ABSTRACT BOOK

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International Neuroscience Conference, Pécs 2024

INTERNATIONAL BRAIN IBRO RESEARCH ORGANIZATION

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Plenary lectures

L1 The Hungarian Neuroscience Society founded 30 years ago

László Lénárd

Institute of Physiology, Pécs University Medical School, Pécs, Hungary

In these days we are celebrating the 30th anniversary of foundation of the independent Hungarian Neuroscience Society (MITT – HNS). This presentation is a historical overview of the circumstances and preceding events leading to the foundation. The development of interrelationship with the IBRO, the ENA and the FENS will also be discussed. The foundation of the HNS has been preceded by number of events. In the few decades after the World War II, the Hungarian Physiological Society (HPhS) became the most attractive community and scientists favoured this Society over their strict disciplinary classification. Members of the HPhS enjoined the privilege to show their results in separate thematic sections on the yearly conferences. Soon, different sections have been formed within the framework of the HPhS, among others the Neuroscience Section.

Another event that preceded the foundation of the HNS was that the Hungarian Academy of Sciences launched a new program entitled Regulatory Mechanisms of Life Processes. Neurobiologists welcomed this opportunity and they thought the best way to join this project was to organize meetings to show the level of advancement in neurobiology research. Therefore, the organization of Neurobiology Colloquia was decided. The First Colloquium was held in Tihany, in 1974 and followed by others in different cities of Hungary. These series of meetings set a kind tradition that wintertime, usually at the end of January, neurobiologists convened and these conventions gradually became transformed into the Conference of the Neuroscience Section of the HPhS dealing with the Regulatory Mechanisms of Life Processes. In the forthcoming years the conferences of the Neuroscience Section were regularly held in different cities with rotation until 1993.

In the Visegrád Conference in 1992, the Board has contemplated that the time has arrived to start with the preparation of founding an independent HNS. After the Conference in Visegrád the president of the Board sent out a circular to the members requesting help in formulating the statutes. The statutes were accepted by the members and the foundation of the HNS was officially announced in January 21, 1993, in the Conference held in Veszprém. The first HNS Congress was held in Pécs, between January 27-29, 1994. During the past 30 years the number of HNS members highly increased, and the Hungarian neuroscience meetings became the well-recognized forum for neuroscientists in Hungary and abroad.

L2 Evolution of thalamocortical development

Zoltán Molnár

Department of Physiology, Anatomy and Genetics, University of Oxford, Oxford, UK

Conscious perception in mammals depends on precise circuit connectivity between the cerebral cortex and thalamus; the evolution and development of these structures are closely linked. During the wiring of reciprocal connections between cortex and thalamus, thalamocortical axons (TCAs) first navigate forebrain regions that had undergone substantial evolutionary modifications. In particular, the organization of the pallial subpallial boundary (PSPB) diverged significantly between mammals, reptiles, and birds. In mammals, transient cell populations in internal capsule and early corticofugal projections from subplate neurons closely interact with TCAs to enable PSPB crossing. Prior to TCA arrival, cortical areas are initially patterned by intrinsic genetic factors. TCAs then innervate cortex in a sensory modality specific manner to refine cortical arealization and form primary sensory areas.

Here, I shall review the mechanisms underlying the guidance of TCAs across forebrain boundaries, the implications of PSPB evolution for TCA pathfinding, and the reciprocal influence between TCAs and cortical areas during development.

L3 Translating migraine from man to animal – Buzsáki Lecture

Jes Olesen

Department of Clinical Medicine University of Copenhagen, Copenhagen, Denmark

Translational research usually means that discoveries in basic science are translated to humans. For migraine the opposite has been the most successful. Migraine poses the unique possibility that attacks can be provoked in patients in a fully ethical fashion. Carotid arteriography induces aura in patients and measurements of regional cerebral blood flow have made it almost certain that cortical spreading depression is the mechanism of the aura and a valid animal model. Nitric oxide (NO) donors induced migraine and a non-selective nitric oxide synthase (NOS)-inhibitor was effective in migraine treatment. A valid mouse model uses repeated injections of NO donors. Calcitonin gene-related peptide (CGRP) induced migraine attacks and this was crucial for the development of modern CGRP antagonistic treatments of migraine that now revolutionize migraine treatment. Pituitary adenylate cyclase activating peptide (PACAP) induced migraine and a monoclonal antibody against PACAP is currently in clinical trial. A mouse model showed that the pathway for PACAP induced migraine is independent of CGRP. Prostanoids and several other signaling molecules, most importantly openers of ATP sensitive potassium channels (Katp) also induced migraine. Mechanisms of provoking molecules have been analyzed in detail in rodent models of migraine. They suggest that migraine induction as well as migraine treatment take place outside of the blood-brain-barrier, that CGRP antagonistic drugs and triptans are not additive, that NO mechanisms and CGRP mechanisms are closely interrelated. Katp channel blockers were effective in rodent models and may represent a new target for migraine treatment.

L4 The Central Amygdala as a Motivational Hub: Paving the Way for Personalized Therapies in Behavioral Disorders

Ewelina Knapska

Laboratory of Emotions Neurobiology Nencki Institute of Experimental Biology, Warsaw, Poland

The current impasse in developing mechanism-based therapies for neuropsychiatric disorders can be overcome by adopting a symptom and circuit-specific approach. The complexity of neuronal circuits involved in controlling behaviors necessitates a focused approach targeting specific brain regions that serve as hubs of high connectivity. One such hub is the central amygdala (CeA), which plays a crucial role in motivation.

We identified specific neuronal circuits within the CeA that are critical for initiating and maintaining social interaction, as well as recognizing negative emotional states in others. We also identified the circuits involved in modulation of food motivation. Interestingly, the social- and food-related circuits only partially overlap. Importantly, our studies have demonstrated the involvement of the human CeA in processing social and food-related stimuli.

These findings provide a promising avenue for developing therapeutic interventions that target specific circuits within the CeA. By focusing on the unique roles of these circuits in various behaviors and emotional processes, researchers can potentially develop circuit-focused treatments for motivation disorders such as depression or autism spectrum disorder.

Symposium

Symposium I

Neuroscience and Philosophy

Chairs: **János Boros** (Professor, Faculty of Cultural Sciences, Education and Regional Development, University of Pécs, Pécs) & **György Buzsáki** (Professor, Neuroscience Institute, School of Medicine, New York University, New York)

Neuroscientists and philosophers will present their latest research. György Buzsáki (New York University) will talk about his experimental results presented in Scientific American, which show that the brain is not designed to represent the world, but to ensure the survival of the whole organism through continuous interaction with the environment. According to Patricia Churchland (University of California, San Diego), recent research in neuroendocrinology suggests that moral behaviour is significantly influenced by changes in the oxytocin-vasopressin system. Christof Koch (Allen Institute) argues that scientific theories of consciousness, in addition to explaining conscious experience and neural correlates, must also explain why we feel the way we do, the extent of space, the passage of time, the taste of food. Computational functionalism offers a way to explain. Joseph LeDoux (New York University) interprets the human being as a set of four domains of existence: biological, neurobiological, cognitive and conscious. The domains are interdependent and have a biological basis. Stratification helps us understand the differences between individuals, groups and cultures. According to János Boros (University of Pécs), György Buzsáki's book The Brain from Inside Out (Oxford 2019) proposes a new philosophical perspective for contemporary brain research based on experiments. This challenges the a priori nature of philosophy. He celebrates the birth of philosophy by claiming that the brain is much more complex than its environment - so that the nature of interaction is fundamentally determined by itself, while going far beyond it. Brain research points to the neurological basis of human culture. In this framework, we can find the thinking of Descartes, Kant, Hegel, Dewey, Davidson and Rorty, among others.

S1.01 Ways to Think About the Brain: Emergence of cognition from action

György Buzsáki

Neuroscience Institute, New York University, School of Medicine, New York, USA

Current neuroscience is largely fueled by an empiricist philosophy that assumes the brain's goal is to perceive, represent the world, and learn the "truth". An inevitable consequence of this framework is the assumption of a decision-making homunculus wedged between our perception and actions. In contrast, I advocate that the brain's fundamental function is to induce actions and predict the consequences of those actions to support the survival and prosperity of the brain's host. Only actions can provide a second opinion about the relevance of the sensory inputs and provide meaning for and interpretation of those inputs. In this "inside-out" framework, the brain comes with a preconfigured and self-organized dynamic that constrains how it acts and views the world. In the brain's nonegalitarian organization, preexisting nonsense brain patterns become meaningful through action-based experience. I will show recent experiments which illustrate brain mechanisms that sele¬ct experiences for lasting memory are not known (aka "credit assignment" problem in AI).

S1.02 What Neurobiology Teaches Us About Morality

Patricia Churchland

University of California, San Diego, USA

Evolutionary biology may suggest that if other-caring behavior entails the carer incurs a cost, then caring will be disadvantageous in the long run; it will be selected against. Thus, “the selfish gene” account by Richard Dawkins. Nevertheless, caring and sharing are in fact typical in highly social species (e.g. wolves, orca, and marmosets). In the last few decades, research in neuroendocrinology has revealed that the ancient peptides, oxytocin and vasopressin play a critical role in mammalian and avian social behavior including caring and sharing. These discoveries have profoundly altered the traditional understanding of the nature and origins of moral behavior in humans and in other mammals. I shall review the benchmark results of the research, and how alterations in the oxytocin-vasopressin system can explain species-typical variations in forms of sociality (e.g. monogamy versus polygyny).

S1.03 What Does a Theory of Consciousness Need to Explain

Christof Koch

Meritorious Investigator, Allen Institute, Seattle, USA
Chief Scientist, Tiny Blue Dot Foundation, Santa Monica, USA

Any scientific theory of consciousness needs to not only explain the relationship between any one conscious experience and its substrate, the neural correlate of consciousness, but also why different experiences feel the way they do – why space feels spatially extended, why time flows and why colors feel different from an infected tooth or the taste of Nutella. Most contemporary theories of consciousness are based on computational functionalism. Integrated Information Theory, is based on different assumptions, and takes a purely operational approach. It argues that the neuronal correlates of consciousness, the maximum of intrinsic cause-effect power, are the posterior hot zone, and that certain type of meditative or psychedelic experiences may go hand-in-hand with a silent cortex. I will discuss experimental progress achieved in locating the footprints of such experiences to the posterior part of the cerebral cortex and in reliably detecting the presence of covert consciousness in patients with Disorder of Consciousness.

S1.04 Our Four Realms of Existence

Joseph LeDoux

The Emotional Brain Institute, NYU, USA
Neural Science and Psychology, NYU, USA
Psychiatry and Child & Adolescent Psychiatry, NYU Langone, USA

Humans have long thought of their bodies and minds as separate spheres of existence. The body is physical—the source of aches and pains. But the mind is mental; it perceives, remembers, believes, feels, and imagines. Although modern science has largely eliminated this mind–body dualism, people still tend to imagine their minds as separate from their physical being. Even in research, the notion of a "self" that is somehow distinct from the rest of the organism persists. But such ideas are increasingly barriers to discovery and understanding, and a new framework is needed. I propose that a human being can be characterized as a composite or ensemble of four fundamental, parallel, entwined realms of existence that reflect our evolutionary past and account for our present ways of being—biological, neurobiological, cognitive, and conscious. All four are, deep down, biological. But the neurobiological realm transcends the mere biological, the cognitive transcends the mere neurobiological, and the conscious transcends the mere cognitive. We each exist uniquely within our own realms every moment of adult life, and together our realms account for all of what and who we are. The four realms also give us a novel understanding of how we, as an individual person, social group, culture, or species, are similar to, and different from, other individuals, social groups, cultures, and species.

S1.05 The mind in the brain from inside out
Reflections on György Buzsáki, The Brain from inside out, Oxford University Press, 2019.

János Boros

University of Pécs, Pécs, Hungary

„Can we somehow put ourselves outside our subjective world and use objective methods to make valid judgments without being influenced by our pasts?" (Buzsáki 34.)

Buzsáki stresses that empirical research goes hand in hand with theory building. Indeed, theory building precedes all empirical research and measurement. A brain researcher has hiddeen or preliminary theories about metaphysics or the nature of reality, about nature itself, about perception, reasoning, logic, epistemology, mathematics, physics, chemistry, the structure of the brain, and so on. When he designs an experiment or measurement, he has a hypothesis loaded with theory, and this allows him to build up his practical work, "a human activity" (xi).

Again and again Buzsáki makes it clear that he is not satisfied with the current use of scientific terminology and that neuroscience is not on the right track. The right paradigm is clearly missing, neuroscientists seem to be repeating old questions without really moving forward. He examined the relationship between thought and language, looked for the origins of neuroscientific terms, and found that most terms were developed, even "invented", before contemporary neuroscience. „I came … to the realization that the general practice in large areas of neuroscience follows a misguided philosophy". (xii.) From experiments in the physical world, Buzsáki deduces the functional primacy of the brain, which produces rationality and logic, over its immediate environment. In doing so, he indirectly confirms the increasingly popular assumption that in science, rationality and logic supersede the physical world, and epistemology supersedes ontology. He performs an epistemological turn of neurophysiological origin.

I try to formulate some brief philosophical reflections on György Buzsáki's book. I refer to Aristotle: we always philosophize, even if we claim not to philosophize.

Symposium II

Preclinical Examination Of Autism Spectrum Disorder

Chairs: **Kristóf László** (Associate Professor, Institute of Physiology, Medical School and Centre for Neuroscience, University of Pécs, Pécs) & **Attila Tóth** (Senior Lecturer, Institute of Physiology, Medical School and Centre for Neuroscience, University of Pécs, Pécs)

The preclinical examination of autism spectrum disorder (ASD) is pivotal for advancing our understanding and treatment strategies. By studying ASD in different animal models, we can uncover underlying biological mechanisms and identify potential biomarkers and therapeutic targets. The ASD research also facilitates the testing of novel interventions, contributing to the development of more effective treatments. Overall, preclinical studies bridge the gap between basic science discoveries and clinical applications, enhancing our ability to address the complexities of ASD.

S2.01 A functional brain network approach to study valproic acid caused autism in rodents and the use of alternative model species in autism research.

Gergely Zachar

Department of Anatomy, Histology and Embryology, Semmelweis University, Budapest, Hungary

Autism spectrum disorder (ASD) defies attempts to attribute its etiology to a singular gene, neurotransmitter system, or specific brain region. Despite the recurrent presence of key symptoms such as diminished sociability, delayed and impaired vocal communication, and resistance to environmental changes, the spectrum's inherent nature and considerable variability in the disorder's severity suggest a broad involvement of neural systems. A widely employed pharmacological model for inducing an autism-like phenotype in animals involves embryonic treatment with valproic acid (VPA). Even this model, relying on VPA's histone deacetylase inhibiting effect, manifests a relatively nonspecific impact on the developing nervous system.

In our investigation, we assessed immediate early gene activity in male mice during social stimulation to pinpoint regions primarily affected by embryonic VPA treatment. Notably, numerous brain regions exhibited distinct activation patterns following social stimulation in VPA-treated individuals. Employing a network-based methodology grounded in correlations between observed brain regions, we identified a functionally connected array of nuclei most affected by the treatment. Functional (activational) connectivity, overall, demonstrated higher levels in VPA-treated animals, implying an aberrant development of connectivity resulting in indiscriminate widespread activation.

Further categorizing the observed array of 36 brain regions into three functional sub-networks based on existing literature data, we observed heightened functional connectivity in autistic mice compared to controls in both the mesolimbic reward network and a cluster of regions associated with stress and anxiety. In contrast, the social behavioral network exhibited lower intercorrelation. We posit a plausible mechanism for increased nonspecific connectedness by demonstrating that VPA induces a defasciculated development of the mesolimbic dopaminergic pathway.

To further study various aspects of the autistic phenotype, laboratory mice may prove insufficient as a model. Consequently, we advocate for the advantages of employing rats, newly hatched domestic chicks, and zebra finches to investigate the effects of valproic acid on adolescent playfight, group preference, and vocal learning, respectively.

S2.02 Functional and morphological alterations of striatal neurons in the autism-related model

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Alterations in dopaminergic brain regions are hypothesized to play a role in the development of autistic symptomatology. However, the changes in the structural or functional characteristics of dopaminergic neurons originating from the midbrain and projecting to the striatum, crucial for both the formation of normal social interactions and the abnormalities observed in autism spectrum disorders, remain poorly understood. Therefore, the aim of the present study was to examine the structural and electrophysiological features of primary dopaminergic neuronal cell cultures isolated from Shank3-deficient transgenic mouse, known for exhibiting autism-like symptomatology. Additionally, we evaluated the expression of selected synaptic proteins in the brain areas relevant for social behavior in wild type (WT) and Shank3-deficient mice. Our immunocytochemical data from primary dopaminergic neurons isolated from the midbrain and striatum indicate significant changes in neurite outgrowth in Shank3-deficient versus WT animals. In the striatum, we also observed a decrease in the expression of synaptic proteins (synaptophysin, SVG40). We found a higher frequency of excitatory spontaneous postsynaptic currents in striatal neurons isolated from Shank3-deficient mice compared to WT neurons. Moreover, striatal neurons isolated from Shank3-deficient mice showed increased absolute amplitude of excitatory postsynaptic currents. These results show ultrastructural and functional changes of dopaminergic neurons, which may explain autistic symptomatology in the used model. In a wider context, these results provide a basis for understanding the etiology of neurodevelopmental diseases.

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S2.03 Autism spectrum disorder associated behavioral symptoms and their relationship with the microbiome

Kitti Mintál[1,2], Attila Tóth[1,2], Edina Hormay[1,2], Béla Kocsis[3], Kristóf László[1], Anita Bufa[4], Tamás Marosvölgyi[4], Renáta Cserjesi[5], Zoltán Vizvári[2,6], László Lénárd[1,2], Zoltán Karádi[1,2]

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Autism spectrum disorder (ASD) is a complex neurodevelopmental disorder where difficulties with social interactions have been supposed to be the most severe symptoms. The escalating use of antibiotics and the consequent disruption of the gastrointestinal microbiome has been implicated in the development of various neurological and psychological symptoms through the microbiome-gut-brain axis. Therefore, changes in the microbiome community are supposed to influence the central regulation processes and affect brain functions which ultimately leading to behavioral alterations.

The first aim of the present study was to assess whether depletion of the gastrointestinal microbiome could induce ASD-like behavioral symptoms. The second goal was to demonstrate the benefits of a probiotic mixture of ours on ASD-like behavioral symptoms.

The impact of the alterations on the social behavior were examined in adult male Wistar rats. Animals have been divided into six groups - 1. antibiotics treated; 2. antibiotics and probiotic treated; 3. probiotic treated; 4 valproic acid treated; 5. valproic acid and probiotic treated; 6. control groups. As antibiotics treatment, rats were given broad spectrum antibiotics mixture dissolved in their drinking water for 4 weeks. Probiotic treated groups daily received our probiotic mixture (containing beneficial bacterial species) with their food for 14 days. Valproic acid treated groups were created as pregnant rats received a single dose of valproic acid on the 12.5th day of gestation and then their male pupils were used in the experiments. Social behavioral test was conducted following the respective modifications of the microbiota.

Our findings demonstrate significant group-differences in the social behavioral test. Antibiotics-induced microbiome alterations during adulthood triggered severe deficits in social behavior similar to those seen in the valproic acid treated rats. However, the probiotic treatment was able to alleviate the antisocial behavior both in the antibiotics- and the valproic acid treated rats.

The present findings well demonstrate that the gastrointestinal microbiome plays important role in the organization of social behavioral processes, and also substantiate that our specific probiotic mixture can alleviate both the antibiotics and the valproic acid generated antisocial behavioral symptoms.

This work was supported by Proof of Principle PTE/101413-1/2019; PTE ÁOK KA 2013/34039/1; EFOP-3.6.1-16-2016-00004; EFOP-VEKOP; TKP2; PTE-ÁOK-PD-2018-10-2017-09; ÚNKP-20-5-PTE-480 New National Excellence Program of the Ministry for Innovation and Technology and PTE ÁOK KA-2020-06.

S2.04 The intraamydaloid oxytocin ameliorates some autistic-like symptoms in valproate-induced autism rodent model

K. László[1,2,5], D. Vörös[1,2], O. Kiss[1,2], B. László[1,5], T. Ollmann[1,2,3,5], L. Péczely[1,3,5], K. Mintál[1,4,5], A. Tóth[1,2,4,5], A. Kovács[1,2,5], O. Zagoracz[1,2,3,5], E. Kertes[1,2,3,5], V. Kállai[1,2,3,5], B. Berta[1,2,3,5], Z. Karádi[1,4,5,6], L. Lénárd[1,4,5,6]

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Autism spectrum disorder (ASD) is a lifelong neurodevelopmental disorder affecting about 1.5 % of children and its prevalence is still increasing. Patients with ASD have impaired social interaction, reduced social motivation which impact social cognition. Anxiety is one of the most common co-morbid sign of ASD. In order to develop new therapeutic approaches, the valproate (VPA) induced rodent model of autism can be an appropriate tool.

