stimuli. Both tests consisted of a learning phase and a test phase, the latter further divided into retrieval and generalization parts. Additionally, the SoundPolygon test performance of 30 migraine patients was compared with that of an age-, gender-, and education-matched control group.

Migraine patients performed significantly better ($p < 0.05$) on the SoundFace test than the SoundPolygon test in all phases and for all cognitive measures assessed. Reaction times were also significantly longer in all phases of the SoundPolygon test. However, no significant differences were observed between migraine patients and healthy controls in the measured cognitive variables of the SoundPolygon test.

The performances of migraine patients across the two tests revealed a trend similar to that described in healthy controls. Thus, simpler visual stimuli altered the audiovisual associative learning in patients suffering from migraine. On the other hand, the performances of migraine patients in the SoundPolygon test were not significantly different from those of healthy controls. This finding is in line with previous studies conducted in migraine patients during implicit learning and could be explained by various cortical compensatory mechanisms of suboptimal subcortical functions.

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Behavioral consequences of astrocyte overstimulation in the pedunculopontine nuclei of mice

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The predunculopontine nucleus (PPN) is a part of the reticular activating system (RAS) and the mesencephalic locomotor region (MLR), regulating sleep-wakefulness cycles, locomotion and sensory gating. We previously showed that various neuromodulatory mechanisms exert their actions on the nucleus partially via the involvement of astrocytes. In this project, we aimed to assess the in vivo significance of our previous findings on in vitro preparations.

Mice were injected with virus vectors carrying plasmids encoding mCherry fluorescent tag alone or with hM3D chemogenetic actuator under GFAP promoter. The chemogenetic actuator was chronically activated by administeration of clozapine N-oxyde (CNO) into the drinking water for 2 weeks. The acoustic startle reflex, the activity wheel test, the Barnes maze test and the footprint test was performed prior and after the CNO consumption. After the experiments, mice were sacrificed and the injection site, the area expressing mCherry tag and the number of astrocytes, cholinergic and non-cholinergic neurons were evaluated.

We found that mice expressing hM3D had a decreased acoustic startle amplitude compared to the control group expressing only mCherry. Mild alterations of the circadian rhytm and voluntary movements were also seen. The time spent in rest was increased in the active period and decreased in the resting period. The speed maximum and the average distance ran was also increased in the resting period. The footprint test revealed an increase in the width of hindlimbs and an increase in hind/front width ratio. No change was seen in the Barnes maze test. The number of cholinergic neurons and astrocytes was decreased during hM3D activation.

In summary, chronic astrocytic activation elicited symptoms resembling the brainstem-related symptoms of progressive supranuclear palsy. With this experimental arrangement, we might provide an animal model for this disease.

Reduced visual stimuli elicit no altered associative learning performances in migraine patients compared to those of healthy controls

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Equivalence learning is a type of associative learning where pair building occurs between two seemingly unrelated stimuli if they have the same outcome. This process is linked to the basal ganglia and the hippocampus. The semantic meanings and verbalizability of the applied stimuli can significantly influence the effectiveness of learning. In migraine, cognitive alterations have been observed not only during the attacks, but also in the interictal phases. Furthermore, structural and functional changes of the hippocampus, are well known in these patients. Given this, a key question arises: How does the application of simplified stimuli influence learning performance in migraine patients compared to healthy individuals?

In our study, we collected data from 33 patients with migraine without aura during the interictal phase, and compared the performance of migraine patients with that of an age-, gender-, and education-matched healthy control group. The Rutgers Acquired Equivalence Test (RAET) is commonly used to assess equivalence learning. To reduce the semantic features and the verbalizability of the visual stimuli, a new test was developed (Polygon). Compared to the original RAET, where the visual stimuli are drawn faces and fish, we applied blank two-dimensional geometric shapes. The Polygon test follows the same structure as the RAET, including an acquisition phase and a testing phase, which includes both retrieval and transfer parts. We analyzed the number of trials needed to complete the acquisition phase, as well as error rates and reaction times across all three parts of the test.

No significant differences ($p > 0.05$) were found in any of the analyzed measures, including the acquisition phase and either part of the testing phase, between the performance of migraine patients and the healthy control group.

These findings indicate that unlike the original RAET — where adult migraine patients demonstrated poorer performance compared to matched healthy controls — the patient's performance did not differ significantly in visual associative learning when the test was more implicit, probably due to the use of stimuli with reduced semantic meaning. Our findings may be explained by cortical compensatory mechanisms, which could help mitigate the effects of hippocampal dysfunction in migraine patients. Supporting evidence from neuroimaging studies suggests that this compensation might be related to the involvement of frontal lobe circuitry.

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Development of Edinger-Westphal area(EWcp) specific conditional TRPA1 KO mice

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Targeting the complex neuro-immune interactions and neuroinflammation could be a new gateway in the pharmacotherapy of neurodegenerative diseases. Our previous studies demonstrated that TRPA1 KO mice show reduced memory loss in a model of senile dementia (Borbély et al., 2019) and attenuated cholinergic fibre and cell body loss in a model of the Alzheimer's Disease (AD) (Payrits et al. 2020).

We recently also demonstrated that the centrally projecting Edinger-Westphal area(EWcp) is the site of the strongest Trpa1 mRNA expression in the mouse central nervous system that is localized in the peptidergic, UCN1-containing neurones (Kormos et al 2022). Considering that the EW is affected by AD, and the TRPA1 is highly expressed here, we presumed that TRPA1 plays a role in the AD-associated neurodegeneration of the peptidergic neurones. We have also presented evidence, that TRPA1 may participate in stress adaptation, mood regulation, as well as in the smell loss which is an early sign of several neurodegenerative disorders.

In the present study we aimed to investigate the site-specific deletion of TRPA1 in the urocortinergic EWcp neurons in a mouse model of AD.

We have constructed an adeno-associated virus vector (AAV) that carries an UCN1 promoter-driven Cre recombinase (Ucn-Cre-AAV). The AAV vector was injected into the EW nucleus of the stopflox-td-Tomato mice. The Trpa1 mRNA expression was determined by RNA scope in situ hybridization technique and the urocortin protein was labelled by immunohistochemistry.

In the brain of the stopflox-td-Tomato reporter mice, we have proven that Ucn-Cre-AAV was introduced into urocortinergic cells, where the promoter activated the Cre recombinase and td Tomato, turning them red. By also performing the RNA scope technique on the sections, the complete co-localization is clearly visible, showing that the virus is specific for TRPA1 expressing urocortinergic cells.

On the basis of histological findings in the stopflox-td-Tomato reporter mice, we can say, that our Ucn-Cre-AAV construct is available to create the EW specific conditional TRPA1 KO mice. These mice are suitable to investigate the role of the TRPA1 receptor expressed in EWcp in mouse models of neurodegenerative diseases.

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Single versus multi-task measurement of non-human primate short-term memory

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Human cognitive test batteries involve multiple tasks to achieve multidimensional characterization of cognition or a specific cognitive function. However, in preclinical translational research test batteries are more frequently used as a set of tasks measured and analyzed separately. The present research aims to explore the challenges and opportunities of a systematic multi-task approach with non-human primates.

In a pilot project 3 adult male rhesus macaques were introduced to a day-by-day alternation of 3 different touchscreen tasks covering object memory (delayed matching to sample, DMTS), location memory (self-ordered spatial search, SOSS) and associative object-location memory (paired associates learning, PAL). The animals had been previously trained and tested in these tasks, but only one task was used for an extended period of time. Thus, we asked the following questions: 1) how fast can the animals reach their target performance level in previously trained tasks, 2) whether day-by-day alternation deteriorates performance in contrast to single-task measurement, 3) whether any combination in the order of tasks interferes with performance, 4) whether any convergent error patterns can be observed by analyzing tasks together.

In SOSS animals were shown 4-7 identical stimuli that they had to touch in an arbitrary order once, and only once. Each touch was followed by a 1-2 s long delay period for which all stimuli disappeared from the screen then reappeared at the same location. In DMTS animals were shown a sample stimulus, then after a 1-14.7 s delay period, the sample and 3-5 very similar, distractor stimuli were shown. The animals had to select the earlier shown sample stimulus from them. In PAL animals had to recall the locations of 3-8 distinct, sequentially presented schematic visual stimuli from a large 800-item stimulus set.

Preliminary results suggest that the more complex the task was, the more time animals needed to reach target level (SOSS ~ 4 days, DMTS ~ 34 days, PAL ~ 69 days) yet, once they reached target, day-by-day alternation did not deteriorate performance. Analyzing the tasks together we also observed similar error patterns: in SOSS and in PAL errors typically occurred at later phases of the choice sequence. As a next step, within-session multi-task designs will be introduced to probe task switching costs and to establish the adequacy of such design for behavioral pharmacological experiments in a translational setting.

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The modulation effect of art painting content on saccade-evoked perceptual processes: A high-density EEG Study

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Art perception involves complex neural mechanisms, with specific responses tied to the elements and content of paintings. The process of artistic appreciation involves processes at difference time scale; early perception of content within few hundred milliseconds followed by style recognition that leads to a later, contemplative stage after a few seconds. This study investigates group-level power differences in the earliest stage of EEG activity during the perception of four distinct categories of paintings: Faces, Geometry, Townscapes and People. Participants of the experiment were presented with a series of Abstract and Figurative paintings in random order. To simulate natural viewing conditions, each painting was displayed for 8 seconds, followed by a 4-second blank screen for a key-press response ("like" or "dislike") and a 1-second cue. Eye movements were registered with a Tobii Pro Fusion eye-tracking device. Brain activity was recorded using a 128-channel Biosemi ActiveTwo EEG system at a sampling rate of 2048 Hz. After pre-processing (high-pass filtering at 1 Hz, low-pass filtering at 40 Hz and down-sampling to 512 Hz), we performed Independent Component Analysis (ICA) to isolate neural and non-neural sources of activity. One of the ICA components showed distinct saccade-related lambda waves, i.e. transient potential peaks appearing ~100 ms after saccade onsets, which allowed us to identify individual saccades and visual responses in freeviewing settings, and consequently perform single-trial analysis. The 8-second EEG data window of painting viewing were segmented into lambda-peak centred epochs (-150 ms to 150 ms), resulting in 400–550 epochs per content category. Power spectral density was computed in the alpha and beta frequency bands using the FieldTrip EEG Toolbox, then the relative power differences were calculated between the painting categories (e.g. Faces vs. Geometry, Faces vs. Townscapes). The preliminary results indicate different topography of activations, mainly in the alpha band, distinguishing geometry/townscapes and faces/people contents. It also demonstrates the utility of this lambda peak-based epoching and analysis method in situations where eye-tracking data and saccade information are unavailable. This study confirmed that content modulates early saccade-evoked perceptual processes and the results contribute to the better understanding of the neural mechanisms related to art perception.

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Cortical coding of sex information in case of unfamiliar faces

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The different aspects of a face are coded at different locations and latencies in the ventral stream. The temporal dynamics of cortical sex coding of a face have been investigated with M/EEG, using MVPA/RSA techniques, which typically resulted in a 100-700 ms poststimulus time window. Based on previous research we suspect, this long time window results from the emerging familiarity due to the high repetition of one facial identity. To confirm this, we used a face stimulus bank with a high identity number and low stimulus repetition to observe the cortical coding of facial sex information in case of unfamiliar faces.

We recorded 64-channel EEG from 38 subjects (20 females, mean age=23.3±4.5y), while exposed to a stimulus sequence. The sequence consisted of 6 repetitions of 100 identities and 10% were target stimuli for an unrelated detection task. After preprocessing, LDA was used to determine decoding accuracy from cortical activity during the first second of the stimulus presentation. Four k-fold cross-validation (CV) was used to describe the cortical patterns. In the case of within-subject CV, LDA was trained on one subject's data, of which one facial identity was left out and was used for testing. Between-subject CV meant that the train set was all the subjects except one, whose data was later used for testing. Next, we divided the sample population into two based on gender. Within-gender CV was carried out similarly to between-subject CV in a given subpopulation, while in the case of between-gender CV, the training set was one subpopulation and the algorithm was tested on the data of one subject from the other subpopulation.

Within-subject CV showed an early significant time window (0.095-0.365 s) associated with the occipital areas. Between-subject CV showed a similar, shorter time window (0.095-0.285 s), similar to within-gender CV. The shortest time window with the greatest latency resulted from between-gender CV, where only a short-lived activity arose (0.125-0.195 s).

These results suggest that facial sex decoding is highly affected by the familiarity of the face and previous research often observed coding activity elongated by the used paradigm. Our results further elaborate on the cortical activity of face processing. Between-subject and between-gender results imply that previously observed gender differences cannot be assigned to early cortical processes and that cortical patterns exude some generality among the population.

The effect of the applied visual stimuli with reduced semantic content in associative learning among migraine patients

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In the Rutgers Acquired Equivalence Test (RAET), participants learn association pairs of visual stimuli (face and fish) during the acquisition phase. In the subsequent test phase, they are required to retrieve and generalize the learned associations. The semantic meaning and verbalizability of the stimuli can influence learning performance. To investigate this, we developed a new test, the Polygon test, which uses simpler visual stimuli with reduced semantic content, while maintaining the same structure as the original RAET. In this study, we aimed to assess how the complexity and semantic content of visual stimuli affect the performance of migraine patients.

We compared the psychophysical performance of 41 migraine patients across the two tests. In the RAET, the antecedents were drawn faces, and the consequents were drawn fish in various colors. In contrast, the Polygon test used grayscale circles as antecedents and blank, two-dimensional geometric shapes as consequents.

Contrary to our earlier findings in healthy individuals, there were no significant differences in performance between the two tests during the acquisition phase for migraine patients. However, reaction times were significantly longer in the Polygon test. Similarly, in the retrieval and generalization phases, performance between the two tests was significantly not different, but reaction times were still significantly longer in the Polygon test.

These results suggest that, unlike in healthy adults, migraine patients show no significant difference in performance during the acquisition phase between the two tests. However, the longer reaction times in the Polygon test imply that reduced complexity in visual stimuli may make learning more challenging for migraine patients. One possible explanation for these findings is the involvement of compensatory mechanisms.

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Fronto-Temporal Causal Network of Phase-Amplitude Coupling in Working Memory

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Working memory (WM) arises from interactions between the prefrontal cortex (PFC) and other associative cortical areas. In WM, these cortical interactions are coordinated by phase-amplitude coupling (PAC) between neuronal populations. However, the causal mechanisms underlying PAC remain unclear. Our study aims to investigate the causal cortical network involved in PAC during fronto-temporal interactions.

High-density electrocorticography (ECoG) recordings were obtained from the PFC and temporal cortex (TE) of two macaque monkeys performing a delayed color recall task, which required them to recall colors associated with grayscale images.

Task-specific oscillatory activity was dynamically modulated in both temporal and prefrontal cortices. Notably, significant PAC emerged within a localized region of the TE during the delay period, specifically between low (delta-theta) and high (beta-gamma) frequency bands. Using frequency-dependent Granger causality, we identified the PAC site in the TE as being causally linked to specific subregions of the PFC during mnemonic processes, particularly in the theta band. Neural decoding further illuminated the functional role of this causal network, revealing how specific cortical regions represented different aspects of the task.

Our findings highlight the critical role of PAC in working memory and demonstrate the PFC's regulatory function in coordinating cortical interactions.

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Revealing a novel pontine reward center in mammals

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Rewards are vital for motivation, decision-making, memory, and mental health. Using viral tract tracing, immunohistochemistry, light- and fluorescent microscopic imaging, fiber photometry recording and optogenetic behavioral experiments in transgenic mice, we investigated a previously unrecognized gamma-aminobutyric acid (GABA)-ergic cell population in the pons that we named the subventricular tegmental nucleus (SVTg). These SVTg neurons localized beneath the fourth ventricle and establish inhibitory synapses in LHb, a brain center involved in aversion and depression. SVTg neurons were activated by rewarding and reward-predicting experience. Mice self-stimulated SVTg neurons, suggesting its role in reward-seeking behavior. Furthermore, we found that special AT-rich sequence-binding protein-1 (Satb1) is a highly specific marker of these SVTg neurons. Therefore, using Satb1 immunohistochemistry, we could locate the SVTg under the fourth ventricle in the pontine central grey both in rat, in rhesus macaque monkeys and in humans. Our results suggest that a GABAergic pontine nucleus (SVTg) has a major role in reward processing shedding new light on a fundamental brain mechanism and potentially providing a therapeutic target for mood disorders.

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Studying the effect of Cariprazine in induced neurons directly reprogrammed from Huntington's disease's patient's fibroblasts

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Huntington's disease (HD) is an uncurable autosomal dominant progressive neurodegenerative disorder. The role of the dopaminergic system in the development of HD symptoms is crucial, as the central dopaminergic pathways are overactivated in HD. The dopaminergic overactivity can be reduced by several drugs. However, their effectivity on psychiatric symptoms is limited. Moreover, the treatment of apathy and cognitive symptoms still remains challenging in HD. Cariprazine, a third-generation antipsychotic is acting as a dopamine D3 and D2 receptor agonist. Previous results shown positive effect in HD patients after cariprazine treatment. Clinical studies indicated positive effects in early-stage HD patients after cariprazine treatment in some psychiatric symptoms such as depressed mood, apathy and cognitive function in patients. Moreover, cariprazine also improved dopamine imbalance in the prefrontal cortex. Aims: In this project, we aim to study the effect of cariprazine in a novel in vitro model system of HD using donor-derived aged-induced neurons. Our goal is to understand the putative beneficial effects of cariprazine in HD patients and to better understand its mechanism of action by focusing on autophagy. Using reverse translational strategy, we use cariprazine treatment in induced neurons directly reprogrammed from ctrl, HD drug-naive and cariprazine-treated HD patients' fibroblasts. For detection, we use immunocytochemistry (ICC) followed by high-content automated microscopy (HCS). We suppose that the described abnormal neurite morphology and the neurite-specific impairment of subcellular autophagy are positively altered following cariprazine treatment.

Induction of transient neurocognitive impairment by chemogenetic silencing of subcortical brain areas in rats: implications for potential preclinical disease models

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Designer Receptors Exclusively Activated by Designer Drugs (DREADD) is a chemogenetic method which is based on inducing the expression of genes for modified receptors in our cells of interest. These receptors can be reversibly activated exclusively by so-called actuators. The technology is suitable for transiently affecting, either silencing or activating certain regions of the brain, however, literature about its potential preclinical use is so far limited.

The aim of our experiments was to develop novel translational animal models of neurocognitive disorders, showing their pathological behavioural symptoms. For this purpose, we silenced the nucleus basalis magnocellularis (NBM) and the ventral tegmental area (VTA) using DREADD technology and assessed the induced cognitive disturbances using behavioural pharmacological tests.

Stereotaxic surgery was used to inject adeno-associated viral vectors expressing hM4D(Gi) receptor and mCherry reporter gene into the NBM or VTA brain area of rats (n=5/group). Animals were treated subcutaneously with 3 different actuator doses of deschloroclozapine (DCZ) or vehiculum at 30 min before the experiments in a randomized, within-subject design. Six non-operated animals were used as controls. Arousal and motivation were assessed in the rat psychomotor vigilance task (PVT). Declarative memory was tested in the novel object recognition test (NOR). Spatial memory was assessed by the Morris water maze test (MWM) and the presence of anxiety was assessed by the elevated zero maze (EOM) test after a single high dose of DCZ treatment.

In the PVT task, the increase in the number of missed trials in the NBM group shows the presence of decreased motivation, whereas the high number of premature responses in the VTA group shows the presence of impulsivity. In addition, in the NOR test, DCZ treatment impaired the long term declarative memory in the NBM group, whereas it was improved in the VTA-silenced animals. In the MWM test, compared to controls, both NBM and VTA animals showed impaired learning curves and also spent significantly less time in the target quadrant during the probe trial. Based on the null results of the EOM test, a confounding role of anxiety and reduced locomotor activity seems unlikely.

We successfully induced brain area-specific cognitive impairments by chemogenetic silencing, that could be further evaluated as potential animal models of human neurocognitive disorders.

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Molecular basis of Sindbis virus replication and pathogeneis in human neuoroblastoma cell line

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Alphaviruses that cause mosquito-borne erythrogenic diseases are human pathogens. SINV circulates in an enzootic cycle between its vectors (Culex/Aedes spp.) and birds. In humans, the infection usually results in mild symptoms, however, few studies are available about the virus's impact on the nervous/immune systems. SINV often infects the brain, consequently, understanding how brain cells respond to the infection is crucial. SH-SY5Y cells are derived from neuroblastoma and are used in neurobiological/virological studies. We aimed to study the mechanism of viral infection in vitro and examine early immune markers employing SINV infection by SH-SY5Y cells.

Due to the lack of adequate knowledge of the SINV, optimization was necessary for all experimental designs. After the virus stock preparation on SH-SY5Y cells, the median tissue culture infectious dose was used to determine the multiplicity of infection (MOI). MOI 0.005 and MOI 0.001 were applied during experiments where only early signs of infection were visible. On the virus side, Taqman-probe-based RT-qPCR was designed to SINV nucleic acid, and immunofluorescence-based ds-RNA antibody staining was also used to reinforce virus replication. Cells were continuously inspected using an inverted-light microscope to distinguish virus-induced cytopathogenic effect. To monitor some immune-genes expression (pattern recognition receptors (PRRs), regulator gene, cytokines) in a time-dependent manner (3/6/12/16/24/30 hr), RT-qPCR was also performed.

In vitro SINV adaptation and optimization to SH-SY5Y cells were efficient. SINV intensively infects cells, and after 24 hr, a cytopathogenic effect and virus replication could be observed. We have also documented the mRNA expression of PRRs, - (TLR-3/7, RIG1/MDA5) a regulator (b-catenin), - inflammatory (IL-1b, IL-6, TNFa), - and antiviral (IL-10, IFNβ) genes over time. Most of the investigated genes evidenced a consistent induction up to 12/16 hr, then by the end of the 30 hr, their expression mainly (e.g., RIG1/MDA5, IFNβ) decreased compared to controls. We can conclude that in vitro the SINV can be transmitted even in small quantities and an inflammatory environment can be developed, which later leads to cell death.

These obtained preliminary results allow us to gain insight into SINV–SH-SY5Y interactions, which are better understood in much more molecular detail and important novel mechanisms of action have been elucidated.

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Investigation of oxygen deficiency in the retina with an optimal ischemic retinopathy mouse model

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The retina is one of the highest metabolically active tissues with a high oxygen consumption, so insufficient blood supply leads to visual impairment. The incidence of related conditions is increasing; however, no effective treatment without side effects is available. Therefore, finding new therapeutic targets and drugs for hypoxia-caused retinopathies is a focus of research interest. Furthermore, the pathomechanism of these diseases is not fully understood. The aim of our study was to develop an optimal ischemic retinopathy mouse model and to investigate the consequences of hypoxia with morphological methods in a time-dependent manner.

Retinal ischemia was induced by bilateral common carotid artery occlusion (BCCAO) for 10, 13, 15 or 20 min, or by right permanent unilateral common carotid artery occlusion (UCCAO) in CD1-IGS mice. Morphological analysis of the retinal layers was performed in vivo by spectral domain optical coherence tomography (OCT). OCT measurements were performed preoperatively and 3,7,14,21 and 28 days later. After standardizing the results, a linear random effect mixed model was applied to compare the thickness of the layers depending on the duration of the ischemia and the time since the surgeries. The extent of damage was also analyzed separately in the central and peripheral regions. Molecular biology methods were used to investigate the consequences of oxygen deprivation at the cellular level.

During the experimental period, the total retinal thickness decreased, and significant changes were observed in the nerve fiber layer, in the retinal pigment epithelium and in the photoreceptor layer, among others, but the severity of damage did not differ between central and peripheral regions according to our OCT measurements. In contrast, a more significant decrease in ganglion cell number was observed in the peripheral regions of the retina, a result confirmed by other immunohistochemical studies. The increased expression of GFAP after UCCAO and the severity of photoreceptor damage also confirmed the other findings.

Our results suggest that the 20 min BCCAO is a good model to investigate the consequences of ischemia and reperfusion in the retina in a time-dependent manner, while the UCCAO causes more severe damage in a short time, so it can be used for testing new drugs. The damage probably starts in the periphery and is more severe there, but this difference has not yet been detected by imaging, which is also used in clinical practice.

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A plant hormone as a potential therapeutic option in the treatment of ischemic retinopathy

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Retinal ischemia can easily lead to visual impairment. The prevalence of hypoxia-caused eye diseases is increasing, but effective, non-invasive treatment options are not available. Abscisic acid (ABA) is a plant hormone with anti-inflammatory and antioxidant effects. ABA is also present in various mammalian tissues and plays an important role in metabolic processes. Although many protective effects of ABA have been described in ischemic conditions, little is known about its role in the eye. Based on our previous experiments, ABA treatment in the form of intravitreal injection could attenuate the release of pro-apoptotic factors. We therefore aimed to investigate the potential protective role of ABA eye drops in ischemic retinopathy.

Retinal ischemia was induced by permanent unilateral common carotid artery occlusion (UCCAO) in CD1-IGS mice. Half of the animals received ABA eye drops three times a day for two weeks. Optical coherence tomography (OCT) was used for following the changes in retinal thickness. OCT measurements were taken before the surgeries and 7 and 14 days later. Retinas were then isolated, and immunohistochemistry was performed. Retinal ganglion cells were labeled with Brn3a on whole retinal preparations and photoreceptor staining was also performed.