In the present study we investigated the possible role of intraamygdaloid oxytocin (OT) on anxiety, social interaction and reinforcement using VPA treated rats in elevated plus maze test, in social interaction test and in conditioned place preference test. Wistar rats were stereotaxically implanted bilaterally with guide cannulae and received intraamygdaloid microinjections.

Our results show that intraamygdaloid OT has anxiolytic effects, increases the time spent with social interaction and it has positive reinforcing effects in VPA-induced autism rodent model. These effects are OT receptor specific.

This work was supported by the University of Pécs, Medical School, Pécs, Hungary (PTE ÁOK KA-2020-06), the National Brain Research Program (NAP 3.0) of the Hungarian Academy of Sciences, New National Excellence Program of the Ministry for Innovation and Technology (ÚNKP-21-5-PTE-1333)

Symposium III

Molecular Biology of Stress Disorders

Chair: **Zsuzsanna Tóth** (Department of Anatomy, Histology and Embryology, Semmelweis University, Budapest)

Stress is a primary cause of many illnesses, such as anxiety, depression and PTSD. These increasingly common illnesses lead to poor quality of life and overburden the health system. Unfortunately, modern treatment methods are often not effective enough. A detailed understanding of the underlying molecular neural mechanisms is essential to develop new, more effective treatments. The aim of the symposium is to present the latest national and international research in the field and to provide a forum for scientific exchange and possible collaborations. The symposium will provide a multi-faceted view of the topic by bringing together speakers, who will give complementary insights into the different mechanisms involved in stress disorders.

S3.01 Dopamine and stress-related disorders: Translation from studies in neonates to searching the molecular mechanisms in animal models

Daniela Jezova

Institute of Experimental Endocrinology, Biomedical Research Center, Slovak Academy of Sciences, Bratislava, Slovakia

Dopamine is a neurotransmitter closely related to neuroendocrine responses under stress conditions. In neonatology, dopamine, and its analogs are also known as a useful treatment to overcome cardiovascular and general emergency states in pathological neonates. We have tested the hypothesis that the neonates of mothers suffering from stress-related diseases require dopamine blood pressure support shortly after birth more often than the neonates of healthy mothers. The results obtained in a sample of 427 mainly preterm newborns showed a lack of association between maternal cardiometabolic stress-related diseases in general and the requirement for dopamine blood pressure support. However, blood pressure support was more frequently required in neonates of mothers with hypertension. These results motivate further research into the molecular mechanisms behind dopaminergic transmission. An interesting animal model is the hyperdopaminergic state observed in rats lacking a functional gene for dopamine transporter (DAT-KO). It has been reported that DAT-KO rats exhibit aberrant cardiovascular responses (heart rate, pulse distension) during restraint stress (Illiano et al. 2020). Consistently with this work, we have observed signs of chronic stress in DAT-KO rats under non-stress conditions including adrenal hypertrophy, a rise in circulating corticosterone, and the cardiovascular hormone aldosterone. Our study in DAT-KO rats focused on the functional interaction of endocrine and immune systems with monoamine and glutamatergic neurotransmission in the mechanisms leading to behavioral alterations. An atypical behavioral response to the NMDA antagonist MK-801 (dizocilpine) was observed in rats lacking the DAT. As a stress stimulus, we used an immune challenge with repeated increasing doses of bacterial lipopolysaccharide (LPS). Interestingly, concentrations of plasma high mobility group box 1 (HMGB1) protein were significantly higher in LPS-treated DAT-KO than in control wild type rats. The gene expression of interleukin-6 in the anterior pituitary increased under stress conditions in the WT but not in the DAT-KO rats. Thus, the interaction between dopamine and immune mechanisms is a promising field for further studies.

Supported by the bilateral Mobility project HAS-SAS and ERANET project 01EW1911 UNMET.

S3.02 Brain area-specific interactions between effects of early life adversity and chronic variable mild stress in the three hit model of depression

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Major depression is a common multifactorial disease with high impact on the affected person, family, the healthcare systems and even economy. The complexity of the condition comes from its background including genetic components, altered stress adaptation capacity related to long-lasting epigenetic changes caused by significantly early life events and finally chronic environmental stress. Based on these, the complex functional and morphological changes that occur in depression throughout the brain may be explained by the three hit concept of depression. This postulates that the co-incidence and interaction of these factors may precipitate the symptoms of depression. Based on this concept, we created a mouse model and aimed at investigating how do the predisposing factors interact with each other and with the efficacy of fluoxetine therapy. We anticipated that the pattern of neuronal activity and epigenetic marker expression will be affected by the interaction of maternal deprivation and stress exposure in the stress adaptation response-involved brain areas.

Pituitary adenylate-cyclase activating polypeptide mutant mice modeled the genetic predisposition. Litters of these mice were subjected to maternal deprivation to induce the epigenetic changes by early life adversity. Later, adult offspring was exposed to chronic variable mild stress vs. controls. Behavioral tests were applied to assess the animals' mood state. Physical and endocrinological parameters were used to assess the stress efficacy. Functional-morphological assessment was performed in limbic forebrain and brainstem stress centers.

Physical and endocrinological parameters approved the efficacy of the model. Marble burying and tail suspension tests supported that the fluoxetine treatment was effective. We found that the interaction between the quality of maternal care and stress exposure affected the FOSB/ΔFOSB neuronal activity in the corticotropin-releasing hormone-producing cells of the central amygdala and also their neuropeptide content. The epigenetic marker H3K9ac revealed that the history of maternal deprivation reverses the effect of stress and fluoxetine treatment in the cornu Ammonis 1 and 3 areas as well as in the dentate gyrus of hippocampus moreover in the Edinger-Westphal nucleus.

The complex, brain area-specific alterations in multiple stress-recruited areas support the predictive validity of our mouse model for depression and support the role of these centers in mood control.

This project (TKP2021-EGA-16) has been implemented with the support provided from the National Research, Development and Innovation Fund of Hungary, financed under the TKP2021-EGA funding scheme.

S3.03 Neuromodulators in stress disorders: emergence of prolactin-releasing peptide

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Sleep and stress are closely linked and related disorders are ruining the lives of millions of people worldwide. Monoaminergic antidepressants represent a first-line treatment for stress-related mental disorders, although treatment resistance is a major problem in significant proportion of cases. Neuropeptides modulating central monoaminergic signaling are promising targets for the development of alternative therapeutic strategies. We found in male rats that prolactin-releasing peptide (PrRP) dysfunction may underlie stress-related disorders. PrRP is produced by the medullary A1/A2 noradrenaline (NA) cells, which are the first interface between the vagus-mediated stress signals and the brain, and by stress-sensitive non-NA cells in the hypothalamic dorsomedial nucleus. PrRP and PrRP-NA cells innervated melanin-concentrating hormone (MCH) neurons in the dorsolateral hypothalamus (DLH), which play a crucial role in the regulation of sleep and mood. PrRP inhibited MCH neurons both ex and in vivo, and enhanced hyperpolarization of MCH neurons by NA ex vivo. In animal models of depression (learned helplessness and inflammation), we observed that failure to cope with chronic/repeated stress was associated with impaired PrRP signaling and altered MCH expression in the DLH. Exposure to stress in PrRP-insensitive period led to increased passive coping with stress in forced swim test. Normal PrRP signaling thus appeared to provide protection against stress-related disorders. Furthermore, using *post-mortem* brain tissue samples, we showed that PrRP signaling was altered in DLH from suicide victims, where we found down-regulation of the PrRP receptors compared to controls. We suggest that repeated/chronic stress leads to PrRP overload, dysfunction of the PrRP system and dysregulation of MCH activity, which consequently increases the risk of developing stress-related mental disorders. Our human data supporting this hypothesis highlight the translational relevance of our study.

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S3.04 Stress related metabolic changes, implication in stress disorders

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Acute traumatic- and chronic stress is a major precipitating factor of various pathologies, including metabolic diseases. Therefore, it is important to understand the details of the metabolic stress responses. However, little is known about the bodily mechanisms that follow the cessation of stressful events. In this study we directly compared metabolic effects of acute stress with chronic repeated- and chronic unpredictable stress in mouse models. Following the stress period, both control and stressed mice were transferred to TSE metabolic cages and their metabolic and locomotor activity were continuously followed for one day. Body composition was measured by Echo MRI. All types of adversities increased energy expenditure, chronic stress exposure decreased body weight gain, locomotor activity and differentially affected fuel utilization. During chronic exposure to variable stressors, carbohydrates were the predominant fuels, whereas fatty acids were catabolized in acutely and repeatedly restrained animals. Chronic exposure to variable stressors in unpredictable manner provoked anxiety. In conclusion, our data emphasize the metabolic differences in response to acute- repeated- and chronic stressors. These results could contribute to the understanding of coping behaviour and stress-induced metabolic and psychopathologies.

Symposium IV

Functional Investigations In Human Brain Sample

Co-organized by HCEMM Nonprofit Kft.

Chairs: **Viktor Szegedi** (Assistant Professor, Department of Physiology, Anatomy and Neuroscience, University of Szeged, Szeged) & **Gábor Molnár** (Department of Physiology, Anatomy and Neuroscience, University of Szeged, Szeged)

The human brain is a network of unprecedented complexity consisting of billions of nerve cells and is particularly interesting to neuroscience. Recent years have witnessed a surge of studies that aim to characterize the human cortical microcircuits functionally. To directly study the electrical activity of human microcircuits, neurosurgical resections are used for in vitro experiments enabling functional readout of human microcircuits. The symposium will focus on electrophysiological recordings of human neurons performed at multiple levels: from dendritic and somatic recordings to multichannel recordings in healthy and diseased human brains. Featured topics will cover active dendritic conductances, how epilepsy alters neuronal performance, and the presentation of atypical spontaneous depolarizing events. Complex human behavior is based on the properties of the human cerebral cortex, which uses simple functional units to achieve yet unknown microcircuit processes.

S4.01 Differences in actions of NMDA receptor mediated neocortical astrocyte-neuron communication between mice and humans

Andrea Csemer[1,2], Adrienn Kovács[1], Baneen Maamrah[1,2], Krisztina Pocsai[1], Kristóf Korpás[1], Álmos Klekner[3], Péter Szücs[4], Péter P. Nánási[1,5], Balázs Pál[1,2]

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Activation of neuronal extrasynaptic NMDA receptors is an important action of glutamate released by astrocytes as gliotransmitter. This activation generates 'slow inward currents' (SICs) which provide phasic and synchronized excitation of neighboring neurons. Besides phasic excitation, NMDA receptor mediated tonic excitation is also observed. In the present study, we sought differences or similarities between the actions and age-dependence of these excitatory phenomena on murine and human neocortical samples.

Human samples were collected from neurosurgery of patients aged between 39 and 75 with primary brain tumors or metastases. Similar to the study on human samples, mice with different ages were employed for comparison.

It was found that SICs generate synaptic plasticity, with features resembling spike timing dependent plasticity in mice. In contrast, only synaptic potentiation was found in humans. Overall SIC activity showed a clear decline with aging in humans and completely disappeared above the age of 70, whereas only a mild decline of the phenomenon was seen in mice. Tonic NMDA receptor mediated inward currents are also affected by aging, as they turn to outward currents above a certain age.

In conclusion, although the NMDA receptor mediated astrocyte-neuron communication exists both in mice and humans, its impact on synaptic plasticity is different. Furthermore, this phenomenon on the two species is differently affected by aging. In mice, the capability of SICs to alter synaptic strength declines, whereas SICs themselves disappear in humans. These age-related changes might contribute cognitive decline of the elderly and has impact on other brain pathologies.

S4.02 From viral injection to electrophysiology and imaging within 24 hours using Semliki Forest Virus.

Albert Gidon

Humboldt-Universität zu Berlin, Charite, NeuroCure, Berlin, Germany

The ability to introduce transgenes into neurons and circuits has revolutionized neuroscience. Typically, the time lag between viral injection and gene expression spans days to weeks and, thus, imposes a limitation on the speed of experimental progress. In my presentation, I will introduce an 'all in one go' protocol utilizing the Semliki Forest viral (SFV) vector to overcome this limitation. I will show how we utilized SFV to rapidly express fluorophores, opsins, and calcium indicators to make electrical recording, imaging, and optogenetic experiments feasible within 24 hours of *in vivo* or ex *vivo* injection. This work introduces a promising new avenue for studying the functional organization of brain circuitry, particularly in short-lived preparations like the human ex vivo brain tissue.

S4.03 Role of excitatory and inhibitory circuits in the generation of synchronies emerging in the human neocortex, in vitro.

Lucia Wittner

Research Centre for Natural Sciences, Budapest, Hungary

Epilepsy is one of the most common neurological disorders characterized by the generation of epileptic seizures and interictal spikes. The imbalance between excitatory and inhibitory circuits is a well-known hypothesis to explain the generation of such hypersynchronous events. Although physiological and anatomical changes have been thoroughly investigated in animal models, considerably less is known about the human disease. We examined the role of both excitatory and inhibitory signalling in the initiation of synchronous population activity (SPA) spontaneously emerging in the human neocortex, in vitro.

Both epileptic and non-epileptic postoperative tissue slices exhibited SPA. NMDA-type glutamate receptor antagonization reduced SPA recurrence only in epileptic tissue, whereas further blockade of AMPA/kainate receptors reversibly abolished SPA emergence regardless of epilepsy. Firing rates and burstiness of excitatory and inhibitory cells remained mainly unchanged during blockade of glutamatergic receptors, except for layer IV neurons in epileptic tissue, which showed increased firing rates, and for inhibitory cells in epileptic tissue showing lower burstiness. Selective antagonism of perisomatic inhibitory cells had slight effect on the generation of SPA, however, parvalbumin (PV)-positive perisomatic interneurons had stronger influence than cannabinoid receptor 1-positive neurons.

Quantitative electron microscopy showed a considerable epileptic synaptic reorganisation in the excitatory circuit. Increased number of smaller synapses, more perforated synapses, and higher ratio of spines among the postsynaptic targets characterized the epileptic tissue. The somatic inhibitory inputs of neocortical pyramidal cells remained unchanged in focal cortical epilepsy, but the size of PV-stained synapses increased, and their number decreased in epileptic samples, in synchrony generating regions.

These findings highlight discrete alterations in both the excitatory and inhibitory systems. Our data suggest that NMDA-dependent glutamatergic signalling and the excitatory synaptic machinery are perturbed in epilepsy. The larger and consequently more efficient inhibitory somatic synapses might account for a higher synchrony in neocortical pyramidal cells. This, together with the excess excitation provided by the modified excitatory circuit might contribute to the initiation of hypersynchronous events, and make a cortical region predisposed to generate epileptic activity.

S4.04 Modulation of Giant Depolarizing Potentials (GDPs) in Human Large Basket Cells by Norepinephrine and Acetylcholine

Dirk Feldmeyer

Forschungszentrum Jülich, Germany

Rhythmic brain activity has been implicated in many brain functions and it is sensible to neuromodulation, but so far very few studies have investigated this activity on the cellular level in vitro in human brain tissue samples. In this study we revealed and characterised a novel rhythmic network activity in human neocortex. To further characterise these events we used intracellular patch-clamp recordings of human cortical neurons to study the large rhythmic depolarisations (LRDs). These LRDs are intricate events made up of multiple depolarising phases and are driven by the release of the excitatory neurotransmitter glutamate, but not by the inhibitory neurotransmitter GABA. LRDs occurred in a low frequency band (~ 0.3 Hz) and displayed large amplitudes and long decay times. Despite these findings in human tissue, under identical experimental conditions, layer 2/3 (L2/3) of the rat neocortex showed no such rhythmic activity. LRDs were predominantly observed in a subset of L2/3 interneurons, that received substantial excitatory inputs and are considered to be large basket cells based on their morphological features. In addition, LRDs are highly sensitive to noradrenaline (NA) and acetylcholine (ACh), two neuromodulators known to modulate network dynamics. NA increased the frequency of the LRDs by enhancing β-adrenergic receptor activity while ACh decreased LRD frequency through M4 muscarinic receptor-activation. Multi-electrode array (MEA) recordings demonstrated that NA promoted and strengthened synchronous oscillatory network activity while the application of ACh led to a desynchronisation of neuronal activity. The distinct modulation of LRDs by NA and ACh exerts a specific modulatory control over the human neocortex and its interaction with subcortical structures.

Symposium V

Information Processing In The Early Visual System; Retina And Retinorecipient Brain Centers

Chairs: **Béla Völgyi** (Professor, Institute of Biology, Department of Neurobiology, University of Pécs, Pécs) & **Ildikó Telkes** (Institute of Physiology, Medical School and Centre for Neuroscience, University of Pécs, Pécs) & **Tamás Kovács-Öller** (Research Fellow, Institute of Biology, Department of Neurobiology, University of Pécs, Pécs)

Talks of this symposium will detail recent advances in visual science covering topics of both color and night vision of human, primate and non-primate mammalian species. The talks focus on the early visual system revealing details of the microcircuitry of the retina and retinorecipient visual brain centers including the lateral geniculate nucleus and the superior colliculus.

S5.01 The Dark Side of Vision: Detection of Single Photons from the Retina to Perception

Petri Ala-Laurila

Department of Biosciences, University of Helsinki, Helsinki, Finland
Department of Neuroscience and Biomedical Engineering, Aalto University, Espoo, Finland

Perception of the dimmest lights relies on several fine-tuned neural mechanisms of the vertebrate retina. I will discuss what retinal mechanisms play a crucial role in the detection of the dimmest lights and shadows and how these retinal mechanisms drive retinal output signals at extremely low light levels. I will also describe how behavioral detection of the dimmest light increments and light decrements ("quantal shadows") relies on the most sensitive ON and OFF type retinal ganglion cells. Finally, I will compare the sensitivity limits of image-forming vision to that of non-image forming vision as assessed by measuring the pupillary light responses of humans at extremely low light levels.

S5.02 Retinal ganglion cell diversity in humans and non-human primates

Ulrike Grünert

Ophthalmology and Visual Science, Faculty of Medicine and Health, Clinical Ophthalmology and Eye Health, Save Sight Institute, Sydney, Australia

Ganglion cells are the output neurons of the retina. We have studied the spatial distribution, molecular properties and central projections of retinal ganglion cells in humans, macaques and marmosets. Our findings suggest that retinal ganglion cells involved with colour vision target the lateral geniculate nucleus exclusively, whereas other ganglion cell types target multiple brain areas.

S5.03 Ganglion cell gap junctions subserve the detection of approach motion in the retina

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The visual field is comprised of distinct image components (color, contrast, different movement types). Each of these components are handled by separate retinal microcircuits and encoded by different types of retinal ganglion cells (RGCs; Roska and Werblin 2001) whose spike outputs reach the brain. Reconstructing these micro-circuits is essential for understanding how visual elements are encoded and transmitted via the optic nerve to image forming brain centers [Dräger 1980]. Following the signal decoding and perception, the brain first applies filters (attention, decision making) and then provides a motor command to best react to the specific stimulus. Life threatening stimuli require faster actions thus signals from the retina also reach subcortical areas to induce species-specific hereditary behavioral patterns, reflexes. One such motor program is the escape behavior initiated by the transient OFF alpha cells (T_{OFF} Alpha) in the retina and processed in the Superior Colliculus as a response to an approaching object (Wang 2021; Munch 2009). By using a combination of *in vitro* Ca^{++}-imaging, electrophysiology, and cell injections we show that gap junctions (GJs) serve to synchronize light response kinetics for T_{OFF} Alpha cells within the GJ coupled array. In addition, we also prove that T_{OFF} Alpha cells utilize GJs to perform a priming excitation to alpha cell neighbors. This priming is robust from cells in the epicenter of the approach stimulus towards alpha cell neighbors and thus counteracting their lateral motion initiated fast inhibition. Without such priming signal, neighboring cells sense the stimulus as a lateral moving object and thus they are inhibited. By this neighbor priming process on the other hand, a single approach stimulus evokes response from an entire population of T_{OFF} Alpha cells and not only from a single cell in the stimulus epicentrum. Conversely, when a GJ blocker was applied through the recording pipette and thereby blocking GJs for the examined T_{OFF} Alpha cell (but not for the rest of the retina) the priming was lost. Finally, we used GJ blockers administered via eyedrops in both in-vivo behavioral tests and in-vivo extracellular Superior Colliculus recordings and demonstrated that disruption of GJs blocks the approach stimulus evoked escape behavior. Thus, we conclude that GJs are essential to form a population code to detect approaching objects and this task is performed *via* the T_{OFF} Alpha RGC population in the mammalian retina.

S5.04 Physiology of standard and non-standard afferent visual pathways in primates

Paul Martin

Faculty of Medicine and Health, Clinical Ophthalmology and Eye Health, Save Sight Institute, Sydney, Australia

Textbooks describe the retino-geniculo-cortical afferent visual pathway as delivering simple visual signals using standard centre-surround receptive fields (parvocellular and magnocellular pathways). I will describe experiments where we targeted the lesser-explored koniocellular cortical afferent pathway and its subcortical visual connections, in extracellular recordings made in anaesthetised marmoset monkeys. We found that many koniocellular receptive fields show properties such as strong orientation selectivity, binocularity, and visual context dependence (extraclassical receptive field). We conclude that properties normally attributed to cortical visual circuitry are present at sub-cortical and, possibly, retinal levels of visual processing in primates.