Based on OCT measurements, ischemic retinopathy was successfully developed. As in our previous results, the retinal layers showed different sensitivities to ischemia. The number of retinal ganglion cells was evaluated in the central and peripheral regions. The ganglion cell number decreased significantly after UCCAO ($p=0.04$) in the central region of the retina. However, ABA treatment could moderate the damage. We experienced more severe ganglion cell loss in the peripheral region. The ganglion cell number decreased after UCCAO ($p=0.001$), but ABA eye drops could prevent this damage.

In conclusion, ABA eye drops may represent a new potential therapeutic option for the treatment of ischemic retinopathy.

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The effect of cholinergic cell manipulation on learning and memory consolidation in female triple transgenic Alzheimer model mice

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Alzheimer's disorder (AD) is a neurodegenerative disorder characterized by progressive cognitive decline, affecting learning and memory. The cholinergic system, particularly the medial septum (MS), plays a central role in modulating these cognitive functions. Although disturbances of MS cholinergic cells are deeply implicated in AD, the process is not fully understood. Therefore, our aim was to observe the consequences of stimulation or inhibition of MS cholinergic neurons.

We utilized our recently developed mice model, the 4xTg-AD mice, which was created by crossbreeding the triple transgenic 3xTg-AD line with ChAT-Cre animals, bearing a Cre recombinase enzyme in the cholinergic cells and showing progressive AD-related pathology. Targeting the MS cholinergic cells we used chemogenetic with stimulatory and inhibitory DREADD (designer receptor exclusively activated by designer drug) sequences delivered by the help of an adenoassociated viral vector (AAV). After 1-month recovery the 9-month-old female mice were tested for their short term (y-maze) and spatial memory (Morris water maze, MWM) problems.

We confirmed that in our test subjects the DREADD receptors were successfully expressed. Significant genotype and treatment effects were noted across both behavioural tasks, suggesting that the impact of chronic manipulation on AD-associated pathology within hippocampal regions is connected to the MS.

These findings highlight the role of MS cholinergic neurons in AD pathology and their potential contribution to cognitive decline, offering insights for future therapeutic targets.

Effect of the histone deacetylase inhibitor SAHA on the gene expression of brain endothelial cells after ischemic injury

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Ischemic stroke is a leading cause of mortality and disability worldwide, resulting from the sudden interruption of blood flow to the brain. This condition leads to extensive neuronal damage and dysfunction of the blood-brain barrier (BBB), which is responsible for maintaining of the brain homeostasis. The breakdown of the BBB during ischemic stroke exacerbates brain injury by allowing the infiltration of harmful substances and immune cells into the brain parenchyma. There are currently no approved therapies specifically aimed at restoring BBB integrity after ischemic stroke. This highlights an urgent need for innovative treatments to mitigate BBB disruption and improve patient outcomes. According to our ongoing study, a histone deacetylase inhibitor: suberoylanilide hydroxamic acid (SAHA) is able to prevent the BBB functions by increasing the resistance, and decreasing the permeability of marker molecules across the BBB.

To gain a more detailed understanding of the mechanisms of SAHA, we conducted RNA-Seq analysis to explore its specific impact on the human stem cell-derived brain endothelial cells which were collected from four parallel groups: normoxia; oxygen-glucose deprivation (OGD); reoxygenation after OGD (OGD/R) and SAHA treatment during reoxygenation (OGD/R+SAHA). The results showed that SAHA treatment during reoxygenation enhanced the expression of genes of basement membrane components and modulated key angiogenesis pathways by inducing Wnt signaling and inhibiting the DLL4-NOTCH signaling pathway, which balance vascular growth and branching. After morphological analysis, we also showed that SAHA treatment led to a more elongated and differentiated brain endothelial phenotype.

Our findings suggest that SAHA could be a promising therapeutic approach for protecting brain endothelial cells after ischemic stroke.

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MiR-146a-5p and TGF-β Collectively Regulate Brain Endothelial Paqr5 and Angiogenesis in Response to Tumour-derived Extracellular Vesicles

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Triple negative breast cancer (TNBC) disseminates frequently to the brain, the prognosis of cerebral metastases being very poor. Development of secondary brain malignancies depends on the interaction of cancer cells with the neurovascular unit. Tumour-released small extracellular vesicles (sEVs) take a central role in this process; however, the mechanisms remain largely unknown.

In order to gain an insight into the tumour–endothelial communication during brain metastasis formation, we used sEVs released by TNBC cells to treat cerebral endothelial cells (CECs). We observed internalization of the sEVs and consequently increased amounts of miR-146a-5p in CECs. Among the tested mRNA-targets of miR-146a-5p, all five (Traf6, Irak1, Med1, Numb, and Paqr5) decreased in CECs in response to the sEVs; however, expression of Paqr5 (progestin and adipoQ receptor family member 5) was only slightly affected by the miR-146a-mimicking synthetic RNA, suggesting auxiliary mechanisms.

Expressed predominantly in endothelial cells in the brain, Paqr5 is encoding a membrane progesterone receptor, which is suppressed by TGF-β in peripheral tumours. Since TGF-β is carried by sEVs, we tested changes of Paqr5 in CECs treated with sEVs in the presence and absence of a TGF-β1 receptor inhibitor. We demonstrated that beside miR-146a-5p, TGF-β is also involved in the sEV-induced downregulation of Paqr5, but not of Irak1.

We have also shown TNBC-induced decrease in Paqr5 mRNA and PAQR5 protein expression in CECs in a mouse brain metastasis model. Finally, as a functional outcome, we revealed decreased angiogenesis in Paqr5-silenced CECs, underlining the angiogenesis-independent growth of breast cancer brain metastases.

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Phase-locked transcranial Intersectional Short Pulse (ISP) stimulation in terminating epileptic seizures

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Transcranial Electric Stimulation (TES) has emerged as a promising non- or minimally-invasive therapeutic approach for drug resistant epilepsy treatment. However, the optimal timing of stimulation relative to the ongoing brain oscillations remains to be established for optimizing efficiency.

This study investigates the phase specificity of transcranial ISP stimulation in terminating epileptic seizures, using both animal models and clinical data. By analyzing deep-brain activity during seizures in rats and post-hoc analysis of clinical data from human patients underwent closed-loop ictal stimulation, we unveil the optimal timing for intervention.

In this study, we recorded intracranial EEG (iEEG) signals in electrically kindled rats, and delivered phase targeted ictal transcranial stimulation by Intersectional Short-Pulse stimulation (ISP), allowing to map and understand the underlying neuronal activities at the seizure onset zone and their responses to the ISP stimulation.

Our findings consistently demonstrate that closed-loop stimulation is effective in shortening both the seizure lengths and the proportion of the generalized segments, emphasizing the potential clinical impact of phase-dependent intervention. Additionally, stimulating at, or near the peak of seizure oscillations yield the most significant reductions in seizure duration. This phase-specific effect is further validated through data from our first-in-patient clinical study performed to demonstrate the feasibility of closed-loop seizure termination in human patients.

In conclusion, these insights not only refine therapeutic strategies, but also underscore the translational potential of preclinical models, in guiding personalized epilepsy care. Ultimately, understanding the phase dependence of closed-loop neurostimulation represents a critical step towards advancing epilepsy treatment.

Changes of midbrain tyrosine hydroxylase immunoreactive elements in the valproate-induced autism model

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Prenatal valproate (VPA) exposure increases the risk of autism in humans. The offspring of valproate-treated female rats are used as a preclinical model of autism spectrum disorder (ASD). Based on literature data, there is a correlation between the dopaminergic system and the symptoms of autism spectrum disorder. Dopamine plays an important role in cognitive and motor control, as well as in emotional regulation and social behavior. In the midbrain, dopaminergic neurons are in three cell groups: A8 cells in the retrorubral field (RRF), A9 cells in the substantia nigra (SN), and A10 cells in the ventral tegmental area (VTA). In our research, we examined whether dopaminergic cells in the midbrain region show changes in the valproate-induced model of autism.

Pregnant female Wistar rats were treated with 500 mg/kg of valproate on day 12.5 of gestation, and behavioral tests on their male offspring were performed to confirm autistic traits. Treated and control animals (n = 9) were perfused at the age of 2 months, and tyrosine hydroxylase (TH) was detected by fluorescent immunohistochemistry on 50 µm-thick parasagittal brain sections. The density of TH-immunoreactive cells was measured on confocal images using ImageJ software.

Based on our analysis, the densities of TH-immunoreactive cells in the VTA ($p = 0.037$) and RRF ($p = 0.016$) areas were lower in the VPA model animals, while the density in the SN area was higher ($p = 0.029$) than in the control animals.

Our results point to a morphological correlate of the phenotype of the VPA autism model in the TH-immunoreactive cells of the midbrain regions. The findings suggest opposite structural changes in the meso-cortico-limbic and nigrostriatal dopaminergic pathways as a result of prenatal VPA treatment.

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Stress-induced mechanical and thermal pain sensitisation mediated through NLRP3 inflammasome activation

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Chronic stress is known to play a role in both the development and the exacerbation of several chronic pain states, for example diseases like fibromyalgia, where drug therapy is not satisfactory. Neuroinflammation and NLRP3 inflammasome-derived interleukine-1 (IL-1) proinflammatory cytokine-release is involved in stress and inflammatory pain. In our preclinical findings, IL-1 KO mice did not develop chronic restraint stress-induced pain. The NLRP3 inflammasome is a multi-protein complex within inflammatory cells, regulating the processing and secretion of IL-1. Here, we investigated the potential analgesic effect of the NLRP3 inflammasome antagonist MCC950 in a mouse model of stress-induced pain.

The animals were subjected to chronic restraint stress (CRS), placed in a well-ventilated, move-restricting tubes for 6 hours daily for 2 weeks. From the beginning of the CRS protocol, MCC950 or vehicle was administered intraperitoneally daily. The mechanical pain threshold, and the cold tolerance of the hind paw was measured weekly.

CRS induced 15-20% mechanical hyperalgesia developed for the second week. MCC950 prevented the formation of mechanical sensitisation of the hind paw compared to the vehicle treatment. 70-80% cold threshold drop developed by the first week of the CRS protocol. In response to stress, cold hyperalgesia was similar in vehicle-, MCC950-treated animals.

Based on our results, NLRP3 inflammasome play a crucial role in the development of chronic stress-induced pain. MCC950 successfully attenuated the mechanical sensitization caused by CRS, further strengthening the potential of NLRP3-IL-1 pathway as a potential drug target for the treatment of stress-induced pain states, like fibromyalgia.

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Investigating the effects of 3.5 GHz 5G electromagnetic field exposure on heart rate and heart rate variability in healthy young adults

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As fifth-generation (5G) mobile telecommunication technology continues to integrate into everyday life, it raises concerns regarding public health. Due to the novelty of 5G deployment and the controversial findings from previous studies, there is a clear need to further investigate this area and understand the potential effects of 5G radiofrequency on human health. This study, part of the GOLIAT project, aimed to investigate the potential health effects of exposure to non-ionizing electromagnetic field (EMF) from 3.5 GHz fifth-generation (5G) telecommunication technology on the heart and its autonomic innervation. The study included 31 healthy young-adult university student volunteers (21 female, 10 male), aged between 19 to 27, and was conducted in a double-blind sham-controlled randomized crossover design. Exposure (3.5 Ghz TDD 5G NR, 1 W/kg SAR, or none for sham) was conveyed by a patch antenna mounted near the right ear of the participant. Electrocardiogram was recorded identically in both sessions during the 3 blocks of exposure period lasting 26 minutes, and 2-2 blocks of pre-exposure and post-exposure periods, each 17 minutes long. Eyes-open and eyes-closed phases from each block were analysed. Data preprocessing in MATLAB using the HEPLAB toolbox within EEGLAB involved manual artifact and ectopic beat detection, semi-automatic R peak detection from the raw ECG signals and the extraction of normal-to-normal (N-N) intervals. Heart rate (HR), and heart rate variability (HRV) indexes, specifically RMSSD (Root Mean Square of Successive N-N Interval Differences), SDNN (Standard Deviation of N-N Intervals) were calculated from the N-N data in the HRVAS (HRV Analysis Software) MATLAB toolbox. HRV calculation involved detrending using the smoothness priors method with 4* oversampling and □=125. The RMSSD and SDNN indexes were selected because they are widely recognized reliable and standardized indicators of autonomic nervous system activity and cardiovascular health. Statistical analysis was conducted in Jamovi using t-tests comparing the sham and real exposure conditions. Preliminary results show no significant differences between sham and real exposure regarding HR, RMSSD or SDNN. In conclusion, there were no detectable physiological effects of 5G EMF exposure on HR and HRV.

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Correlating in vivo hippocampal and amygdala volumes with emotion regulation parameters in depressed patients with childhood maltreatment

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Numerous studies demonstrate alterations in the volume of the hippocampus and amygdala of depressed patients. A few studies report associations between the volume of these brain areas and emotional functioning of individuals. To further investigate this relationship, we studied associations between volumes of the hippocampal subfields and the amygdala, and emotion regulation in depressed patients with or without childhood maltreatment.

Depressed patients (n = 18), depressed patients with childhood maltreatment (n = 21), matched healthy controls (n = 21) were involved. Childhood maltreatment was assessed using the Hungarian version of the 28-item Childhood Trauma Questionnaire (H-CTQ). Emotion regulation was evaluated using the Cognitive Emotion Regulation Questionnaire (CERQ), Difficulties in Emotion Regulation Questionnaire (DERS), Reading the Mind in the Eyes Test (RMET), and the Toronto Alexithymia Scale (TAS). In vivo structural magnetic resonance imaging was done to determine the volume of hippocampal subfields and amygdala. One-way ANCOVA was run to find differences in volumes between groups. Multilinear regression analysis was used for calculating the relationship between volumes and emotion regulation.

In the maltreated depressed group, we observed predominantly positive correlations between subscales of CERQ, H-CTQ–Emotional abuse, and H-CTQ–total score, and the volumes of hippocampal subfields and amygdala ($p<0.05$). Negative associations were found between DERS–Goals ($p=0.034$), CERQ–Clarity ($p<0.05$), and hippocampal subfields. In the depressed group, we observed only positive relationships between CERQ–Positive refocusing, subscales of DERS ($p<0.05$), difficulty identifying feelings subscale of TAS ($p=0.049$), and volumes of hippocampal subfields and amygdala. In controls, we detected mainly negative relationship between hippocampal subfields and CERQ–Acceptance ($p=0.008$), DERS–Awareness ($p=0.017$), CERQ–Positive refocusing, and CERQ Adaptive sum ($p<0.05$). However, RMET was positively associated with hippocampal subfields volume (bilateral subiculum, molecular layer, CA1; $p<0.05$).

Here we provide further evidence that parameters of emotion regulation correlate with in vivo volumetric data of the hippocampus and amygdala both in healthy and depressed individuals.

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Gene expression analysis in the parahippocampal cortex of individuals who died by suicide

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Suicide is strongly linked to various neuropsychiatric disorders and it is characterized by disrupted connectivity in resting-state networks. Notably, dysfunction within the default mode network (DMN) has been associated with suicidal ideation. The parahippocampal cortex (PHC), a critical component of the ventral hub of the DMN, mediates communication between resting-state networks and the medial temporal lobe. The PHC plays a role in visuospatial navigation, memory processing, and mood regulation. Abnormal activation of the PHC has been observed in individuals who have attempted suicide. Our previous proteomic analysis implicated mitochondrial dysfunction and elevated glutaminolysis in the PHC as key factors in the pathogenesis of suicidal behavior, however, the broader molecular mechanisms remain to be elucidated.

The objective of this study is to investigate the molecular basis of suicidal behavior through RNA sequencing (RNA-seq) to identify gene expression alterations in the PHC of individuals who died by suicide. Postmortem brain samples from 11 control individuals and 11 suicide decedents were processed. Differential gene expression analysis was performed using the DeSeq2 pipeline.

A total of 171 downregulated and 201 upregulated genes were identified, with statistically significant changes in gene expression using log2 fold change > ± 1 and adjusted p-value < 0.05 criteria. The STRING protein-protein interaction (PPI) network analysis revealed a significant overexpression of chemokine genes associated with neuroinflammation among the top differentially expressed hub genes. Furthermore, in conjunction with the Gene Ontology (GO) enrichment, the PPI network analysis identified oxidative phosphorylation as a central process among the upregulated hub genes, underscoring the pivotal role of mitochondrial ATP synthesis. The GO enrichment and Reactome Pathway analysis suggested that G-protein coupled receptor signaling pathways are pivotal in upregulated genes involved in neurotransmitter signaling and cellular communication. In contrast, the PPI network and GO enrichment analysis of the downregulated genes revealed disruptions in oligodendrocyte differentiation and impaired myelination, leading to disrupted neuronal communication in the PHC of individuals who died by suicide.

The findings suggest that alterations in gene expression take place within the PHC in suicidal behavior and potentially contribute to its pathophysiology.

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Comparison of different "delayed non-matching to sample" learning paradigms as models of working memory in rats

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Examination of working memory in animal experiments is an important tool in understanding the functioning of our brain and the pathomechanism of certain cognitive disorders. One of the frequently used testing methods is the "delayed non-matching to sample" (DNMTS) paradigm, where the animal is presented with a sample object, then a delay period occurs, after which the same ("matching") and another similar object ("non-matching") are presented. The animal receives a reward if it is able to recall which one of the objects it has already seen and chooses the non-matching one. This task is usually performed in a Skinner box with two retractable pedals, however, due to certain methodological problems, the use of other apparatuses should be considered.

In our experiment, the DNMTS task performed in three different test environments was taught to 16 male Hannover Wistar rats with the aim of selecting the most appropriate model. The apparatuses used were the Skinner box, the 5-hole box and the Touchscreen apparatus. The test environments' most important differences were in the number of possible locations of the objects (2, 5, and 12, respectively) and whether the object was continuously physically present or not (in the first two cases yes, but not in the Touchscreen).

In the Skinner box, we were able to demonstrate a significant dependence of the correct ratios on the delay, which was in accordance with findings in the literature. In the Touchscreen and 5-hole box, the animals performed well as long as only the non-matching object could appear in a random location, but they were unable to give sufficient correct responses when the appearance of the sample object was also unpredictable; therefore, we could not examine a delay dependence here. We attributed this phenomenon to the increased demand for working memory, which may have exceeded the cognitive capacity of the animals. In accordance with this assumption, in the non-matching to sample paradigm (without delay) animals still showed better performance in the 5-hole box than in the Touchscreen.

Regarding human translation, the Skinner-box version still can be practical for the examination of working memory, as it is suitable for detecting both improvement and deterioration, and the delay dependence is clearly observable – but the results should be evaluated with reservations. The use of the 5-hole box can be a sensitive method for testing possible working memory improving agents.

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Kynurenic acid shifts astrocyte activation

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Astrocytes, integral components of the central nervous system, fulfill diverse roles such as providing structural support, maintaining the blood-brain barrier, participating in neurotransmitter uptake and recycling, and regulating synapses and neural signaling. Injuries or pathological conditions which evoke pain are concurrently leading to a reactive astrocyte state, which is characterized by a variety of changes in the morphological, molecular, and functional properties of these cells including their involvement in neuroinflammation. Distinguished into A1 and A2 types, astrocytes showcase pro-inflammatory and anti-inflammatory actions, respectively. A1, pro-inflammatory astrocytes release inflammatory mediators, including cytokines and chemokines, modulating neuronal activity and contributing to the recruitment of microglia and peripheral immune cells. However, astrocytes exhibit versatility in functions such as neuroprotection (A2 type), releasing anti-inflammatory growth factors. Understanding this spectrum of abilities is pivotal for developing targeted therapeutic approaches to regulate immune responses, emphasizing the delicate balance between pro and anti-inflammatory roles in maintaining nervous system health.

Our study aimed to investigate the activation of spinal astrocytes using glutamate, kynurenic acid, ATP, and LPS. Employing western blotting, we detected inflammasome components (NLRP2, ASC), revealing the intricate molecular responses. Pro-inflammatory cytokines were quantified using ELISA, providing insights into the inflammatory milieu. To gauge pyroptotic cell death and gasdermin pore formation, a Lactate Dehydrogenase Assay was conducted.

Our findings unveiled that kynurenic acid, a glutamate receptor antagonist, demonstrated a remarkable ability to attenuate pro-inflammatory cytokine production by spinal astrocytes. This modulation was associated with the influence on NLRP2 expression. The cleavage and detection of the gasdermin pore further highlighted the nuanced regulatory mechanisms impacted by kynurenic acid. These results contribute to a deeper understanding of astrocyte dynamics and offer potential avenues for targeted interventions in neuroinflammatory conditions.

The nonsteroidal anti-inflammatory drug meclofenamate mitigates kainic acid induced seizures via TRPM4 inhibition

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TRPM4 is a Ca2+-activated non-selective cation channel regulating diverse physiological functions of excitable cells. It has been shown previously that TRPM4 is present and functionally active in hilar mossy cells and modulates seizure susceptibility. Using a battery of in vivo and in vitro electrophysiological and histological experiments we studied the effect of TRPM4 channel blockade during kainic acid induced seizures.

Here we demonstrate that in vivo application of meclofenamate a novel antagonist of TRPM4 before kainic acid injection reduces the frequency and duration of seizures in mice. Furthermore, we showed that mossy cell loss is reduced selectively in the ventral hippocampus upon meclofenamate treatment following seizures. Interestingly, we have found higher expression of TRPM4 in mossy cells from the ventral hippocampus pointing to inhomogeneity of mossy cells. Moreover, using patch clamp recordings, we proved that meclofenamate modulates spontaneous activity and AP dynamics of MCs. Finally, we detected TRPM4 expression in human mossy cells as well.

This data indicates that pharmacological blocking of TRPM4 may reduce seizure frequency and therefore protects mossy cells.

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Functional and electrophysiological analysis of aging in induced neurons reprogrammed from adult human dermal fibroblasts

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There is a great need to study in detail human specific functional properties during physiological aging in human neurons, which is challenging due to the lack of accessibility. Although neurons from other species commonly used as models share many similarities with human neurons, the latter display unique electrophysiological characteristics, including distinct input resistance and action potential threshold voltage. Our goal is to develop a robust measuring system that will enable reliable, high throughput functional characterization of aged human neurons suitable for drug-screening.

To this end we are using an inducible lentiviral system which consists of a doxycycline driven transactivator coupled with a transcription factor ASCL1. This enables us a highly efficient methodology to reprogram human dermal fibroblasts into induced neurons (iN). During the transdifferentiation cells don't go through a pluripotent phase. iNs keep the genetic and epigenetic, aging signature of the donors. This makes iNs uniquely adequate to study aging and age-related diseases in neurons from a functional perspective.

First, we started to optimize the transdifferentiation methodology to whole-cell patch-clamp recordings coupled with current-step measurements. Our first conversion identified 3 subpopulations in iNs after 25 days of conversion: (i) non-differentiated cells with no neuron like membrane potential, (ii) differentiated-passive cells with neuron like membrane potential, and (iii) differentiated cells, with rectification of injected current and spikelets. We are currently repeating our recordings and further optimizing our conversion methodology by increasing the cellular density to engage synapse formation and by optogenetic training expressing ChR2-channel rhodopsin in the iNs. After successfully defining the best culturing conditions we plan to study functional differences in iNs derived from young and old donors. Identifying age-related changes in electrophysiological properties, such as in ion-channel response, action potential shape and duration, membrane potential will be an important step to understand healthy and non-healthy aging, while also giving us potential targets for intervention.

Investigating the effects of 5G mobile phone technology on human resting state EEG activity

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As 5G mobile technology becomes ubiquitous, understanding its potential neurophysiological effects remains a priority. Resting-state EEG provides a valuable window into brain activity and a sensitive measure for detecting potential subtle neurophysiological effects of electromagnetic fields (EMFs). Based on our previous research using 3G/4G technology, we specifically probed whether alpha oscillations were modulated by near-field exposure to 3.5 GHz 5G EMFs.

EEG recordings were obtained from 18-27 years old college student volunteers (n=25, 18 female) in a double-blind, sham-controlled design during resting-state with eyes open and closed. Each session included pre-exposure (17 mins, two blocks), exposure (real or sham, 25 mins, three blocks), and post-exposure (17 mins, two blocks), with each block consisting of an audio stimulation phase (not analyzed here) followed by eyes-open and eyes-closed phases. In the beginning of the exposure block, a patch antenna was mounted on the subject's head near the right ear, and a Bird GH-60 signal generator was turned on that transmitted the 3.5 GHz TDD 5G NR signal either to an absorber or to the antenna via a blinding switch. The SAR of the exposure was 1 W/kg. EEG was recorded using an ActiChamp amplifier (Brain Products GmbH.) in DC mode via 64 active electrodes (ActiCap Slim), alongside with ECG, respiration belt, skin resistance and skin temperature. The primary outcome was EEG alpha power (8–12 Hz) over the entire scalp.

Alpha power was significantly higher in the eyes-closed compared to the eyes-open condition. Alpha power also significantly increased with time-on-task, an expected correlate of gradual decrease of subject arousal during the recording session. Preliminary analysis showed no differences in the power or scalp distribution of alpha oscillations between the real and sham exposure conditions, regardless of eye condition.

Consistent with prior evidence from older technologies, our findings indicate that acute exposure to 5G EMFs at 3.5 GHz does not significantly impact alpha oscillatory activity in resting-state EEG. These results contribute to reassuring evidence about the safety of 5G technology and underscore the importance of rigorous scientific assessment in addressing public concerns.