Symposium VI

Neuropeptides in Health and Disease

Chairs: **Krisztina Csabafi** (Adjunct Professor, Department of Pathophysiology, Albert Szent-Györgyi School of Medicine, University of Szeged, Szeged) & **Zsolt Bagosi** (Associate Professor, Department of Pathophysiology, Albert Szent-Györgyi School of Medicine, University of Szeged, Szeged)

Neuropeptides are short-chain proteinic substances synthesized mainly in the central nervous system (CNS) playing the role of a neurohormone or a neurotransmitter. Neuropeptides can modulate physiological processes, such as stress response, food and water intake, social and sexual behavior, but they can also be involved in the pathogenesis of diseases, such as anxiety, depression and pain. In this symposium entitled ”Neuropeptides in Health and Disease”, we would like to present recently discovered roles attributed to classical neuropeptides, including corticotropin-releasing factor (CRF), urocortins, kisspeptins, arginine vasopressin (AVP), oxytocin, ghrelin and obestatin.

S6.01 Unraveling the Complexity: The Multifaceted Significance of CRF and Urocortins in Stress and Psychiatric Disorders

Tamás Kozicz

Department of Clinical Genomics, Mayo Clinic, Rochester, MN, USA

The significance of corticotropin-releasing factor (CRF) and urocortins in the context of stress is multifaceted, encompassing their fundamental role in orchestrating the stress response and their involvement in stress-related psychiatric disorders. The significance of CRF and urocortins in stress research continues to evolve, challenging traditional paradigms and revealing the intricate mechanisms underlying stress adaptation. This presentation underscores the critical aspects of CRF system neuropeptides and their cognate receptors in the context of stress adaptation response.

Traditionally, it was believed that the stress response's initiation and recovery were coordinated by the mainly opposing yet well-balanced actions of CRFR1 and CRFR2. This dualistic view suggested that CRF/CRFR1 controlled the initiation of stress, while urocortins/CRFR2 mediated stress recovery, thereby maintaining both physical and mental well-being. However, recent literature challenges this dualistic perspective, suggesting that stress recruits CRF system components in a brain area and neuron-specific manner to promote adaptation as conditions dictate.

CRF and urocortins are widely distributed throughout the brain, regulating cognitive, emotional, and behavioral responses to stressors. Dysregulation of the CRF and urocortins system has been implicated in the pathophysiology of numerous stress-related disorders, including anxiety, depression, and post-traumatic stress disorder (PTSD). Consequently, understanding the CRF system's role in stress holds substantial clinical implications, offering potential therapeutic targets for these debilitating conditions.

S6.02 Urocortins modulate social behavior through different CRF receptors and pathways

Zsolt Bagosi

Department of Pathophysiology, Albert Szent-Györgyi School of Medicine, University of Szeged, Szeged, Hungary

Since corticotropin-releasing factor (CRF) was first isolated from ovine brain, new, structurally similar, but pharmacologically different neuropeptides have been discovered and termed urocortins (Ucn1, Ucn2 and Ucn3). The effects of CRF and the urocortins are mediated through two distinct receptors (CRF1 and CRF2) and inhibited by a CRF-binding protein (CRF-BP). Originally, CRF and CRF-related peptides were presumed to have dualistic roles in the central nervous system (CNS), with CRF and Ucn1 inducing stress, anxiety and depression via CRF1, and with Ucn2 and Ucn3 producing stress-coping, anxiolytic and antidepressant effects via CRF2. However, lately it was proposed that the effect of CRF1 and CRF2 activation is not a matter of simple dualism, but depends on the brain regions and neuronal populations being activated. In the present study, we review the experiments investigating the role of CRF and the urocortins in the social behavior of rodents, with a special focus on the sociability and the preference for social novelty of mice. In general, these experiments demonstrate that CRF, Ucn1, Ucn2 and Ucn3 play important, but different roles in social interactions, that are mediated through distinct CRF receptors. In addition, we suggest possible brain regions and pathways expressing CRF and urocortins that might be involved in social behavior.

The present study was sponsored by SZAOK-KKA-SZGYA: 2023.02.01.-2025.01.30.

S6.03 Kisspeptins activate the hypothalamus-pituitary-adrenal axis and induce anxiety

Krisztina Csabafi, Katalin Eszter Ibos, Éva Bodnár, Júlia Szakács, Zsolt Bagosi

Department of Pathophysiology, Albert Szent-Györgyi Medical School, University of Szeged, Szeged, Hungary

We aimed to investigate if kisspeptins, the main regulators of the reproductive axis, might play a role in the regulation of the hypothalamic-pituitary-adrenal (HPA) axis and stress-related behavior.

After intracerebroventricular cannulation, adult male Wistar rats were injected with kisspeptin-13 (KP-13) or a synthetic derivative, kisspeptin-8 (KP-8), 30 min after of which, trunk blood was obtained for corticosterone measurement, or the following behavior tests were performed: open-field test (OF), elevated plus maze (EPM) test, and telemetric registration of general locomotor activity and body temperature. A different set of animals received CRF or V1 receptor antagonist pretreatment before the KP-13 challenge, then an OF test or plasma corticosterone measurement was performed. Furthermore, the expressions of the corticotropin-releasing factor *(Crf, Crfr1, Crfr2)* and arginine vasopressin *(Avp, Avpr1a, Avpr1b)* systems were measured in the amygdala and hippocampus, as well as CRF and AVP protein content. In the case of KP-8, the GABA release in the nucleus accumbens (NA), dopamine release in the ventral tegmental area (VTA), and the gene expression of dopamine receptors and neuropeptide FF receptors *(Drd1, Drd2, Npff1r* and *Npff2r)* were determined in the NA.

KP-13 increased the plasma corticosterone level, that was inhibited by the CRF and V1 receptor antagonists. It also evoked anxiety-like behavior, which the V1 receptor blocker antagonized. Telemetric experiments revealed that KP-13 increased the locomotion and core temperature. In the amygdala, KP-13 induced *Avp* and *Avpr1b* upregulation, and *Crf* downregulation; in the hippocampus, *Crf* upregulation and *Avpr1a* downregulation. A rise in AVP protein content was also detected in the amygdala. KP-8 also activated the HPA axis and induced anxiety-like behavior, however, in contrast to KP-13, it caused a hypolocomotion in the OF test and the telemetric study. Core temperature was increased by both KP-13 and KP-8. Furthermore, KP-8 increased the GABA release and decreased the expression of *drd1, drd2,* and *npff2r* in the NA.

In conclusion, both KP-13 and KP-8 activated the HPA-axis, induced anxiety-like behavior, and increased the core temperature. KP-13's stress-evoking effect might be mediated by an altered AVP and CRF signaling in the amygdala. In addition, KP-13 and KP-8 have an opposing effect on locomotion. Our results suggest that KP-8 suppresses locomotion by modulating the VTA-NA dopaminergic circuitry.

SZAOK-KKA-SZGYA: 2023.02.01.-2025.01.30.; EFOP-3.6.2-16-2017-00006

S6.04 The role of obestatin in depression-like behavior in mice

Júlia Szakács, Katalin Eszter Ibos, Éva Bodnár, Krisztina Csabafi

Department of Pathophysiology, Albert Szent-Györgyi School of Medicine, University of Szeged, Szeged, Hungary

Ghrelin and obestatin are both products of preproghrelin precursor, isolated first from the GI tract. Ghrelin is a well studied orexigenic hormone with pleiotropic effects on anxiety and depression-like behaviors, among others. In several experimental studies, obestatin was originally identified as an anorexigenic peptide and antagonist of ghrelin, however its behavioral effects have been far less elucidated yet. We have first demonstrated that it exerts anxiogenic-like behavior in mice, mediated through ghrelin receptor- and HPA axis activation, by elevating corticosterone levels. In the present study we investigated the influence of obestatin in the forced-swimming test (FST), a rodent behavioral assay for depression-like behavior. We also explored the effect of this neuropeptide on the Corticotropin-Releasing Hormone (Crh) and arginine vasopressin (Avp) gene expression in the amygdala and hypothalamus.

Male, CFLP mice were treated with acute intracerebroventricular (icv) obestatin injections, while other groups, prior to the administration of obestatin received pretreatment with the ghrelin receptor antagonist [D-Lys3]-growth hormone releasing peptide-6 ([D-Lys3] GHRP-6 or the CRH type 1 receptor antagonist antalarmin. After the different treatments, the animals were tested in the FST and the gene expression studies were performed.

According to our results, treatment with icv obestatin induced a depression-like behavior, by increasing the immobility time and decreasing the swimming time in the FST. This effect was blunted by the pretreatment with antalarmin or with the ghrelin receptor antagonist which both decreased the immobility score, and increased the swimming score. Gene expression of Avp and Crh were upregulated after central obestatin administration in the amygdala.

In line with our previous findings on anxiety-like behavior, here we demonstrated that obestatin can also induce a depressive-like effect. The possible mechanisms involved might be HPA axis activation, ghrelin signaling, as well as AVP release. Although our current results are pioneering, further careful investigations in this field will be required.

This research was funded by SZAOK-KKA-SZGYA: 2023.02.01.-2025.01.30.

S6.05 Oxytocin Receptor Expression In Primary Sensory Neurons: Unveiling How Oxytocin And Its Receptor Contribute To The Sensory Processing And The Modulation Of Pain

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The majority of pain is caused by musculoskeletal and neuropathological diseases related to inflammatory processes or neuropathies, leading to transcriptional alterations in primary sensory ganglion neurons. Oxytocin, a hypothalamic hormone, has been reported to be involved in nociception by binding and activating its receptor. Nevertheless, the modulatory mechanism of this nonapeptide and its receptor in the sensory pathway has yet to be fully understood. We conducted immunohistochemistry, enzyme-linked immunosorbent assay, and RT-qPCR analysis to assess changes in the neuronal expression of the oxytocin receptor in rats following peripheral nerve lesions or adjuvant-induced inflammation. A cobalt-uptake assay was performed to observe the effect of oxytocin on capsaicin-induced cultured DRG neurons. Oxytocin receptor immunoreactivity was detected in trigeminal and spinal ganglia, both in peptidergic and non-peptidergic neurons with various sizes, as well as in vitro cultures in the chemo-sensitive pain sensor, small-diameter, transient receptor potential vanilloid member 1 (TRPV1)-positive cells. Both nerve lesion or tissue inflammation led to a time-dependent increase in its protein and mRNA expression. The expression peaks were observed 3 days after the interventions and slightly downturned by the 7th day of survival. *In vitro* analyses showed that cells responsive to capsaicin display a brownish cobalt precipitate. Administration of oxytocin for 3 days prior to capsaicin activation resulted in a decrease in the proportion of neurons exhibiting cobalt staining, which could be prevented by co-administration of the oxytocin receptor antagonist Atosiban. Our results provide evidence for injury- and inflammation-induced upregulation of oxytocin receptor. It is suggested that oxytocin modulates nociception by enhancing their signaling capacity due to the elevated expression of its receptor in the sensory ganglion cells, and as targets, may provide new therapies for pain relief. Our study is consistent with the concept that oxytocin has modulatory role through oxytocin receptors and may bear of significance in the nociceptive and local regulatory/sensory-efferent functions of chemo-sensitive primary sensory neurons.

GINOP_PLUSZ-2.1.1-21-2022-00083

Poster Session 1
Disorders, disease models

P1.01 A1 adenosine receptors modulate the beneficial effect of exogenous ketone supplements on isoflurane-induced blood glucose elevation in WAG/Rij rats

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It has been demonstrated that isoflurane-induced hyperglycemia may be in association with different side effects, such as immunosuppression and infectious complications. Exogenous ketone supplements (EKSs), such as KEMCT (mix of ketone ester/KE and medium chain triglyceride/MCT oil in a 1:1 ratio) can decrease the blood glucose level and increase R-β-hydroxybutyrate (R-βHB levels, leading to ketosis). Ketosis can enhance extracellular adenosine concentration, which adenosine can modulate the effects induced by EKSs and isoflurane via A1 adenosine receptors (A1Rs). Thus, we investigated whether KEMCT applied alone or in combination with the selective A1R antagonist DPCPX (1,3-dipropyl-8-cyclopentylxanthine) modifies the isoflurane-generated increase in blood glucose level in WAG/Rij rats. To investigate the influence of KEMCT on isoflurane anesthesia-evoked changes in blood glucose and R-βHB levels, rats were fed with standard rodent chow, which diet was supplemented with KEMCT (3.0 g/kg/day, gavage) for 7 days (STUDY 1 and STUDY 2). Isoflurane (3%) anesthesia was induced for 20 minutes on the 7th day of water (control) or KEMCT gavage. Subsequently, blood levels of glucose and R-βHB were also measured from blood taken from the tail vein of male WAG/Rij rats (STUDY 1). Moreover, we investigated the effect of DPCPX (i.p. 0.2 mg/kg) alone or in combination with KEMCT on isoflurane (3%) anesthesia-generated changes in blood glucose and R-βHB levels in female WAG/Rij rats (STUDY 2). We demonstrated that KEMCT gavage not only increased blood level of R-βHB, but also abolished the isoflurane anesthesia-generated increase in blood glucose level in both male and female WAG/Rij rats (STUDY 1 and STUDY 2). Furthermore, DPCPX abolished the KEMCT-evoked alleviating effect on isoflurane-generated increase in blood glucose level in female WAG/Rij rats (STUDY 2). Our results suggest that ketone supplements (e.g. KEMCT) are able to decrease the isoflurane anesthesia-evoked increase in blood glucose level efficiently likely via A1Rs at least in WAG/Rij rats.

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P1.02 Neuroinflammation in the Huntington's disease Midcingulate Cortex

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Huntington's disease (HD) is a neurodegenerative condition that can result in motor, mood and cognitive symptoms. HD mood symptomotology correlates with neuronal death in the cingulate cortex. Neuroinflammation, involving reactive glial cells and inflammatory mediators in the brain parenchyma, may influence HD pathophysiology. Accumulation of mutant Huntington (mHTT) aggregates has also been linked to neuroinflammation and neuronal loss. Importantly, the degree to which these neuroinflammatory changes are detrimental to neurons and contribute to HD pathology progression is not well understood.

Using fluorescent immunohistochemistry, we labelled HD and control post-mortem human midcingulate cortex tissue with HLA DP/DQ/DR, an inflammatory marker, and Iba-1, labelling microglia. We qualitatively and quantitatively assessed activation and morphology changes, indicating neuroinflammation, and mHTT levels - linking neuroinflammation and mHTT burden.

We found increased activated microglial morphologies across all HD cases (53.82%), and increased ramified microglia in control cases (67.41%). HD cases showed a decreased number of ramified and ameboid microglia. Activated microglia were localised close to neurons containing mHTT aggregates in HD cases, which positively correlated with mHTT burden. The total microglia number did not increase in HD cases.

Total microglia number remaining constant between HD and control cases suggests ramified microglia change to activated states in HD, increasing neuroinflammation. We show an association between mHTT burden and neuroinflammation in HD. These findings further the understanding of neuroinflammation in Huntington's disease, a necessary step for developing inflammation-targeted therapeutics.

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P1.03 Dimethyl-tryptamine reduces microglia activation in primer cell cultures

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The activation of microglia cells in ischemic brain injuries is an early response which initiates inflammatory processes. Sigma-1 receptor which is also expressed in microglia, is involved in the regulation of inflammatory processes in nerve tissue. In our experiments, we searched for the answer to how the neuroprotective Sig-1R agonist N,N-dimethyltryptamine (DMT) affects microglial activation.

Isolated microglia 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) or DMT (Sig-1R agonist) alone (5-10-20-50 µM), or in combination with LPS for 24 h. Microglial activation was evaluated by Iba1 immunolabeling (degree of arborization expressed by a transformation index - TI) and Western blot analysis, and the visualization of phagocytotic activity with fluorescent microbeads.

Microglia displayed decreased area, perimeter and TI in response to the LPS challenge, indicative of amoeboid transformation and activation (TI: 2,08±0,48 vs. 3,62±1,33 LPS vs. control). When the LPS-challenged cell cultures were treated with DMT, significantly more ramified cells were seen (TI: 2,08±0,48 vs. 9,14±5,17 LPS vs. LPS+20 µM DMT). Increased Iba1 signal intensity in Western blot analysis confirmed microglial activation due to LPS treatment (integrated optical density, IOD: 121,08±63,31 vs. 100±0, LPS vs. control), which was increased particularly by DMT (IOD:121,08±63,31 vs. 212,64±201,37 LPS vs. LPS+20 µM DMT). The proportion of phagocytic microglia in the culture was increased by the addition of LPS (65 vs. 48 %, LPS vs. control), which was counterbalanced by DMT treatment (25 vs. 65 %, LPS+20 µM DMT vs. LPS).

DMT effectively reduced microglial activation. We assume that DMT exerted its protective effect by modifying the stress response of the endoplasmic reticulum (ER) and the Ca2+ homeostasis of microglia, since Sig-1R is expressed on the membrane section of the ER associated with mitochondria and regulates Ca2+ transport between the ER and mitochondria.

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P1.04 Post mortem analysis of synapse numbers in the hippocampus of depressed patients and control subjects

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Major depressive disorder (MDD) is a common multifactorial disorder, characterized by depressed mood, diminished interests, impaired cognitive function and vegetative symptoms. Despite decades of intensive research, the exact pathophysiology is still unknown. Numerous in vivo and post-mortem studies document volumetric and cellular changes in the hippocampus of depressed patients. Neuroimaging studies report on reduced hippocampal volume whereas, post-mortem histopathological analyses document altered morphology and changes in cell numbers of both neurons and glia. Chemical synapses are key functional units of the central nervous system through which neurons communicate with each other. Earlier studies found reduced number of synapses in the prefrontal cortex of depressed patients and such changes of synapse numbers is likely to have detrimental impact on neural network functioning and essentially on complex cognitive and emotional functions. Here, we performed a quantitative electron microscopic analysis and investigated synapse numbers in post-mortem hippocampal tissue samples from depressed patients. Hippocampal samples were received from two different brain banks: 1) from the collection of the University of Mississippi Medical Center, Jackson (MS), USA and from the Institute of Experimental Medicine, Budapest, Hungary. We compared samples from MDD patients (n = 11) and samples from control subjects (n = 10). Control subjects were accidentally deceased individuals without any mental disorder. Ultrathin sections were examined and photomicrographs were taken using a JEOL JEM 1400 FLASH transmission electron microscope. Systematic quantitative analysis was performed on the dentate gyrus, CA3 and CA1 areas using unbiased quantification principles. So far, we could not detect any significant differences in synapse densities between MDD patients and controls subjects. The exact data and potential explanations will be presented on the poster.

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P1.05 Developing and validating a new model for mouse subretinal haemorrhage

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Subretinal hemorrhage (SRH) is due to accumulation of blood between the retina and the retinal pigment epithelium (RPE), or between the RPE and the choroid. It can occur spontaneously as a result of changes in the elasticity of blood vessels, but is often caused by hypertension or trauma. Subretinal blood is toxic to the retina and untreated SRH can lead to rapid tissue damage accompanied by photoreceptor and RPE degradation, resulting in visual impairment, and possible blindness.

In our research, we aim to create a new mouse model for SRH to better delineate the exact area of the bleeding itself and its effects regarding cellular survival and immune cell activation. Also, in our model system we show a possible treatment option that can alleviate the effects of secondary injuries due to the spreading of death signals and inflammation next to the bleeding site.

We created 4 injection groups of C57BL/6 mice with 24-hour survival times. For the SHAM group, filtered PBS was injected subrationally, in group I native, autologous blood from the tail vein of the animals were injected, in group II Cholera toxin B-AlexaFluor594 conjugate was co-injected with the autologous blood, while in group III fluorescently labelled GADPH siRNA was co-injected with blood to test it's effectivity in gene regulation. We used Iba1, GFAP and Casp3 markers for posthoc IHC staining and to show the level of immune activation in relation to SRH As far as we know it, our method is the first to induce and treat SRH with native blood and siRNA to not only reach a deeper insight to the pathology but to alleviate the effects as well.

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P1.06 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 current project, in parallel with the FELL-HD trial, we are testing felodipine drug efficacy in induced neurons (iN) directly reprogrammed from the FELL-HD cohort's skin fibroblasts. Transdifferentiated iNs keep the genetic and aging signatures of the donor bypassing any stem cell or neuroprogenitor phase during conversion. We converted 8 control and 18 HD patients' fibroblasts with mild symptoms to iNs with the same conversion efficiency and purity. Our previous results indicated an accelerated aging in HD-iNs defined by DNA methylation. Therefore, we are investigating in the current cohort the presence of any accelerated aging using the Horvath epigenetic clock. Felodipine cell survival measurements showed no toxicity. We used 0.1µM and 1 µM felodipine treatment for 24h and 72h. After 28 days of conversion in 96-well plates iNs will be counterstained with neuronal and autophagy markers to determine neuronal morphology and subcellular autophagy changes using high-content automated screening microscopy.

These preclinical results will be directly compared and correlated with FELL-HD trial outcome and the patients 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.

P1.07 Manipulating the cholinergic neurons in Alzheimer's disease: validation of a mouse model

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Alzheimer's disease (AD) is an increasing health and social problem ranking 7th among the most common causes of mortality worldwide. The cholinergic system is its most affected neurocircuit, therefore, it is a common therapeutical target. However, revealing its exact role requires further studies.

A genetical mouse model was created, that represented the progression of AD and, additionally, expressed Cre recombinase enzyme in cholinergic neurons allowing their targeted manipulation. Here we aimed to validate the model.

Two strains were cross-bread: B6;129-Tg(APPSwe,tauP301L)1Lfa Psen1tm1Mpm/Mmjax) and B6;129S6-Chattm2(cre)Lowl/J. After serial genotyping, a colony, homozygote for four genes (PSEN1, APPSwe, tauP301L and Cre; 3xTg-ChAT-Cre) was established. To test the functionality of the Cre enzyme a stimulating DREADD virus (AAV8-hSyn-DIO-hM3Dq-mCherry) was injected unilaterally into the nucleus basalis magnocellularis (NBM) and clozapine-N-oxide-induced c-Fos activation was compared between the two hemispheres. For behavioral characterization different tests were performed: Y-maze, single pellet skilled reaching (SPSR), fox odor test (FOT), splash test (ST) and social discrimination test (SDT). The progressive appearance of Aβ plaques and pTau aggregates were confirmed by immunohistochemistry.

Immunostainings confirmed the expression of Cre-dependent fluorophore in ChAT positive cells as well as the appearance of the pathological hallmarks (Aβ and pTau). The c-Fos activity was significantly increased at the virus injected hemisphere. In the behavioral tests 3xTg-ChAT-Cre mice showed decreased locomotor activity (Y-maze, SDT, FOT), increased anxiety (FOT, ST) and weaker fine motoric skills (SPSR) compared to control animals.

The newly created animals have a functional Cre recombinase enzyme in cholinergic cells. Additionally, the animals showed the pathophysiological hallmark of AD in specific brain areas and kept the typical behavioral alteration found in 3xTg-AD mice before. Thus, this strain seems to be appropriate for further studies.

P1.08 Age-dependent changes in nmda-induced excitotoxicity and neuromodulatory effects of kynurenic acid and its analogues in mouse brain slice

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Kynurenic acid (KYNA) is the main neuroprotective substance of the kynurenine pathway. KYNA plays an important role in various neurodegenerative diseases, such as Alzheimer's disease, Parkinson's disease, and Huntington's disease. Although KYNA has proven neuroprotective effects, it cannot be used as a peripherally administered drug because it cannot cross the blood-brain barrier. To address this problem, chemically modified KYNA analogues are currently being tested: SZR72 and SZR104 are such analogues and have been shown to be protective in various animal models. Glutamate-induced excitotoxicity plays a key role in the neurodegenerative diseases mentioned above. Therefore, we used the N-methyl-D-aspartate (NMDA)-induced excitotoxicity model to investigate the neuromodulatory agents.

Using acute hippocampal slices from mouse brain, we investigated the excitotoxic effect of NMDA and the neuromodulatory effect of KYNA and its analogues against excitotoxicity in animals of different ages. The degree of tissue damage was evaluated by biochemical and histological methods.

In young animals (1- and 4-week-old), NMDA treatment did not cause any changes and the cells also proved to be more resistant. However, in older animals (8-week-old and 1-year-old), the treatment caused significant damage. KYNA and SZR72 exerted neuroprotective effects compared with NMDA treatment at all ages, although this was not statistically significant in all cases. SZR104 treatment did not produce significant changes over NMDA-induced damage in any age group.

In summary, our results provide important data on the harmful effects of NMDA treatment and the neuroprotective effects of various neuroactive compounds in mouse brain tissue under *in vitro* conditions. Further investigation of KYNA and its analogues is expedient, as they have therapeutic potential in neurodegenerative diseases.

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P1.09 Neurodegeneration in a transgenetic rat model of Alzheimer's disease

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Alzheimer's disease is the most common form of dementia. The incidence of the disease increases with age, affecting 1% of 60-year-olds, and 30% of 85-year-olds, altogether approximately 55 million people worldwide (WHO data). Several studies provide evidence that abnormally folded amyloid beta and tau proteins that accumulate in amyloid plaques are responsible for the neurodegeneration, causing progressive deterioration of the nervous tissue and subsequent behavioral disturbances and memory-loss. It is well documented that the aggregation of the β-amyloid leads to neuro-inflammation and neuronal cell death, however the progression and exact mechanism still remains to be clarified.

Here, we investigated neuronal and glial changes in the brains of a transgenic Alzheimer's rat model, the TgF344-AD rats. This model shows the overexpression of human amyloid precursor protein (APPsw) and presenilin 1 (PSEN1E9), which play an important role in the progression of the disease. TgF344-AD rats express 2.6 times more human APP and 6.2 times more human PSEN1 in the brain. With post-mortem immunohistochemistry, we labelled amyloid plaques, microglial cells and astrocytes, as well as GABAergic interneurons in the hippocampus. Systematic, unbiased cell counting was carried out to assess putative cellular changes.

Our preliminary data indicate a pronounced neuro-inflammatory response in the hippocampus of the TgF344-AD model, involving mainly microglial cells, but among the GABAergic neurons only in the number of cholecystokinin-positive cells were altered.

Contrary to expectations, we could did not detect a correlation between amyloid plaque load and the neuronal and glial changes.

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P1.10 The long-term impact of early life stress on resting state functional connectivity in depressed patients

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Early life stress, such as childhood maltreatment (CM), is a major risk factor for developing major depressive disorder (MDD). However, it is not clear exactly why maltreated individuals are more susceptible to develop psychopathology and how adverse childhood experiences influence the development of brain architecture. To gain a better insight into how early life stress influence brain functions, we performed resting state functional magnetic resonance imaging (fMRI) in maltreated and non-maltreated depressed patients. Afterwards, we analyzed the fMRI data to detect putative between-network functional connectivity (FC) differences as a consequence of early life stress.

We recruited 37 depressed patients, n=18 maltreated and n=19 non-maltreated, together with 20 matched healthy controls. History of maltreatment was assessed using the Hungarian version of the 28-item Childhood Trauma Questionnaire. Functional connectivity differences between the groups were investigated using CONN Connectivity Toolbox.

We found several between-network functional connectivity alterations in the connections of the default mode network with the executive control, salience and cerebellar networks. The strongest differences (Fals discovery rate corrected $p < 0.00001$) were the increased resting state functional connectivity strengths between the basal ganglia network and salience, executive control and sensorimotor networks, and between sensorimotor and cerebellar, visual and default mode networks in maltreated patients compared to the non-maltreated depressed group.

Our results confirm that depressed patients with a history of early life stress have numerous alterations of between-network FC strengths not only in their fronto-limbic circuits, but also in sensorimotor, visual, auditory and cerebellar networks compared to non-maltreated depressed patients.

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P1.11 Examination of pituitary adenylate cyclase-activating polypeptide in multiple myeloma

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Pituitary adenylate cyclase-activating polypeptide (PACAP) is a multifunctional neuropeptide with well-known antiapoptotic, anti-inflammatory and antioxidant effects. The antitumor effect of PACAP in multiple myeloma (MM) has been demonstrated in numerous studies. PACAP inhibits the growth of myeloma cells, regulates osteolytic bone destruction, and protects proximal tubule cells in various models of MM. The peptide also has an immunomodulatory effect and may influence the complex cytokine network of the bone marrow microenvironment.

The aim of our study was to investigate the plasma PACAP-38 levels of patients with MM using ELISA method (n=66; control: n=10). We correlated the changes of PACAP levels with various clinical and laboratory parameters.

Lower PACAP levels were measured in treated MM patients compared with the healthy control group, but this difference disappeared if the patient achieved better response than partial response after therapy. Significantly higher PACAP-38 levels were seen in younger individuals with lower stage, lower plasma cell fraction in bone marrow, lower tumor markers and in patients after lenalidomide therapy. Higher PACAP-38 levels in newly diagnosed MM patients predicted longer survival and higher probability of response to treatment.

Based on our findings, we suggest that this peptide may play an important role in the pathophysiology of MM and PACAP could be used as a valuable noninvasive alternative biomarker. However, further studies are needed to describe the exact patomechanism of the protective effect in this disease.

P1.12 Anti-Parkinson therapy does not reverse the functional-morphological changes in the corticotropin-releasing hormone and urocortin-1 expressing neurons in the rotenone model

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Parkinson's disease (PD) is a neurodegenerative disorder with motor (tremor, rigor, hypokinesia) and non-motor (e.g. depression, anxiety) symptoms. Our group investigates the background of mood disorders of PD. We have previously found a correlation between damage to urocortin-1 (UCN1)-containing cells of the centrally projecting Edinger-Westphal nucleus (EWcp) and mood disorders in the rotenone model of PD, in the rat. An inverse correlation between corticotropin-releasing hormone (CRH) and UCN1 expression levels has been previously found, which also raises the question whether there are deficits of the CRH-containing systems in the PD rotenone model.

Therefore, we aimed to investigate functional morphological changes in the urocortinergic neurons of the EWcp, and the CRH cells of the hypothalamic paraventricular nucleus (PVN), central amygdala (CeA) and the bed nucleus of stria terminalis (BNST) upon rotenone treatment, and upon anti-PD treatment consisting of levodopa/benserazide.

Six weeks of subcutaneous rotenone treatment was applied to induce a PD-like state. Control rats received vehicle injections. Half of the treated rats also underwent levodopa/benserazide anti-PD therapy. The animals' locomotion was analyzed by rotarod test, the anhedonia by sucrose preference, and anxiety level by open field test. Morphological changes were assessed by a combination of RNAscope in situ hybridization and immunofluorescence.

The rotenone-induced motor deficits improved on levodopa/benserazide treatment, in contrast to non-motor symptoms. We reproduced the previously observed UCN1/EWcp neuronal death, which was not affected by the therapy. Surviving cells exhibited higher UCN1 peptide content and lower Ucn1 mRNA expression, which remained unaffected by drug treatment. Rotenone treatment did not induce remarkable CRH neuron loss in any of the regions studied, but Crh mRNA levels decreased, which was not affected by treatment.

The inverse change in UCN1 peptide and mRNA content suggests inhibited neuropeptide release from the cells. This may be due to the PD-like state and concomitant energetic deficit induced by rotenone. Our results indicate that the benserazide/levodopa treatment is ineffective in treating mood-related non-motor symptoms. We provide further indirect evidence that the impairment of the EWcp contributes to the mood disorder in PD because no significant CRH neuron death occurred in the rotenone model of PD.

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P1.13 TRPA1 ion channel in the centrally-projecting Edinger-Westphal nucleus may modulate the symptoms of posttraumatic stress disorder in mouse models

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Transient receptor potential ankyrin 1 (TRPA1) is a non-selective cation channel expressed in the urocortin 1 (UCN1) positive neurons of the centrally projecting Edinger-Westphal nucleus (EWcp) both in mice and humans. The EWcp is implicated in stress adaptation response. As post-traumatic stress disorder (PTSD) is a stress adaptation disorder and the EWcp/UCN1 neurons have modulatory impact on the stress response we hypothesized the regulatory role of EWcp/UCN1/Trpa1 neurons in PTSD.

Male TRPA1 wild-type (WT) and knockout (KO) mice were exposed to two different models of PTSD: single prolonged stress (SPS) paradigm and a repeated electrical foot-shocks combined with acoustic startle stimuli. Time spent immobility was measured in forced swim test (FST) in the SPS model, as an indicator of depression-like behaviour. In the shock paradigm, the jumping behaviour was assessed as a characteristic parameter for hyperarousal. *Trpa1* and *Ucn1* mRNA expressions as well as UCN1 peptide contents were measured in the EWcp/UCN1 neurons applying RNAscope *in situ* hybridization combined with immunofluorescence.

Enhanced immobility was detected in stressed WTs compared to the controls, however similar change was not observed in the KO groups in SPS model. In the shock paradigm, elevated stress-induced jumping behaviour was detected in KO mice in comparison with the WT group. *Trpa1* mRNA was downregulated in stressed groups of both models. Using SPS, significantly higher UCN1 peptide content was observed in stressed WT mice compared to the controls, while similar change was not detectable in KO mice. The basal *Ucn1* mRNA level was higher in KO mice, however the expression was not altered in any SPS-exposed groups. Upon foot-shock, elevated *Ucn1* mRNA expression was observed in WT but not in KO animals, compared to the controls, furthermore, the UCN1 peptide content was significantly higher in WT mice, compared to the KOs.

Reduced *Trpa1* mRNA expression in WT animals, as well as unaltered UCN1 turnover in KO mice together with the absence of depression-like behaviour upon SPS and increased hypervigilance upon foot-shock may suggest the role of TRPA1 in the development of depression-like behavior and extinction of hyperarousal in PTSD.

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P1.14 Neuroanatomical evidence and a mouse CGRP model in line with human fMRI data support the recruitment of peptidergic Edinger-Westphal nucleus in migraine

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Urocortin 1 (UCN1)-expressing neurons of the centrally projecting Edinger-Westphal (EWcp) nucleus are recruited by acute pain stress. The EWcp projects to several migraine-related brain areas including the spinal trigeminal nucleus (STN) and dorsal raphe nucleus (DRN). 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 calcitonin gene-related peptide (CGRP) receptor (Cgrp) in the EWcp and DRN of mice and humans. Anterograde and retrograde tracing study was performed to identify possible urocortinergic projection from EWcp to the STN. Functional connectivity matrix of Edinger-Westphal (EW) was examined using fMRI in control humans and interictal migraineurs. A CGRP injection model of migraine was applied and validated by light-dark box test in C57BL6 mice. Immunostaining was performed to assess the expression of acute neuronal activity marker (FOS) 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 and serotonin (5-HT) as well as tryptophan hydroxylase 2 (TPH2) content of the DRN.

We proved the presence of Cgrp receptor mRNA in DRN and EWcp of mice and human. We also identified a direct urocortinergic projection arising from EWcp to the STN. A positive functional connectivity between EW and STN as well as DRN has been identified by fMRI in humans. CGRP treatment induced photophobic behavior in mice and increased the number of FOS positive neurons in the TRG and lPAG, suggesting the validity of the model. In response to CGRP, the expression of Ucn1 mRNA, FOS and UCN1 peptide in the EWcp/UCN1 neurons increased, both 5-HT and TPH2 levels in the DRN decreased.

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

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P1.15 Investigation of the indole pathway of tryptophan metabolism in the cuprizone mouse model

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Multiple sclerosis (MS) is an immune-mediated, chronic inflammatory and demyelinating disease of the central nervous system. It is characterized by demyelination, lesions with loss of axons and degeneration of neurons [1]. Cuprizone (CPZ) toxin can be used to investigate the mechanisms of demyelination (DEM) and remyelination (REM) of the MS in lack of peripheral immune response. Some metabolites of the tryptophan metabolism show alterations in different neurological disorders, including MS [2]. Furthermore, the abnormal state of the gut microbiome may influence the progression of the disease [3]. Recently, we found significant differences in the concentrations of certain kynurenine metabolites in the cuprizone model [4].

The aim of this study was to investigate the alterations caused by the CPZ feeding in the levels of metabolites of the indole pathway.

The animals (n = 12) were fed with 0.2% cuprizone mixed into a grounded standard rodent chow for 5 weeks. Every treatment week, urine samples were collected from the animals (CPZ n = 6, CO n = 6). At the end of the five week treatment period, the animals (CPZ n = 6, CO n = 6) were sacrificed. The animals were investigated with ultrahigh-performance liquid chromatography with tandem mass spectrometry (UHPLC–MS/MS) technique.

Ultrahigh-performance liquid chromatography with tandem mass spectrometry (UHPLC–MS/MS) measurements showed a significantly increase in tryptamine levels in urine samples from the first week of CPZ treatment ($p < 0.01$ DEM vs. CO), which remained until the end of the intoxication. Furthermore, in the end of CPZ intoxication, we experienced a significant increase in the level of indoxyl-sulphate ($p < 0.05$ DEM vs. CO), and a decrease in indole-3-acetic acid concentration ($p < 0.05$ DEM vs. CO) in the CPZ treated group (DEM) compared to the control (CO).

Our results suggest, that cuprizone intoxication significantly affects the concentration of certain metabolites in the indole pathway of tryptophan metabolism.

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P1.16 Unravelling the Dynamics of Thermoregulation in the Triple Transgenic Mouse Model of Alzheimer's Disease

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According to the literature low body temperature is considered a risk factor for Alzheimer's disorder (AD) and may contribute to a significant worsening of its histological pathology. Less is known about the alteration of the thermoregulation in AD subjects. Therefore, our aim was to identify it in the popular triple transgenic mouse model (3xTg-AD) of AD. In addition to continuous telemetric monitoring of circadian temperature changes, we used cold and warm stimulation as well as an NK3 agonist (senktide)-induced drop in core body temperature modelling post-menopausal heat waves. Six-month-old female animals are compared to wild-type (WT). As the important thermoregulatory centrum, the medial preoptic area is rich in oestrogen-sensitive cells, the stimuli were used also after ovariectomy (OVX). No difference was found after cold stimulation (neither in the forced swim test in cold water), while there was a marginal difference between genotypes after warm stimulation. The senktide was effective (drop in temperature) in WT animals but not in 3xTg-AD animals. So far, OVX did not influenced the outcome. Our results confirm that the 3xTg-AD animals have disturbed thermoregulation, which was more sensitive to external heat (both warm stimulus and senktide-induced increase in tail temperature). The one week OVX does not seem to be long enough to substantially influence the thermoregulation. The ineffectiveness of senktide in 3xTg-AD animals might have a potential as new biomarker for AD, however, further studies needed in this aspect.

P1.17 The role of SIRT3 in cerebral ischemia and its effect on sirtuin expression

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Stroke is the second most common cause of death worldwide, from which 70% of incident strokes are ischemic. During ischemic stroke, the blood flow decreases at a particular brain area, which results in neuronal injury. This involves irreversible brain damage in the core area, while in the penumbra region the neuronal apoptosis may be potentially reversible. The most of ischemic stroke is connected to the middle cerebral artery (MCA). Several pathways are implicated in ischemic stroke, among with sirtuin family. Sirtuins (SIRT1-7) are NAD+-dependent histone deacetylases, they can improve ischemic stroke-caused damage via neuroprotection, while other sirtuins can contribute to stroke pathophysiology. SIRT3 has important role in the mitochondrial functions regulation, sustaining mitochondrial integrity, but its role in ischemic stroke is contradictory in literature. The aim of this study was to investigate the effect of SIRT3 and examine the other sirtuin gene expression changes during ischemic stroke.

Middle cerebral artery occlusion (MCAO) is widely used to model focal ischemic stroke in rodents. MCAO was performed on 12-weeks-old male wild-type (WT) and SIRT3 knockout (SIRT3KO) mice (n=25). We involved SHAM-operated animals as a control group (n=25). MCA was occluded for 60 minutes. The infarct size was examined after 24h and 72h reperfusion with TTC staining. Image J software was used to calculate the percentage of infarct size. From the brain tissues, we analysed SIRT1-7 relative mRNA expression in the ipsilateral core region with real-time PCR. We used a 5-point scale to measure the animals' neurological deficit.

Our results showed that after 24h and 72h reperfusion SIRT3KO MCAO animals displayed larger infarct volume (24h: $p<0,05$ SIRT3KO MCAO vs WT MCAO), moreover 30% of the animals have expired. After 24h reperfusion in WT MCAO groups SIRT4 expression has decreased significantly (SIRT4: $p<0,05$ WT SHAM vs WT MCAO), while in SIRT3KO MCAO groups remained unchanged. After 72h reperfusion SIRT3 declined (SIRT3: $p<0,01$ WT SHAM vs WT MCAO) in WT animals, while in SIRT-3KO groups SIRT1 increased and SIRT5 decreased significantly (SIRT1: $p<0,05$; SIRT5: $p<0,05$ SIRT3KO SHAM vs SIRT3KO MCAO).

We provided evidence that SIRT3 has a protective effect in ischemic stroke. Additionally, the expression level of SIRT3 decreases in ischemic stroke over time and has impact on other sirtuin expression.

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P1.18 The response of serotonergic dorsal raphe neurons to chronic variable mild stress is modulated by the lack of TRPA1 ion channel

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Transient receptor potential ankyrin 1 (TRPA1), a cation channel predominantly expressed in primary sensory neurons, has a well-established role in peripheral pain sensation, however, its distribution and role in the central nervous system remains unclear. Recently, we proved the presence of TRPA1 in the urocortin 1 (UCN1)-expressing Edinger-Westphal nucleus (EW), playing a role in stress adaptation. Urocortinergic fibers innervate the serotoninergic neurons of dorsal raphe nucleus (DRN), that also contribute to mood control, stress adaptation as well as depression. Our aim was to examine the presence of *Trpa1* mRNA in the DRN, moreover, to characterize the Trpa1-expressing cells. We also put forward to examine if the lack of TRPA1 affects serotoninergic DRN in response to chronic variable mild stress (CVMS) model of depression.