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Mouse functional-neuromorphological evidence in line with human fMRI data support the involvement of peptidergic Edinger-Westphal nucleus in migraine

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Urocortin 1 (UCN1)-expressing neurons of the centrally projecting Edinger-Westphal (EWcp) nucleus project to several migraine-related brain areas, moreover, they are activated by acute pain stress. EWcp is regulated by the circadian rhythm, hormonal changes, stress exposure and pain that are known to trigger migraine. Therefore, here we aimed at investigating the possible role of EWcp in the neurobiology of migraine.

RNAscope in situ hybridization (ISH) combined with immunostaining was used to examine the expression of all calcitonin gene-related peptide (CGRP) receptor components in the EWcp of mice and humans. Anterograde and retrograde tracing studies were performed to identify possible urocortinergic projection from EWcp to the spinal trigeminal nucleus (STN). Functional connectivity matrix of Edinger-Westphal nucleus (EW) was examined using fMRI in humans. The CGRP injection model of migraine was applied and validated by von Frey and light-dark box behavioral tests in C57BL6/J mice. Immunostaining was performed to assess the expression of acute neuronal activity markers (FOS, P-CREB) in the EWcp, lateral periaqueductal gray matter (lPAG), trigeminal ganglia (TRG) and STN. RNAscope ISH and immunostaining was used to measure UCN1 mRNA and peptide content of the EWcp.

We proved the presence of CGRP receptor components in EWcp of mice and human. We identified a direct urocortinergic projection arising from EWcp to the STN. This was further supported by the strong positive functional connectivity between EW and STN as well as DRN in humans by fMRI. CGRP treatment induced photophobia and pain-related behavior in mice and increased the neuronal activation in the TRG, STN and lPAG, supporting the efficacy of CGRP-induced migraine-like state. In response to CGRP, the expression of Ucn1 mRNA, FOS and UCN1 peptide in the EWcp/UCN1 neurons increased.

The presence of CGRP-receptor components, increased expression of Ucn1 mRNA, FOS and UCN1 peptide in EWcp neurons upon CGRP treatment, moreover, their urocortinergic projection to the STN strongly suggest the regulatory role of EWcp/UCN1 neurons in migraine with high human translational relevance.

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Age-dependent FOS, FOSB/ΔFOSB responsiveness of medial prefrontal cortex in acute and chronic stress male rat models

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FOS proteins family members (FOS, FOSB, ΔFOSB) in medial prefrontal cortex (mPFC) play pivotal role in manifestation of stress-induced major depressive disorder (MDD). The MDD incidence shows two main temporal peaks: one in the pubertal-young adulthood period, and another during in the elderly. Since MDD may affect may occur anytime during the life, therefore, it is essential to understand the age dependent neural (re)activity of mPFC. There is limited information about the age-dependency of acute restraint stress (ARS) or chronic variable mild stress (CVMS) induced FOS, FOSB, ΔFOSB products in mPFC, therefore we aimed to semi-quantify the ARS or CVMS induced FOS, FOSB/ΔFOSB content in anterior cingulate (AC), prelimbic (PL) and infralimbic (IL) regions of the mPFC.

ARS model was designed with eight Wistar rat age groups (1-month-old [M], 1.5M, 3M, 6M, 12M, 18M, 24M), whereas the CVMS model we used six age stress groups (2M, 3M, 6M, 12M, 18M, 24M) versus their age matched controls. The stress efficacy was verified by measurement of the total body, thymus, and adrenal glands' weight. Diamino-benzidine FOS or FOSB/ΔFOSB immunolabelling was performed on the mPFC coronal sections.

The IL and PL showed ARS-induced FOS rise in the pubertal-young adult periods only but not later. The CVMS-induced FOSB/ΔFOSB content did not change, while the basal activity (content) of FOSB/ΔFOSB show a slow decline in the elderly. Both FOS and FOSB/ΔFOSB contents peaked at 3M. FOS activity strongly decreases in ARS or in control animals, whereas FOSB/ΔFOSB shows less impressive decrease in mPFC with ageing.

The amount of inducible FOS occurrence is function of age that limited to younger age periods. The mPFC shows delayed neural FOS, FOSB/ΔFOSB activity peak during the life compare to subcortical areas. FOSB/ΔFOSB do not show any rise upon ARS nor CVMS, but in CVMS animals its presence maintained during life. The mPFC (re)activity assessed by FOS, FOSB/ΔFOSB shows an age-matched hyper- (youngerhood) or hypo- (re)activity (elderly) with the incidence peaks of MDD. Further investigation needed to better understand the age-characterized changes in the stress-orchestrated machinery.

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Expressional changes of claudin-5 and PDGFRbeta, two key blood brain barrier proteins, in a culture model of ischemic stroke

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The most frequent in vitro ischemic brain injury model is oxygen-glucose deprivation. Under this pathological condition several characteristics of the blood-brain barrier show endothelial and pericyte dysfunction. One of the key features suggesting a reduction in blood-brain barrier tightness is a decrease in the expression of tight junction proteins, including claudin-5. The impairment of blood-brain barrier integrity may also be reflected by a disturbance in endothelial cell-pericyte crosstalk, in which platelet-derived growth factor β is a key participant.

In our first series of experiments we investigated whether the decreased expression of claudin-5 induced by oxygen-glucose deprivation can be increased by treating the cells with a small-molecule cocktail, called cARLA, which is known to enhance blood-brain barrier properites of endothelial cells in several models. The effect of a drug named SAHA (Suberoylanilide hydroxamic acid), which is a histone deacetylase inhibitor having anti-inflammatory effects, was also tested on the expression of claudin-5. The localization and staining pattern of claudin-5 was studied using immunocytochemistry on mouse brain microvascular endothelial cells co-cultured with pericytes. Relative claudin-5 protein expression levels were analyzed by western blotting. Protein samples were obtained as whole cell lysates of endothelial cells cultured with pericyte conditioned medium. Our results show that both treatments induced an increase in claudin-5 expression. Moreover, cARLA treatment resulted in an increase in active β-catenin levels too, indicating the involvement of the Wnt/β -catenin pathway in its mechanism of action.

In our second series of experiments relative expression levels of PDGFRβ protein were analyzed following oxygen-glucose deprivation challenge and SAHA treatment. Protein samples for western blotting were prepared from whole cell lysates of pericytes cultured with endothelial cell conditioned medium. Our results indicate that oxygen-glucose deprivation did not reduce PDGFRβ levels, but SAHA treatment resulted in a significant decrease in PDGFRβ levels both under control and hypoxic conditions.

Our findings suggest that claudin-5 expression can be rescued by both cARLA and SAHA treatments, which is likely to increase blood-brain barrier tightness. The crosstalk between endothelial cells and pericytes, however, may be disrupted due to SAHA treatment.

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Investigating the blood-brain barrier in acute pancreatitis: a clinical and cell-culture study

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Acute pancreatitis (AP) is one of the most common inflammatory gastrointestinal disease needing urgent hospitalization. During AP about 4% of all patients develop disturbance in consciousness. Besides this sypmtom among the severe AP cases 10 % show serious neurological involvement manifesting in pancreatic encephalopathy. Our research group showed blood-brain barrier (BBB) permeability elevation in a rat non-invasive AP model earlier. Now our goal was to identify potential BBB injury in pancreatitis patients. For this study we used serum from mild, moderate and severe AP patients to identify BBB opening by measuring neuron specific enolase (NSE) and S100β presence in the blood. Furthermore, cultured brain endothelial cells were also treated with 20% human sera. Functional tests like permeability, transendothelial electrical resistance (TEER) and ROS/NO production were analyzed. Morphological investigation on PECAM-1 adherent junction protein performed. In our results we found elevated NSE and S100B levels in the serum of patients with mild, moderate and severe AP reflecting BBB integrity damage. The serum treatment decreased the resistance and elevated permeability of the brain endothelial cell layer, and interendothelial junctional morphology was also affected. ROS and NO production was also increased. Our results show, that treatment with the AP patient sera influences many important BBB properties such as barrier integrity, level of oxidative stress and junctional morphology. The fact that BBB leakage markers were found in the blood of patients from all AP severity groups draws the attention to the serious neurological side effects of the disease and the importance of further investigations.

Morphological investigation of meningeal macrophages (BAMs) and mast cells in inflammatory reactions following experimental subarachnoid hemorrhage

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Subarachnoid hemorrhage (SAH) is bleeding between the brain and the overlying arachnoid membrane. Glia limitans, a special layer formed by astrocytes, serves as an important barrier separating the brain parenchyma from the meningeal compartments. In the parenchyma, astrocytes and microglial cells, while in the meninges, the border-associated mactophages (BAMs) and mast cells function as immune cells of the central nervous system. We aimed to study the impact of meningeal immune cell activation on the activation state of parenchymal glial cells and the integrity of cerebral barrier systems following SAH.

Experimental SAH was induced in rats by a single intracisternal injection of autologous blood. One group of animals received an intraperitoneal injection of a mast cell degranulator, compound48/80 4 days prior to SAH induction. 72 hours post-SAH, the animals were perfused transcardially, frozen brain sections were cut and the dura mater was used as total preparation. Morphological evaluation of meningeal mast cells was performed using toluidin-blue staining, while Iba1-immunohistochemistry was used to detect BAMs. Immunohistochemical examination of OX42- and GFAP-antibodies was used for the assessment of parenchymal microglial cells and astrocytes, respectively.

Our findings indicate that SAH is accompanied with the degranulation of meningeal mast cells, the infiltration of Iba1-immunopositive cells and a phenotype switching of BAMs. Notably, the GFAP-positivity was significantly reduced on the parenchymal surface (thickness of the GFAP-immunopositive layer: 12.24±0.67 vs. 7.78±0.74 μm) after SAH, however, this effect was not observed in animals pretreated with compound48/80. (12.92±2.45 μm). Moreover, a marked increase in OX42 staining intensity was observed in the parenchyma (0.21±0.04 vs. 0.54±0.16 OD), an effect that was also absent following depletion of mast cells with compound48/80.

Our results suggest a significant meningeal inflammatory reaction characterized by mast cell activation, which plays a pivotal role in the regulation of glial cell status and in maintaining the integrity of the glia limitans.

Effects of maternal smoking on retinopathy of prematurity

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Premature birth is often linked to various disorders that can impact future quality of life. One such condition is retinopathy of prematurity (ROP), a neurovascular disease affecting the retina. Oxygen-induced retinopathy (OIR) is a well-established animal model of ROP, characterized by vascular abnormalities such as vaso-obliteration and neovascularization. Several factors, including maternal smoking during pregnancy, are known to contribute to premature birth. This study aimed to investigate the effects of maternal smoking (MS) on OIR using in vivo imaging and immunohistochemical techniques.

A pigmented strain of laboratory mice (C57BL/6) was used in this experiment. During pregnancy, mice were exposed to smoke twice a day for 30 minutes in a specialized chamber. To induce retinopathy, pups were subjected to 75% oxygen (±2%) from postnatal day (PD) 7 to PD12, before returning them to room air. On PD17, under anesthesia, imaging with optical coherence tomography (OCT) was performed to analyze the retinal layers' thickness. Following the animals' decapitation, their eyes were removed and retinal whole-mounts were prepared and immunolabelled with glial fibrillary acidic protein (GFAP) to visualize Müller glial cell stress.

Measurements with OCT showed that in the case of ROP retinas the thinning of the total retinal thickness and several layers (such as inner nuclear and inner plexiform layer) was significant compared to the controls. Glial cell labeling revealed a trend of increased cell stress in the ROP-affected groups, with a further elevation in the ROP+smoking group.

Our results indicated the possibility that maternal smoking has negative effects on OIR. These findings suggest that maternal smoking may exacerbate retinal damage in ROP, thus prevention and screening of this disease can be considered essential in preterm infant care.

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PAC1 Receptor Activation by a PACAP Fragment Alleviates Anterior Segment Inflammation in Endopthalmitis

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Endophthalmitis is a serious intraocular inflammatory disorder resulting from infection and may lead to irreversible blindness. Due to the rapid progression of the disease early detection and appropriate treatment application are crucial to preserving vision. The potential therapeutic effects of pituitary adenylate cyclase activating polypeptide (PACAP) have already been investigated in multiple eye diseases. Our research group discovered that the activation of the PAC1 receptor can reduce the extent of inflammation in case of bacterial keratitis. A short PACAP fragment has been identified, that exclusively stimulates the PAC1 receptor and able to penetrate through the cornea via eyedrops. Our aim was to investigate the anti-inflammatory effects of PACAP fragment in a mouse model of endophthalmitis, focusing on the anterior segment of the eye, using a non-invasive imaging technique.

Systemic inflammation was induced in CD1-IGS mouse strain via intraperitoneal injection of lipopolysaccharide (LPS). The animals were treated 6 times within 24 hours with eye drops containing PACAP fragment. At the peak of the inflammation, non-invasive optical coherence tomography (OCT) was used to obtain high-resolution cross sectional images of the anterior segment of the eye. To assess morphological changes OCT images were analysed by ImageJ and Matlab programs.

OCT image analysis revealed cornea swelling, evidenced by increased central corneal thickness. Due to the inflammation enhanced corneal reflectivity with increased mean pixel intensity of the epithelial and stromal layer were observed. Anterior cell infiltration was detected in the anterior segment of the eye. PACAP fragment treatment reduced the severity of inflammation, as indicated by mild OCT findings: reduced corneal thickness and lower corneal reflectivity.

The PACAP fragment exhibited anti-inflammatory effects via PAC1 receptor activation, highlighting its potential as a therapeutic approach for endophthalmitis.

Gamma-aminobutyric-acid and glutamine/glutamate concentration differences in the hippocampus of febrile seizure subjects with and without epilepsy

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Febrile seizure (FS) is a convulsive condition in young children caused by fever and could be associated with subsequent epilepsy in later ages. γ-Aminobutyric acid (GABA) and glutamine/glutamate (Glx) are the main inhibitory and excitatory metabolites in the human brain and play a crucial role in seizures development. The objective of this study was to identify differences in hippocampal GABA and Glx levels between FS subjects with and without epilepsy, and to determine the effect of age at onsets and years of education on GABA and Glx concentrations.

18 FS subjects without epilepsy (FS; 13 females; 36.61 ± 10.05 years), 21 age-matched healthy controls (FS–HC; 14 females; 39.04 ± 12.30 years), 12 epilepsy patients with febrile seizure (EPI-FS; 6 females; 43.83 ± 13.22 years) and 11 age-matched healthy controls (EPI-FS–HC; 6 females; 42.09 ± 12.66 years) were enrolled. Data were collected using a 3T Siemens MR scanner with MEGA-PRESS sequence to the measure GABA and Glx concentrations. Statistical analyses were conducted by unpaired t-tests and correlations.

No significant GABA and Glx differences were found between the FS and FS–HC groups and between the EPI-FS and EPI-FS–HC subjects. We did not find any significant differences in GABA and Glx levels between the left and right hippocampus of the examined groups. Neither epilepsy nor febrile seizure onset was related to GABA and Glx concentrations. The levels of GABA/creatine ($p=0.006$) and GABA/water ($p=0.05$) were negatively correlated with years of education in the left hippocampus of FS subjects.

These findings suggest that hippocampal GABA and Glx concentrations are not associated with febrile seizure and epilepsy onset. Although there was no difference in years of education between groups, the negative association between GABA level and education attainment indicates that time spent studying may be related to hippocampal GABA levels, which differ between subjects with and without FS.

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The histone deacetylase inhibitor SAHA protects the blood-brain barrier against ischemic injury

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During an ischemic event, blood flow interruption and the subsequent oxygen-glucose deprivation (OGD) cause blood-brain barrier (BBB) disruption and neuronal death, which may have life-threatening consequences. Protection of the BBB as a therapeutic target is a novel concept in medicinal strategies to treat ischemia-reperfusion injury. Our aim was to investigate the possible protective effect of a clinically used histone deacetylase inhibitor, suberoylanilide hidroxamic acid (SAHA), against BBB disruption in the cell culture model of ischemic stroke.

We cultured human brain endothelial cells and brain pericytes under three different conditions: normoxia, OGD, and reoxygenation after OGD (OGD/R) and then tested the effect of SAHA on BBB dysfunctions. SAHA promoted BBB protection against OGD/R by enhancing several barrier functions: it increased transendothelial electrical resistance (TEER), decreased the permeability of trans- and paracellular tracers, increased the protein level of the important tight junction protein claudin-5 measured by immunofluorescent intensity, and also increased the intensity of the glycocalyx constituent syalic acid. We also observed that SAHA had barrier-strengthening effects only in the presence of brain pericytes, suggesting that pericytes have a pivotal role in the protective mechanism of SAHA.

Our results suggest that SAHA has beneficial effects on the integrity of the BBB after OGD and may prove useful as a therapeutic drug in the treatment of ischemia-reperfusion injury.

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Statistical determination of major factors determining RNA quality of postmortem microdissected human brain samples collected in the SE Human Brain Tissue Bank

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Recent advancements in RNA sequencing have highlighted the importance of human brain tissue samples in studying transcriptional alterations associated with neurological and psychiatric diseases. However, RNA stability is a critical concern, as degradation can occur due to RNases and chemical decomposition, influenced by factors such as post mortem time, storage conditions, and biological and clinical parameters of the tissue of the deceased persons. This study systematically evaluated RNA quality in 480 brain tissue samples from 107 individuals stored in the Human Brain Tissue Bank of Semmelweis University.

Using microdissected brain tissues, RNA was isolated via a TRIzol-columnar hybrid method, and RNA quality was assessed with RNA integrity numbers (RIN). First, the technical and then the biological and clinical parameters were tested using a generalized linear model. Our results revealed that RNA quality is well-preserved up to 12 hours post mortem, though some degradation occurs over time. Technical factors, including storage duration and sample dissection parameters had no impact on RNA integrity except for the very short post mortem interval surgical samples. In contrast, biological and clinical factors, such as the age of the patient, post mortem delay, Alzheimer status, and the cause of death, significantly influenced RNA quality. Samples from older patients and from those who experienced hypoxia-related conditions exhibited greater RNA degradation.

This study provides the first comprehensive analysis of RNA quality in human brain tissue samples, demonstrating that high-quality RNA can be extracted from post mortem and surgical samples for advanced transcriptomic studies. However, to ensure reliable data, RIN values should be determined before analysis, and attention should be given to biological and clinical factors influencing RNA stability.

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The sex-dependent effect of short- and long-term training on cognitive functions and the gene expression pattern of the brain-muscle axis

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Regular exercise training is an efficient non-pharmacological intervention to ameliorate the deleterious effect of aging on cognitive function. The effects of physical activity on brain health may depend on several factors, such as biological sex, or the type of exercise, however, the cellular and molecular mechanisms behind this phenomenon are not completely understood. Therefore, we aimed to investigate the sex-dependent effects of short- and long-term running on the expression levels of genes involved in muscle-brain communication and regeneration, and on changes in cognitive functions.

During our experiments, we examined the short- (1-7 days) and long-term (7 months) effects of medium-intensity treadmill training on male and female C57BL/6 mice. The long-term studies were performed on both healthy and hyperlipidemic animals. The changes in the spatial memory were analised by Barnes maze test, while gene expression patterns of the hippocampus and m. quadriceps femoris tissues were studied by qPCR.

According to our results, short-term, medium-intensity running promotes the expression of neurotrophic factors and heat shock proteins in the hippocampus, in both sexes. In addition, running also stimulates the expression of heat shock proteins and myokines in skeletal muscle, however in a sex-dependent manner. The higher expression of heat shock proteins and the macrophage marker Cd68 in the skeletal muscle of male animals may indicate that the same type of training is more strenuous for males than females. Long-term regular running resulted in significantly improved cognitive functions in both healthy and hyperlipidemic female animals, but no such effect was observed in males. Moreover, the gene expression of heat shock proteins, myokine Il-6, and other factors regulating muscle work, integrity, and metabolism showed remarkable sex differences.

Our results confirm that regular training improves cognitive functions, which can be associated with the altered regulation and production of myokines, heat shock proteins, and neurotrophic factors during exercise. However, this significant improvement in cognitive functions showed a sex-dependent effect, underlining the importance of personalized training. A better understanding of these processes may promote the therapeutic use of personalized exercise in the prevention or treatment of neurological diseases.

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Excessive fructose intake aggravates inflammation and may lead to brain damage in mice with obesity

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In the last decades, dietary fructose consumption increased notably, which may play a significant role in the rising prevalence of obesity. Given that fructolysis is not regulated by cellular energy status, it may provide excess substrates for lipogenesis, leading to excessive fat accumulation in the white adipose tissue. Consequently, the released pro-inflammatory factors induce a low-grade systemic inflammation, which appears to be responsible for the development of not only metabolic disturbances but also neurodegenerative disorders. However, the molecular mechanisms underlying these effects are not fully understood. Therefore, our aim was to examine inflammation and pathological changes related to obesity in mice using two diet induced obesity models. Male mice were fed a high-fat diet (HFD), an HFD supplemented with 30% fructose solution (HFD+FR) or a normal chow for 5 months. Although obesity-related pathology was observed in mice fed HFD alone, HFD+FR diet led to significantly greater weight gain and glucose intolerance, as evidenced by oral glucose tolerance tests. Obesity was accompanied by systemic inflammation in both models, revealed through single-cell phenotyping of major immune cell populations and the analysis of cytokine levels. However, fructose supplementation led to a further increase in the gene expression of pro-inflammatory cytokines in visceral white adipose tissue, and serum TNFα concentration. This systemic inflammation observed in the HFD+FR group extended to the brain, with modest elevations in pro-inflammatory gene expression. Surprisingly, despite these changes, increased neurogenesis was detected in certain animals in the HFD+FR group, as indicated by immunostaining of doublecortin positive neurons. We hypothesize that this neurogenesis represents a compensatory response to diet-induced brain injury. Our results confirm that the inflammatory and metabolic alterations induced by obesity affect the entire body, including the brain, highlighting the complex relationship between metabolic and neurological dysfunction. Further research in this area may promote the development of more effective therapeutic strategies for obesity-related pathologies, including those affecting the brain.

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Protective effects of dehydroepiandrosterone on glia cells and the cholinergic sytem in a neurotoxic Alzheimer's disorder mouse model

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Alzheimer's disorder (AD) is the most common neurodegenerative disease. Pathological protein accumulation in the brain, such as Aβ and pTau, impacts not only neurons (presumably cholinergic cells), but also microglia and astrocytes. This alters their morphology and heightened glial activity. Dehydroepiandrosterone (DHEA) and dehydroepiandrosterone sulfate (DHEAS) are endogen steroids that are hypothesized to have neuroprotective effects.

To investigate the protective effects of DHEAS on the cholinergic system and on the microglia and astrocytes.

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 DHEAS or vehicle (0.9% saline) was performed. The animals were transcardially perfused 12 days later, and immunohistochemical stainings (ChAT, AChE, Iba-1, GFAP,) were performed to investigate cholinergic cell, microglia and astrocyte morphology, respectively.

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. Furthermore, activated the microglia and astrocytes in the injection site. DHEAS had a protective effect on the cholinergic fibers, decreasing the Aβ induced neurotoxicity. The treatment also decreased the glia 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.

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Age-related changes in dopaminergic areas of the mesencephalon in wild-type and PACAP gene knockout mice

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The neuroprotective effect of pituitary adenylate cyclase-activating polypeptide (PACAP) has been demonstrated in several Parkinson's disease models, and its absence accelerates aging in PACAP gene knockout (KO) mice. In previous studies, we investigated dopaminergic regions in wild-type and PACAP KO mice up to 8 months of age. In the substantia nigra (SN), we observed no dopaminergic cell loss but noted an increase in microglia, whereas in the ventral tegmental area (VTA), significant dopaminergic cell loss was seen with aging, particularly in KO mice, along with a continuous decrease in microglia until 8 months.

In this study, we compared the SN and VTA regions in 4-month-old and 1.5-year-old wild-type (n=5-5) and PACAP KO (n=8-9) mice. Dopaminergic neurons were labeled with tyrosine hydroxylase (TH) and microglia with Iba1, categorizing their activity based on morphology. Additionally, we assessed the expression of the PACAP-specific PAC1 receptor.

Our results showed a significant age-related reduction in dopaminergic cells in the SN, with notably fewer TH+ cells detected in both regions of 1.5-year-old KO mice compared to wild-type controls. The number of both active and inactive microglia increased significantly in aged KO mice, while in wild-type mice, age-related microglial increases were confined to the VTA. Expression of the PAC1 receptor was minimal in all groups.

The pronounced reduction in dopaminergic cells in older PACAP KO mice suggests increased vulnerability to age-related neurodegenerative processes, similar to those seen in Parkinson's disease. The observed increase in number and activity of microglia in aged KO mice may result from the loss of PACAP's immunosuppressive effects, contributing to dopaminergic cell death. These findings indicate that, in the absence of endogenous PACAP, age-related morphological changes associated with Parkinson's disease advance more rapidly, highlighting PACAP's potential role in the disease's pathomechanism.

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Thermoregulatory Impairments in Alzheimer's Disease: Comparative Effects of Senktide and Rolipram in 3xTg-AD Male 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.

Felodipine efficiency analysis on induced neurons derived from Huntington's disease FELL-HD clinical trial patients

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Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder, caused by CAG expansions in the huntingtin gene (HTT), which results in the aggregation of the mutated huntingtin protein (mHtt). HD is uncurable, and after disease onset around 30-40 years of age patients die within the next 10-20 years. Autophagy, a lysosomal degradation pathway ensuring cytoplasmic homeostasis is dysfunctional in HD, contributing to insufficient mHTT protein removal. The Fell-HD clinical trial is based on repurposing Felodipine, an already licensed L-type calcium channel blocker and antihypertensive drug with a low chance of side effects. Felodipine significantly increases autophagy in animal models of HD and subsequently reduces the level of toxic mHTT, neurodegeneration, and disease symptoms, like tremors and loss of motor coordination.