We performed RNA scope *in situ* hybridization combined with immunostaining to examine the expression of *Trpa1* mRNA in the DRN of intact C57BL6 mice. The CVMS model of depression was applied for 3 weeks involving TRPA1 wildtype (WT) and knockout (KO) male mice. This was followed by behavioral tests for depression and anxiety, as well as evaluation of physical and endocrinological parameters to validate our model. Semi-quantitative immunofluorescence was performed in the DRN to measure the tryptophan hydroxylase (TPH) and serotonin (5-HT) content of the neurons. Chronic neuronal activation was assessed by FOS immunolabeling.

Trpa1 is expressed in non-serotonergic, vesicular glutamate transporter 3 *(Vglut3)*-positive DRN neurons. CVMS increased 5-HT and TPH content in the DRN of WT mice. Interestingly, in KO mice, an elevated basal 5-HT and TPH content was observed, which did not increase further in response to CVMS. CVMS increased the FOS neuronal activity in non-serotoninergic cells in both genotypes, but KO mice showed a blunted response.

Morphological findings suggest that non-serotonergic Vglut3/DRN cells that carry TRPA1 are involved in the control of stress (mal)adaptation. Further research is needed to clarify how the urocortinergic EWcp inputs and/or the glutamatergic interneuronal afferents are integrated by DRN serotonergic cells and how these contribute to the mood control in health and disease.

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P1.19 In-vitro investigation of single-unit activities in the human epileptic subiculum

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Mesial temporal lobe epilepsy (MTLE) – a neurological disorder with high ratio of pharmacoresistant cases – is characterized by unpredictable seizures. The hippocampal formation, and especially its output region, the subiculum is closely associated with the generation of the seizures, resulting from an imbalance between excitatory and inhibitory neuronal firing. In this study we analysed the firing properties of single subicular neurons in human postoperative hippocampal samples, with special focus on the role of the different neuron types in synchrony generation.

Recordings with a linear 24-channel microelectrode were made in the subiculum, in vitro, in slices derived from epileptic patients (n=6), in physiological solution. Python-based spike sorting algorithms (trisdeclous and spykingcircus2) followed by manual verification was used to cluster single neurons. Bursting and regular firing pyramidal cells and inhibitory neurons were classified based on their action potential shape and autocorrelogram, and the firing rate and the burstiness index were determined.

Spontaneous synchronous population activity (SPA) was detected in n=7/12 slices. 133 neurons were clustered in recordings with SPA, and 165 cells without SPA (no-SPA). Neurons in records without SPA had a higher firing rate (6.1±4.4 Hz vs. 2.4±2.9 Hz) and higher burstiness index (17.6±22.9 vs. 10.7±20.9) than cells in the vicinity of SPA. The firing rate and burstiness showed a correlation of 0.35 in the no-SPA group, but not in the SPA group. Higher ratios of bursting pyramidal cells (bPC) and interneurons (IN) were found in the no-SPA group (bPC:36.9%, IN:16%), compared to the SPA group (bPC:7.5%, IN:9%). The firing rate and the burstiness of these neuron types did not differ between the two groups.

Unexpectedly, very high numbers of bursting pyramidal cells and higher firing rates were found in human epileptic subicular slices not generating SPA. Other factors, than the presence of bursting neurons and elevated discharge rate are necessary to the initiation of synchronous activity in the human subiculum.

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P1.20 Investigation of the centrally projecting Edinger-Westphal nucleus in a mouse model of Alzheimer's disease

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Alzheimer's disease (AD) is the most common form of dementia, associated with a progressive loss of memory and cognitive function. The neurodegeneration of the basal forebrain cholinergic cells is known in AD, however less attention has been paid to the role of other cholinergic brain areas. The cholinergic neurons of the Edinger-Westphal nucleus (EW), part of the oculomotor complex, may be affected early in the disease, causing pupillomotor dysfunction. However, no data are available on the involvement of the peptidergic neurons of the centrally projecting EW (EWcp) in AD. Both our own data and those in the literature suggest a role for the transient receptor potential ankyrin1 (TRPA1) ion channel in neurodegenerative diseases. We recently demonstrated that the EWcp area is the site of the strongest *Trpa1* mRNA expression in the mouse central nervous system that is localized to the peptidergic, UCN1-containing neurons. 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 neurons of EW.

3xTg (amyloid precursor protein, presenilin-1 and tau protein overexpressing) mice were used as a model of AD. Two, 12 and 18 months old 3xTg mice and C57BL6 mice of the same age as controls were studied. *Trpa1* RNAscope *in situ* hybridization was combined with UCN1 immunofluorescence labeling in the EWcp to measure *Trpa1* mRNA expression and UCN1 peptide content.

Significantly higher *Trpa1* expression was observed in 2-months-old controls than in age-matched 3xTg mice. *Trpa1* expression decreased by age in the C57BL6 strain. 3xTg mice showed lower *Trpa1* expression that was not affected by the course of aging. The UCN1 peptide level peaked at 12 months of age in both genotypes compared to their 2-month-old counterparts, followed by a trend of decline by 18 months of age.

Altered age-related dynamics of *Trpa1* expression in the urocortinergic neurons of AD mice suggest that the peptidergic neurons may also be affected by AD.

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P1.21 The TRPA1 ion channel is downregulated in the centrally projecting Edinger-Westphal nucleus in a mouse model of chronic alcohol consumption

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The centrally projecting Edinger-Westphal nucleus (EWcp) contributes to control of alcohol consumption by its urocortin 1 (UCN1) and cocaine- and amphetamine-regulated transcript (CART) co-expressing peptidergic neurons. Ethanol and its metabolites activate the transient receptor potential ankyrin 1 (TRPA1) ion channel. We recently showed that the urocortinergic EWcp is the primary seat of central TRPA1 cation channel mRNA expression in mice. In this study we first aimed to test whether TRPA1 is also present in the human urocortinergic EWcp neurons. Next, we aimed to examine the role of EWcp/TRPA1 a mouse model of chronic alcohol consumption. We hypothesized that chronic alcohol exposure influences the EWcp/UCN1/CART neurons *via* TRPA1.

RNAscope *in situ* hybridization was performed on perfused human EWcp sections (n=3) combined with UCN1 immunostaining. Male *Trpa1* knockout (KO) and wild-type (WT) mice (n=10-12) were compared in a free-choice-dark-phase chronic alcohol (10%) consumption model in a 3-month period. EWcp chronic neuronal activity was assessed by FOSB immunostaining combined with CART immunofluorescence to semi-quantify the CART peptide content. *Trpa1* and *Cart* mRNA expression was examined by RNAscope *in situ* hybridization.

We first proved that TRPA1 is expressed in the human EWcp/UCN1 neurons also. In WTs, both the *Cart* and *Trpa1* mRNAs were downregulated upon chronic alcohol consumption in line with the reduced CART peptide immunoreactivity. This, associated with the absence of FOSB activation suggests reduced CART release. In KO mice, the lower basal *Cart* mRNA density was further reduced upon alcohol exposure, however this was associated with increased FOSB neuronal activity.

The *Cart* mRNA and CART neuropeptide content of the EWcp is known to correlate positively with alcohol preference. In chronic alcohol consumption, the reduced *Trpa1* and *Cart* mRNA expression as well as CART peptide content suggest the regulatory role of TRPA1 in CART release. The presence of TRPA1 in the human EWcp/UCN1 suggests the translational value of our finding with possible future clinical relevance in alcohol addiction.

Alcohol and its metabolites may influence the release of CART from EWcp/UCN1/CART neurons via TRPA1 channels suggesting the role of the ion channel in alcohol abuse.

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P1.22 CARiNg-HD: Studying the effect of cariprazine in induced neurons directly reprogrammed from Huntington's disease patients' 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 have shown positive effects 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.

P1.23 Investigation of the antidepressant and anti-anxiety effects of DMTS mediated by the TRPA1 ion channel

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Dimethyl trisulfide (DMTS) is a polysulfide found in garlic and used as food additive. In our previous experiments we showed that DMTS inhibited spontaneous motor activity and respiration in mice. We wanted to investigate the effect of DMTS on anxiety and depression-like behavior caused by chronic unpredictable mild stress (CUMS). To explore the mechanism of action, we used gene knockout mice for the TRPA1 ion channel gene likely to be involved in the process.

The three-week CUMS paradigm consisted of 4 mid-day stressors and 3 different overnight stressors to induce depression-like behavior. We used 8–10-week-old male TRPA1 wild-type (WT) and knock out (KO) mice on C57B1/6 background. The animals were divided into 12 treatment groups.: stressed and non-stressed groups, within these untreated, vehicle-treated and DMTS-treated subgroups.

Five well-established behavioral tests were used to verify depression-like behavior and to test the impact of DMTS: open field test (OFT), marble burying test (MBT), sucrose preference test (SPT), tail suspension test (TST) and forced swim test (FST).

In wild type mice, stress exposure significantly reduced the time spent in open area in every group in the OFT. It increased the number of marbles hidden in the MBT. Inactive duration in the FST and TST was longer in naïve stressed animals. Sucrose preference was reduced in stressed animals. Relative adrenal weight was larger and thymus weight was reduced after exposure to chronic stress. In knock out mice, stress-exposure did not lead to depression-like behavior and anxiety.

In wild type mice, DMTS treatment significantly reduced time spent in the open area and increased the number of marbles hidden in non-stressed animals. The treatment reduced immobility anhedonia in stressed animals. DMTS administration increased relative adrenal weight and relative weight of the thymus in stressed mice.

DMTS treatment seemed to relieve depression-like behavior in stress-exposed wild type mice. DMTS administration increased anxiety in non-stressed wild type animals, but reduced anxiety in chronic stress-exposed animals. Neither chronic stress nor DMTS treatment had a significant effect on the animals' behavior.

According to our results, DMTS might be an ideal candidate for further study as a dietary supplement for the complementary treatment of depression and TRPA1 ion channel may be involved in mediating the effects of DMTS.

P1.24 Individuality of rats in large home-cage condition with environmental enrichment

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As interindividual differences are a key determinant in several diseases, disclosing their mechanisms becomes important. Wide-range behavioral disturbances were observed in acute behavioral tests of a multiple-hit schizophrenia rat model (named Wisket), but the results might have been influenced by environmental factors leading to stress. The goal of this study was to characterize the behavior of control Wistar (C) and Wisket (W) rats for prolonged period at group, subgroup and individual levels.

A special large home-cage system (named Home-Manner; HM) with environmental enrichment and food delivery device was constructed. Rats (n=9/group) were housed in the HM for 13 days. The cage was supplied with two feeding devices for delivering rewards in large (LD) and small (SD) doses (3 vs 1 pellet), if the animal touched a tray. As an animal learnt to prefer the LD side, delay discount design was provided to evaluate impulsive behavior. Parameters were automatically recorded, including exploratory activity, eating time (time until the feeder runs out) and delay time. These data were analyzed to reveal the cognitive functions and impulsivity of the animals.

Based on the exploratory pattern of the animals, the rats were classified into four subgroups (SGs):

SG1: Activity at both trays (SG1_LD, SG1_SD; C:5; W: 4);
SG2: Activity only at the LD tray (SG2_LD; C:1; W:2);
SG3: Activity exclusively at SD tray (SG3_SD; C:2; W:1);
SG4: No activity at either trays (C:1; W:2), no detailed analyses were possible.

Altogether data obtained from four active sides (subsets) of the 4 SGs could be analyzed.

Significant differences were obtained between the subsets, but only a few parameters showed alterations in the Wisket animals compared to controls within the different SGs. Thus, the W rats showed high level of exploration activity in SG3. The cognitive functions seemed to be impaired in the W group within the SG1, and these animals showed enhanced impulsivity compared to C animals. The personalized analyses in this test revealed high behavioral variability in both groups.

This study highlights the importance of categorized and personalized analyses of behaviors in rodents to improve a translational value of preclinical schizophrenia models. The moderate changes in behavior observed in Wisket animals in HM suggest that the lower stress level may blunt the schizophrenia-like behavioral impairments.

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P1.25 Neuronal preservation and gliosis in human brain organotypic slice cultures

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Several types of models are available for studying the properties of the central nervous system, from simple cell cultures through in vitro slices to living animal models. However, to understand the human brain and its neurodegenerative diseases, as well as to develop appropriate treatments, we need to investigate human tissue. Human organotypic culture provides an excellent opportunity to examine cellular and network-level activities, as well as molecular changes at long-term, as this technique enables the maintenance of the brain tissue for several weeks with a preserved tissue structure and cellular connections. In this study, we explored neuronal degeneration and loss, as well as the degree of gliosis along the culture period.

We evaluated preservation of the cultures in artificial culturing medium (AM, n=5) and human cerebrospinal fluid (hCSF, n=13) as well as the effect of distinct antibiotics+/-micotics (penicillin-streptomycin (PS)-/+amphotericin (AA), n=7 vs. n=10) in preventing infections. Neuronal densities were calculated based on the neuronal marker NeuN staining, Fluoro-Jade C (FJC) staining-based pixel intensity histograms showed the amount of degenerating cells, while astrocytic involvement, including soma and cell process coverage of the samples were relieved by anti-GFAP staining.

Baseline cell density was 1100 cells/mm^2, and day-based pairwise values were 651 in AM and 578 in hCSF, differences being insignificant, while FJC histogram-derived values showed a higher pixel mean intensity (hCSF 85 vs. AM 43, p=.0003) and lower black pixel ratio (hCSF 0.43 vs. AM 0.8, p<.0001). The average glial process coverage of the slices was 0.36 (AM) vs. 0.66 (hCSF), p<.0001. The differences were more pronounced when comparing 1-6 vs. 10+ day coverage values (0.82 vs. 0.49, p=.03). The choice of bacterial and fungal prevention combinations did not significantly influence the neuronal density (PS 584 vs. AA 728 cells/mm^2), nor glial process coverage (0.61 vs. 0.58). In the categories above, glial cell bodies did not show significantly different coverage patterns, however, there was an r=-0.89 between glial cell processes and cell body coverage values.

Contrary to the seemingly straightforward categorisation of tissue samples, a complex set of culturing conditions influences tissue preservation, yet, choosing the suitable culturing medium seems to bear the highest impact.

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P1.26 Various approaches for enhancement of astrocytic Glu/GABA exchange mechanism against convulsive and non-convulsive seizures

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We have previously revealed the endogenous Glu/GABA exchange mechanism, in which astrocytes exchange excitatory Glu for inhibitory GABA through a synergistic action of glial Glu and GABA transporters. Glu/GABA exchange constitutes a negative feedback mechanism that efficiently combats seizures. Here we explored whether pharmaceutical enhancement of this endogenous mechanism may be a viable 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 shortens seizure-like events (SLEs) in the low-[Mg^{2+}] in vitro model of temporal lobe epilepsy by increasing desynchronization. In addition, PUT also significantly decreased seizure duration 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 commonly used anti-epileptic medication that has been demonstrated to be effective in seizure control. Its effective mechanism of action, however, is 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, implying that the Glu/GABA exchange mechanism is a significant contributor to the antiepileptic effect of LEV.

In summary, we provide various pathways by which intensification of the Glu/GABA exchange mechanism can effectively inhibit seizure generation in both convulsive and non-convulsive seizure models.

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P1.27 Involvement of CRHR2 in behavioral and molecular-biological outcomes in animal model of PTSD

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Post-traumatic stress disorder (PTSD) is a mental disorder that can be triggered by experienced traumatic event. It is characterized by dysregulated fear and stress responses in which corticotropin releasing hormone (CRH) system plays an important role. The CRH acts via two receptors, CRHR1 that plays role in stress responding and anxious arousal and CRHR2 that is responsible for dampening the stress response. In PTSD altered CRH and glucocorticoid (GC) levels were detected. Involvement of GC has been underlined by changed levels in FK506 binding protein 51 (FKBP5), a key regulatory factor in GC signaling. Our goal was to assess if intranasal application of CRHR2 agonists (urocortin 2 (Ucn2) and urocortin 3 (Ucn3)) suppresses negative behavioral and molecular-biological outcomes related to PTSD in animals. We used the single prolonged stress (SPS) paradigm to induce PTSD related symptoms in animals. Vehiculum, Ucn2 or Ucn3 were applicated once immediately after SPS exposure. After one week of sensitization period animals were exposed to elevated plus maze, open field (OF) and 24 h afterwards decapitated. In the plasma we measured corticosterone (CORT) levels and in the hypothalamic paraventricular nucleus (PVN) we measured gene expression of selected markers connected to stress response. In the OF PTSD animals spent less time in the central zone and were more immobile but this effect was suppressed by Ucn3. PTSD animals had decreased levels of plasma CORT what Ucn3 reversed again. In the PVN animals exposed to SPS exhibited increased level of CRH mRNA and decreased levels of GR, CRHR1 and FKBP5 mRNA. Ucn3 reversed effect of SPS only in case of FKBP5. Obtained results indicate that intranasal administration of Ucn3 affected changes induced by PTSD in animals. On behavioral level Ucn3 suppressed PTSD-induced anxiety-like behavior, however, in the PVN results were ambiguous. Therefore, further studies will be needed to elucidate the mechanisms through which Ucn3 affects anxiety-like behavior in animals.

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P1.28 Exploring excitability changes in *ex vivo* and *in vitro* prefrontal cortical networks in a rodent model of autism

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Individuals with ASD experience social interaction and communication skill deficits, as well as repetitive behaviours. These impairments are related to modifications in neural network connectivity and excitability in various brain areas, including the prefrontal cortex. which is as a "social hub," responsible for interpreting others' emotions and evaluating social situations. Neuronal alterations behind idiopathic ASD can be investigated in rodents using the valproate (VPA) model. In this model, a single dose of VPA is administered during pregnancy to induce ASD-related alterations in offspring. In this study, we focused to investigate established alterations in prefrontal cortical networks in brain slices from VPA-treated adult rats.

During the experiments, Valproic acid was administered to rats dams on the 12.5th day of pregnancy. The treatment resulted in substantial postnatal development delays of pups and impaired their social behaviour, suggesting that VPA has led to cellular and/or network-related alterations.

Field potential studies were conducted on acute prefrontal slices to characterise the network activity of both young and adult rodents of both sexes. Our findings indicate a significant increase in excitability among the male subjects in the treatment group. While underlying factors remain unclear, this finding suggests a potential alteration in network-level activity. Further exploration of cellular activity and network development is warranted.

To investigate alterations in prefrontal cortical cells and networks during early development, which result in a modified state in adulthood, we aim to develop an organotypic slice culture model. Organotypic prefrontal slices from 6-day-old mice were prepared for experimentation. We can analyse active and passive membrane properties of neurons, as well as spontaneous network activity, using whole-cell patch-clamping. Recordings of slices at different ages in vitro demonstrate parallel development with the behaviour of acute slices from young animals.

Thus, the slice culture exhibits expected changes related to maturation. These preliminary results indicate that the model is suitable for characterising the VPA-induced ASD-related changes during early development stages.

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P1.29 Hippocampal local field potentials evoked by 4-aminopyridine in valproate rat model of autism spectrum disorder

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Autism is a neurodevelopmental disorder described by several major symptoms for example impairment of sociability, impaired communicational skills, stereotypic and repetitive behaviors. According to a leading hypothesis about the background of autism, the disequilibrium of the excitation/inhibition processes within neuronal networks plays a key role. This imbalance may be caused by the hyper- or hypoactivation of the glutamatergic system or the GABAergic system. During my experiments, I compared local field potentials (LFPs) in hippocampal areas ex vivo using rat brain slices of control and in utero valproate (VPA) exposed animals.

The rodent valproate autism model was applied: pregnant Wistar rat dams were treated with VPA on the 12.5 gestation day, autistic traits were confirmed with behavioral testing and later 6-week-old and 3-month-old male and female offspring was used for electrophysiological experiments. I used multi-channel microelectrode array (MEA) system to detect synchronous activity in the hippocampus which was evoked by 50 µM 4-aminopyridine (4-AP) perfusion, a potassium channel blocker used as a convulsant in several epilepsy models. Local field potentials were recorded in 400 µm thick horizontal rat brain slices.

Preliminary analysis of local field potentials revealed alterations in hippocampal excitability of VPA males, namely both 6-week-old and 3-month-old male treated subjects showed deviation from their control counterparts. The hippocampal area of 6-week-old treated males seems to be more excitable than the control 6-week-old males, as the frequency of LFPs was increased, although the amplitude of local field potentials was lower. On the contrary, the hippocampus of 3-month-old treated males was less excitable while having similar amplitude of LFPs as the control 3-month-old males. On the other hand, females do not exhibit similar differences. Additionally, a spatial propagation of field potentials were observed in both groups from the CA3 region of the hippocampus towards the CA1 region.

Interestingly, even control females and males show differences. Recordings from 6-week-old control female hippocampus revealed higher frequency of LFPs compared to the 6-week-old control males, suggesting that the control, untreated animals also have gender-related differences, which could be altered by the VPA treatment.

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P1.30 Downregulation of PACAP and PAC1 receptor in the basal ganglia, substantia nigra and centrally projecting Edinger-Westphal nucleus in the rotenone model of Parkinson's disease

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Parkinson's disease (PD) has been extensively studied using various *in vitro* and *in vivo* models, highlighting the role of pituitary adenylate cyclase-activating polypeptide (PACAP) and its specific receptor, PAC1R, in neuroprotection. Previous research in a macaque model of PD demonstrated a decrease in PAC1R protein content in the basal ganglia, partially restored by levodopa therapy. This study aims to investigate whether similar observations occur in the rotenone-induced rat model of PD.

The primary objective of this study was to assess the impact of rotenone administration on PACAP and PAC1R expression in the rat brain, with a focus on the caudate-putamen (CPu), globus pallidus, substantia nigra pars compacta (SNpc), and the centrally projecting Edinger-Westphal nucleus (EWcp). Additionally, we aimed to explore the potential therapeutic effects of benserazide/levodopa (B/L) on the observed molecular changes.

We employed the rotarod test to evaluate motor skills, the sucrose preference test for depression levels, and the open field test to assess anxiety in rats exposed to rotenone vs. vehicle-injected controls. Dopaminergic cell count in the SNpc, dopaminergic fiber density in the CPu, and peptidergic cell count in the EWcp were quantified to confirm the efficacy of rotenone treatment. RNAscope in situ hybridization was utilized to analyze PACAP *(Adcyap1)* and PAC1R mRNA *(Adcyap1r1)* expression in various brain regions.

Rotenone administration led to a decline in motor skills, increased the depression level, and heightened anxiety in rotenone-treated rats. Furthermore, it resulted in reduced dopaminergic cell count in the SNpc, diminished dopaminergic fiber density in the CPu, and decreased peptidergic cell count in the EWcp. RNAscope analysis revealed downregulation of PACAP and PAC1R mRNA expression in specific brain regions. B/L therapy partially mitigated the observed molecular changes, with a notable effect on *Adcyap1* expression in the CPu.

Our findings support the evolutionary conserved role of the PACAP/PAC1R system in neuroprotection and its involvement in the development as well as progression of neurodegenerative states such as PD. The observed molecular changes in response to rotenone and the partial reversal with B/L therapy underscore the potential therapeutic significance of targeting this pathway in PD treatment.

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P1.31 Is gut dysbiosis associated with the motivational deficit observed in Wisket animals?

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Increasing evidence suggests a bidirectional relationship between the gut microbiome and the central nervous system, thus dysbiosis may also play a key role in the etiology of neurodevelopmental disorders, including schizophrenia. Studies revealed that dysbiosis may also affect cognitive performance and memory, impaired in schizophrenia. The rats of the triple-hit Wisket model (repeated ketamine treatment; post-weaning social isolation; selective breeding) show various schizophrenia-like behavioral phenotypes, such as decreased locomotor activity and cognitive deficit. Thus, we aimed to determine the correlation between gut microbiome composition and cognitive behavior in Wisket model rats.

Three-month-old male and female, control (n=8) and Wisket (n=10) rats were involved in the study. The food-rewarded Ambitus test was used to assess the animal's behavior. The Ambitus apparatus is a rectangular corridor system that includes side boxes with food rewards. Rats performed two sessions per day three hours apart. In session 1, all inside and outside boxes were baited (16 rewards), whereas in session 2, only the inside boxes were baited (8 rewards). Based on the number of collected rewards and completion time the motivation index was calculated, which predicts the cognitive performance. After behavioral testing, fecal samples were collected to verify the microbiome composition by using deep sequencing of bacterial 16S rRNA. Behavioral changes and fecal microbiome composition were correlated by multiple regression analysis.

A motivational deficit was indicated in the Wisket group by the significantly lower motivation index by group ($F(1,16)=7.95$; $p<0.05$), by session ($F(1,16)=52.60$; $p<0.001$), and group and session interaction ($F(1,16)=5.86$; $p<0.05$). The relative abundance of the families *Bifidobacteriaceae, Clostridiaceae, Erysipelotrichaceae, Lachnospiraceae, Lactobacillaceae, Oscillospiraceae,* and *Sutterellaceae* positively; while that of the families *Acidaminococcaceae, Eubacteriaceae,* and *Prevotellaceae* negatively correlated with the motivation index.

The present results suggest that targeting the microbiome may provide a promising opportunity for the development of new therapies to alleviate or prevent schizophrenia-related cognitive symptoms.

P1.32 The Firmicutes/Bacteroidetes ratio in the triple-hit Wisket model rats of schizophrenia

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Accumulating evidence suggests that human gut microbiota is associated with both gastrointestinal tract diseases and psychiatric disorders, including schizophrenia. Some studies reported that the most prominent changes in fecal microbiota involve the high level of *Bacteroidetes* (Gram-negative) and reduced *Firmicutes* (Gram-positive) composition in patients, however, data are inconsistent. A complex schizophrenia rat model (Wisket) was generated using the 'three-hit' approach, which included post-weaning isolation rearing, ketamine treatment, and behavior-based selective breeding. Wisket rats display numerous behavioral traits that are comparable with schizophrenia, such as impaired sensory gating, cognitive deficits, altered social behavior, reduced pain sensitivity, or even alterations in autonomic functions.

The present study aimed to examine the gut microbial composition of Wisket rats and Wistar control rats. Twelve-week-old male and female control (n=8) and Wisket (n=10) rats were involved in the study. The fecal microbiota diversity was assessed by deep sequencing of bacterial 16S rRNA.

Total of 13 different phyla were identified; the most abundant bacteria in both groups were *Bacteroidetes* (control: 42.6±2.63%, Wisket: 47.1±2.16%) and *Firmicutes* (control: 49.0±3.08%, Wisket: 43.5±1.73%). Regarding the *Firmicutes/Bacteroidetes* ratio Wisket rats demonstrated decreased rate compared to the control, but this result did not reach statistical significance. Fecal samples of Wisket rats demonstrated significantly reduced proportion of *Firmicutes* phyla [i.e. *Limosilactobacillus* ($p<0.005$), *Lactobacillus* ($p<0.005$), *Lacticaseibacillus* ($p<0.05$), *Levilactobacillus* ($p<0.05$), *Lactiplantibacillus* ($p<0.05$), and *Streptococcus* ($p<0.005$)], while in *Bacteroidetes* [i.e. *Prevotella* ($p<0.01$) and *Prevotellaceae-UCG-003* ($p<0.05$)] significantly increased compared to the control animals.

Our results suggest that the 'three-hit' schizophrenia rat model may be effective for examining complex abnormalities at the microbiome level, which were also seen in patients. Additionally, the use of prebiotics as an adjunct therapy may be a useful way to assess the contribution of changes in the gut microbiota to psychiatric symptoms.

P1.33 Protective effects of Dehydroepiandrosterone in a neurotoxic Alzheimer's disease mouse model

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The prevalence of neurodegenerative diseases is becoming an increasingly significant societal problem. Current therapies are not curative, can only slow the progression of the disease, further emphasizing the importance of the research. Alzheimer's disease (AD) is the most common type of dementia. The degeneration of neuron cells can be caused by the accumulation of pathological proteins (amyloid-β and p-tau). In the neuropathology of AD, the dysfunction of the cholinergic system plays an important role, characterized by the degeneration of choline acetyltransferase (ChAT) positive cells, along with a reduction in acetylcholinesterase (AChE) fibers. Dehydroepiandrosterone (DHEA) and dehydroepiandrosterone sulfate (DHEAS) are endogen steroid hormones that are hypothesized to have neuroprotective effects, what we aimed to test.

We used a neurotoxic AD model induced by amyloid-β microinjection into the cholinergic substantia innominata/nucleus basalis magnocellularis (SI/NBM) region. The stereotaxic delivered was done in C57BL6/J mice, followed by treatment with 10mg/kg DHEAS or vehicle (physiological saline) 1 hour later. The animals were transcardially perfused, and ChAT and AChE immunohistochemical stainings were conducted to examine the cholinergic system.

The results confirmed the suitability of the neurotoxic model for our investigation, as amyloid-β injection decreased the number of ChAT-positive neurons in the SI/NBM region and the number of AChE-positive fibers in the somatosensory cortex. DHEAS treatment improved the extent of fiber degeneration without affecting ChAT cell count.

Overall, DHEA(S) compounds may provide new insights into understanding the pathology of AD and could represent a new therapeutic target.

P1.34 Role of microglia in SORL1-dependent neurodegeneration and Alzheimer's disease

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Understanding the mechanisms of neurological diseases is one of the most urgent challenges of medicine. In recent years, the contribution of microglia, the main regulator of immune processes in the brain to homeostatic and neuroprotective processes has gained increasing interest. Alzheimer's disease (AD) is a progressive neurodegenerative disease and the leading cause of dementia worldwide. In this study, we explore the involvement of microglia in the pathogenesis of AD, focusing on microglia-neuron somatic junctions through which microglia monitor and shape neuronal function. Specifically, we study the role of microglia in the context of the sortilin-related receptor SORL1, a key risk gene in hereditary AD.

In this translational study, we have collected fixed tissue, native brains and cerebrospinal fluid of triple-transgenic AD-model (PSEN1//App_swe//tauP301L) and control mice, from three different age groups (60-80 days, 220-240 days, 490-520 days) and from both sexes. We have also developed a new CRISPR/CAS9 targeting strategy combined with in utero electroporation to achieve genetic deletion of Sorl1 in a subpopulation of cortical neurons in transgenic mice. Measurement of inflammatory biomarkers and high-resolution immunofluorescent imaging have been performed to study the effects of SORL1 deletion on cellular- and inflammatory responses. We also obtained 3 groups of human CSF samples: SORL1-mutation carrying AD patients, age-matched non-SORL1-associated AD and CTRL patients, as well as fresh frozen human tissue from these groups.

Our results show significant alterations of microglial morphology and function during the aging of mice with chronic neurodegeneration, as well as marked, related changes in the expression of SORL1. Proteomic analysis of human samples revealed a strong neuroinflammatory background to SORL1-associated AD, suggesting the involvement of microglial actions. Our ongoing investigations may shed light to some novel inflammatory mechanisms underlying the pathogenesis of AD.

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P1.35 COVID-19 leads to focal microglial dysfunction and vascular inflammation through central and systemic inflammatory processes

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Since the outbreak of the coronavirus pandemic, several studies have shown that COVID-19 has respiratory and non-respiratory manifestations, including multi-organ failure, systemic inflammation and induction of procoagulant states. SARS CoV-2 infection is also associated with diverse neurological abnormalities, which predict poor outcome in patients. However, the mechanisms whereby infection-induced inflammation could affect complex neuropathologies in COVID-19 are unclear. We hypothesized that microglia, the resident immune cells of brain, are centrally involved in this process. To investigate associations between microglial dysfunction and inflammatory processes, we developed an autopsy platform allowing the integration of molecular anatomy-, protein- and mRNA data sets in post-mortem mirror blocks collected from multiple brain areas and peripheral organs from 11 COVID-19 cases. Tissue samples were either immersion fixed or snap-frozen on dry ice. Inflammatory- and metabolic signatures were assessed by nanoscale microscopy, qPCR, single-cell RNA sequencing, proteomics and analysis of proinflammatory cascades by cytometric bead array. We observed focal loss of microglial P2Y12R at sites of virus-associated vascular inflammation together with dysregulated microglia-vascular-astrocyte interactions, Cx3Cr1-fractalkine axis deficits and mitochondrial failure in severely affected medullary autonomic nuclei and other brain areas. We show that microglial dysfunction occurs at sites of excessive synapse- and myelin phagocytosis and loss of glutamatergic terminals. While central and systemic viral load is strongly linked in individual patients, the regionally heterogenous microglial reactivity in the brain correlated with the central and systemic inflammation related to IL-1 / IL-6 via virus-sensing pattern recognition receptors (PRRs) and inflammasome activation pathways. Single-cell RNA sequencing revealed dysregulated cell-cell interactions and mitochondrial failure in severely affected autonomic nuclei in the medulla. We identified microglia as a key candidate to modulate central and systemic inflammatory processes in COVID-19. SARS-CoV-2-induced central and systemic inflammation might lead to a primarily glio-vascular failure in the brain, which could be a common contributor to diverse COVID-19-related neuropathologies.

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P1.36 Kleefstra syndrome results in accelerated maturation of human iPSC derived neurons

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Kleefstra Syndrome (KS) is a rare neurodevelopmental disorder associated with intellectual disability, hypotonia, dysmorphic features and autism spectrum disorder (ASD). 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 compared the in vitro differentiation and functional maturation of neurotypic human neurons with those affected by Kleefstra syndrome. Neural progenitor cells (NPCs) were derived from reprogrammed peripheral blood cells of a young adult neurotypical female donor (NT) or from a young female Kleefstra syndrome patient (KS-ASD). Neuronal differentiation was induced by BDNF, GDNF growth factors. We performed weekly patch clamp measurements in whole-cell configuration to monitor the maturation of the induced NT and KS-ASD neuronal cultures for nine weeks. Our analysis of 171 NT and 169 KS-ASD cells revealed that neurons in KS-ASD cultures exhibited a more mature and active phenotype already during the first or second week of induction, and electrically active neurons were more abundant until the fourth week compared to those seen in NT cultures. Active membrane properties (high amplitude and rapid action potentials, “kink”) were already detectable from the first week in KS-ASD neurons, while in NT neurons their appearance was delayed until the third week. We also followed the development of the axon initial segment (AIS) by ankyrin G immunostaining. KS-ASD neurons exhibited a more proximal AIS localisation from the first week on while in NT neurons, AIS shifted closer to the soma gradually during the first 3 weeks of maturation. In agreement with patch clamp studies, MEA measurements revealed more prominent network activity of KS-ASD cultures compared to NT cultures during the first month. Importantly, neural activity of KS-ASD cultures was decreased following the fifth week of maturation.

Based on our morphological and electrophysiological studies, Kleefstra syndrome clearly accelerates neural maturation but leads to a more rapid decline in neuronal network activity, indicating that premature or excessive neuronal maturation may hinder further network integrative function.

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P1.37 Altered gene expression of barrier molecules in a rat model of posttraumatic stress disorder contributes to vulnerability

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Traumatising events can lead to development of posttraumatic stress disorder (PTSD), and 20-30% of the victims prove to be vulnerable. Our previous study showed dysbiosis in the gut with increased relative abundance of *Akkermansia municiphila* in the faeces samples of vulnerable rats, which could negatively affect the protective mucin layer of the intestines. In our follow-up experiments, we explored the mRNA levels of barrier proteins of the gut and the brain.

Based on the overall PTSD-like behaviour (z-score), adult, shocked male Long-Evans rats were divided into stress vulnerable and stress resistant groups. With real time quantitative polymerase chain reaction (RT-qPCR), we measured the mRNA levels of connective proteins (zona occludens-1, occludin, claudin-1, -8 and -12) in the intestines as well as in the prefrontal cortex (PFC), which is a known regulator of fear-related behaviour.

In the gut gene expression of claudin-1 of resistant rats was lower than in controls. No differences were found in the expression of other molecules investigated. On the other hand, in the PFC, zona occludens-1, claudin-1 and -11 mRNA were more expressed in the vulnerable animals. This was also confirmed by positive correlations between the severity of PTSD-like behaviour and the mRNA levels. Interestingly, the abundance of *A. municiphila* showed strong, positive correlations with numerous connective proteins in the PFC (zona occludens, claudin-11).

In summary, our study showed that alongside with behavioural changes after traumatising footshock, there is a marked change on the gene expression level of barrier proteins in the PFC, with possible consequences on blood-brain barrier function contributing to vulnerability.

P1.38 Impairment of AgRP neurons influence body weight, lifespan, and behaviour in calorie restricted mice

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Hypothalamic agouti-related peptide (AgRP)-expressing neurons are exclusively located in the arcuate nucleus of the hypothalamus and form the basis of the melanocortin system, a set of CNS circuits regulating energy homeostasis. Besides an important role in driving food intake, AgRP neurons are also involved in modulating complex, non-feeding behaviors. However, it is unknown how AgRP-dependent feeding influences general exploratory behavior. Using the $AgRP^{DTR}$ mouse model with impaired AgRP neuronal functions, we show here that perturbation of AgRP neuronal function leads to sex-dependent body weight changes, lifespan, and altered behavioral responses. Interestingly, neonatal loss of AgRP neurons led to significant sex-specific differences in bodyweight under AL diet. In addition, reduced AgRP function also reduces overall activity of animals of both sexes, revealing that AgRP neurons might regulate bodyweight and food intake through different mechanisms in males and females. Our findings highlight the pivotal role of the AgRP neurons, which likely play a fundamental, sex-dependent role in determining longevity, an also in the regulation of complex behaviors and show that AgRP neurons are critical for complex behavioral adaptations.

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P1.39 Working Toward the Development of a Feasible Neurovascular Uncoupling Model in Rats

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Our goal was to develop a pharmacologically induced model of neurovascular uncoupling (NVU) in rats, aiming for a translationally valid representation of cognitive decline observed in humans. A pharmacologically induced NVU model with resulting neurological and cognitive impairments has been documented in mice (Tarantini, 2015). However, such a procedure had not yet been reported in rats.

In our previous study (E1), we used 28 male Hannover Wistar rats. NVU was induced by intraperitoneal administration of a pharmacological "cocktail" consisting of N-(methylsulfonyl)-2-(2-propynyloxy)-benzenehexanamide (MSPPOH, 5 mg/kg), L-NG-nitroarginine methyl ester (L-NAME, 10 mg/kg) and indomethacin (1 mg/kg) and injected twice daily for 8 consecutive days. In a follow-up experiment (E2) with 17 rats, we repeated the same protocol, using halved doses.

In E1, animals were tested in Morris water-maze and fear-conditioning assays, whereas in E2, they performed in novel object recognition, lever-press and spontaneous alternation tasks. Blood pressure (BP) was monitored by tail-cuffs. NVC was measured in the barrel cortex in a non-recovery operation. A laser Doppler probe was used to detect changes in cerebral blood-flow (CBF), while the contralateral whisker pad was stimulated. Brain and small intestine tissue samples were collected postmortem. Samples from E1 were processed for prostaglandin E2 (PGE2) level determination, while measurement of brain EET levels were determined for E2.

Animals treated with the "cocktail" showed no impairment in their performance in any of the cognitive tasks. However, in E1, they showed ~50 % less increase in CBF and an overall increase in systolic BP (12 Hgmm). Intestinal bleeding and ulcers were found in some of them, and ELISA assays revealed significantly decreased levels of PGE2 in the brain (-70%) and small intestine (-86%). In E2, we did not observe significant changes in BP, intestinal autopsy, CBF or EET levels.

We could evoke NVU by the applied mixture of pharmacons in E1, but the treatment also induced hypertension and intestinal alterations. By halving the doses, we could avoid these side effects but also lost efficacy. Thus, further refinements are needed to develop an applicable model, mostly with regard to finding the appropriate dosing regime and learning assays. Another set of treatment and measurements were carried out this summer (E3) with old experienced Long Evans male rats. The results are currently under evaluation.

P1.40 Changes of amygdalar tyrosine hydroxylase and parvalbumin immunoreactive elements in the valproate-induced model of autism

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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). The amygdala is part of the so-called social brain, and its role in the pathogenesis of autism has been postulated for some time. Dopaminergic pathways and interneurons containing parvalbumin (PV) are directly involved in autism and its preclinical models, but their changes in the amygdala have not been investigated. We were interested in whether catecholaminergic afferents and PV-containing interneurons show changes in the central and basolateral amygdala in the valproate-induced model of autism.

We treated gestating female Wistar rats with 500 mg/kg valproic acid on day 12.5 of gestation and we tested their male offspring for the presence of autistic-like behaviour using 3-compartment social interaction test and elevated plus maze test for anxiety. The control group was the offspring of sham-treated females. The brains of treated (n=5) and control (n=3) animals were fixed at the age of 2 months, and tyrosine-hydroxylase (TH) and PV were detected using fluorescent immunohistochemistry on 50 µm thick parasagittal sections. Regions of the sections containing the amygdala were imaged on a Nikon Eclipse Ti2-E microscope as montages of confocal images. Basolateral, central and intercalated amygdaloid nuclei were delineated based on anatomical location and TH and PV staining. The density of TH-immunoreactive fibres was measured in the Ce and BL using the scan line method. TH immunoreactivity in the intercalated nuclei was quantified by the mean labelling intensity. PV-immunoreactive cell bodies were counted in BL and their areal density was calculated.

TH-positive fibres were mostly found in the central amygdala and in dense clusters of the intercalated amygdaloid nuclei. PV-immunoreactive cell bodies and neuropil were characteristic of basolateral amygdala. However, neither of the measured morphometric parameters were significantly different between control and VPA-induced autistic animals.