In this study, conducted in parallel with the FELL-HD trial, we evaluated the efficacy of felodipine in induced neurons (iNs) directly reprogrammed from the skin fibroblasts of participants in the FELL-HD cohort. The transdifferentiated iNs retained the genetic and aging signatures of the donors, bypassing the stem cell or neuroprogenitor phases during conversion. Fibroblasts from seven control individuals and 18 HD patients with mild symptoms were successfully converted into iNs with comparable efficiency and purity. The HD-iNs showed accelerated aging according to multiple epigenetic clocks. Basal autophagy and felodipine-induced autophagic responses in TAU+ neuronal cells were examined at two-time points and across two concentrations using autophagy markers LC3, p62, and LAMP1. Changes in HTT expression in response to felodipine were quantified via RT-qPCR. The findings delineated three distinct groups: a control-like group, an HD-specific group, and an HD-specific group exhibiting accelerated aging. Felodipine's effects varied across these groups, with observed reductions in HTT expression and improvements in neuronal and autophagic.

These preclinical results will be directly compared and correlated with FELL-HD trial outcomes and the patient's cognitive and motor scores. This project using an in vitro preclinical iN model can potentially provide predictive information about drug effectiveness, opening a new dimension in clinical trial optimization and personalized medicine.

The potential protective effect of PACAP on the vertical signal transduction pathway in type 2 diabetic retinopathy

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Diabetic retinopathy (DR) is the leading cause of vision impairment and permanent blindness in one-third of the working-age diabetic population. Our previous research has shown that the pituitary adenylate cyclase-activating polypeptide38 (PACAP38) has potent retinoprotective effects. The aim of the present study was to investigate the protective effect of PACAP focusing on the vertical information processing pathway in type 2 diabetic retinopathy (T2DR).
Three-month-old male Wistar rats were divided into control and diabetes groups. To induce T2DR, animals were injected with streptozotocine (STZ) (i.p. 30mg/kg) and were maintained on a high-fat diet. Systane (vehicle) and PACAP38 eye drops were administered twice a day to the experimental groups, accordingly (Control+Systane, Control+PACAP, Diabetes+Systane, Diabetes+PACAP). After 6 months of diabetes duration, rats were sacrificed, and their eyes were collected for further immunohistochemical and western blot analysis.

Our immunohistochemistry results confirmed the severe damage in the vertical signal transduction pathway in diabetic retinas, evidenced by the breakage of the outer and inner blood-retinal-barriers (BRB), the serious destruction of photoreceptor cells, the decreased rod bipolar cells and ganglion cells. Western blot analysis confirmed the significant reduction of ZO-1 expression. However, PACAP38 treatment moderated the damage to all three-order neurons, their morphological appearance, and the density of these cells.

Our results have been disclosed that PACAP38 has a potent neuroprotective effect in type 2 diabetic retinopathy, therefore PACAP38 can be a potential therapeutical candidate against T2DR.

Increased firing activity, decreased presynaptic neurotransmission and altered transcriptome profile of GnRH neurons in middle-aged female mice of menopause model

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The role of aging GnRH neurons in the process of perimenopause has not been fully characterized. Therefore, we studied the electrophysiological performance and gene expression of GnRH neurons in middle-aged (MA: 420-440 days) female mice, an animal model of perimenopause. In acute brain slices of MA mice, whole cell clamp recordings from GnRH-GFP neurons revealed a heterogenous population exhibiting an increase in spontaneous firing rate (3.4±0.40Hz) in comparison with that of young (Y) mice (1.7±0.33Hz). GnRH neurons of MA mice exhibited alterations in AP (MA:96±2.40mV;Y:77±4.00mV), AHP (MA:-37±1.80mV;Y:-14±1.90mV) and DAP amplitudes (MA:4.2±0.37mV;Y:1±0.50mV), and AP duration (MA:1.2±0.052ms;Y:2±0.31ms). In most MA GnRH neurons (71%), the mPSCs were either absent or showed an attenuated frequency.

Blockade of GABA-A-, glutamate and ACh receptors had no effect on the increased firing rate. Their ligands, however, facilitated it, indicating functional receptor assemblies and that the lack of presynaptic transmitter release is responsible for the absence of mPSCs. MA GnRH neurons responded to estradiol (E2) (10-200pM) with decreased firing rate, indicating the existence of the negative E2 feedback mechanism. Arresting the GPCRs in GnRH neurons decreased the firing rate (from 3.3±0.61Hz to 0.94±0.34Hz). Antagonizing GnRH-Rs with Antide had no effect on cell firing. In contrast, kisspeptin (KP, 200nM) increased the firing rate (from 4.1±1.1Hz to 6.6±1.7Hz), while the KP receptor antagonist KP-234 diminished it (from 4.6±1.2Hz to 3.0±0.87Hz).

The expression profile of MA GnRH neurons showed heterogeneity, 33% of MA animals showed only the molecular signature characteristic for aging neurons. Compared to young mice, 233 of the differentially expressed genes (458) were downregulated. The altered main molecular pathways included mRNA processing, GPCRs, oxidative phosphorylation, electron transport chain and estrogen signaling, among others. Differential expression of genes encoding neurotransmitter receptors (Chrnb4,Gabrb1), ion channels (Scn4a,Kcnmb2,Kcnj10) and neuropeptide receptors (Npffr2,Nmur2,Mc5r) in MA GnRH neurons showed further altered regulatory elements.

The findings indicate that MA GnRH neurons show an increased firing rate, absence of mPSCs, responsiveness to negative estradiol feedback and alertness to kisspeptin signaling. The transcriptome profile recognized the thumbprint of aging in 33% of MA animals with downregulation of vital pathways.

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Age-dependent expression of cocaine- and amphetamine-regulated transcript and urocortin 1 in the centrally projecting Edinger-Westphal nucleus of male rats

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Earlier we demonstrated that the expression of hypothalamic neuropeptides that contribute to the control of energy homeostasis show age-dependent dynamics in the rat. We recently demonstrated that the peptidergic centrally projecting Edinger-Westphal nucleus (EWcp) plays a role in the control of energy homeostasis. The EWcp cells co-expresses cocaine- and amphetamine-regulated transcript (CART) and urocortin 1 (UCN1). Considering the anorexigenic properties of these neuropeptides arose the question whether an aging-related dynamics exists in the EWcp, similar to the hypothalamic peptidergic systems. Therefore, in this study we aimed at investigating the expression of these neuropeptides both at mRNA and peptide level in the course of aging. We hypothesized that the Cart and Ucn1 mRNA, moreover the cells' CART and UCN1 content will be a function of age.

To test this, we perfused 3, 6, 12, 18 and 24-months-old male Wistar rats for histological examinations. In coronal sections of the EWcp, we performed RNAscope in situ hybridization for Cart and Ucn1 as well as immunofluorescence for CART and UCN1. Confocal microscopic images were subjected to morphometry and the fluorescence signal was semi-quantified using Image J software.

We found that the EWcp cells express both Cart and Ucn1 mRNAs, but the Cart signal appeared to be stronger. Both neuropeptide mRNAs showed age-dependent dynamics: the expression was the highest in young adult 3-months-old rats. At 6 months the expression was the lowest. Cart mRNA expression increased at 12 months, and did not change significantly in 18 and 24 months old rats. Ucn1 mRNA showed similar dynamics, but the expression increased in 18 months old rats. At protein level, CART showed a tendency to increase in 12 and 18 months old rats that was followed by a significant decline in 24 months old rats. The UCN1 peptide content of the EWcp appeared to be constant till 18 months of age, but at 24 months we observed a significant decline. We did not observe ageing-related cell loss in the number of peptidergic cells of the EWcp.

Our results suggest that the EWcp produces these neuropeptides in a higher rate in young adults. In middle age, the mRNA expression declined while the peptide content remained unchanged suggesting a lower activity. The expression increased again in older rats, except for the oldest group. Further studies will determine how these alterations modulate the energy homeostasis in the course of aging.

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Prokineticin receptors are expressed in GnRH neurons and mediate excitatory effects in adult female mice

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Prokineticins (PK1, PK2), produced in the brain, exert their effects via two G protein-coupled receptors, prokineticin receptor 1 and 2 (PKR1, PKR2) PKs are involved in the central regulation of diverse functions including reproduction. Besides acting as chemoattractant for guiding migration of GnRH neurons during embryonic life, their role seems to be important also in adult, cycling females. Antagonizing prokineticin receptors has been shown to arrest the estrous cycle and blunt the circulating luteinizing hormone (LH) level.

The aim of the current study was to reveal whether GnRH neurons express either or both PK receptors and respond to prokineticin 2 in in vitro slice preparation.

Highly sensitive RNAscope hybridization was employed to detect mRNAs for PKR1 and PKR2 in neurons expressing GnRH mRNAs in 25 µm-thick vibratome sections. Confocal microscopic analysis of the signals revealed that most of the GnRH neurons were positive for PKR1 transcripts. PKR2 mRNAs were also expressed, at a lower level, present in one third of the GnRH neurons (32.4% ±1.5%).

Immunohistochemical double labeling confirmed the expression of PKR2 in GnRH neurons indicating that even low levels of mRNA are translated to receptor proteins.

Whole-cell patch clamp measurements revealed that GnRH-GFP neurons from acute brain slice of adult female mice responded to prokineticin-2 (PK2; 26 nM) with increased firing rate (from 1.5±0.30 to 1.9±0.34 Hz, p=0.0324, n=11, paired t-test), which was eliminated both by the PK-R antagonist, PRKA7 (from 0.87±0.28 to 0.81±0.25 Hz) and the intracellularly applied, membrane impermeable G-protein blocker GDP-beta-S (from 1.2±0.31 to 1.1±0.3 Hz). PK2 also increased the frequency of mPSCs in GnRH neurons (from 0.83±0.1 to 1.1±0.17 Hz, p=0.0457, n=19, Wilcoxon paired test).

These results indicate that PKs can directly regulate GnRH neurons in adult mice. Further studies are required to identify the source of PKs targeting the GnRH neurons and elucidate the physiological and behavioral consequences of PK activation in vivo.

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Perinatal BPA exposure alters body weight and composition in male offspring

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Environmental chemicals with estrogenic effects have been hypothesized to cross the maternal-fetal barrier and interfere with the endocrine/metabolic regulatory systems of the developing embryo/fetus. Previous studies brought the exposure to these chemicals in association with the prevalence of various cardiometabolic diseases including obesity, metabolic syndrome, type 2 diabetes, and cardiovascular diseases. The present study investigated the effects of bisphenol A (BPA) and 17α-ethinylestradiol (EE2) on the metabolic functions of male offsprings following exposure of their mothers during gestational days 9-21, and the following breast-feeding days 0-21. BPA, a chemical widely used in polycarbonate plastics and epoxy resins and EE2, a synthetic compound of contraceptive pills, both present in increasing concentration in the environments. To mimic a potential environmental exposure, BPA and EE2 were chronically administered (32-33 days) for mothers through osmotic minipumps at environmentally relevant doses, and metabolic parameters of the adult male offsprings were determined.

Male offsprings reaching the post-pubertal age (60-80 day old) were investigated for basic metabolic parameters i.e. total and lean body weight, food and water consumption, as well as glucose levels after 24h fasting. Their calorie expenditure, respiratory exchange ratio and locomotor activity were monitored during the circadian day to reveal potential alteration in these parameters compared to the offsprings of vehicle-treated mothers.

Offsprings of mothers exposed chronically to environmental dose of BPA during gestation and lactation showed a reduced body weight, fat ratio and 24h fast blood glucose levels compared to control and EE2 offsprings. The circadian pattern of motor activity of the experimental animals shows parallelism/synchrony with the peaks of food intake, but with obvious differences present among the groups in both locomotor (X-Y direction) and rearing (Z direction) activity. Calculation of the respiratory exchange ratio revealed reduced values for the BPA and more severally the EE2 offsprings, manifesting mainly during the night phase.

These observations indicate a long-lasting effect of environmental exposure of mothers to BPA or EE2, resulting in an altered circadian pattern of some metabolic processes. This may explain the vulnerability of the offsprings of exposed mothers for cardiometabolic diseases.

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The central effects of PACAP on the hypothalamic-pituitary-gonadal (HPG) axis in male mice

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Introduction: Pituitary adenylate cyclase-activating polypeptide (PACAP) is a member of the vasoactive intestinal peptide (VIP) neuropeptide family that is involved in the regulation of several releasing hormones and trop-hormones by stimulating intracellular cAMP production. The hypothalamic-pituitary-gonadal (HPG) axis regulates the synthesis and the release of sex hormones and the gametogenesis in all mammals. Although the effect of PACAP on fertility is well documented, the mechanism of the effect of PACAP on hypothalamic GnRH and kisspeptin neurons, which are key elements at the highest regulatory level of the HPG axis, is not known in detail. In our previous study, we demonstrated hypothalamic changes that could contribute to the irregular estrous cycle observed in PACAP knockout (KO) female mice.

Aim: In this study our goal was to explore potential structural changes in the hypothalamus of male PACAP KO mice that might underlie reported fertility problems.

Methods: We performed experiments in brain samples obtained from wild-type (WT) and PACAP KO animals using immunohistochemistry, immunofluorescence staining and RNAscope in situ hybridization techniques. Immunohistochemical staining was applied to determine the number and the fiber density of GnRH neurons. Using the RNAscope technique, kisspeptin mRNA-positive cells were counted in the rostral periventricular region of the third ventricle (RP3V) and arcuate nucleus (ARC). Finally, the mRNA and protein expression of estrogen receptor alpha (ERα) and androgen receptor (AR) were also examined in several hypothalamic regions.

Results: In our experiments, we found that in PACAP KO animals the body weight was increased. Immunohistochemical staining of the hypothalamus showed that the number and fiber density of GnRH neurons decreased in the medial preoptic area. Furthermore, the number of kisspeptin neurons increased in the RP3V and the mid-portion of the ARC. The amount of ERα mRNA elevated in both the anteroventral periventricular nucleus (AVPV) and the ARC, in regions where kisspeptin neurons regulating GnRH neurons are located. Interestingly, the number of AR+ cells decreased while the number of ERα+ cells increased in the medial preoptic area (MPOA).

Conclusion: Our results suggest that the observed changes in the hypothalamus might be involved in the development of fertility problems in PACAP-deficient males by abnormal alteration in the function of the HPG axis.

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Hypothalamic orexigenic and anorexigenic neuropeptides in the rotenone model of Parkinson's disease, in the rat

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Parkinson's disease (PD) is a progressive neurodegenerative disorder that involves both motor and non-motor symptoms. The motor symptoms are associated with damage of the dopaminergic neurons of the substantia nigra, while the neurobiological background of non-motor symptoms such as disturbances of the metabolic balance is not fully understood.

Our primary aim was to characterize the changes in the expression of hypothalamic anorexigenic and orexigenic neuropeptides in a rat model of rotenone-induced Parkinson's disease. In the arcuate nucleus (ARC), we focused on cocaine- and amphetamine- regulated transcript mRNA (Cart) and peptide, proopiomelanocortin mRNA (Pomc) and alpha melanocyte-stimulating hormone (alpha-MSH) as well as neuropeptide Y mRNA (Npy) and peptide (NPY). Orexin-1 mRNA and peptide was examined in the lateral hypothalamus (LH). We aimed to explore the possible therapeutic effects of benserazide/levodopa (B/L) and fluoxetine on the observed molecular changes.

The Parkinson's disease-like state was induced by six-week subcutaneous rotenone administration, vs. vehicle-injected controls. From the fourth week on, one-third of the neurotoxin-treated group received B/L treatment, one third underwent fluoxetine therapy combined with B/L medication, while the last subgroup received no further therapy. The motor coordination was assessed by rotarod test, the anxiety by open field test, while anhedonia was examined in sucrose preference test. RNAscope in situ hybridization and immunofluorescence were used to examine the hypothalamic neuropeptides.

Rotenone administration resulted in impaired motor skills, elevated depression level, and increased anxiety. B/L improved motor performance, unlike anxiety and anhedonia. Fluoxetine had a positive effect on anhedonia in the antidepressant-treated group. Rotenone treatment reduced Pomc, alfa-MSH and Cart mRNA while elevated the NPY peptide content in the ARC. In the LH, orexin-1 decreased at both the mRNA and peptide level. B/L treatment resulted in decreased Npy mRNA expression. In animals subjected to combined therapy, Cart mRNA and alfa-MSH peptide density decreased.

Our results suggest complex regulatory alterations in orexinergic and anorexinergic neuropeptides in the rotenone model of Parkinson's disease, that is modulated by the therapy. Disturbed orexinegic and anorexigenic peptidergic signaling may contribute to the metabolic aspects of PD.

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Characterization of pheromone-responsive ventral premammillary neurons in male rats

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In male rodents, sexual behavior is primarily driven by female pheromones. Pheromones are detected by the vomeronasal organ, which is located in the nasal cavity and projects to the accessory olfactory bulb. From this point, the information is transmitted to the posterior medial amygdala (pMeA) and the bed nucleus of the stria terminalis, which project to the ventral premammillary nucleus (PMv) in the hypothalamus. The PMv integrates metabolic and reproductive signals and controls reproduction via its direct connections with gonadotropin-releasing hormone (GnRH) and kisspeptin neurons. According to its integrative role, the PMv expresses a range of neuropeptides and receptors. In the present study, we aimed to further characterize the pheromone-sensitive neurons located in the PMv.

Sexually inexperienced male rats were maintained in individual cages, isolated from females for a period of one week. On the day of the experiment, the rats were divided into three groups. One group served as naive control, while the other two groups received either an intraperitoneal (IP) injection of insulin or a physiological saline solution. Ten minutes later, the rats in the first group were provided with fresh bedding, while the rats in the second and third groups were provided with female-soiled bedding. All rats were sacrificed 90 min later. Furthermore, in an independent experiment, naive rats were fasted overnight and received leptin or saline treatment into the right ventricle via a previously implanted cannula. The rats were then sacrificed 45 minutes later. Coronal hypothalamic brain sections were analysed by immunohistochemistry and in situ hybridization.

The majority of pheromone-activated neurons (Fos-positivity) were nesfatin-1 immunopositive cells. The number of Fos+ neurons was significantly reduced by hypoglycemia. Nesfatin-1 and Fos double-labeled neurons formed synapses with urocortin3-immunoreactive terminals, which primarily originate from the pMeA. Leptin-induced a robust cell activation (pSTAT3 positivity) in the PMv, but not in the nesfatin-1 neurons. However, many nesfatin-1 neurons expressed GPR10, a cognate receptor for the anorexigenic prolactin-releasing peptide. Examination of the nesfatin-1 peptide and mRNA expression in the PMv during postnatal development revealed an early onset of expression that reaches its strongest level after sexual maturation.

Our data demonstrate the complex role of the PMv in the metabolic control of reproduction.

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The role of uncoupling protein 2 (UCP2) in adaptation to stress

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In today's fast-paced world, stress places a significant burden on the body, contributing to various disorders such as anxiety and depression. Adaptation to stress heavily relies on the hypothalamic-pituitary-adrenal (HPA) axis, which requires energy generated at the mitochondrial level. Uncoupling protein 2 (UCP2) plays a crucial role in cellular stress responses and mitochondrial bioenergetics. In rodents, UCP2 is expressed in the paraventricular nucleus (PVN) of the hypothalamus, a central component of the HPA axis, and in the anterior pituitary. In the hypothalamus, UCP2 co-expresses with corticotropin-releasing hormone (CRH), a key regulator of stress, while in the pituitary, it colocalizes with proopiomelanocortin (POMC), the precursor of adrenocorticotropic hormone (ACTH). CRH stimulates ACTH secretion, thereby contributing to stress response regulation. Our study aimed to explore the role of UCP2 in stress adaptation using wild-type (WT) and UCP2 knockout (KO) rats. Behavioural responses to mild ("splash" test) and intense (foot shock) stressors were assessed. After a single foot shock, we measured corticosterone levels in the serum and examined the mRNA expression of stress-regulating neuropeptides in the PVN, pituitary, and adrenal glands. Our results revealed genotype-, sex-, and stress-dependent behavioural differences. Molecular analyses highlighted significant changes in neuropeptide expression, emphasizing the critical role of UCP2 in stress regulation in KO animals. These findings enhance our understanding on the involvement of UCP2 in stress adaptation, offering insights into its potential impact on stress-related disorders.

NUCB2 is involved in the control of AVP neurons in the supraoptic nucleus of rats

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An increase in blood osmolality prompts the release of vasopressin (AVP) from the supraoptic nucleus (SON). AVP facilitates water reabsorption in the kidneys, thereby restoring the normal osmotic concentration. The release of AVP is under precise control, with the dendritic release of AVP and oxytocin playing a significant role. Nesfatin-1, a biologically active fragment of the NUCB2 prohormone, acts through dendritic release. Its role in regulating fluid homeostasis has been suggested previously. Given that nesfatin-1 is highly coexpressed with AVP, we postulated that it may participate in the regulation of AVP neurons.

In Experiment 1, rats were high-salt loaded (2% NaCl, SL group) or received tap water (NL group). The NL group was further divided into an ad libitum-fed group and a pair-fed (PF) group. After four days, the rats were sacrificed and mRNA levels of NUCB2 and AVP were measured in the SON by RT-PCR. In Experiment 2, small hairpin RNA (shRNA) was employed to suppress NUCB2 expression in the SON, in conjunction with salt-loading over a seven-day period, or water deprivation (WD, 24h) and subsequent rehydration. Urine samples were collected from the same subjects at baseline, in WD state, as well as at two-hour intervals following rehydration. Plasma samples were collected at baseline and following WD, as well as one hour after water readministration. The expression of AVP and nesfatin-1 was analyzed in the supraoptic nucleus (SON) by immunohistochemistry.

NUCB2 and AVP mRNA levels were elevated in the SL group in comparison to the NL and PF groups. The SL-NUCB2sh conditions resulted in an increase in AVP immunoreactivity in the ventral dendritic zone of SON, with the greatest effect observed in the combination of the treatments. The level of AVP immunoreactivity within the perikarya was found to be reduced in the SL-scrambled (SCR) sh, NL-NUCB2sh, and SL-NUCB2sh groups when compared to the NL-SCRsh group. The perimeter of the AVP-positive somata was increased in the SL groups, whereas NUCB2sh treatment had no effect on this parameter. Urine osmolarity after WD was lower, while plasma osmolality was higher in the NUCB2sh group than in the SCRsh group. Our data suggest that NUCB2/nesfatin-1 plays an important role in the regulation of AVP neuronal function.

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Modeling approaches ATP induced Ca2+ transients in different types of cochlear supporting cells

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Physiology of supporting cells in the cochlea are studied mostly in young and still deaf rodents, because of the easier preparation of the bony capsule of the organ. However, the developmental noise is remarkable in the case of the hearing organ of mice. The receptors of the purinergic system are spread in the supporting cells and their roles in spontaneous activity during the development have been recognized, but our knowledge about the presence and role in older mice is much sparse.

We have investigated the ATP induced Ca2+ transients (via the activation of P2X and P2Y receptors) with both experimental and modeling approaches.

The Ca2+ concentration elevation were measured in Deiters' (DCs), Hensen's (HCs) and Claudius' cells (CCs) in the BALB/c mouse hemicochlea preparation (from 14 days old to 21 days old). From this time the hair cells respond to external sound stimuli. The evoked Ca2+ transients have different characteristics (in duration and amplitude) depending on the cell-type indicating different receptors and Ca2+ handling mechanisms.

We have set up a model to simulate the mechanisms of the ATP-induced responses (activate both the intracellular stores and the extracellular influx of Ca2+ through the ionotropic P2X receptors).

Our model was emulated reliably the Ca2+ transients measured by the functional imaging. The models suggest that the parameters of Ca2+ removing mechanism (especially in parameters of SERCA function) and the P2X receptors have the biggest differences among the cells. The results indicated by the model are suitable to initiate new experiments to decipher the precise role of the intracellular Ca2+ regulation in DCs, HCs and CCs in the function of hearing and hearing sensitivity.

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Distinct Signaling Properties of Lateral and Medial Entorhinal Cortical Axons Terminating in the Hippocampus

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The lateral entorhinal cortex (LEC) and medial entorhinal cortex (MEC) convey distinct types of information to the hippocampus via the lateral and medial perforant pathways. The LEC primarily transmits object-related information, whereas the MEC is the source of basic spatial information, such as grid cell activity. Even though the fundamental anatomical features of the two pathways are similar, the differences in the type of information they carry are reflected in the strength and dynamics of their synaptic connections.

Here we investigated axonal excitability properties in the two perforant pathways to reveal mechanistic differences that can potentially contribute to their distinct functionality.

First, we performed direct patch-clamp recordings from anatomically identified perforant pathway axons to measure their passive membrane properties and action potential (AP) characteristics. We found that the APs in LEC axons were unusually wide and were followed by a large after-depolarization. In contrast, APs in MEC axons were narrow and lacked after-depolarization. The subthreshold properties were also different. Particularly, LEC axons exhibited a prominent sag, which is usually mediated by hyperpolarization-activated cation channels. The sag was less pronounced in MEC axons. Interestingly, this axonal observation is the opposite of the known somatic sag properties of their parent cells, as MEC cells exhibit a large sag but it is much less in LEC somatas.

To compare axonal properties using an independent approach, we expressed the Voltron sensor specifically in the two perforant pathways and performed voltage imaging in their hippocampal termination zones. The imaging results confirmed that LEC axon APs were consistently slower than MEC APs and had a significant after-depolarization.

Our Patch-Seq data from retrogradely labeled LEC and MEC cells revealed differences in potassium channels that potentially underlie their different AP signaling properties. We took advantage of the stability of Voltage imaging to pharmacologically evaluate the contribution of distinct types of potassium channels.

Overall, our results highlight key differences in the signaling mechanisms of LEC and MEC axons that can support their distinct roles in processing object-related and spatial information. Specifically, the wider action potentials in LEC axons help ensure reliable transfer of object information.

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Examination of mRNA-loaded solid lipid nanoparticles in human cell cultures

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The blood-brain barrier (BBB) is a strictly controlled biological barrier that not only prevents harmful substances from entering the brain tissue from the bloodstream but also blocks the passage of many drugs and therapeutic agents. Brain endothelial cells with special properties play a crucial role in the formation and maintenance of the BBB. Transfection of brain endothelial cells—particularly for gene therapy purposes—is extremely challenging because these cells are resistant to conventional transfection methods, including viral and non-viral vectors. Solid lipid nanoparticles (LNPs) are a promising tool for non-viral gene therapy as they can efficiently deliver various therapeutic mRNAs while minimizing immune responses and cytotoxicity. LNPs provide protection against mRNA instability and facilitate its targeted delivery into specific cells, particularly hard-to-reach targets like brain endothelial cells.

In our experiments, we aimed to investigate the transfection efficiency of LNPs containing green fluorescent protein (GFP) coding mRNA in cultures of HeLa cells and human brain endothelial cells. The size and charge of the nanoparticles were determined using dynamic light scattering measurements. The uptake efficiency of LNPs with varying mRNA concentrations was assessed by measuring GFP fluorescence intensity in the subconfluent and confluent cell cultures high content imaging system.

Our results showed that the LNPs did not affected the viability of the cells and were able to deliver mRNA in significant amounts to tumor-derived HeLa cells, while a lower but measurable transfection efficiency was observed in brain endothelial cells. The cellular uptake of LNPs was concentration-dependent, and the transfection efficiency was significantly higher in the subconfluent cells compared to the confluent cell layers.

Based on our findings, LNPs could be safe vectors for gene therapy-focused transfection of human brain endothelial cells, potentially opening new therapeutic possibilities for treating brain diseases.

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Species-Specific T-Type Calcium Channel Contributions to Spike Precision in Human Parvalbumin Interneurons

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Parvalbumin-expressing interneurons (PVINs) are crucial in regulating neocortical gamma oscillations, which are essential for precise neuronal timing. Disruptions in these oscillations have been linked to various disorders, including schizophrenia, epilepsy, and autism spectrum disorder. To address the limitations of mouse models for translation to human biology, we investigated species-specific differences in the excitability and spike timing of PVINs. Our research revealed that human PVINs exhibit a unique subthreshold membrane voltage deflection ("hump"), which is absent in mice. This hump is driven by T-type calcium channels and enhances the precision of spikes. By introducing this conductance into mouse cells using a dynamic clamp, we could improve spike precision, demonstrating that the hump plays a critical role in the temporal synchrony of neuronal activity. Single-cell patch sequencing indicated that the gene encoding the CaV3.1 subunit of T-type calcium channels (CACNA1G) is expressed at a higher level in human PVINs, which correlates with distinct bursting and accommodating firing patterns of these cells. In contrast, mouse PVINs exhibit non-accommodating behavior. Furthermore, confocal imaging showed that Cav3.1 channels are enriched in the dendrites of human PVINs, a feature not found in their mouse counterparts. These findings highlight the importance of T-type calcium channels in fine-tuning the excitability and spike timing of human PVINs, providing valuable insights into species-specific mechanisms with significant implications for translational brain research.

Single-nucleus transcriptome analysis of the human arcuate nucleus

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The arcuate nucleus (AN), situated within the mediobasal hypothalamus, plays a pivotal role in endocrine and metabolic regulations. The AN is comprised of diverse neuronal populations, including POMC, AgRP, GHRH, TIDA, and KNDy neurons, integral to energy balance and pituitary hormone regulation. Despite their importance, their heterogeneity, molecular profiles, and spatial organization in humans remain largely unexplored. Advances in single-nucleus RNA sequencing (snRNA-seq) have facilitated a comprehensive examination of the AN's transcriptomic landscape, offering insights into its diverse roles and intercellular communication.

We performed snRNA-seq on pooled human AN samples from eight healthy donors, identifying 19 distinct cell clusters, including well-characterized glial and ependymal populations. Differential gene expression analysis of neuronal population revealed the predominance of GABAergic neurons and smaller subsets of dopaminergic neurons, with a minimal expression of excitatory markers. Further subclustering of the neurons delineated 25 neuronal subpopulations, revealing significant heterogeneity in neurotransmitter and neuropeptide expression. Neuropeptides such as TENM1, NPPC, and IGF1 exhibited distinctive expression patterns, suggesting the presence of potentially novel neuronal subtypes. These findings highlight the molecular complexity of the arcuate nucleus and underscore its diverse roles in maintaining homeostasis.

Ligand-receptor interaction analysis provided further insights into intercellular communication, unveiling extensive signaling networks between neuronal populations. It is noteworthy that particularly strong interactions involving TAC3- and IGFBP3-expressing neurons, CRHR2- and PTH2R-expressing populations, and neurons defined by NPFFR2 and VIPR2 expression were identified suggesting a functional connection between these cell types.

This study presents the first comprehensive single-nucleus transcriptomic map of the human arcuate nucleus, elucidating its molecular diversity, intercellular communication, and distinctive subpopulations. These findings advance our comprehension of the AN’s critical role in homeostasis and open new avenues for exploring therapeutic targets in endocrine and metabolic disorders.

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DETERMINATION OF VISUAL INPUTS OF THE POSTERIOR INTRALAMINARY NUCLEI

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The posterior intralaminar complex (PIL) of the thalamus is a triangular area in the thalamus and its main role to regulate social behavior. The PIL receives auditory- and visual inputs indirectly via the inferior- and superior colliculus (SC) of the midbrain, respectively. Although, the retina-SC signaling stream has been studied in detail, relatively little is known about visual input components the SC cells that convey signals from the retina to the PIL. The retina breaks down surrounding visual input into its elementary components (color, contrast, movement, etc.) and retinal ganglion cells (RGCs), the outputs of the retina, encode this stereotypic information into a spike train and passes towards visual centers of the brain, including the dorsal lateral geniculate nucleus (dLGN) and the SC. The various RGC subtypes can be identified based on the morphology and stratification level of their dendritic arbor. In this study, we took advantage of this latter feature and attempted to determine the RGC subtypes and corresponding visual features that are conveyed through the retina-SC-PIL signaling route. Moreover, using a combination of GMO mouse lines (VGAT, VGLUT specific Cre lines) and viral tracing methods we identified RGCs providing either excitatory (glutamatergic) and/or inhibitory (GABAergic) inputs to PIL projecting SC cells. Collected data served to determine the type of visual information integrated by the PIL.

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Network activity alterations by common antipsychotics in cultures of mouse primary hippocampal neurons

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A wide variety of antipsychotics are used to treat mental disorders, including schizophrenia, bipolar disorder and chronic depression. A common feature of such drugs is a negative effect on dopaminergic signaling. Dopamine, one of the key neurotransmitters and modulators have been shown to regulate the activity of both excitatory and inhibitory neurons, thus reduced dopamine signaling can have a strong effect on network activity of neurons expressing dopamine receptors.

Our present project focuses on a novel antipsychotic drug, cariprazine (CAR), and its effect on neural function. We decided to investigate chronic functional effects of CAR in a well described model system of cultured mouse primary hippocampal neurons. Taking advantage of the high-throughput recording capabilities of the advanced multielectrode array technique, we recorded network dynamics and synchronization properties of such developing neuronal populations. Long-term recordings were performed using the Axion Maestro system allowing the simultaneous monitoring of neuronal networks in 24 independent wells, each containing 16 electrodes. Cariprazine (0.1 and 1 μM) was applied either chronically (48-hour) or acutely. Additionally, to compare the effectiveness of the drug, we used three more compounds – aripiprazole, haloperidol, pramiprexole – in acute conditions.

Chronic CAR administration at 1 μM concentration improved network synchronization and regularity of bursts generated by the neurons. More pronounced effects were observed upon acute application of increased concentration of the drug, namely, firing rate in all wells were markedly reduced with improved synchronization among the neurons. In contrast with CAR, haloperidol and aripiprazole completely eliminated firing in all cultures, pramipexole displaying similar, although less dramatic reduction of activity. All effects were reversible following washout.

Our experiments have shown that compounds that mainly act on D2 receptors (haloperidol and aripiprazole) exert a strong negative effect on neuronal activity, while drugs that prefer D3 receptors (cariprazine and pramiprexole) are moderately able to reduce hippocampal network activity in vitro. In addition to mental illnesses, cariprazine is also a used medication in Huntington's disease (HD), thus our next step is to investigate the physiological effects of the drug on induced neuronal cultures generated by direct reprogramming of human fibroblasts from HD patients.

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TRPM3 regulates fear memory encoding and seizure susceptibility in the lateral amygdala

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TRPM3 is a member of the Transient Receptor Potential (TRP) family of ion channels expressed in sensory neurons and other cells and is mainly recognized as a peripheral pain sensor. However, recently, several studies have reported central nervous system mutations of TRPM3 in patients, resulting in increased channel activity. Associated pathologies include autism spectrum disorder and developmental and epilepsy-associated encephalopathies. These recent findings suggest an important role for the channel in the CNS. However, the cell specific expression pattern and function of TRPM3 in the brain is almost completely unknown. In our preliminary experiments we showed for the first time that Trpm3 can be found in the lateral nucleus (LA) of the amygdala, a structure that plays an important role in the neural coding of fear and affected in different brain pathologies including temporal lobe epilepsy. In view of these findings, clarification of the function of TRPM3 in the LA is of particular importance.

Here we report that Trpm3 knockout mice exhibit impaired fear memory formation while their basal anxiety-related behaviors are unchanged. Furthermore, we showed that Trpm3 knockout mice is characterized by less severe seizures in the kainic acid epilepsy model. Finally, we showed that TRPM3 is functionally active in neurons of the LA and activation of the channel leads to membrane depolarization.

Taken together our results provide direct evidence for the role of TRPM3 in LA related physiological and pathophysiological conditions.

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CHARACTERIZING TAC4 EXPRESSION PATTERNS IN CNS REGIONS ASSOCIATED WITH MOTOR CONTROL

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Hemokinin-1 (HK-1), encoded by the Tac4 gene, is the youngest member of the tachykinin family. It is widely expressed in the immune, digestive, cardiovascular and central nervous system (CNS); however, its precise role in the CNS remains not fully elucidated. We investigated its role in locomotor coordination and muscle function during ageing. Our results suggest that HK-1 may play a complex regulatory role in locomotor coordination in old age; however, it does not affect muscle strength. Based on these results, our aim was to characterize the gene expression profile within the affected brain regions.

Due to the high structural similarity between HK-1 and substance P, no specific antibody is available. Therefore, we used the highly sensitive RNAscope in situ hybridization technique to examine Tac4 gene expression in mice and human samples. For neuronal characterization vesicular glutamate transporter 1, glutamate decarboxylase 1, choline acetyltransferase RNAscope probes were used in case of glutamatergic, GABAergic and cholinergic neurons, respectively and anti-tyrosine hydroxylase antibody for dopaminergic neurons.

Our results indicate that Tac4 is significantly expressed across all CNS regions involved in motor coordination in mice. In the motor cortex, where the planning, controlling and executing of voluntary movements occur, the mRNA is expressed in both glutamatergic and GABAergic neurons. Furthermore, in various nuclei of the basal ganglia, which regulate the initiation and smooth execution of voluntary movements, Tac4 is detectable in GABAergic and dopaminergic cells. The excitatory and inhibitory neurons in the cerebellum, which play a key role in fine-tuning and coordinating motor movements, also exhibit expression of the gene. Not least, Tac4 mRNA is expressed in cholinergic motor neurons in the lumbar spinal cord. Although in smaller quantities, TAC4 is also present in human samples, e.g motorcortex.

In summary, due to its widespread expression, HK-1 encoded by the Tac4 gene may play a complex role in the regulation of motor coordination, both locally and in the interregional communication of the brain regions and the periphery.

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Glutamatergic neurons of the precuneiform nucleus form a transitional group among the neighboring areas of the mesencephalic locomotor system

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The mesencephalic precuneiform nucleus (PrCN) is located rostrally from the cuneiform (CN) and dorsally from the pedunculopontine nuclei (PPN). The region is known as the mesencephalic locomotor region (MLR) with various roles in movement regulation. The CN and PPN are characterized by several morphological, ex vivo or in vivo studies, but little is known about the PrCN.

In this study we provided somatodendritic morphological and ex vivo functional analysis of PrCN glutamatergic neurons and a comparison with the glutamatergic neuronal populations of the neighboring regions. We showed that PrCN neurons possess dendritic morphology similar to the PPN but distinct from the CN. Most PrCN neurons have A-current and display delay of the first spike of action potential trains. According to depolarization-dependent changes of spike adaptation, in contrast to the other nuclei, the majority of PrCN neurons do not adapt and fire with a significantly higher frequency than PPN and CN neurons. This high frequency of action potential firing is related to TTX-sensitive high threshold membrane potential oscillations. These oscillations have a higher power amplitude and frequency than in the CN and PPN. On the basis of the presence or absence of the A-current and the persistent sodium current, functional subgroups might be defined in the PrCN.

In conclusion, glutamatergic neurons of the PrCN share several morphological and functional similarities with the neighboring MLR nuclei, but distinct from them in firing rate, adaptation properties and properties of the A-current. These differences might let us predicting its possible differential contribution to regulation of locomotion and to obtain a better design of in vivo experiments.

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Ectopic neurons in the cerebral cortex in human temporal lobe epilepsy

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Temporal lobe epilepsy (TLE) is the most common form of pharmacotherapy-resistant epilepsies. Hippocampal sclerosis (HS), malformation of cortical development (MCD) and intracranial tumors are the most common causes of TLE. During cortical development, neuronal migration can be altered resulting in the presence of ectopic cells in various positions. The aim of this study was to examine and characterize ectopic neurons in the human archicortical dentate gyrus and in the temporal neocortex. Our aim was to explore possible association between the presence of different ectopic cells in different location and the different etiologies that caused TLE.

Ectopic cells were detected by immunohistochemistry based on the expression of neurochemical markers including calcium binding proteins, such as calretinin (CR), parvalbumin (PV) and calbindin (CB) in neurosurgically removed sections of the cerebral neocortex and hippocampal formation of patients with TLE. Patients were grouped based on preoperative MRI diagnosis.

CR-immunoreactive (IR) ectopic cells were found in the dentate gyrus, between the end of the ventral blade of the granule cell layers and the fimbria fornicis. Ectopic CB-IR were present in the hilus of the dentate gyrus, in addition to the dispersed granule cells. Ectopic PV-IR neurons were detected in the molecular layer of the dentate gyrus. Patients in the HS group had the largest density of ectopic CR-immunoreactive (IR) and PV-IR neurons in the dentate gyrus. Significantly larger number of ectopic CR-IR cells were in those patients who had dispersed granule cells. No significant correlation was found between density of ectopic CR-IR and PV-IR neurons, as well as between the density of ectopic PV-IR neurons and the morphology of the granule cell layer. No significant correlation was detected between the densities of different ectopic neurons in the dentate gyrus and in the deep white matter of the cerebral neocortex.

We can conclude that in HS, as well as in MCD, large number of different ectopic neurons are present in the dentate gyrus and in the deep white matter of the cerebral neocortex. The lack of correlation between the appearance and density of different ectopic neuronal groups suggests that the appearance of ectopic PV-, CR-, and CB-IR cells occurs by different mechanisms.

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NEUROCHEMICAL CHARACTERIZATION OF THE LATE BORN NEURONS IN THE SPINAL DORSAL HORN OF MICE

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The spinal dorsal horn (SDH) receives diverse somatosensory information from the periphery and following complex neuronal processing it is transmitted to the higher brain centres. Neurons in the SDH are derived from the late born neuronal population and differentiate into an excitatory and an inhibitory group. Besides the main neurotransmitters (glutamate, GABA and glycine), they express a set of enzymes, calcium-binding proteins or receptors restricted to distinct subpopulations. Concerning the neurochemical properties, there is a large heterogeneity among the superficial neurons. In contrast, these neurons develop from a relatively homogeneous late born postmitotic neurons. Therefore, our aim was to characterize and quantify neurochemical phenotypes of these neurons born within a short time interval revealed by in utero electroporation (IUEP) followed by immunocytochemistry in mice.

The immature migrating GFP-labelled cells had neither excitatory nor inhibitory fates. We found that majority of the GFP-positive late born neurons was calbindin- or calretinin-positive (24-18%) that are mostly excitatory cells in the superficial dorsal horn. Another group of GFP-labelled cells expressed the transcription factor Pax2 (17%) that is required for the development of the GABAergic neurons. A small portion of the GFP-labelled neurons were positive for either PKCg (14%) or NK1 receptor (14%), markers of excitatory neurons with distinct laminar distribution. The smallest group of GFP-positive cells (12%) showed prodynorphin (Pdyn) immunoreactivity that is characteristic for the inhibitory neurons in the superficial laminae of the spinal dorsal horn.

Our results indicate that neurons populating the spinal dorsal horn born together in a short time interval from a unique progenitor population differentiate into a large variety of cells that may be due to more extrinsic over intrinsic factors defining their neurochemical, morphological and functional properties.

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High frequency astrocytic calcium signals influence slow wave activity and memory consolidation

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One of the major breakthroughs of neurobiology was the identification of distinct ranges of oscillatory activity in the neuronal network that were found to be responsible for specific biological functions. Astrocytes, physically coupled by gap junctions and possessing the ability to simultaneously modulate the functions of surrounding synapses, are perfectly positioned to introduce synchronised oscillatory activity into the neural network. However, astrocytic somatic calcium signalling has not been investigated to date in the frequency ranges of common neuronal oscillations, since astrocytes are generally considered to be slow responders in terms of Ca2+ signalling. Using high frequency two-photon imaging, we reveal fast Ca2+ oscillations in the soma of astrocytes in the delta (0.5–4 Hz) and theta (4–8 Hz) frequency bands in vivo in the rat cortex under ketamine–xylazine anaesthesia, which is known to induce permanent slow-wave activity (SWA). These high frequency oscillations appear to be linked to the induced SWA, as they were absent under fentanyl anaesthesia. We also demonstrate that these fast astrocytic Ca2+ signals, previously considered to be exclusive to neurons, are present in a large number of astrocytes and are synchronised at the astrocytic network level.

Furthermore, we have investigated the link between astrocytes and SWA on the cellular, network, and behavioural level. Astrocytic synchronization was modified by activating gap junctions using trimethylamine (TMA), or by blocking them with an astrocyte-specific Cx43 antibody. High frequency two-photon imaging of both astrocytes and neurons shows that synchronization of both cell types at frequencies characteristic of SWA (0.5 –2 Hz) is strongly diminished following gap junction blockade. In contrast, gap junction activation resulted in increased synchronization in both cell networks. Since SWA is known to be involved in memory formation, we investigated whether the activation or inhibition of gap junctions influences memory performance in the novel object recognition memory test. We demonstrated that the working memory of rats can be enhanced by TMA, while treatment with the Cx43 antibody causes memory impairment. We believe that large-scale synchronization in the astrocyte network through gap junctions plays a previously unrecognized, essential role in higher cognitive functions and may open up new avenues in the therapy of cognitive disorders.

Novel astrocytic targets in epilepsy utilizing the Glu/GABA exchange mechanisms

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Astrocytes are considered promising targets for new epilepsy treatments due to their pivotal role in regulating ion and neurotransmitter homeostasis. This insight opens new avenues for the development of more effective anti-epileptic drugs for patients with drug-resistant epilepsy. We previously revealed that the uptake of Glu during seizures triggers GABA release from astrocytes through GAT-3 transporter, and this negative feedback mechanism effectively suppresses epileptic seizures. Here we investigate the potential of pharmacologically enhancing this mechanism as a promising strategy for antiepileptic drug development.

Since astrocytic GABA is synthesized from putrescine (PUT), we opted to initially enhance the Glu/GABA exchange pathway by applying exogenous PUT. We observed that PUT indeed shortens seizure-like events (SLEs) in the low-[Mg2+] in vitro epilepsy model by increasing desynchronization. Moreover, in the presence of PUT, the disappearance of characteristic high frequencies in the tonic phase of SLEs indicated that PUT specifically impacts depolarization-induced tonic desynchronization. We also observed that PUT produced a significant depression of spontaneous depolarizing potentials (dSPs). The inhibitory synaptic potentials (hSPs), on the other hand, were unaffected, suggesting that the PUT-derived GABA acts on extrasynaptic receptors. Furthermore, PUT significantly reduced the duration of seizures in vivo in WAG/Rij rats, a genetic model of absence epilepsy. Even more importantly, inhibiting the conversion of PUT to spermidine, therefore increasing the astrocytic pool of PUT for GABA synthesis, completely blocked seizure generation in the same in vivo model.

Levetiracetam (LEV) is a widely used antiepileptic drug, though its mechanism of action remains poorly understood. LEV is known to increase the surface expression of astrocytic GABA transporters, suggesting that it may enhance the Glu/GABA exchange mechanism. Indeed, we demonstrated that the anti-epileptic effect of LEV can be blocked by a specific glial GABA transporter inhibitor, indicating that the Glu/GABA exchange mechanism plays a key role in the long-term antiepileptic effects of LEV. In the short term, however, LEV appears to exert its effects by regulating synaptic inhibition.

In summary, we propose several pathways by which intensifying the Glu/GABA exchange mechanism can effectively suppress seizure generation in both convulsive and non-convulsive seizure models.

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Group I mGluR mediated changes in neuronal excitability and synaptic strength show cell-type specificity

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Group I mGluRs are perisynaptic receptors composed of mGluR1 and 5 that are predominantly activated following intense presynaptic firing. They have been implicated in various physiological processes such as regulation of REM and NREM sleep as well as numerous neurodegenerative diseases such as Alzheimer's and Fragile X syndrome. However, their specific effects on different cell types and synaptic connections remain poorly understood in the human brain.

To investigate the effects of mGluRs on excitatory synaptic transmission and neuronal excitability, we used dual whole cell patch clamp to simultaneously record mono- and disynaptically connected human supragranular cortical pyramidal cells (PC) and interneurons (IN) before and after pharmacological activation of mGluR1/5 by DHPG.

We separated fast-spikers (FS) from non-fast spikers (non-FS) based on their electrophysiological properties. We observed the potentiation of excitatory postsynaptic current amplitudes (EPSCs) in the majority of FS cells, while synaptic strength of non-FS Ins remained mostly unaffected by the agonist. These results in monosynaptic transmission did not translate into significant modulation of disynaptic connections. We found differences between neuron populations in their membrane potential changes measured either directly or through monitoring of holding currents. In human FS INs, the agonist caused a non-significant reduction in holding currents indicating the depolarization of these INs, while in non-FS INs, it led to a non-significant increase in holding currents. Additionally, in human PCs, DHPG induced a significant depolarization, which is consistent with our results from the rodent cortex.

Our results point to the cell-type specific modulation of group I mGluR in the human cortex, which alters the role and function of GABAerg neurons within L2/3 microcircuits, with possible implications for both basic research and potential therapeutic strategies targeting these receptors.

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Properties and activity-dependent plasticity of excitability of neurons in a learning and memory circuit of octopus

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The cognitive capacities of octopi exceed those of many mammalian species. Due to their divergence at an early stage of the evolution, cephalopods employ distinct brain structures for these sophisticated neuronal functions. Therefore, understanding the shared and differing features can identify the minimum requirements for a neuronal network to perform higher-order cognitive functions. Using somatic whole-cell patch clamp recordings in acute slices, we investigated the cellular properties of neurons in the Superior Frontal Lobe (SFL) and Vertical Lobe (VL) of Octopus bimaculoides, regions which form a network that is known to be critical for associative learning.

Both regions contain enormous numbers (2M and 25M) of small neurons, whose unipolar soma is electrotonically isolated from neurites that perform neuronal computations. We did not detect Na+ currents in the soma of SFL neurons, but distally initiated action potentials can be evoked by strong somatic depolarization. Somatic recordings showed two types of K+ currents in SFL neurons: a low voltage-activated, inactivating transient type and a slow, TEA-sensitive current type. Most SFL neurons are known to send glutamatergic synapses to the VL region. In contrast to SFL, VL cells have substantial amounts of somatic Na+ currents. However, it was not possible to initiate action potentials from their soma, even with extremely strong depolarization. VL neurons show a single type of voltage-dependent K+ current.

After establishing that the somatic voltage-activated currents of VL and SFL neurons have consistent properties, we investigated their activity-dependent changes following stimulation of the VL region with strong, natural activity patterns. We found that 5-25 hours after the long 1 Hz stimuli, the excitability of VL cells in the vicinity of the stimulation site was enhanced, as their Na+ currents and input resistance increased. Intriguingly, we also observed specific excitability changes in the SFL neurons distal to the stimulation site. Specifically, the slow K+ current component decreased, whereas the fast K+ currents remained unchanged. Thus, neurons in both regions became more excitable, but their plasticity is mediated by different mechanisms.

Our results reveal the previously unknown cellular excitability properties of the SFL-VL network and provide the basis for further investigations of genetic changes underlying the region-specific activity-dependent plasticity of neuronal excitability.