Our results suggest that there is no morphological correlate of the VPA autism model phenotype in TH+ and PV+ neural elements of the amygdala.

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P1.41 Potential analgesic effect of fractalkine receptor (CX3CR1) antagonist in mouse model of chronic stress-induced pain

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Chronic psychosocial distress plays a role in the development/exacerbation of several painful diseases (e.g. fibromyalgia, neuropathy). Drug treatment for these diseases has not been resolved, so it is important to examine the pathomechanism more precisely to identify new therapeutic targets. The role of neuroinflammation and the fractalkine receptor (CX3CR1), which is primarily expressed on microglial cells in the brain, has already been proven in stress and inflammatory pain. Based on our unpublished results, we were able to demonstrate the role of the receptor in CX3CR1 knockout mice in the development of pain caused by chronic immobilization stress (chronic restraint stress: CRS). In this research, we investigated the potential analgesic effect of the fractalkine receptor antagonist AZD8797 in a mouse model of stress-induced pain.

Male C57Bl/6J wild-type (WT) mice were exposed to CRS for 2 weeks. From the beginning of the stress protocol, AZD8797 (1 mg/kg) or vehicle was administered intraperitoneally twice daily. The mechanical pain threshold was measured with a dynamic plantar aesthesiometer, and the cold tolerance of the hind paw was measured weekly with the withdrawal latency from icy water test. At the end of the second week, behaviour tests were performed.

Significant mechanical hyperalgesia developed for the second, cold hyperalgesia for the first week. However, mechanical sensitization of the hind paw did not develop in the presence of the antagonist. Cold hyperalgesia was developed to the same level in both vehicle and AZD8797-treated animals in response to stress. In the forced swim test, time spent immobile was decreased due to stress, but only in vehicle-treated animals. In the open field test, animals treated with AZD8797 spent significantly more time in the centre compared to the vehicle-treated group regardless of stress application.

Based on our results, the fractalkine receptor may play an important mediating role in the development of chronic stress-induced pain. Acting on the CX3CR1 receptor, AZD8797 successfully attenuated the mechanical sensitization caused by CRS, further strengthening the potential use of CX3CR1 as a drug target.

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P1.42 Osteosarcoma-induced bone pain is mediated by microglia activation, but not capsaicin-sensitive nociceptive neurons: a complex functional and morphological characterization in mice

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Bone tumor-induced chronic pain is a significant clinical problem, and its treatment has not been solved. Neuropathic components play a crucial role in it, however the exact mechanisms are barely known. Translational models are important for mapping complex neuro-immune interactions and defining new therapeutic targets. We characterize here a mouse bone cancer model induced by intratibial injection of K7M2 osteosarcoma cells using an integrative approach and investigate the role of capsaicin-sensitive peptidergic sensory nerves and neuroinflammation

K7M2 osteosarcoma cells were injected into the tibia of male balb/c mice. The mechanical pain threshold, limb load, spontaneous pain-related behaviors, knee diameter change, and the bone structure of the tibia (micro-CT) were examined in 14-28 day experiments. Microglia (Iba1) and astrocyte (GFAP) immunohistochemistry was performed in pain-related central nervous system areas and in tha spinal cord. Capsaicin-sensitive peptidergic sensory neurons were defunctionalized with resiniferatoxin (RTX) pretreatment.

The mechano-nociceptive threshold and spontaneous loading of the tumor limb decreased significantly, in addition to lifting and sparing the limb, the knee diameter increased and microarchitectural changes characteristic of osteoplastic tumors developed. After 14 days, a significant microglia activation appeared in the dorsal horn of the spinal cord, and there were no glial changes in the periaqueductal gray matter and the sensory cortex. Knocking out the function of capsaicin-sensitive nociceptive neurons did not affect any of the parameters.

We described a complex functional and morphological characterization of this mouse osteosarcoma model, suggesting that bone cancer-related chronic pain is likely to be mediated by central sensitization including microglia activation, but not the capsaicin-sensitive sensory neuronal system.

TKP2021-EGA-16; TKP2021-EGA-13; RRF-2.3.1-21-2022-00015; ELKH-PTE, Hungarian Research Network (Chronic Pain Research Group), Pécs, Hungary

P1.43 The effect of Kisspeptin-13 on spatial learning and memory in a rat model of Amyloid-beta induced neurotoxicity

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The Hypothalamic-pituitary-gonadal axis may affect the development and the progression of Alzheimer's disease (AD). Kisspeptin (Kp) is a key regulator of the reproductive axis. Kp and its receptors are expressed in many regions of the nervous system, such as dentate gyrus of hippocampus. Previously, the role of Kp has been implicated in AD as well as in learning processes. The aim of this study was to investigate the effect of Kp-13 on spatial learning and memory in Amyloid-beta (1-42) induced neurotoxicity.

6-week-old male and female Wistar rats were used in our experiments. First, intracerebroventricular cannula were implanted, then Amyloid-beta in a dose of 4µg/4µl was injected. After a recovery time, spatial memory was tested by Morris Water Maze test and Kp-13 (2µg/2µl) was injected on the last day. Afterwards, the hippocampi were isolated and the Kp-13 (1µg/1ml) induced Glutamate (GLU) release was measured via a superfusion system. Finally, hippocampi and prefrontal cortexes were isolated, and expression of c-Fos, Egr-1 gene levels were determined by RT-qPCR.

Results showed that the AD group's learning ability was significantly decreased compared to the control group, which was blunted in females by Kp-13 treatment. Superfusion study revealed that AD hippocampal slices secreted significantly more GLU than control and this was antagonized by Kp-13. Finally, Kp-13 is shown to inhibit Amyloid-beta induced Egr-1 gene expression in females. In summary, our results indicate that specifically in female rats, the Kp-13 has a positive effect on Amyloid-beta induced neurotoxicity.

EFOP-3.6.2-16-2017-00006

P1.44 Chronic kidney disease may evoke anxiety by altering CRH expression in the amygdala and tryptophan metabolism in rats

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One of the systemic complications of chronic kidney disease (CKD) is anxiety; however, its exact mechanism is not well understood. Therefore, the aim of the present study was to assess the effect of moderate CKD on anxiety in rats. Two-staged 5/6 nephrectomy was performed in male Wistar rats to induce CKD. 7 weeks after the operation, anxiety-like behavior was assessed by elevated plus maze (EPM), open field (OF), and marble burying (MB) tests. At weeks 8 and 9, urinalysis was performed, and blood and amygdala samples were collected, respectively. In the amygdala, the gene expression of *Avp,* as well as the gene and protein expression of *Crh, Crhr1,* and *Crhr2* were analyzed. Furthermore, the plasma concentration of corticosterone, uremic toxins (indoxyl-sulfate and p-cresyl-sulfate), and tryptophan metabolites were measured by UHPLC-MS/MS. The laboratory tests confirmed the development of CKD. In the EPM test, CKD evoked an increase in the closed arm time and central time, whereas the total number of entries decreased. In the OF, a reduction in rearing, central distance and time were detected in the CKD group, and fewer interactions with marbles were registered during MB. In the amygdala, CKD evoked an upregulation of gene expression of *Crh, Crhr1,* and *Crhr2,* but not *Avp.* However, no alteration was found in protein expression. In the CKD group, plasma concentrations of p-cresyl-sulfate, indoxyl-sulfate, kynurenine, kynurenic acid, 3-hydroxykynurenine, anthranilic acid, xanthurenic acid, 5-hydroxyindoleacetic acid, picolinic acid, and quinolinic acid increased. However, the levels of tryptophan, tryptamine, 5-hydroxytryptophan, serotonin, and tyrosine decreased. In conclusion, moderate CKD evoked anxiety-like behavior that might be mediated by the accumulation of uremic toxins and metabolites of the kynurenine pathway, but the contribution of the amygdalar CRH system to the development of anxiety seems to be negligible at this stage.

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P1.45 Disruptive nature of ischemic spreading depolarization to neurovascular coupling after global cerebral ischemia in mice

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Neurovascular coupling (NVC) becomes significantly impaired upon acute ischemic stroke (AIS). The mechanistic basis of NVC dysfunction might be the evolution of spreading depolarization (SD), that causes vasoconstriction and lesion progression in AIS. Here, we show that SD occurrence during ischemia predicts reperfusion failure and the concomitant disruption of NVC.

Male C57BL/6 mice (n=25) were anesthetized with isoflurane (0.6-1.2%). Cerebral blood flow (CBF) variations were captured using laser speckle contrast imaging (LASCA). After recording a baseline of 10 min, transient (45 min) unilateral (1VO) or bilateral (2VO) common carotid artery occlusion was performed to create ischemia. Reperfusion was initiated by the release of the occlusions and was monitored for 60 min. Subsequently, NVC function was evaluated under isoflurane (0.1%)-medetomidine (0.1 mg/kg) anesthesia by whisker stimulation (~2Hz). SHAM operated animals served as control.

Low CBF (<20%) early under ischemia favored spontaneous SD evolution (CBF <20% vs. >30% vs. ~60%, 2VO SD vs. 2VO no SD vs. 1VO; 29 SDs in 15 mice). SDs occurred in both hemispheres in 14 mice and in one hemisphere in 1 mouse (n=15, 2VO SD) and were absent in 3 mice (n=4, 2VO no SD) in the 2VO group. No SD was detected in the 1VO and SHAM groups. The relative amplitude of functional hyperemia in the 2VO no SD and 1VO hemispheres was similar to that seen in the SHAM group (16±19 vs. 45±35 vs. 40±25%, 2VO no SD vs. 1VO vs. SHAM). However, functional hyperemia was impaired in the 2VO SD hemispheres (10±8%).

Our data demonstrate that SD evolution impairs NVC after AIS. SD is known to trigger long-lasting vasoconstriction, known as spreading oligemia that might diminish NVC function beyond recanalization. We propose the pharmacological attenuation of spreading oligemia to improve NVC function after AIS.

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P1.46 Brain regions activated during inflammatory and non-inflammation phases of chronic pain in a rheumatoid arthritis mouse model

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The K/BxN serum-induced arthritis mouse model is widely used as a human rheumatoid arthritis (RA) model, in which characteristic symptoms including persistent pain occur. Previous results demonstrated that after the arthritogenic K/BxN serum injections joint edema and inflammation are pronounced for 9 days, then decrease, while hyperalgesia persists without inflammatory signs. Chronic pain is suggested to involve central sensitization mechanisms via altered nociceptive processing. Therefore, here we investigated the activation of brain regions of the "pain matrix" on days 9 and 21 reflecting the inflammatory and non-inflammatory phases of this mouse arthritis model.

Activity of brain areas was measured using manganese-enhanced magnetic resonance imaging (MEMRI). Mn^{2+} enters via voltage-sensitive Ca^{2+} channels, accumulates intraneuronally, and reduces T1 relaxation times in a concentration-dependent manner. MRI was performed before and 24 hours after MnCl2 infusion. T1 maps were calculated, T2-weighted images were applied for identifying brain structures. Waxholm Space atlas of the C57BL/6J mouse brain was used to segment brain areas: cortex, thalamus, striatum, nucleus accumbens, globus pallidus, amygdala, central gray of the midbrain, and lateral ventricles.

Approximately 40% mechanical hyperalgesia developed in all mice following K/BxN serum administration on day 2 and lasted for 21 days, edema declined after day 9. No significant differences in baseline T1 relaxation times were observed on days 9 and 21. On day 9, T1 relaxation time was reduced in the globus pallidus, nucleus accumbens and thalamus after MnCl2 administration, compared to the non-arthritic control group. The T1 relaxation time also decreased in these brain regions on day 21, and also in the amygdala in comparison with the controls. Reduced Mn^{2+} accumulation can be attributed to altered transport through the blood-brain barrier and/or to Ca^{2+} dyshomeostasis associated with neuroinflammatory processes. Reduced activity of the thalamus, amygdala, nucleus accumbens and globus pallidus might explain hyperalgesia, presumably via decreased pain control mechanisms.

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P1.47 Capillary pericytes regulate vascular tone and local blood flow in inflammation

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Pericytes are the only contractile cells in cerebral capillaries. However, their role in the regulation of capillary diameter, microvascular tone and local cerebral blood flow is far from being completely understood. Furthermore, a large number of CNS disorders is accompanied by inflammatory processes. Therefore, in our present study, we investigated the role of pericytes in the maintenance of capillary tone and how inflammatory mediators could regulate pericyte contractility.

Using primary human pericytes in an in vitro collagen contraction assay, we could demonstrate that TNFalpha, IL-6 and CCL2 induce a significant pericyte contraction. In order to prove that inflammatory mediators have similar effects in vivo, we used two photon microscopy in mice with labelled pericytes. Inflammatory mediators were administered in the vicinity of identified pericytes using microinjection techniques under continuous monitoring. TNF-alpha induced a slow but significant reduction in the capillary diameter. In addition, using line scan technology, we could show a decrease in red blood cell velocity and a reduction in the number of red blood cells passing the capillary segment in the neighborhood of the injection. Furthermore, careful ablation of pericytes using the two-photon laser led to a late onset (after 24 hours) dilation of the capillary segment belonging to the ablated pericyte.

Our results indicate that pericytes may have an important role in the maintenance of the capillary tone, and may regulate capillary flow under inflammatory conditions.

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P1.48 Complex examination of the pathophysiological mechanisms of pain in fibromyalgia-a human clinical study and rodent experiment

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Fibromyalgia (FM) is a common primary chronic pain condition associated with widespread musculoskeletal pain, predominantly in women. The mechanisms are unclear, but psychosocial distress and anxiety are likely to be the main etiological factors. The therapy is unsatisfactory; therefore, it is an *"unmet medical need".* Studies in translationally relevant animal models are important to identify key mediators. Although an optimal rodent FM model does not exist, chronic restraint stress (CRS)-induced pain in mice characterized earlier by our team seems to be appropriate to investigate the pathophysiological pathways and mechanisms. In the present study we perform unbiased transcriptomic investigation on PBMC and metabolomic analysis on plasma samples of CRS-exposed mice compared to non-stressed controls.

12-week-old female and male C57BL/6 mice are exposed to CRS in ventilated centrifuge tubes for 6 h/day for 2 weeks, age and sex-matched animals without stress kept under standard circumstances serve as controls (n=12-15). During the CRS protocol their general health parameters (appearance, fur condition, stool and urine) are monitored daily and their weight is measured every other day. Pain threshold tests (dynamic plantar aesthesiometry, cold sensitivity in icy water) are performed before the protocol and repeated after the exposure to CRS. Blood samples are collected under deep anaesthesia via cardiac puncture, PBMC is isolated for next generation sequencing, and plasma for mass spectrometry. The data are analysed by complex bioinformatic tools, differentially expressed genes and metabolites are identified, potential pathways, signalling processes and networks are determined. The experiment was approved by the Animal Welfare Committee at the University of Pécs.

CRS induces approximately 20-25% mechanical and cold hyperalgesia on the paw after 2 weeks without any anxiety, depression-like behaviour or locomotor disturbances demonstrating the development of stress-induced pain. The analysis of the samples is still ongoing, the results are planned to be completed by March of 2024. We parallelly perform a study on FM patients with similar unbiased methodological approaches and the final aim is to compare the animal experimental data with the clinical results. We would like to determine the translational value of the mouse model for the investigation of potential pharmacological interventions.

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P1.49 GABAergic synaptic abnormalities in olfactory brain regions of mice with autism-like symptoms

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Dysfunctional sensory systems, including altered olfactory function, have recently been reported in individuals with autism spectrum disorder (ASD). Disturbances in processing odors may be linked to γ-aminobutyric acid (GABA)ergic synaptic abnormalities in the olfactory brain regions. At GABAergic synapses, gephyrin is a key scaffolding molecule that contributes to the maintenance of appropriate excitatory and inhibitory balance by regulating GABA receptor trafficking. However, the precise molecular mechanism by which GABAergic transmission affects the olfactory system in ASD is not fully understood. Therefore, the present study aimed to evaluate selected components of the GABAergic system in olfactory brain regions and primary olfactory neurons isolated from *Shank3*-deficient ($^{-/-}$) mice, which exhibit autism-like behavioral phenotypes. *Shank3* deficiency led to a significant reduction in GEPHYRIN/$GABA_AR$ colocalization in the piriform cortex and primary neurons isolated from the olfactory bulb. Gene expression analysis revealed significantly lower mRNA expression of presynaptic *GABA transporter 1* in the olfactory bulb and *Collybistin* in the frontal cortex of Shank3$^{-/-}$ mice than in WT mice. A similar trend of reduction was observed in Somatostatin expression in the frontal cortex of *Shank3*$^{-/-}$ mice. Overall, it appears that *Shank3* deficiency is associated with changes in GABAergic synapses in brain regions that are important for olfactory information processing, which may represent a basis for understanding the functional impairments in ASD.

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P1.50 Potential and limitations of rat models of trigeminovascular activation

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Primary headache diseases such as migraine are widespread debilitating pain conditions affecting a large population. The pathophysiological mechanisms are not fully understood, but activation and sensitization of trigeminal sensory nerves and neurogenic inflammation via the release of pro-inflammatory neuropeptides in the dura mater play important roles. Since optimal translational models do not exist, it is necessary to characterize and compare different in vivo paradigms to identify key mediators and novel therapeutic targets. Inflammatory and neuropathic orofacial or periorbital pain models are accepted surrogate models for investigating pain mechanisms related to primary headaches. Here we performed functional studies in three rat models of different origin.

Chronic orofacial inflammation and consequent allodynia was induced by subcutaneous injection of Complete Freund's Adjuvant (CFA) into the right whisker pad of adult male Sprague-Dawley rats. Meningeal inflammation and periorbital allodynia was evoked by supradural infusions of an "inflammatory soup" (15 µl) containing 2 mM histamine, bradykinin, serotonin, and 0.2 mM prostaglandin E2. Neuropathic allodynia was induced by partial ligation of the infraorbital nerve (pIONL model). The mechanonociceptive threshold values were measured using von Frey filaments.

In the CFA model approximately 60% of all rats showed allodynia, the threshold values decreased from 18.30 g to approximately 5 g, which lasted for 9 days. In case of the inflammatory soup infusion approximately 45% of the rats exhibited periorbital allodynia shown by mechanonociceptive threshold decrease from 18.30 g to around 12 g, but it was relatively stable throughout the 35-day experiments. In the pIONL model only approximately 30% of the animals developed orofacial allodynia (threshold decrease from 18.30 g to 13 g) and the degree of sensitisation varied greatly between measurements lasting for 16 days.

Trigeminovascular activation-induced allodynia can be tested in all the 3 models, therefore, they can all be used for investigating the pathophysiological mechanisms. Since CFA induced the most severe and stable pain behaviour in the highest proportion of animals, this seems to be appropriate for testing the effects of potential novel antimigraine drugs.

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P1.51 The fractalkine receptor 1 (CX3CR1) mediates hyperalgesia and neuroinflammation the passive transfer-trauma mouse model of complex regional pain syndrome

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Complex Regional Pain Syndrome (CRPS) is a severe, chronic pain condition, which develops after a small injury. The most common symptoms are hyperalgesia, edema and autonomic disorders. Autoimmunity, complex sensory-immune-vascular interactions and neuroinflammation are involved in its pathophysiology. Since its therapy is unsatisfactory, it is necessary to identify novel therapeutic targets. We investigated the role of the fractalkine inflammatory chemokine receptor 1 *(CX3CR1)* expressed on microglia and macrophages in our previously developed and characterized CRPS passive transfer-trauma mouse model.

Female C57Bl/6 mice were treated i.p. daily with purified IgG from CRPS patients or healthy volunteers. Plantar skin-muscle incision was performed to model the microinjury. The role of the CX3CR1 receptor was investigated by gene-deficient mice and the selective receptor antagonist AZD 8797 (80 µg/kg i.p/day). The paw mechanonociceptive threshold was measured by dynamic plantar aesthesiometry and volume by plethysmometry, astrocyte and microglia in pain-related central nervous system regions by glial fibrillary acidic protein (GFAP) and ionized calcium-binding adapter molecule 1 (Iba1) immunohistochemistry.

CRPS IgG significantly increased plantar incision-induced mechanical hyperalgesia by 40-50% throughout the 7-day experiment. Both CX3CR1 deficiency and antagonist treatment significantly reduced CRPS IgG-induced mechanical hyperalgesia-increase. Genetic deletion of CX3CR1 reduced microglia activation and increased astrocyte activation in the periaqueductal gray matter and the somatosensory cortex in both treatment groups compared to wild-type mice. Antagonist treatment did not affect the levels of reactive microglia, but reduced the number of astrocyte cells in the periaqueductal gray matter.

CX3CR1 activation mediates CRPS-associated chronic pain presumably via neuroinflammation. Therefore, CX3CR1 inhibition may represent novel analgesic perspectives in this primary chronic pain condition.