Aging-associated weakening of the action potential in 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; however, the exact cellular mechanisms behind this deterioration in humans are poorly elucidated. Recordings in human acute brain slices prepared from tissue resected during brain surgery enable the investigation of neuronal changes with age. Although neocortical fast-spiking cells are widely implicated in neuronal network activities underlying cognitive processes, they are vulnerable to neurodegeneration. Herein, we analyzed the electrical properties of 147 fast-spiking interneurons in neocortex samples resected in brain surgery from 106 patients aged 11–84 years. By studying the electrophysiological features of action potentials and passive membrane properties, we report that action potential overshoot significantly decreases and spike half-width increases with age. Moreover, the action potential maximum-rise speed (but not the repolarization speed or the afterhyperpolarization amplitude) significantly changed with age, suggesting a particular weakening of the sodium channel current generated in the soma. Cell passive membrane properties measured as the input resistance, membrane time constant, and cell capacitance remained unaffected by senescence. Thus, we conclude that the action potential in fast-spiking interneurons shows a significant weakening in the human neocortex with age. This may contribute to the deterioration of cortical functions by aging.

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Electrophysiology and Morphology of Human Cortical Supragranular Pyramidal Cells in a Wide Age Range

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The basic excitatory neurons of the cerebral cortex, the pyramidal cells, are the most important signal integrators for the local circuit. They have quite characteristic morphological and electrophysiological properties that are known to be largely constant with age in the young and adult cortex. However, the brain undergoes several dynamic changes throughout life, such as in the phases of early development and cognitive decline in the aging brain. We set out to search for intrinsic cellular changes in supragranular pyramidal cells across a broad age range: from birth to 85 years of age and we found differences in several biophysical properties between defined age groups. During the first year of life, subthreshold and suprathreshold electrophysiological properties changed in a way that shows that pyramidal cells become less excitable with maturation, but also become temporarily more precise. According to our findings, the morphological features of the three-dimensional reconstructions from different life stages showed consistent morphological properties and systematic dendritic spine analysis of an infantile and an old pyramidal cell showed clear significant differences in the distribution of spine shapes. Overall, the changes that occur during development and aging may have lasting effects on the properties of pyramidal cells in the cerebral cortex. Understanding these changes is important to unravel the complex mechanisms underlying brain development, cognition, and age-related neurodegenerative diseases.

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Epigenetic Regulation and Molecular Mechanisms of Burn Injury-Induced Nociception in the Spinal Cord of Mice

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Epigenetic mechanisms, particularly histone post-translational modifications (PTMs), are crucial in regulating pain perception and the pathophysiology of burn injury. Nevertheless, the epigenetic regulation and molecular mechanisms that underlie pain induced by burn injury have yet to be thoroughly explored.

Spinal dynorphinergic (Pdyn) neurons are involved in heat hyperalgesia following severe scalding burn injury through p-S10H3-dependent signaling. In addition to this modification, burn injury can affect other histone H3 post-translational modifications. Our analyses revealed significant hypermethylation at the H3K4me1 and H3K4me3 sites and hyperphosphorylation at S10H3 in the spinal cord. Additionally, Pdyn neurons in the spinal dorsal horn showed evidence of chromatin activation with increased p-S10H3 immunoreactivity.

We performed RNA sequencing (RNA-seq) analysis to compare the effects of burn injury and formalin-induced inflammatory pain on spinal cord transcriptomic profiles. We identified 98 differentially expressed genes (DEGs) for burn injury and 86 for formalin-induced pain, with few overlaps indicating different central pain processing mechanisms. KEGG pathway analysis revealed that burn injury activates the Wnt signaling pathway.

This study enhances our understanding of burn injury mechanisms and identifies distinct pathways in different pain models.

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Studying neuronal autophagy in human ageing using induced neurons directly reprogrammed from adult human fibroblasts

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Age is the major risk factor for most neurodegenerative diseases (NDDs), such as Alzheimer's (AD). The molecular mechanisms that underlie aging and its contribution to NDDs remains largely unknown. Autophagy, a key pathway that controls the cytoplasmic homeostasis, declines with age and plays an instrumental role in age-related NDDs. The purpose of this project is to understand, using directly reprogrammed neurons obtained from human fibroblasts, how autophagy is affected during neuronal aging. We will analyze induced neurons (iNs) obtained from 63 healthy donors of young and old ages and perform detailed molecular, biochemical investigations of aging and the alterations of autophagy. Our preliminary results indicate that iNs obtained using direct reprogramming retain age-associated features. The age of the donor strongly correlates with the predicted epigenetic age of the iNs. Our preliminary findings indicate that the transcriptional and posttranscriptional control of autophagy is altered with age in iNs. We went further studying basal and stress (starvation) induced autophagic mechanism, first results indicate different effect of starvation and autophagy response in iNs derived from differentially aged donors. This project will provide new insights into human neuronal aging, thereby opening for the design of new treatment strategies to restore autophagy alterations in aged neurons from patients with NDDs.

Cellular and molecular footprint of aging in a defined neuronal network encoding associative memory

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Due to the complexity of the central nervous system (CNS), the study of aging processes in vertebrates is not an easy task at the level of neural circuits and individually identified neurons. As a result, aging research heavily relies on invertebrate model organisms. One such model is the great pond snail (Lymnaea stagnalis), which has been used extensively for decades to study aging and age-related memory impairment with a characteristically integrative top-down approach.

We made the neuronal transcriptome assembly of Lymnaea and identified several evolutionarily conserved homolog sequences to genes involved in aging, age-related memory impairment, and neurodegenerative diseases (e.g., Parkinson's disease, Alzheimer's disease) of vertebrates including humans. We hypothesize that the proteins encoded by these sequences are involved in age-related impairments of learning mechanisms in Lymnaea by targeting the identified components (e.g., NMDA receptor) of the signalling pathways of long-term memory formation. Using young (3-4-month) and old (11-12-month) snails, we investigated the age-related cellular and molecular changes in the whole CNS and in an identified key interneuron of implicit learning, the Cerebral Giant Cell (CGC). In the whole CNS, the expression of 960 transcripts significantly changed during aging. Highlighting, the expression of several key molecules of learning, such as NMDA receptor and CREB-binding protein, showed an age-related decline. In the CGC, the expression of 143 transcripts showed an age-dependent manner. Using LC-MS-based untargeted lipidomics, we identified 291 lipids in the whole CNS. The levels of 79 lipids significantly changed during aging. Notably, polyunsaturated fatty acids increased, diacylglycerols decreased, and a phospholipid-lipophospholipid shift was observed, indicating age-related alterations in membrane fluidity and certain signal transduction pathways. Our LC-MS-based proteomics investigations also revealed proteins that significantly changed during aging (data evaluation is ongoing).

The identified cellular and molecular changes both at the system and single-cell levels during aging which may contribute to age-related memory impairment. The investigation of molecular processes underlying age-related memory decline in more detail leading to the discovery of novel mechanisms operating not just in molluscs but also in higher organisms.

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Modelling of ATP induced intracellular Ca2+ concentration changes in Deiters cells

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Hearing loss is a sensory disorder that affects many people. The cochlea, which contains the organ of Corti, has a spiral and bony structure, making the investigation of the inner cellular mechanisms challenging. Our knowledge of the hair cells is growing intensively but their supporting cells have received little attention. One of these supporting cells are the Deiters cells. Their close contact with the outer hair cells makes them potentially important in the development and regeneration of the hair cells, as well as in the formation of the auditory threshold. In order to facilitate cochlear preparation, most studies use young mice (younger than three weeks old), preceding the end of cochlear ossification. However, it should be noted that results obtained before the 14th postnatal day may not be representative of adult mouse hearing, as the onset of hearing is at two weeks of age.

Mathematical modelling is a tool to supplement laboratory work. There are currently no models established specifically for Deiters cells, so our aim was to test whether a model developed for another cell type could accurately describe the calcium handling mechanism of a Deiters cell. The chosen model was set up for astrocytes by Taheri et al. (2017). Functional calcium imaging recordings were used for the model validation (15-day-old BALB/c strain mice, n = 34 cells prepared from basal, middle and apical turns, induced with 100 µM ATP). In order to identify the parameter combinations that best characterise the calcium handling of the Deiters cells, broadly 800 000 parameter combinations were tested. The model was found to be a suitable foundation for a more specific Deiters model.

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Improving real-time epileptic seizure detection using light-weight deeplearning

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Many nervous system diseases are characterized by oscillatory disturbances at the neural network level. In the case of refractory epilepsy, a promising therapeutic approach involves the application of transcranial electrical stimulation (TES) during the early phases of seizures to disrupt their propagation. Although previous research from our group has demonstrated the potential of closed-loop TES, its success hinges on the real-time detection of abnormal neural activity. Accurate seizure detection presents an inherently difficult challenge, as neural patterns characterizing seizures are highly variable across patients, the surface EEG (electroencephalogram) data has low signal-to-noise ratio and is strongly imbalanced (seizure vs. background periods). Furthermore, implementing closed-loop stimulation requires the development of a computationally efficient model capable of running in real-time on a low-power device.

In this study, we adopt a solution based on Convolutional Neural Networks (CNNs), a state-of-the-art machine learning method for EEG-based seizure classification. The implemented CNN model is designed to extract spatio-temporal features from the EEG relevant for identifying the early stages of epileptic activity. To improve detection performance, we apply transfer learning, which helps exploit features learned from recordings of different patients. We evaluate the proposed methodology against conventional features-based methods, including the Random Forest Classifier, a standard benchmark for seizure detection, and XGBoost, another ensemble tree algorithm. The models are tested on the publicly available CHB-MIT dataset, the most widely used dataset by the field, and on our clinically recorded subgaleal EEG dataset.

The CNN model outperformed both tree-based models across nearly all clinically relevant event-based metrics for both datasets. Real-time detection capability was validated by running the model on an average laptop during online streaming of EEG data. Furthermore, the model's compact size and low computational requirements make it suitable for deployment on low-power microcontrollers, making it an ideal candidate for real-time seizure detection in a closed-loop system.

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Characterization of a new human stem cell based blood-brain barrier and brain organoid lab-on-a-chip model

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The blood-brain barrier (BBB) protects the brain and provides oxygen and nutrients for the central nervous system (CNS), but it also restricts the entry of pharmaceutical drugs into the brain. Cell culture models became essential to investigate cerebral drug delivery. Microfluidic chip devices allow complex and physiological modelling of the BBB. Induced pluripotent stem cell (iPSC) based technologies, the formation and use of human brain organoids provide simplified 3D modeling. Our aim was to (1) create and optimize a new, dynamic cell culture lab-on-a-chip model by the co-culture of a BBB model and human midbrain organoids, and to (2) examine BBB properties and functionality in the presence of organoids. Human stem cell derived endothelial cells and brain pericytes were co-cultured to establish the BBB model (Cecchelli et al., 2014). Human midbrain organoids were differentiated from healthy donor iPSCs (Nickels et al., 2020). The barrier integrity of the BBB model was investigated in the presence of midbrain organoids in a dynamic setup by the measurement of impedance and permeability for fluorescent markers. The morphology of brain endothelial cells was examined by immunostaining for tight junction proteins. Functionality of the model was tested by the passage of nanocarriers across the BBB and by characterizing the uptake into the organoids. We examined the indirect negative effect of clinically used contrast agent iopamidol on brain organoids. We found appropriate BBB integrity in the presence of midbrain organoids. Nanoparticles crossed and entered the organoids effectively. Iopamidol was proved to open the blood-brain barrier and damage the neuronal network. This complex organ-on-a-chip system can be a valuable tool for further experiments in toxicological, pharmacological and pathology testing.

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CAMKIIα-GFP MOUSE LINE PROVIDES A NEW TOOL FOR MICROSCOPIC AND ELECTROPHYSIOLOGICAL ANALYSIS OF HIPPOCAMPAL NEURONS

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CaMKIIalpha-GFP mouse line expresses GFP in a cell-specific manner under the control of CamKIIalpha promoter (Wang et al., 2013). In this work, we analyzed the expression of the endogenous CamKIIalpha gene as well as the CaMKIIalpha-GFP transgene in developing embryonic mouse brain and checked whether the cellular morphology and electrophysiological properties of neurons were affected by the long-term expression of GFP within hippocampal organotypic slice cultures and dissociated neuronal cultures. In addition, behaviour tests were carried out to confirm GFP expression does not alter the behaviour of adult rodents.

Our results show that GFP expression begins early in embryonic brain development and reaches a plateau at the third week after birth. Strong GFP expression is detected in the developing cortex and hippocampal formation, especially in the dentate gyrus and CA1 region. Detailed in vitro analyses showed that GFP expression selectively visualized pyramidal neurons. The lack of glutamic acid decarboxylase (GAD65/67) immunopositivity indicated that GFP positive cells are not GABAergic neurons. Using pre- and postsynaptic markers, we did not experience any difference in the maturation of these cultures compared to the control (CD1) ones. Analysis of the passive and active membrane properties also confirmed that expression of GFP did not affect the electrophysiological properties of the neurons

Genetic modification did not influence the behaviour of the animals. Neither spontaneous exploration, nor anxiety related behaviours were affected in the open field tests. The novel object tests suggest unaltered memory and learning in adult life.

Thus, our results indicate that the CaMKIIalpha-GFP transgenic mice could serve as an ideal tool for further electrophysiological or anatomical studies and labeling of pyramidal neurons.

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Gap junction formation is governed by redox-sensitive residues

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The network of astrocytic gap junction channels (GJC) allows rapid progression of Ca2+ signals (Szabó et al., 2017) and their propagation plays a role in synchronized neuronal activities (e.g. slow wave sleep and epilepsy (Szabó et al., 2017, Kékesi et al., 2015)). The lack of subtype-specific inhibitors for GJCs, however, prevented the targeting of GJCs in pharmacological strategies. To find GJC coupling inhibitors, we considered an alternative approach, namely understanding GJC formation in a stepwise manner, and identifying essential components of coupling. GJCs are formed by two connexion hemichannels (HCs), which are coupled by H-bonds farther from the membrane. The structure of HC subunits themselves is largely influenced by three disulphide bonds per chain, that are able to open depending on the redox environment.

Using computer modelling algorithms, we have built a homology model of Cx43 HC and positioned two membrane-embedded Cx43 HCs at 3 Å, 1 Å and 0 Å relative to the physiological distance, followed by a 100 ns molecular dynamics simulation. All simulations were analysed by identifying hubs of interactions, called stabilization centers (SCs) formed between two hemichannels. All these coupling simulations were repeated using an artificial setting, namely with disulphide bonds kept open. Our simulations revealed that the exceptionally high number of conserved Cys disulfide bonds at the extracellular interface play an essential role in HC docking. Explicitly, when Cys disulfide bonds were artificially kept “open” in the Cx43 HC-HC model, we observed the disappearance of trans-GJ stabilization centers (trans-GJ SCs), further from the Cys site. In addition, we have shown that the presence of an adjoining HC contributed to extracellular Cys disulfide formation and consequently to the emergence of trans-GJ H-bonds. In contrast to GJC structure, however, solo HC structure was consistent with open disulfide bonds (Héja et al., 2024).

Analyzing the water-accessible channel of GJCs, we found that the functionally open, permeable channel is attributed to the closed S-S state, while the functionally closed channel is in accordance with the open S-S configuration.

We showed that S-S bonds have an important effect on GJC formation. Our trans-GJ SC interface design may serve the development of subtype-specific inhibition of intercellular HC docking by targeting trans-GJ SCs.

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Modifying effects of testing conditions in metabolic stress studies

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Animal models are frequently used for researching the underlying patomechanisms of metabolic diseases such as diabetes. Similarly to human diagnostics, in rodents a glucose tolerance test can be applied via intraperitoneal glucose injection. For this 120 minute-long test the use of anaesthetics can be considered. Nowdays isoflurane anaesthesia is becoming the most popular choice of anaesthetic in preclinical settings. Based on the litarature, the effect of isoflurane on the hypothalamo-hypophyseal-adrenal (HPA) axis seems contradictory. Moreover sex, fasting and the time of testing can also influence both blood glucose and stress hormone levels. These factors have already been investigated by themselves, but their combined effect is unknown.

We investigated the effect of isoflurane anaesthesia, sex, length of fasting and the testing time on blood glucose and stress hormone levels in Sprague-Dawley rats.

Isoflurane anaesthesia had similar effects on the HPA axis hormones as the well-known stressor, restraint. Longer anaesthesia time in males resulted in a higher adrenocorticotropic hormone-levels (ACTH levels). Sex, fasting and the time of testing combined with a fast acting insulin treatment had no combined effect on blood glucose or stress-hormone levels, however all fasted and insulin-treated groups had significant ACTH elevation. Testing these factors in a glucose tolerance test revealed interesting differences such as a worsened glucose tolerance in males when tested at night (in inactive period) and in females tested during daytime (in their active period).

The extensive results of these experiments might contribute to better experimental designs in rodent metabolic stress studies.

Cell- and Layer-Specific Roles of TRPV1 Ion Channels in Infrared Neurostimulation: Insights from High-Density Laminar Recordings in the Mouse Neocortex

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Infrared neurostimulation (INS) has demonstrated the potential to modulate neural activity, with applications in the treatment of neurodegenerative diseases such as epilepsy. Temperature-sensitive ion channels, particularly TRPV1, play a pivotal role in neuronal excitability. However, their role in IR-induced modulation remains unexplored in vivo. This study aims to investigate the involvement of TRPV1 channels in INS using high-density laminar electrode recordings, thereby providing insights into the biophysical mechanisms underlying this approach.

In this study, the effects of INS on cortical neurons in vivo were investigated using high-density laminar recordings. The neocortex of anaesthetised TRPV1 knockout (KO) and wild-type mice was exposed to pulsed (500 Hz) and continuous wave (CW) infrared light (1550 nm) using an optical fibre. Over 3000 single units were recorded from 10 mice using a Neuropixels probe. Single units from the cortex were identified as putative principal neurons and inhibitory narrow-waveform and wide waveform interneurons with suppressed or increased activity, highlighting cell- and layer-specific responses. The findings revealed that continuous stimulation had a more pronounced impact than pulsed stimulation. In wild-type mice, a greater number of neurons were activated in comparison to TRPV1 KO mice. Local field potential analysis revealed a shift in power across frequency bands, with delta and theta powers increasing and alpha and beta powers decreasing during stimulation. Notably, delta and theta powers were higher in KO mice. Additionally, the temporal dynamics during stimulation trials were analyzed. These times were longer in wild-type mice in comparison to KO mice, with pyramidal cells exhibiting the longest run-up times.

The preliminary results indicate that TRPV1 channels play a pivotal role in regulating neural responses to infrared stimulation. This study offers novel insights into the mechanisms of INS by accurately characterising layer- and cell-type-specific responses. By analysing thousands of neurons, our findings on neuronal sensitivity to infrared irradiation parameters may facilitate the optimisation of future applications.

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A multistep analysis workflow for the classification of cortical LFP events.

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Cortical local fields and oscillatory events are usually analyzed by estimating their spectral characteristics, but these approaches have limited ability to extract all the information carried by the signal. Applying dimensionality reduction methods such as principal component analysis can improve classification performance, help discover hidden patterns, and create new features. We aimed to assemble a multi-step analysis workflow that can transform oscillatory LFP activity into a statistical representation. After filtering and downsampling the LFP signal, waveform segments for the events of interest were collected. The data was projected from the original space to the low-dimensional principal component space. After that, a Self-Organizing Map was trained and used to cluster the segments. Finally, a 2D probability distribution (SOM profile) was calculated for related LFP segments using cluster labels. We applied the workflow to cortical slow wave, theta and spindle oscillations recorded from juxtasomal position of pyramidal cells and interneurons in freely moving rodents. The application of the workflow on the juxtacellular LFP events data set recorded near pyramidal cells (n=65), regular spiking (n=42), and fast spiking (n=33) interneurons (n > 34000 down state events) revealed that the down states express a significant difference in their SOM profiles depending on the type of recorded cell. We conclude that juxtacellular LFP convey cell-type specific information. This suggests that field potentials in the network can be highly compartmentalized and can retain identities of cellular units in space and time, even if neuronal populations are in a silent state.

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Investigating Beta Band and Surface Laplacian For Motor Imagery Brain Computer Interfacing

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In this paper, we propose the transformation of the raw EEG data into spherical spline Surface Laplacian based multichannel 2-D images in order to be classified using deep neural models. For the challenging Pysionet motor imagery dataset, the power spectral density estimates of the Surface Laplacian filtered Mu [8-13 Hz], Beta [13-30 Hz], and low Gamma [30-45 Hz] bands were applied to generate three channel images using azimuthal projection and Clough-Tocher interpolation. These images formulate the input for the Deep Neural Network model (ResNet, CNN). The study shows significant classification improvement of the proposed signal-to-image transformation with deep neural networks framework compared to the baseline method Support Vector Machine which is widely used in the Brain Computer Interfacing field, and that adding Low Gamma band improved the motor imagery classification accuracy significantly. This work opens the door to a new area in which the advantage of the deep neural networks regarding image classification can be exploited to better understand the EEG related problems.

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Current Source Density calculation for stereo EEG data

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The EEG signal originates from the transmembrane Current Source Density (CSD) of active neuronal populations. Source localization techniques aim to determine the spatio-temporal distribution of CSD, providing precise insights into neuronal localization and activity patterns. Existing CSD methods face challenges, especially with the irregular electrode arrangements typical of stereo-EEG (sEEG) measurements. To address these limitations, we present a novel mathematical approach for calculating CSD in non-regular 3D electrode systems, achieving higher precision compared to existing methods.Traditional CSD techniques assume electrodes are arranged in regular 1D, 2D, or 3D grids, using graph Laplacian approximations based on neighboring electrodes. These methods fail with irregular electrode configurations like sEEG, where unmeasured dimensions are neglected, introducing significant errors. Model-based source localization techniques, including dipole fitting and LORETA, can handle irregular arrangements but rely on assumptions that often mismatch actual source locations. Such mismatches produce inaccuracies, particularly when electrode coverage is limited, as in sEEG, leading to disturbances from sources outside the recorded volume.Our Laplace-based method overcomes these issues by utilizing all available electrodes, not just neighboring ones, for local CSD calculation. Simulations with known ground truth CSD distributions confirmed our method's superior accuracy over traditional 1D approximations and model-based inverse methods. By reducing errors inherent in existing approaches, our method offers robust and precise CSD estimation.This enhanced precision holds significant promise for clinical applications, particularly in localizing seizure onset zones for epilepsy surgery, supporting improved surgical planning and patient outcomes.

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Longitudinal Multimodal Access to Neuronal Activity in Large Animal Brains with Functional Ultrasound Imaging

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Studying neuronal function in large-brained species is critical for translational neuroscience but is hindered by the limited availability of animal models. Longitudinal experiments offer a solution by enabling comprehensive analyses of brain function over extended periods, allowing for intra-subject normalization and reducing the variability inherent in cross-sectional studies, all while minimizing animal use. We developed a multimodal protocol combining functional ultrasound imaging (fUSI) with electrophysiology for mesoscale neuronal monitoring in large-brained animals.

fUSI provides ~150 µm spatial and 5 Hz temporal resolution, surpassing traditional fMRI, and is compatible with modern tools such as Neuropixels probes and optogenetics. We performed functional mapping over several months using visual stimulation, while evaluating anesthesia protocols to ensure stable, reproducible data during extended sessions.

Our approach enables stable multimodal recordings over several months in cats, demonstrating the compatibility of fUSI with electrocorticography. Optimal anesthesia protocols were identified to support extended mapping sessions. Data showed high reproducibility and stability, highlighting the feasibility of long-term neuronal monitoring.

This work establishes a robust framework for longitudinal multimodal access to neuronal function in large-brained animal models. By addressing the challenge of model scarcity, our protocol provides a scalable approach for translational neuroscience studies.

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Demonstration of the safe operation and long-term in vivo use of a custom-designed infrared optrode and headstage system in freely behaving rats

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Infrared neuromodulation (INM) has shown promise in numerous studies involving rodents and non-human primates. Although the first human trials have already taken place, the journey toward developing a reliable, potentially wearable therapeutic INM implant remains challenging, primarily due to the incomplete understanding of the long-term physiological effects of this method. In this study, we present the first validation of a custom infrared (IR) optrode and headstage system for use in freely behaving rodents.

The optrode can be implanted into brain tissue and enables both optically induced neuromodulation and multi-site electrophysiological recording through a single silicon shank. All aspects of the implant are tailored for use in freely moving animals. The shank is 5 mm long and has a rectangular cross-section measuring 0.19 × 0.17 mm². It features 16 square-shaped platinum recording sites (30 × 30 µm²) arranged in a linear array, with a 100 µm inter-site distance. To address the challenge of signal quality deterioration over time, we developed a custom-designed, 3D-printed micro-drive (µD). This interface allows controlled adjustments of the optrode's penetration depth, thus maintaining signal quality and enabling the modular retrieval of the optrode after experimentation. A protective headstage surrounds the µD, providing a barrier against external forces generated by animal behaviour while securely attaching the data cable and optical fibre to the optrode. The system was tested on two rats, with the optrode implanted in the somatosensory cortex. Electrophysiological signals were recorded during IR stimulation at a wavelength of 1550 nm. Multi-unit activity was reliably recorded until the eighth day, and local field potential (LFP) signals (1–200 Hz) with a signal-to-noise ratio (SNR) of approximately 10 (mean ± standard deviation: 9.66 ± 3 and 9.55 ± 1.54) were captured up to the fourteenth day. The µD's vertical displacement mechanism was employed to adjust the optrode's depth by 200 µm, which resulted in minimal alterations in amplitude, firing rate, and SNR. Furthermore, consistent neural clusters were observed post-adjustment. We were also able to identify neural units whose firing rates were modulated by the stimulation protocol.

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Investigating the Neurophysiological Effects of Low-Intensity Ultrasound: Development, Simulation and Experimentation

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Transcranial focused ultrasound (tFUS) neuromodulation (NM) is an innovative technology capable of noninvasively modulating the activity of neurons and the nervous system, even through the skull. Research holds FUS NM promising for treating neurological and psychiatric disorders. However, the technology is in an experimental stage, requiring further investigations to fully understand its mechanisms, efficacy, and safety. Our efforts focus on designing and testing a miniature tFUS NM device for exploratory and preclinical studies that can alter brain states of freely moving rodents.

First, we developed an ultrasound device and used in vitro and in silico methods to examine the physiological effects of ultrasound on individual neurons. We also began preparations of an in vivo experimental setup. Hardware and software tools were developed to evaluate the focusability of the pressure field and to study its spatial distribution. These tools helped determine the absorption of ultrasound energy in the focusing lenses. However, our methods currently allow only relative and low-resolution pressure field studies; higher precision measurements will require further advancements.

To investigate the neurophysiological effects of ultrasound, we performed current-clamp measurements on hippocampal cell cultures in synaptically isolated neurons. Voltage responses to current steps were recorded both before and after brief ultrasound exposure. Our findings suggest no immediate changes in the activity of voltage-gated ion channels, but we cannot exclude the possibility of long-term alterations in cell membrane parameters. For further interpretation of the results, we conducted in silico modeling, which confirmed that observed changes may stem from altered membrane permeability. Computational modeling also helped us explore the impact of membrane oscillations on neuronal electrical responses during acute ultrasound exposure, suggesting that such exposure may increase neuronal excitability.

As a proof-of-concept experiment to examine the brain-state-modulating effects of ultrasound, we plan to use the miniature FUS transducer to enhance learning in aged experimental rats. Real-time EEG recordings will detect sleep spindles, which we aim to modulate by increasing their duration and occurrence probability using ultrasonic stimulation. To this end, we tested various sleep spindle detectors in offline and online environments and developed dedicated hardware for our purpose.

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Microcircuits in the marmoset prefrontal cortex with a large volume electron microscopy, ATUM-Blade-TEM

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In recent decades, brain connectomics using large volume electron microscopy (vEM) has been introduced in several neuroscience laboratories. This methodology allows us to elucidate synaptic connections on a large scale, such as cortical columnar wiring. To implement vEM, we used a modified automated tape collecting ultramicrotome (ATUM) to collect large numbers of serial ultrathin sections, and a high-throughput EM imaging system (Blade, Voxa, USA). Firstly, we remodeled the original ATUM to control the timing of cutting to achieve reliable collection of serial ultrathin sections on individual slots of a grid tape. We succeeded in collecting more than 1000 serial ultrathin sections. These sections were placed securely and fairly reliably at similar locations within each grid slot. Secondly, a transmission EM (TEM) equipped with Blade was used for imaging the ultrathin sections on the grid tape. The Blade-TEM system captured high-resolution (2.3 nm/pixel) images of an area 1.1 x 1.6 mm2 in size, and a vEM dataset from the ~1000 serial ultrathin sections (50-nm thick sections) in about a month. Processing imaging data of such a huge size can be challenging. However, their connectivity at synaptic levels remains unclear. We injected combined antero- and retrograde viral tracers into the area 10 area to visualize reciprocal projections between columnar patches in the area 9 area in the marmoset PFC (Watakabe et al., Neuron. 2023; 111(14):2258-2273). To unravel the cortico-cortical circuit within area 9 including axon terminals from area 10, we used confocal laser scanning microscopy of PFC sections in which both axons and dendrites were labeled anterogradely and retrogradely, and acquired a large TEM dataset of the area 9 PFC area. Preliminary results with this correlative light and EM using vEM will be shown.

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A flexible, implantable, bioelectronic electroporation device for targeted ablation of seizure foci in the mouse brain

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The primary method of treatment for patients suffering from drug-resistant focal-onset epilepsy is resective surgery, which adversely impacts neurocognitive function. Radio frequency (RF) ablation and laser ablation are the methods with the most promise, achieving seizure-free rates similar to resection but with less negative impact on neurocognitive function. However, there remains a number of concerns and open technical questions about these two methods of thermal ablation, primarily: 1) Heating, 2) Hemorrhage and bleeding, and 3) Poor directionality. Irreversible electroporation (IRE) is a proven method of focal ablation, which circumvents all three of the primary concerns regarding focal RF and laser ablation. Here, we demonstrate the in vivo application of a flexible implant with organic electrodes for focal ablation of epilepsy foci using high-frequency IRE (H-FIRE) in mice. Our results show that local, targeted ablation is possible in the close neighborhood of the electrode, paving the way for the clinical application in the treatment of focal epilepsy.

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Targeting Large Brains with Gene Therapy Vectors: The Role of Systemic Immune Profiling in Preclinical and Clinical Contexts

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Accurate detection of AAV neutralizing antibodies (NAbs) is critical for safe and effective transgene delivery, ensuring sustained therapeutic effects in gene therapy. Current AAV NAb assays often rely on variable serum concentrations, which can lead to inflated transduction measurements. These inaccuracies may obscure patient stratification and misrepresent suitability for AAV therapy.

The Constant Serum Concentration (CSC) AAV assay addresses these issues by maintaining a fixed serum concentration, creating a stable baseline. This stability allows for more precise and reproducible measurements of NAb levels. Our results show that the CSC assay significantly reduces neutralizing misclassification compared to variable serum assays. In an analysis of 47 human samples, the CSC assay reclassified 42.6% of cases as neutralizing, which were previously considered non-neutralizing by the variable serum assay. This demonstrates the superior accuracy of the CSC assay, improving patient stratification and reducing the risk of immune-mediated therapy failure.

The CSC assay represents a crucial advancement in patient selection for gene therapy, enhancing treatment consistency and expanding the applicability of AAV-based therapies.

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Spatiotemporal backpropagation patterns of human single unit waveforms revealed by intraoperative high-density Neuropixels recordings

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High-density laminar silicon probes have been widely used in rodent electrophysiology but were virtually absent from human research until recently. Clinical research devices have so far been able to detect single-unit activity (SUA) with a poor spatial resolution (due to large inter-contact distances) or they have been limited to recording only the low-frequency component of the extracellular activity (local field potentials). We pioneered a rare opportunity to test state-of-the-art Neuropixels silicon probes in operation room settings during resective surgeries in the cases of tumors or epilepsy or the implantation of a deep brain stimulator (DBS) in patients with Parkinson's disease. These probes feature hundreds of closely packed contact sites enabling multi-channel oversampling of extracellular SUAs. Spatiotemporally resolved morpho-electric properties of single units allow for sophisticated clustering of neural cell types. We focus on a particular spatial feature of cortical neurons called the backpropagating action potential (bAP). Intracellular spikes can propagate backward along the somatodendritic axis. This phenomenon can also be detected extracellularly on spike-triggered averages (STAs) of the corresponding SUAs after spike sorting. These bAPs have been observed in rodents (using standard Neuropixels 1.0 probes) in vivo with distinct spatiotemporal patterns across brain regions, putative cell types, and morphological orientations. Using algorithms and criteria from published rodent literature, we report the first in vivo evidence for human action potential backpropagation from awake and anesthetized human patients. The appearance of human bAP did not depend on the spike amplitude, firing frequency, or spike count of the clustered SUA, but was correlated with the cell type. Putative cell types were predicted by their peak-to-trough ratio on the channel with the largest amplitude. Our preliminary results on multi-channel bAP patterns have shown consistency with the rodent findings, as we were able to identify canonical regular spiking (RS) putative principal cells expressing reliable bAPs (over 5 vertical channels, or over 100 µm), as well as fast-spiking (FS) putative interneurons which completely lack this spatial propagation. These results highlight the importance of high-density sampling in translational human in vivo recordings for disentangling cell types and morphological contributions to the local neocortical microcircuit.

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Effect of electrical microstimulation parameters on in vivo neuronal calcium responses in the visual cortex of mice

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Sensory neuroprostheses aim to restore visual or auditory experiences by precisely targeted electrical microstimulation via implanted neural interfaces. Although significant progress has been made in recent years, there still needs to be a more detailed understanding of precisely targeting and activating specific neurons using intracortical microstimulation. Applying advanced stimulation strategies such as current steering and dynamic stimulation might improve control over neuronal activity and keep the implanted electrodes at a reasonable number. In this study, we developed flexible multi-shank probes containing iridium-oxide microelectrodes to assess the effects of advanced electrical microstimulation patterns on cortical activity obtained using in vivo calcium imaging. The probes were inserted into layer 2/3 of the primary visual cortex (V1) of Thy1-GCaMP6 transgenic mice, including both anaesthetised and awake, head-fixed animals. Electrical stimulation patterns were generated by a custom high-channel-count neural stimulator. To image calcium activity, we used a two-photon laser scanning microscope (laser wavelength: 900 nm; raster scanning at 31 Hz) with a 20x water immersion objective (field of view: 550 µm × 550 µm). We tested a diverse set of stimulation parameters (e.g., current amplitude, pulse duration, stimulation frequency) and patterns (e.g., dynamic current steering), then evaluated the observed spatial and temporal activation patterns of neurons. The obtained calcium imaging datasets were processed using Suite2p and further analysed with custom Python scripts. Here, we report the preliminary findings of the spatiotemporal patterns induced during these in vivo experiments. Our future plans involve further exploring the influence of advanced stimulation patterns on the neuronal activity of V1 and higher-order visual cortex and identifying stimulation strategies that may improve the resolution of state-of-the-art visual cortical prostheses.

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Physiological assessment of the psychological flow state using wearable devices

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Flow is the state of optimal experience which can lead to outstanding performance. Our study demonstrates the feasibility of detecting and monitoring flow using wearable devices.

28 adult Hungarian people took part in the experiment. They performed a computer game on three different levels to induce flow and antiflow states, which we tested by questionnaires. We measured electroencephalography (EEG), heart rate (HR), blood oxygen saturation (SpO2) and galvanic skin response (GSR) signals and head and hand motion. We isolated EEG delta, theta, alpha and beta band power, HR, SpO2 and GSR average and standard deviation, and acceleration and angular velocity standard deviation.

In flow condition, alpha and theta power were the dominant components, in accordance with the transient hypofrontality hypothesis. We also replicated the U-shaped characteristic of the heart rate variability; in addition to this, we propose an inverse U-shaped and a U-shaped characteristic for SpO2 and SpO2 variability, respectively. Based on motion tracking, subjects were the least physically active in flow, signifying a focused state, and the most active in boredom.

Our results support the applicability of lightweight, wearable devices for mental state monitoring that can be utilized for the improvement of well-being at the workplace or in everyday situations.

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A novel technique for classification of neurons

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Neuronal morphology significantly impacts function. Yet, classification methods of neurons usually use either morphological features or functional properties (e.g. action potential firing properties) of nerve cells and do not consider these sets of features at the same time.

Here we report a novel technique for classification of neurons that is based on both morphology and signalling properties of dendrites. Our method uses 10X10 morphofunctional matrices (MFM) to quantify morphofunctional properties of neurons. Elements of the MFM tell the proportions of dendritic surface area with a given path distance from the soma and with a given transfer or delay value of PSPs starting from the dendritic surface considered. MFMs of neurons were subjected to cluster analysis and cluster formations of MFMs were considered as a classification of the respective neurons. We calculated different properties of somatopetally propagating PSPs (steady-state and sinusoidal voltage transfer, current transfer, and delays) in morphologically faithful 3D computer models of neurons. These data served the basis to compute an MFM for each neuron and for each signalling property. Finally, MFMs of a neuron were summed up to create the composite MFM that includes every information on morphology and on multiple signalling properties of the given neuron.

To test this technique, we checked if the MFM method can group neurons as expected intuitively in the trivial case, when pyramidal neurons, Purkinje cells and spinal motoneurons are classified (n=15). Cluster analysis of composite MFMs of these neurons resulted in a 100% correct classification of MFMs into three clusters. Then we considered two non-trivial classifications, where neurons have been previously classified by using different methods that did not involve the use of MFMs. We classified the very same neurons by the MFMs and compared our classification with that obtained earlier by another method. 1) We classified layer II/III pyramidal neurons of age-matched Tg2576 and control mice (n=58) and 2) layer III pyramidal neurons of rTg4510 and control mice (n=51). In both cases classification of mutant and wild-type neurons by the MFM method led to the same results and to the same conclusions as by the more conventional classification techniques.

In conclusion, our novel classification method of MFMs works well and may allow a deeper understanding of the interrelationship between neuronal structure and function in health and disease.

Genetically targeted, long-term stable and low-immunogenic modulation of brain function: promise or reality?

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Whether for circuit dissection or disease modeling: genetic targeting of brain function via transgenic mouse lines has become ubiquitous in experimental neuroscience. In stark contrast, for non-mouse models where a similar diversity of transgenic lines is not available, stable and safe genetically-targeted modulation of brain function remains difficult. Gene therapy vectors, primarily AAVs, can target cell populations but their long-term efficacy and safety can be hampered by humoral and local immune response as well as by variability of transgene expression. Therefore, genetically-targeted functional access to the brain requires the labeling of a sufficient number of cells at stable transgene expression levels with contained immune response.

Our primary objective has been to identify a delivery route and gene therapy construct that yields optimal transgene expression for long-term optical imaging while also remaining safe in terms of local and systemic immune responses. We performed long-term optical imaging on 9 animal cohorts across at least 13 weeks each, evaluated the stability of optical activity readout, labelling efficacy, local and systemic immune responses. We found strong nonlinear relationships between dose,expression levels and immune response.

Our work identifies an optimized gene therapy delivery method that enables stable, long-term all-optical imaging in the brain while minimizing immune response.

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INFERRING HIGHER-ORDER HIDDEN DRIVERS FROM fMRI DATA

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Traditional approaches to analyzing interactions between brain areas have primarily focused on pairwise interactions between measured activities. These include functional connectivity, measured via correlation, and causality analysis, which aims to infer directional couplings between regions of interest. Only a few recent studies have attempted to infer the emergence of higher-order interactions between brain areas, but the underlying mechanisms of such emergence remain unclear.

Here, we introduce a novel method to infer higher-order hidden driver dynamics based on fMRI time series data. These higher-order hidden common drivers – drivers that influence more than two brain areas simultaneously – may underlie the observed higher-order associations. Our method was applied to fMRI time series data to investigate the existence and complexity of hidden common drivers among eight brain regions associated with visuo-motor responding and working memory tasks.

As the first step in the analysis, the pairwise application of the Dimensional Causality (DC) method assigned high probabilities to the existence of hidden common drivers influencing many pairs of the investigated brain areas. Notably, the patterns of these revealed hidden common drivers were characteristic of the specific experimental tasks.

In the second step, our novel method was applied to infer the presence of higher-order hidden common drivers, complementing the pairwise analysis.

During the visuo-motor task, only pairwise hidden common drivers were found to be significant. In contrast, during the working-memory task, higher-order hidden common drivers were inferred, involving triplets and even a multiplets of the relevant brain areas.

Our initial results demonstrate the potential of this method to uncover rich functional structures and hidden dynamics in complex systems like the brain, even when working with relatively imperfect signals such as fMRI.

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Changes in low-frequency cortical activity in response to thermal neuromodulation induced by an intracortical infrared light source

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Infrared neuromodulation (INM) represents an effective and safe technique for modulating local cerebral temperature, which consequently influences neuronal activity within the brain tissue. We conducted in vivo measurements with an optical probe enabling simultaneous stimulation and electrophysiological signal recording with minimal noise in anesthetized rats. Following a 2 minute long baseline period, 2 or 4 minutes of stimulation periods (ON) were initiated, followed by 4 minute long periods without stimulation (OFF). The ON and OFF periods were repeated five times during one measurement. The experimental device used for INM was an optrode with a sharp tip, which incorporates an embedded waveguide that transmits infrared light into the cortical tissue. Wideband recordings were performed with 12 linearly placed recording sites with a 20 kHz sampling frequency across cortical layers in acute in vivo measurements performed in 8 rats. The experiments were grouped based on the depth of the probe insertion, with four cases in which the tip was located in cortical layer 6 (L6) and four cases in which the tip was in layer 5 (L5).

During signal processing and analysis, we found that ketamine/xylazine-induced slow waves were significantly affected by the continuous-wave infrared stimulation, therefore, we examined the changes in the frequency range of delta waves (0.5-4 Hz). The low delta (0.5-2 Hz) and high delta (2-4 Hz) frequency ranges were considered separately to ensure a detailed analysis. In this study, we present the stimulation-related changes in the selected frequency bands, considering of two channel groups, located in cortical layers 2/3 (L2/3) and L5. Our results indicate that during optical modulation, both low and high delta power increases in the supragranular (L2/3) layers and low delta power increases in the infragranular (L5) layers when the stimulation occurs in L6. Infrared stimulation in L5 resulted in a reduction in low delta power in both examined layers, with a slightly more pronounced decrease observed in L2/3. In addition to the spectral analyses, a multiunit activity-based up/down state detection method was performed on these data. We found that the duration of down-states of the cortical slow waves increased during stimulation, while up-states became shorter. The aim of our future studies is to investigate the temperature dependency of the spectral and temporal properties of slow waves in freely moving, naturally sleeping animals.

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Cleaning of autofluorescence in large preclinical mammalian brain samples with quantitative efficacy analysis

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In studies of the nervous system, particularly using optogenetics, labelling cells with fluorescent protein variants provides a convenient biological readout. While fluorescent signals are easily detected in mouse brains, autofluorescence in large animal and human brain tissue complicates signal detection. This study aimed to develop and assess reliable autofluorescence reduction (AFD) methods, including techniques reported in the literature, for application in cat and primate brains as well as human organotypic cultures.

We combined several AFD methods with two- and three-layer fluorescent immunostainings to enhance fluorescent-specific signals relative to autofluorescence. Efficacy was quantified through cell counting, pixel intensity, and object detection analyses. Distinct sources of autofluorescence, such as lipofuscin, hemosiderin puncta, and red blood cells were identified, alongside autofluorescent neurons with bright cell bodies and dendrites. While fluorescent staining significantly improved signal detection, particularly in cell bodies and dendrites, AFD methods often reduced both autofluorescence and fluorescent signals, notably in axons. Quantitative analysis indicated that combining fluorescent immunostaining with copper-sulphate and TrueBlack treatments was the most effective approach for detecting fluorescent-positive cells.

These findings highlight the challenges posed by widespread autofluorescent signals in large animal and human brain tissue. By combining signal enhancement with autofluorescence reduction, fluorescent-positive cells and dendrites can be reliably visualized, facilitating optogenetic studies in translational research.

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In vivo assessment of gamma-aminobutyric acid and glutamine/glutamate concentrations in the brain using modern MR spectroscopy

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γ-Aminobutyric acid (GABA) is the main inhibitory neurotransmitter of the human brain, and GABA-ergic dysfunction has been implicated in a variety of neuropsychiatric disorders. The most widely used sequence for measuring GABA concentrations with magnetic resonance spectroscopy (MRS) in the central nervous system is the so-called MEGA-PRESS (Mescher-Garwood Point Resolved Spectroscopy). However, this method is still technically challenging compared to conventional MR spectroscopy measurements. Our aim was to test this MEGA-PRESS sequence in a control sample and to make recommendations for its future application.

We carried out in total 60 MEGA-PRESS measurements involving 10 healthy subjects. We also performed repeated measurements after 1 week to evaluate test-retest reliability of the method by measuring GABA+ and Glx levels through the brain. The regions of interests were the anterior cingulate cortex, occipital cortex and precuneus cortex. Results were evaluated using Gannet 3.1.5. software. Statistical analyses were made by SPSS (version 29.0.2.0, IBM Inc. Armonk, NY)

Taking all three voxels into account, no significant difference was found between the two measurement time points for either GABA+/Cr or GABA+/water. The highest and considered good positive correlation was seen in the OCC area, both for GABA+/Cr concentration ($\rho = 0.60$) and GABA+/water concentration ($\rho = 0.612$). For GABA+/water, CV values were above 10%. For Glx/water, each voxel showed a significant correlation at both time points. For Glx/Cr concentrations, we found very good test-retest reproducibility in all three regions, better than for GABA+/Cr (CVACC = 8.30%; CVOCC = 9.40% and CVPrC = 6.29%) The fit error rates were 2-3% higher for Glx compared to GABA.

Our data shows that the measurements are fully reproducible in case both GABA+ and Glx neurotransmitter however, numerous factors influence the efficacy of the measurement, including the specific cortical area, internal reference movement and individual neuroanatomical variations.

Keywords: GABA, anterior cingulate cortex, occipital cortex, precuneus cortex, MRS, reliability, reproducibility, Glx, MEGA-PRESS

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Senolysis potentiates endothelial progenitor cell adhesion to and integration into the brain vasculature

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One of the most severe consequences of ageing is cognitive decline, which is associated with the dysfunction of brain microvasculature. Thus, repairing brain vasculature could result in healthier brain function. To better understand vascular repair, we studied the adhesion and integration of endothelial progenitor cells (EPCs) using an early embryonic mouse aorta–gonad–mesonephros endothelial cell (MAgEC 10.5) line in mouse models. We found that the MAgEC 10.5 cells rapidly adhered to brain microvasculature and some differentiated into endothelial cells. MAgEC 10.5 derived endothelial cells integrated into microvessels establishing tight junctions and co-forming vessel lumens with pre-existing endothelial cells within five days. Adhesion and integration was much less pronounced in aged mice, but could be increased by depleting senescent cells via senolysis using abt-263 or a combination of dasatinib and quercetin. Furthermore, we demonstrated that MAgEC 10.5 interaction with brain vessels can be upregulated by ischaemic conditions and the inflammatory mediator TNFα. Thus, even though progenitor cell therapy using MAgEC 10.5 was rendered less effective by aging, it could be improved by modulating EPC-vasculature interactions through senolytics or by activating EPCs before treatment.

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Functional characterization of human iPSC neurons of Kleefstra syndrome origin

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Kleefstra syndrome (KS) is a neurodevelopmental disorder associated with autism spectrum disorder (ASD), intellectual disability and hypotonia. The syndrome is caused by specific mutations in the EHMT1 gene, which plays a crucial role in the formation of heterochromatin, perturbing gene expression. Synaptic plasticity, synaptic scaling, learning and memory formation is severely affected in KS, however, the impact of the functional loss of EHMT1 on the development of neuronal networks in humans remains unclear.

In this study, we modelled neuronal maturation and network formation of human iPSC derived neurons from neurotypical (NT) and young Kleefstra syndrome (KS) patients under in vitro conditions. To study synaptic connections between NT- and KS-derived cultures, voltage clamp measurements were performed weekly for 9 weeks. KS-derived neurons showed spontaneous excitatory postsynaptic currents (sEPSC) from week 1 of development, whereas in NT-derived cultures these appeared only from week 3. AMPA events dominated during maturation, but the proportion of GABA events increased in a time dependent manner in both cultures.

Furthermore, the developmental and maturational dynamics of the networks formed in the cultures were investigated using the Ca-imaging technique during the first four weeks of development. KS cultures demonstrated elevated network activity from the outset, and this level of activity persisted throughout the four-week period. In contrast, the NT cultures showed an increase in activity, reaching a comparable level to the KS cultures by the fourth week of development. To gain further insight into the emerging networks, multielectrode array (MEA) measurements were conducted during the initial six weeks of neural maturation. In the KS arrays, we observed a significant rise in network activity from the first week of induction, as evidenced by an elevated number of firing electrodes and the emergence of burst oscillations. It is noteworthy that the activity in the KS cultures exhibited a decline towards the conclusion of the observation period.

Our findings indicate that Kleefstra syndrome exerts an influence on the network formation properties. In KS cultures, we detected accelerated network activity, which may contribute to the abnormal neuronal network formation observed in ASD.

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Dynamics of neuropeptide expression in the developing mouse Edinger-Westphal nucleus

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The role of the peptidergic centrally projecting Edinger-Westphal nucleus (EWcp) has been investigated in depression models by our research group. Early life adversity such as maternal deprivation causes long-lasting changes in the stress responsivity of EWcp in both mice and rats. Much less is known about the prenatal life stress adaptation capacity of these cells. Only few embryological studies describe the development of the EWcp in close relationship with midbrain dopaminergic cells. EWcp cells express cocaine- and amphetamine-regulated transcript peptide (CART) and urocortin 1 (UCN1). In this study we aimed to determine at what developmental stage do the EWcp cells start to contain Cart and Ucn1 mRNA and/or CART and UCN1 peptides, possibly co-expressed with mesodiencephalic basal plate homeobox genes Shh, Nkx6.1 and Nkx6.2. According to databases, we anticipated that Cart/CART will be produced in the prenatally, while Ucn1/UCN1 appears later in the postnatal period.

Timely pregnant C57Bl6/J mice and their litters were used. Six age groups were defined and embryos on the embryonic (E) 14th and E16th days were collected. The postnatal period (P) was examined in 1, 7, 14 and 21 days old mice. Heads or the isolated brains were dissected, immersion fixed and postfixed. Upon paraffin embedding and sectioning, Cart, Ucn1, Shh, Nkx6.1, and Nkx6.2 mRNAs as well as CART and UCN1 peptide content was assessed by RNAscope in situ hybridization and immunofluorescence.

Cart mRNA-expressing as well as CART immunoreactive neurons were identified in all age groups in a well-defined dense population of nerve cells. At E14 and E16, Cart cells were localized to the mesodiencephalic basal plate and expressed both Nkx6.1 and Nkx6.2, unlike Shh. After birth, they were found in the ventral periaqueductal grey next to the midline in line with location of EWcp cells in the adult brain. In contrast, neither Ucn1 mRNA, nor UCN1 peptide content was observed in the embryonic phases and at P1 and P7. The Ucn1 mRNA, and UCN1 peptide was earliest detectable on P14. From this age on, the EWcp peptidergic cells expressed both neuropeptides in full co-localization.

In utero developing peptidergic EWcp neurons can be identified by their Nkx6.1, Nkx6.2 and Cart mRNA and/or peptide content, while the use of Ucn1/UCN1 for this purpose is not optimal. Further research will determine the neurochemical characteristics of the developing EWcp cells.

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Semaphorin receptor neuropilins are critical for metabolic balance in early postmitotic neuron transcription and translation processes in the chick spinal cord

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The differentiation of post-mitotic neurons from neuroprogenitor cells is a critical process that occurs shortly after neurulation. Once they exit the cell cycle, spinal cord neurons migrate from the ventricular zone to form a highly organized layered structure. Semaphorins are pivotal in neuronal differentiation, particularly in axon pathfinding. Although semaphorin receptors are present in the ventricular zone of the developing spinal cord, their role in the neuronal cell cycle remains unclear. Our recent study explores the potential involvement of secreted semaphorins and their receptors in this process. We employed a dominant-negative approach to disrupt the signaling pathway of secreted semaphorins by targeting their main receptors, neuropilin 1 and 2. Plasmids encoding dominant-negative neuropilin receptors and GFP were introduced into the spinal cords of chicken embryos via in-ovo electroporation. The samples were analyzed using histology, RNA sequencing (RNAseq), and protein-level assessment through simple western assay (WES). Our findings revealed that cells expressing dominant-negative neuropilin 1 or 2 remained in the ventricular zone, unlike control GFP-expressing spinal cords. BrdU labeling and immunohistochemistry against cell cycle markers indicated that most labeled cells were post-mitotic in the case of neuropilin 2 but failed to develop migratory processes in the presumptive dorsal horn and underwent apoptosis. RNAseq analysis showed that metabolic processes, such as oxidative phosphorylation and protein synthesis, were impaired in both downregulated neuropilin 1 and 2 cases. These results provide valuable insights into the role of secreted semaphorins and their receptors in neuronal differentiation.

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Cortical and subcortical neuromodulatory dynamics in variable learning environments

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Dopamine (DA), acetylcholine (ACh), noradrenaline (NA) and serotonin (5-HT) play key roles in learning, attention, rapid adaptation, as well as influencing decision-making and regulating behaviour. Their abnormalities have been observed in major neurodegenerative diseases such as Alzheimer's and Parkinson's, in which cognitive abilities can be severely impaired. Although much is known about these neuromodulators, their exact dynamics during decision-making are not yet known.

Our questions were what is the impact of a continuous adaptive environment on learning? What roles do neuromodulatory systems play in these adaptive mechanisms?

In our experiments, water-restricted, freely moving young female (n=7) and male (n=22) mice are expected to choose between left or right water-delivery ports after light stimulation, which encode water reward with different probabilities. The predictability and variability of the environment were controlled by the difference between the two probabilities and the length of the blocks. We interpret as volatile environments the close probabilities and short blocks, and as predictable environments the distant probabilities and long blocks. Our fluorescence signal-based measurements were performed using DA, ACh, NA, 5-HT biosensors from prefrontal cortex and deep brain nuclei (basolateral amygdala - BLA, ventral striatum - VS).

In volatile environments, we observed 7.4% better performance and 14% faster adaptation compared to a predictable environment. Our results show that DA encodes reward processing and prediction errors. ACh release in BLA scaled similarly, with more pronounced release during decision making. In relation to NA, we also observed elevated levels in the BLA during the stimulus, when the environment was most unpredictable. Like DA, subcortical release of 5-HT plays an important role in reinforcement feedback at a slower time scale. In cortical areas, Ach, NA and 5-HT show a similar oscillatory pattern.

Neuromodulators separately modulate brain functions of reward and behaviour: subcortical dynamics are scaled by the unexpectedness of reinforcement or the unpredictability of the changing environments, while more complex cortical processes are accompanied by different oscillatory release patterns.

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Anatomically heterogeneous pyramidal cells in supragranular layers of the dorsal cortex show the surface-to-deep firing frequency increase during natural sleep

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To explore how supragranular cortical pyramidal cells (Pyrs) are recruited during natural sleep, we applied a method of juxtacellular recording/labelling in freely moving rats. We asked how different Pyrs activate during sleep events such as down-to-up-state transition (DUt), spindle oscillations (SO), and REM sleep-related theta oscillation (ThO). Based on the polarity of the Delta waves (DWs) recorded along with the cell firing, we divided Pyrs as superficial (negative DWs, layer2 (L2), n=35) or deep (positive DWs, layer 3 (L3), n=35). Most of the Pyrs showed very sporadic firing behavior, with a lower average frequency for L2 than for L3 (0.32+/-0.32Hz vs. 0.57+/-0.54Hz). In addition, L2 showed a lower probability of spikes and lower firing frequency within the measured events (DUt:4+/-3.9% vs. 75+/-6.9%; 0.39+/-0.93Hz vs. 0.67+/-0.64Hz; SO: 14.9+/-11.8% vs. 25.9+/-18.2%; 0.53+/-0.55Hz vs. 0.83+/-0.71Hz; and ThO: 0.22+/-0.17Hz vs. 0.82+/-1.25Hz). The number of bursting events and the number of spikes within the bursts was lower than for L3. Interestingly, the L2 didn't show firing preference within the spindle or theta cycle, while 40% of the L3 showed strong phase-related activity, preferring the descending phase or the trough of the spindle cycle. The average firing frequency during slow wave sleep was correlated with the cortical depth of the cells (R=0.424; p=0.007). We conclude that during natural sleep the Pyrs in supragranular layers of the dorsal cortex show a surface-to-deep firing frequency increase. Application of dimensionality reduction methods for the identification of subclasses of Pyrs with different anatomical and electrophysiological characteristics is underway.

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How Global and Local Image Features are Encoded in the Architecture of Visual Cortex

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The primary visual cortex in mammals with high visual acuity exhibits a patterned spatial organization. Neuronal circuits encode multiple stimulus dimensions, such as spatial position, eye dominance, light-dark polarity, orientation, and spatial resolution. Among these, orientation preference has been extensively studied, with neurons tuned to similar orientations forming iso-orientation domains that create smoothly varying patterns across the cortical surface. Despite significant advances in imaging techniques, the three-dimensional organization of these functional maps, as well as the fine interconnections between different feature maps, remain poorly understood. In this study, we employed functional ultrasound (fUS) imaging to explore the 3D organization of the primary visual cortex, focusing on the interplay between retinotopic and orientation maps. High-resolution fUS enabled the identification of functional maps at multiple scales: from the centimeter scale across multiple cytoarchitectonic areas, to the millimeter scale at the pinwheel level, and down to sub-millimeter resolution within iso-orientation domain structures.

Results Our results reveal that local iso-orientation domains are shaped by their embedding within the global orientation map, where systematic biases drive gradual, coherent changes across the cortical volume. This interdependence between retinotopic and orientation representations underscores how large-scale organization constrains local structure, optimizing the balance between global continuity and fine-scale feature processing in the visual cortex.

Conclusion These findings provide insights into the organizational principles of the primary visual cortex, emphasizing the role of global orientation biases in shaping local cortical representations. By leveraging fUS imaging, this study unifies large-scale cortical mapping with fine-scale domain analysis, providing a framework to explore the interplay between cortical maps in encoding the complete visual field. Integrating these scales is essential for understanding the mechanisms underlying sensory processing in the visual cortex.

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The role of microglia in modulating neurovascular processes and functional connectivity

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Microglia are the main immunocompetent cells of the brain with emerging roles that extend beyond immune-related functions. Despite their significant role in shaping neuronal function in both health and disease, their contribution to neurovascular processes has remained largely unclear. We have recently shown that microglia are key modulators of cerebral blood flow (CBF) and neurovascular coupling via purinergic mechanisms. The aims of this study were to investigate the mechanisms of microglia-vascular interactions and the role of microglia in modulation of functional connectivity during resting-state activity and sensory stimulation.

CBF changes were measured by Functional Ultrasound and Laser Speckle Contrast Imaging through the intact skull bone, in real time. Functional connectivity patterns were investigated during resting state and whisker- or visual stimulation using Functional Ultrasound. The role of microglial actions were investigated via elimination of microglia by PLX5622 or by using P2Y12R KO mice.

We found that microglia modulate neurovascular coupling response during visual stimulation in the visual cortex. Furthermore, our results show that there is no significant differences in resting-state functional connectivity in the absence of microglia or microglial P2Y12R. However, we observed that thalamocortical functional connectivity during somatosensory stimulation is impaired both in microglia-depleted and P2Y12R KO mice. These findings suggest that microglia should be considered as an important modulatory cell type involved in physiological and pathological alterations of CBF. Our observations also emphasize the importance of microglia-mediated actions in maintaining normal functional connectivity within the thalamocortical network during somatosensory stimulation. Understanding microglial actions may facilitate the discovery of novel treatment opportunities in common neurological disorders.

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Integrated Electrophysiology and Fiber Photometry Examination of the Prefrontal Cortex in the Mouse Model of Implicit Learning

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Neuromodulators have a key role in mediating cognitive processes such as attention, learning and memory. Disorders of the neuromodulatory systems underlie certain degenerative neurological conditions like Alzheimer's and Parkinson's diseases. While the role of neuromodulators in explicit learning is increasingly in the focus of the research, their participation in implicit learning and memory processes is poorly understood.

To examine this, we have used a previously developed automated training setup in which animals must respond to sequential light flashes at different locations. Following the automatic behavioral pretraining period we implanted a custom-built microdrive with eight tetrode electrodes in the prefrontal cortex which is centrally involved in learning. In addition to electrophysiological recordings, genetically encoded dopamine sensor was injected into the contralateral hemisphere to monitor the release of dopamine through fiber photometry. When the animals were able to follow the sequence stably, we introduced blocks of trials in which the stimuli followed each other in a random order. During the sequential learning task, behavioral, electrophysiological and photometric data was collected synchronously.

By comparing the animals' behaviour between sequential and randomized blocks, we found that the reaction time was shorter while the accuracy was higher in the sequential blocks, indicating successful implicit learning. Recorded neurons could be categorized into distinct groups based on characteristic firing patterns, all of which were represented in both the cingulate and prelimbic corticies. Furthermore, both dopamine levels and a subset of single-unit activities precisely reflected the animal's current stage in the sequence—that is, how many steps remained to receive a reward. Interestingly, the activity of neuronal clusters were correlated differently with the dopamine signal during the execution of the task. Additionally, 'poke-in' events enhance alpha activity – which is associated with cognitive performance - and induce phase resetting.

The combination of electrophysiology and fiber photometry measurements can help to understand the neural basis of implicit learning processes and neurodegenerative diseases.

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Investigating connectivity of enkephalinergic interneurons in the rodent spinal dorsal horn

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It has long been known that the functioning of neuronal networks responsible for sensory signal processing in the spinal cord is influenced by descending pathways originating from various regions of the brainstem. These descending modulatory pathways primarily target local interneurons located in the dorsal horn of the spinal cord, which regulate normal sensory signal transmission through pre- and postsynaptic mechanisms. Among these interneurons are endogenious opioid-containing enkephalinergic neurons.

Our research group previously demonstrated that selective activation of enkephalinergic neurons enhances feed-forward inhibition originating from primary afferents and that inhibitory and excitatory enkephalinergic interneurons can establish monosynaptic connections with one another.

In our work, we aimed to investigate the presynaptic and postsynaptic effects elicited by selective optogenetic activation of enkephalinergic neurons on neurons in the superficial dorsal horn, a region crucial for nociceptive signal processing.

Our experiments were conducted on 1–3-month-old PENK::ChR2 hybrid mice, in which enkephalin-containing neurons express channelrhodopsin-2, a light-sensitive non-selective cation channel. With whole-cell patch-clamp recordings, we analyzed neurons in the superficial dorsal horn. Primary afferent inputs to these neurons were activated electrically, while enkephalinergic neurons were selectively stimulated using 470 nm wavelength light pulses.

Based on our results, the majority of randomly recorded dorsal horn neurons received direct postsynaptic enkephalinergic inputs, targeting either the soma or dendrites. These inputs were predominantly excitatory and exhibited signs of various forms of plasticity. The amplitude of primary afferent inputs to the neurons was differently modulated by selective activation of enkephalinergic neurons. Interestingly, the previously observed feed-forward inhibition was not detected in any case, suggesting a cell-type-specific nature of this phenomenon.

Our findings highlight the significant role of enkephalinergic neurons in the ecosystem of the superficial dorsal horn.

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From simple tasks to complex challenges: exploring implicit learning in mice through advanced sequential protocols

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The brain employs various learning strategies to adapt to a changing environment, enhancing individual fitness and survival. Implicit sequential learning involves subconsciously acquiring an order, with skills like cycling, playing basketball, or speaking partially resulting from this process. Our research investigates the neural mechanisms behind sequential learning, focusing on dopamine (DA).

Our goal is to develop a model comparable to human studies for cross-species analysis, aiming to understand how DA encodes learning, with potential implications for treating neurodegenerative diseases.

To address this, we trained mice in two different sequential learning protocols, where they learned a four-part sequence, while we measured DA release by fiber photometry. Each trial involves four ports equipped with LEDs, water tubes, and sensors, with water as the reward. In the alternating protocol, the sequence is interspersed with random elements. Animals must correctly identify the illuminated port by poking their nose, they are rewarded after every fourth correct response. Additionally, there are random blocks, when the ports light up in a fully randomized order. In the uncued protocol, all four LEDs are illuminated, and animals must determine the correct sequence independently. Here every correct response is rewarded. Once the sequence is learned, cues are removed, and animals must complete the sequence autonomously.

In the alternating protocol, mice displayed consistent accuracy across both sequential and random blocks. Reaction times were notably longer during the random blocks compared to the sequential blocks. A negative correlation was identified between accuracy and reaction time, as mice became more accurate, their reaction times tended to decrease during sequential blocks. The uncued protocol results show that animals successfully learned the sequence. Photometric data revealed distinct patterns of DA release in the ventral striatum (VS) both with and without visual cues.

In the alternating protocol, mice performed with similar accuracy during both sequential and random blocks, though their reaction times were longer in the random blocks. This pattern closely mirrors findings from the human version of the task. In the uncued sequence protocol animals could learn and execute a specific sequence in the absence of external cues, relying on internal representations with associated changes in DA activity reflecting task learning and performance.

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Dendritic synaptome of calcium-binding protein containing GABAergic neurons in the cortex

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Complete morphometric characterization of synaptic inputs of GABAergic interneurons (INs) is a key component in exploring their regulatory roles to make predictions in cortical function. For resolving the different structural components of synapses established on the surface of neurochemically distinct IN subtypes, high-resolution serial-section transmission electron microscopy (ssTEM) is an adequate tool.

Here we provide a synaptic input database, dendritic synaptome, for dendrites of calcium-binding protein containing INs suh as parvalbumin (PV), calretinin (CR), calbindin-D28K (CB). The modified correlated light- and electron microscopy was employed (mirror-technique, Talapka et al., 2021). Nine dendrites and all presynaptic boutons (n=XXXX) terminating on the dendritic surface were traced and reconstructed in three-dimensions (3D). The following basic parameters of the synapses were determined: relative ratio of symmetric (ss) and asymmetric (as) synapses; number of synapses per unit length of dendrite; surface area and volume of presynaptic boutons; area of the active zones; frequency distribution of synapse types along the dendrites. Significant differences in the morphometric parameters of excitatory, but not in inhibitory inputs were detected between the three IN subtypes. Surface extent and the number of synaptic inputs on PV+ dendrites were multifold compared to the other two subtypes. Interestingly, no difference in the basic parameters of presynaptic excitatory and inhibitory boutons were found ibetween IN subtypes.No obvious clustering of the presynaptic boutons could be observed in either case. The database allowed for estimating the total number of exitatory and inhibitory synapses on dendrites of individual CBP subtypes: PV: XXXX (as: XX%, ss: XX%), CR: XXXX (as: XX%, ss: XX%), CB: XXXX (as: XX%, ss: XX%).

Our findings provide essential structural information to establish a realistic computational models for studying the function of neuronal ensembles in the mouse primary visual cortex.

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Medio-lateral H-current gradient in the medial entorhinal cortex

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The primary gateway between the hippocampus and cortical regions is the entorhinal cortex (EC), however this area not only convey information via the perforant path, but also processes them through functionally significant cells such as grid cells, border cells, and head-direction cells.

The perforant path originates in the lateral and medial EC (MEC) and terminates in different parts of the dentate gyrus. The dentate projecting stellate cells in the second layer of the medial entorhinal cortex exhibit a distinct sag potential and subthreshold oscillations which is caused by H-currents. Previous studies have shown that, H-current in the dorsal part of the MEC is greater than the ventral part and this phenomenon could underlie the different spacing of grid-cell activity.

In our study, using in vitro patch-clamp technique, we found that the H-current change along not only dorso-ventrally, but also medio-laterally as well within the MEC. We hypothesize that this will also result in grid cell spacing differences in a medio-lateral fashion.

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Age-Dependent Changes in the Regulation of Extrasynaptic Glutamate Concentration in Human and Mouse Neocortex

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Glutamate, the most common neurotransmitter in the central nervous system, is not limited to the synaptic cleft but is also present as "ambient" glutamate in extrasynaptic regions. It is primarily taken up by neurons and astrocytes via glutamate transporters (EAATs), with astrocytes metabolizing it into glutamine. In rodents, EAAT1 (GLAST) and EAAT2 (GLT-1) are primarily astrocytic transporters.

Preliminary experiments in our lab showed that inhibition of EAATs by DL-TBOA or the EAAT2-specific WAY213613 induced phasic and tonic excitatory currents in human neocortical pyramidal cells. These tonic NMDA receptor-mediated currents, partially inhibited by GLUN2B-specific ifenprodil and fully blocked by D-AP5, disappear or reverse after age 65. My project investigates the age-related immunohistochemical changes in EAAT transporters underlying this phenomenon.

I prepared formalin-fixed, paraffin-embedded mouse brain sections and optimized antibodies specific to GLAST+GFAP and GLT1+GFAP in IHC experiments. The reactions, visualized with DAB and VIP chromogens, successfully highlighted astrocyte processes (brown) and glutamate transporters (purple). Immunofluorescence with Alexa Fluor 647 further confirmed these results, with images captured using an IF microscope.

Future work will focus on identifying age-dependent changes in EAAT transporters in both human and mouse neocortex, as well as their potential roles in brain pathologies.

GABA-ergic modulation of cortical excitability in awake non-human primates

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Disruption in the excitatory-inhibitory (E/I) balance of cortical networks is well-characterised in age-related neurocognitive disorders (NCDs). Utilising a well-established non-invasive transcranial magnetic stimulation (TMS) protocol, that is similarly applicable in preclinical translational research and human studies, we recorded baseline cortical excitability in awake adult rhesus macaques and assessed basic GABAergic modulation of E/I balance.

Using neuronavigation-guided single pulse TMS targeting the cortical M1 region, we recorded motor-evoked potentials (MEPs) from the right abductor pollicis brevis muscle with surface electromyography. After establishing a reliable individual resting motor threshold (MT) (n=8, within-subject SD: 2.41%, ICC: 0.821), we recorded MEP recruitment curves at nine stimulation intensities (50-150% of MT) with 8 or 10 single-pulses in a semi-random order. Pharmacological validation was performed by systemic (i.m.) application of diazepam (GABAA PAM, 0.1, 0.3 and 1 mg/kg, n=4) and RS-baclofen (GABAB agonist, 1 and 3 mg/kg, n=5). Behavioural sedation effects were scored by two observers blinded to the experimental conditions.

The middle and the highest doses of diazepam shifted the recruitment curve to the right (at 10 min post-administration, showing a pronounced decrease in excitability (main effects of treatment, $F_{3,382}=28.00$, $p<0.001$) with no interaction between treatment and stimulation intensity ($F_{3,382}=1.11$, $p=0.344$). RS-baclofen elicited the expected inhibitory shift only the 3 mg/kg dose, both at 2-hr post-administration (stimulation*treatment interaction: $F_{8,32}=2.62$, $p=0.025$) and at 4-hr post-administration (stimulation*treatment interaction: $F_{6,18}=7.46$, $p<0.0005$), indicating a much less pronounced inhibitory shift of the recruitment curve. The observed mild inhibitory effect was also supported by the sedation scores.

In summary, both diazepam and RS-baclofen decreased motor cortical excitability, as recorded by recruitment curves, though the observed shift was much more prominent for diazepam. Results indicate that TMS-MEP recruitment curves provide valuable insight into GABAergic modulation of the motor cortex, highlighting differences in the characteristics of the effects between GABAA and GABAB receptor modulation, and enabling the system-level testing of the efficacy of potential drug candidates that aim at improving treatment options in NCDs by altering cortical E/I balance.

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Seeing the Whole: Fine-Grained 3D Reconstruction of Functional Mesoscale Domains in Cat Visual Cortex

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In large animals, the visual cortex consists of feature-processing circuits organized into mesoscale patterns. Capturing the function of these intricate functional structures remains challenging, as traditional techniques trade off high spatiotemporal resolution for limited spatial coverage or vice versa. We have developed a 3D functional ultrasound imaging (fUSI) method that we use to reconstruct the functional architecture of the visual cortex in a higher-order mammalian model, the cat.

Results: We captured a 2560 mm3 volume spanning multiple visual cortical areas achieving high resolution in space (150 µm) and time (5 Hz) during stimulation with classical stimuli. We present detailed 3D reconstructions of cortical retinotopy and orientation preference maps across primary and higher visual cortical areas of the cat. Our results reveal distinct organizational patterns within orientation preference maps. Within this high-resolution functional space, we identified and tracked orientation singularities, commonly referred to as orientation pinwheels.

Conclusion: Our fUSI pipeline enables the generation of high-resolution 3D cortical functional maps with unprecedented spatial and temporal resolution. Through the reconstruction of orientation preference maps and identification of feature-space singularities, we demonstrate the pipeline's capacity of capturing the complex 3D structure of cortical functional organization.

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Exploring the Role of Multiple Neuromodulators in Associative Learning and Reward Prediction Error

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Reward prediction error (RPE), defined as the discrepancy between expected and actual rewards, plays a pivotal role in reinforcement learning, guiding value updates and behavioral adaptations. While the dopaminergic system is well-established in encoding RPE, the involvement of other neuromodulatory systems remains less understood. Our study aimed to investigate the contributions of dopamine (DA), acetylcholine (ACh), norepinephrine (NE), and serotonin (5-HT) in associative learning and their relationships with RPE.

We employed an auditory Pavlovian conditioning task with a 50% reward probability, allowing for a clear distinction between positive RPE (reward trials) and negative RPE (omission trials) in mice. Neurotransmitter release was monitored in real time using fiber photometry, with DA measured in both the prefrontal cortex (PFC) and ventral striatum (VS), and ACh, NE, and 5-HT measured in the PFC and basolateral amygdala (BLA). Behavioral responses were assessed by tracking anticipatory licking behavior, which served as an index of reward expectation.

As expected, mice exhibited a decrease in anticipatory licking following omission trials and an increase after rewarded trials. This behavioral pattern was reflected in DA release in both the VS and PFC, with significant correlations between DA levels and licking rates. Similarly, ACh release in the BLA and PFC followed RPE-related changes, indicating its role in updating the value of the stimulus on a trial-by-trial basis. In contrast, NE and 5-HT release showed inverse patterns in the PFC—decreasing after reward and increasing after omissions—while both tracked RPE signals in the BLA.

Our results confirm that DA encodes RPE in both cortical and striatal regions, while ACh signals unsigned prediction errors, updating trial outcomes in a manner similar to DA. The involvement of NE and 5-HT in the BLA suggests their role in learning processes, though their distinct patterns in the PFC may reflect differential innervation from neuromodulatory subregions. These findings highlight the nuanced roles of multiple neuromodulatory systems in shaping reward-related learning.

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Characterisation of the CCK-positive inhibitory cells in the medial entorhinal cortex

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Perisomatic inhibition is considered as one of the most effective regulator in neuronal circuits. Two types of basket cells target the perisomatic regions of principal cells: fast-spiking parvalbumin-expressing and regular-spiking CCK-positive interneurons. In the entorhinal cortex parvalbumin-positive fast-spiking basket cells have been shown to play a major role in forming grid-like firing of layerII principal cells. Little is known, however, about the CCK-positive basket cells in this brain region. This lack of knowledge is mostly due to the heterogeneous expression of CCK throughout different neuronal types, including pyramidal cells. This expression pattern made the transgenic approaches for specific cell-type labeling extremely difficult. Here, with the help of a VGAT-IRES-Cre/BAC-CCK-eGFP-coIN transgenic mice we show the overall distribution of CCK+ interneurons and their specific targets in the medial entorhinal cortex. We found that the targets of the CCK-positive basket cells often show layer selectivity and cell-type specificity. Moreover, we found that layerI CCK-positive GABAergic cells can be divided in different groups based on their electrophysiological properties, one of the groups showing resemblances of human-specific rosehip cells (Boldog et al., 2018; Field et al., 2021). Taken together, we characterized a previously poorly known GABAergic cell-type group, which plays a crucial role in the local entorhinal cortical microcircuit, and we found an interneuron-type, which can be the non-human equivalent of rosehip-cells.

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