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P1.52 Topiramate inhibits adjuvant-induced orofacial allodynia in the rat: pharmacological validation of the inflammatory trigeminovascular activation model

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Orofacial pain is a common condition radiating to the head, face, and neck, which develops due to the sensitization of extra- and intracranial trigeminal primary afferents. Orofacial inflammation activates and sensitizes the peptidergic sensory nerves, which induces mechanical allodynia/hyperalgesia. Since there is no appropriate therapy in a great proportion of patients, investigating the mechanisms and identifying novel therapeutic targets is important in validated animal models. Here we aimed to investigate the effects of the antimigraine drugs sumatriptan (5-$HT_{1B/1D}$ receptor agonist) and topiramate (voltage-gated Na^+ channel blocker), as well as the adjuvant analgesic gabapentin (mainly voltage-gated Ca^{2+} channel blocker) in the rat Complete Freund's Adjuvant (CFA)-induced orofacial pain model.

Orofacial inflammation was induced in adult male Sprague-Dawley rats (300-350 g) by CFA injection (0.5 mg/mL, 50 µL s.c.) into the right whisker pad. Allodynia was measured by von Frey filaments. The effects of sumatriptan (1 mg/kg s.c., 0.1 mL/100 g volume), topiramate (30 mg/kg p.o., 0.5 mL/100 g volume), and gabapentin (30 mg/kg p.o., 0.5 mL/100 g volume) were tested on the mechanonociceptive threshold values (on days 3, 5, and 7), 60, 120, and 180 minutes after the treatments.

Approximately 60% of all rats developed remarkable decrease in mechanonociceptive thresholds after CFA injection, this allodynia was stable and reproducible throughout the measurements. Topiramate showed approximately 60% anti-allodynic effect compared to the vehicle-treated group on all experimental days, which proved to be statistically significant on days 5 and 7. In contrast, neither sumatriptan nor gabapentin altered CFA-induced allodynia in any investigated doses.

The CFA-induced orofacial pain model is appropriate to study allodynia related to chronic inflammatory trigeminovascular activation and to evaluate the effect of novel drug candidates in comparison with topiramate, as the reference compound.

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P1.53 Involvement of spinal cholinergic transmission in tetanus toxin-evoked local muscle spasm

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Propriospinal cholinergic transmission in the ventral horn is represented by C-boutons, cholinergic synapses derived from V0C excitatory interneurons, which synapse onto motoneurons with post-synaptic M2 muscarinic receptors taking part in afterhyperpolarization. This, in turn, sustains the motoneuronal activity during high-performance motor tasks such as swimming. However, its possible role in sustained or intermittent muscle hyperactivity evoked by disinhibition of ventral horn motoneurons has not been examined so far. In present study we examined the modulatory effect of spinal cholinergic transmission on experimental muscle spasm evoked by GABA/glycinergic synapse blocker tetanus neurotoxin (TeNT).

The rats were injected with saline, low TeNT dose (33 pg), and high dose TeNT (100 pg) into the bilateral gastrocnemius. The role of spinal cholinergic neurotransmission was examined by low dose lumbar spinal intrathecal (i.t.) cholinesterase blocker neostigmine (5 µg/10 µL) or M_2 muscarinic antagonist AQ-RA 741 (20 µg/10 µL). The muscle spasm intensity was assessed by measuring resistance to passive dorsiflexion (needed to achieve 90 ° tibiotarsal angle).

The injection of highly purified TeNT into the gastrocnemius resulted in dose-dependent moderate to very strong spastic paralysis. The short-term pharmacological blockage of acetylcholine esterase (AChE) resulted in elevated resistance to ankle dorsiflexion) in control animals and aggravated the pre-existing spasm in low dose TeNT-treated rats (approx 40% elevation; $p<0.05$ compared to saline i.t.). The M_2 blockage exhibited tendency but did not significantly reduce the low -dose TeNT-evoked spasm (n.s.), while it significantly reduced the muscle spasm in high dose TeNT-injected rats (approx. 50 % reduction, $p<0.001$ compared to saline i.t.).

The short-term upregulation of cholinergic transmission by blockage of spinal AChE augments the TeNT-evoked mild spasm, while blockage of M_2 muscarinic receptors results in amelioration of high-dose TeNT-evoked spasm. These results point to the possibility that the spinal cholinergic interneurons participate in the maintenance of increased muscle tone both in normal and spastic conditions. This has important implications for the possible mechanisms of actions of centrally-acting anticholinergic drugs, and botulinum toxin A whose central action could be partly mediated by the spinal cholinergic system.

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P1.54 Bridging in vivo and in vitro recordings in the human epileptic neocortex: patient-wise comparative analysis of single-unit activities

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Epilepsy affords an excellent opportunity to explore cellular electrophysiology in humans, both in vivo and in vitro conditions. While long-term, single-site in vivo recordings might offer advantages in action potential pattern reconstruction and in the preservation of neuronal connections, in vitro electrophysiology provides a broader tissue exploration, but within a restricted space comprising neurons with cut connections. Both paradigms yield valuable insights into cellular electrophysiology, yet coherent investigation of neuronal firing patterns in these distinct conditions is lacking. In this study we compared the firing properties of single neurons recorded in the same patients, both in vivo and in vitro conditions.

An identical recording system was used to conduct in vivo and in vitro intracortical recordings comprising a 24-channel laminar microelectrode with 150 µm distance between contacts, in epileptic patients (n=4) as well as in their postoperative tissue slices. We employed spike sorting algorithms docked by spikeinterface (tridesclous, spykingcircus2, and pykilosort) for single-unit analysis. We performed analysis of unit waveforms, correlograms, firing rates and burstiness index, as well as determined the excitatory and inhibitory feature of the single neurons.

Identifying 330 single neurons in vitro and 22 in vivo, we observed a significantly higher overall firing rate for in vitro (2.738 Hz) than in vivo cells (1.321 Hz, $p<0.0001$). The firing rate of principal cells was not different (2.71 Hz in vitro vs. 2.73 Hz in vivo), while interneurons discharged with a higher rate in vitro, than in vivo (2.93 vs. 0.74 Hz). The burstiness of all cells was significantly higher in vivo (6.986%) than in vitro (3.506%, $p=0.0006$). Patient-wise in vivo and in vitro unit numbers correlated strongly ($r=0.703$). Layer-specific analysis revealed high correlations (0.926 for firing rates, 0.762 for burstiness indices).

Disparities in firing rates and burstiness highlight the differences between in vivo and in vitro conditions. In vitro neurons, especially inhibitory cells with limited synaptic connections show higher activity than those in the intact neocortex. The lower burstiness in vitro might also be linked to the partial loss of synaptic inputs, but the different environment provided by the bathing solution might also account for the observed differences.

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P1.55 Decoding Maladaptive Fear: A Whole-Brain Activity Mapping Approach to Network Alterations

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Traumatic life events can lead to post-traumatic stress disorder (PTSD), a psychiatric condition that manifests as a persistent avoidance, hyperarousal and fear generalization to safe contexts, which are orchestrated by coordinated activity of complex brain networks. Although specific brain regions, i.e. the prefrontal cortex, hippocampus, and the amygdala have been identified as essential brain regions involved in PTSD, understanding complex brain network alterations is still missing, which requires a comprehensive assessment of brain-wide network functions related to maladaptive fear.

In this study, we exposed adult male rats to an uncontrolled series of footshocks as a traumatic experience and measured long-term (4 weeks) fear generalization outcomes in a safe context. We classified vulnerable and resilient subpopulation as rats exhibiting the highest and lowest fear response (upper and lower 25%). To investigate the neuronal activity across the whole brain, we used immunohistochemical labeling with the neuronal activity marker c-Fos, co-labeled with inhibitory and excitatory neuronal markers.

Brain mapping was carried out using custom-generated delineations from the Waxholm Space Atlas, followed by systematic quantification of neuronal activations by region. Besides well-characterized brain regions, our results identified novel major network elements and potential hubs that could contribute to altered fear learning and expression in PTSD, such as the retrosplenial cortex, insular cortex, or reticular thalamic nucleus. Furthermore, by comparing cross-regional activity patterns we could reveal more distinct modularity, suggesting elevated intra-modular functional connectivity inside modules in the case of the resilient group. The graph model generated connectivity possessed higher degree nodes in the resilient group compared to the vulnerable group, which was also characterized by elevated centrality measures. This model may help us understand the complex network alterations underlying fear generalization in PTSD.

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P1.56 Analgesic effect of combined capsaicin-diclofenac containing transdermal therapeutic system (TTS)

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Pain is the leading cause of human suffering and disability worldwide and is one of the most common reasons for seeking medical care. The activation of capsaicin-sensitive sensory nerves via the Transient Receptor Potential Vanilloid 1 (TRPV1) receptor plays an important role in the pathogenesis of several pain conditions. In the medical practice, the TRPV1 agonist capsaicin is usually applied in form of ointment or high-concentration (8%) transdermal patch (transdermal therapeutic system, TTS). However, these formulations cannot ensure precise dosing or cause temporary loss of nerve function, respectively. We have previously developed a silicone polymer matrix-based TTS ensuring not only the precise dosing of the compound but also providing sustained release of that. Moreover, the capsaicin-induced increase of local microcirculation might facilitate the absorption of non-steroidal anti-inflammatory drugs (NSAIDs), e.g. diclofenac. Therefore, we aimed to investigate the analgesic effect of silicone-based polymer matrix TTS containing capsaicin in low-concentration (<1%), or diclofenac or the combination of them in rat models of acute postoperative and inflammatory pain.

Acute postoperative pain was elicited with plantar skin-muscle incision and thermal hyperalgesia was assessed with increasing temperature water bath 18 h after surgery, as well as 2.5 h and 6 h after the application of the TTS. Acute inflammatory pain was induced with carrageenan (3%, i.pl.) and mechanical hyperalgesia was determined with dynamic plantar aesthesiometer 3 h after carrageenan treatment, as well as 2.5 h and 6 h after the application of the TTS.

Thermal hyperalgesia was decreased 2.5 h after the application of diclofenac-containing TTS and 6 h after the application of capsaicin-containing TTS. In the case of combined capsaicin-diclofenac containing TTS, thermal hyperalgesia was reduced both 2.5 h and 6 h after TTS application. Mechanical hyperalgesia was decreased 6 h after capsaicin TTS, as well as 2.5 h and 6 h after diclofenac and combined TTS.

Formulated in the silicone-based polymer matrix TTS, low-concentration capsaicin can alleviate acute postoperative pain, moreover, it can prolong the short-term analgesic effect of diclofenac, if used in combination. Furthermore, combined capsaicin-diclofenac containing TTS is also effective in the relief of acute inflammatory pain. The combined capsaicin-diclofenac containing TTS might be a promising therapeutic tool in various pain states.

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P1.57 The involvement of transient receptor vanilloid 1 (TRPV1) receptor in chronic restraint stress induced pain

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Fibromyalgia (FM) is a chronic pain condition that affects at least 200 million people worldwide. The pathogenesis of the disease is unclear, but chronic stress is a well-known factor in its development. The TRPV1 receptor is a non-selective cation channel expressed mainly in capsaicin-sensitive primary sensory neurons and activated by a variety of stimuli including noxious heat, as well as a number of exogenous and endogenous chemicals.

However, its contribution both in development of chronic stress and stress-induced nociception are not cleared yet. In our study we investigated the involvement of TRPV1 in chronic restrain stress (CRS) induced fibromyalgia-like mouse model.

Female TRPV1 gene-deleted and C57/Bl6 wildtype mice (WT) (10-12 weeks, 18-23 g) were used, on which stress was applied by restraining them for 6 hours daily for 2 weeks. Changes in the cold and mechano-nociceptive thresholds were determined by using cold tolerance test or dynamic plantar aesthesiometry. Behavioral tests (open field (OFT), light-dark box (LDB) and thymus and adrenal-gland weighed were performed at the end of the experiment.

CRS induced 45-50% cold sensitivity in the WT groups, which was significantly lower in the TRPV1 KO mice in both timepoints. Stressed WT animals had a significantly larger mechanical hyperalgesia at week 2 than the stressed TRPV1 KO group. Non-stressed WT mice spent more time in the center zone in the OFT compared to the non-stressed KO animals. No significant alterations were observed in the weight of thymus/adrenal-gland between the groups.

According to our results the mechanonociception and cold sensitivity of the stressed TRPV1 mice were not influenced by the restraining stress. This is strengthened by the results of the behavioral tests, and the weighing of the thymus/adrenal-gland. Our results suggest that TRPV1 might have a role in the pathogenesis of the stress-induced disorder, fibromyalgia.

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P1.58 N,N-dimethyltryptamine shows a protective effect against oxygen-glucose deprivation in a culture model of the blood-brain barrier

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N,N-dimethyltryptamine (DMT) is an endogenous ligand of the Sigma 1 receptor which has *in vivo* cytoprotective properties against hypoxia. The blood-brain barrier (BBB), a dynamic interface between the blood and the central nervous system, plays a crucial role in the protection of the brain. Disruption of the BBB is a key factor in the development of several neurological symptoms in stroke. Earlier studies described a beneficial, protective effect of DMT in a rat stroke model, but BBB functions were previously not studied in the same context. Our aim was to examine the effect of DMT on the functions of neurovascular unit forming cell types following oxygen-glucose deprivation and reoxygenation (OGDR) in a rat primary co-culture BBB system.

To model stroke *in vitro,* OGD was introduced for 6 hours on the BBB model, followed by reoxygenation for 24 hours with or without the addition of DMT. First, impedance measurement was performed to test the effect of OGDR on primary rat brain endothelial cells, glial cells and pericytes. Metabolic activity (MTT) of the endothelial cells after the OGDR was also quantified. To examine the barrier functions of the BBB after OGDR with or without the addition of DMT a triple co-culture model on cell culture inserts was used. Transendothelial electrical resistance (TEER), sodium fluorescein (SF) and Evans blue-labelled albumin (EBA) permeability was measured, and claudin-5 immunocytochemistry performed. After the OGDR treatment supernatants were collected from both compartments to measure cytokine release.

Impedance of brain endothelial cells, pericytes and glial cultures decreased in response to the OGD, but this sensitivity showed different kinetics. Impedance of primary rat brain endothelial cells was the only one recovering to the control level at 24 h reoxygenation. The endothelial cells also showed metabolic changes during the OGDR. Barrier functions were decreased and the proinflammatory cytokine release was increased after the OGDR. The DMT treated groups showed improved barrier functions and a decrease in cytokine release.

In conclusion DMT was protective against OGDR-induced metabolic damage in rat primary brain endothelial monocultures. In the BBB co-culture model OGDR decreased barrier properties as measured by reduced TEER, permeability and junctional discontinuity. DMT treatment showed a protective effect against OGDR-induced barrier damage in the rat BBB co-culture model.

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P1.59 Phosphoproteomic analysis reveals post-translational dysregulation in Huntington's disease patient derived induced neurons

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Huntington disease (HD) is a heritable fatal neurodegenerative disorder with rising prevalence characterized by severe symptoms but no successful treatment available. HD is caused by CAG expansions in the huntingtin gene, leading to protein aggregation. It is challenging to study HD due to the lack of appropriate model systems that can capture human ageing. Our group studies HD using a novel induced neuronal (iN) model which serves as a unique patient-derived cellular system for age-related neuronal disease modeling. iNs uniquely maintain the aging, epigenetic and genetic features of the donor. Impairments in autophagy – an ubiquitinal lysosomal protein degradation pathway indispensable for protein homeostasis and cellular function – seems to play a critical role in the neuronal death in HD. However, understanding of how alterations in autophagy causes cellular dysfunction and death is lacking.

In this study, we established an iN model to study impairments in HD at the proteome level with special interest in autophagy-related pathways.

We performed mass spectrometry (MS) and phospho-MS measurements of iNs generated from HD-iNs and healthy donors (Ctr-iNs). We gained information about protein abundance and activity from MS and phospho-MS data, respectively.

The bioinformatic analysis revealed 21 proteins with no detectable activity in HD-iNs (ON/OFF proteins). These proteins showed no significant differences in the RNA level or in the protein abundance, ensuring that HD specific alteration happened post-translationally. The radical difference in ON/OFF proteins suggest they play critical role in HD. We chose one of the ON/OFF proteins, MXRA8 for further investigation. MXRA8 is significantly more abundant in HD-iNs but shows no detectable activity. Strikingly, MXRA8, which primarily localizes to the cell membrane, shows pronounced nuclear localization in HD-iNs. The function of this protein in neurons is unknown and not yet studied before. We will perform CRISPR modification experiments to silence (CRISPRi) or enhance (CRISPRa) MXRA8 to gain new information about their role in neural function. We further analyze the kinases and phosphatases responsible for MXRA8 phosphorylation to uncover the cause of MXRA8 inactivity in HD-iNs.

Our main purpose in this project is to identify novel key protein targets dysregulated in HD which can serve for future therapeutic strategies.

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P1.60 A mouse model of chronic fatigue syndrome validated by behavioural and hormonal changes

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We have tested a model of chronic fatigue syndrome (CFS) on Bl/6 mice, by modelling the disease with injecting 5 mg/kg of synthetic RNA-analogue poly(I:C) to ten adult male mice and comparing the results to those obtained with ten mice treated with physiological salt solution. To validate the model, we followed the body weight, sucrose solution consumption and voluntary wheel running of the animals in a three-week long experiment, drew blood samples by tail nipping for corticosterone-level determination the day before treatment, half a day, 3.5 days, and 9.5 days after treatment and for β-endorphin-level determination after termination, when we also collected the thymus, hypophysis and hypothalamus for the estimation of long-term changes in corticosterone level and for TSH- and TRH-, along with CRH-level determination, respectively. Time spent on voluntary wheel running in 30-minute experiments carried out in the animals' home cages decreased up to five days after treatment in the treated group, although the body weight normalized to the value measured on the first day for each animal kept increasing throughout the study and we found no significant difference between the treated and the control group' sucrose solution consumption, implying that the activity of the animals treated with P(I:C) decreased while their general condition was not significantly affected. Evaluation of the other collected data to explore changes in the HPA and HPT axes is in progress. In the future, we would like to use this model to study the role of the purinergic P2X7 receptors in CFS, by comparing wild type and P2X7 receptor knockout animals.

P1.61 Retinal cell mosaics in the valproate-induced rat model of autism spectrum disorder

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Valproic acid (VPA) is a widely used antiepileptic drug that is also teratogenic, increasing the risk of neurodevelopmental disorders in the offspring of mothers exposed to VPA. Prenatal VPA treatment is used in an established preclinical model of autism spectrum disorder (ASD). VPA is given at a time of intense early retinal neurogenesis. Moreover, anatomical and functional alterations of the retinas of such animals have occasionally been described in the literature. Therefore, we were interested in whether the density or regularity of horizontal cells, parvalbumin (PV)-positive amacrine cells including AII, or S-cones are altered in adult rats following prenatal VPA exposure. Gestating female Wistar rats were given 500 mg/kg valproic acid on day 12.5 of gestation and their male offspring was tested for the presence of autistic-like behaviour using 3-compartment social interaction test and elevated plus maze test for anxiety. The control group was the offspring of sham-treated females. Whole mount retinas of 2 months old autistic (n=5) and control (n=3) animals were immunolabelled for PV, Prox1 and S-cones and confocal image stacks of the inner nuclear layer (INL) and photoreceptor outer segments were recorded. Within the INL, horizontal cells, AII amacrine cells and PV-positive widefield amacrine cells were identified. Densities of horizontal cells and both types of PV-positive amacrines were significantly correlated with each other (AII, r=0.54; widefield, r=0.57 versus horizontal cells) while S-cones showed independent variation. To account for regional differences in the retina, the covariation of cell densities was included in a general linear model, and the effect of VPA treatment was tested. Here, VPA exposure resulted in significantly lower ($p<0.05$) density of AII amacrine cells. In another analysis, the regularity of each cell mosaic was analysed by using the regularity index (RI). RI is defined as the ratio of the mean nearest neighbour distance to their standard deviation. VPA model rats showed lower RIs for AII amacrine cells ($p<0.05$, two-sample t-test) but not for the other cell types. Altogether, our results suggest that the development of AII amacrine cells is specifically affected in the VPA-induced rat model of ASD. This finding is compatible with a disturbance of the scotopic (rod) system, in which AII amacrine cells are a key element.

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P1.62 Abstract title: Investigation of autism spectrum disorder using principal component analysis in a rat model of autism

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Autism is a neurodevelopmental disorder characterized by impaired social communication, limited interest and repetitive behavior patterns. On the neuronal networks level, autism is associated with loss of excitation-inhibition balance and increased risk of developing epilepsy.

The valproic acid (VPA) rat model is commonly used to study autism-like behaviors. Prenatal VPA exposure increases the risk of developing autism in offspring.

In our study, pregnant Wistar rats received an i.p. injection of VPA on gestation day 12.5 (dose 500 mg/bwkg). Pups were tested for various motor functions and behaviors from day P3 to week 6. Then, electrophysiological studies were performed in two age groups: 6 weeks and 3 months. Brain slices were prepared from the offspring for field potential measurements and parallel detection of intrinsic optical signals. Excitability changes were tested with spontaneous bursts evoked by Mg-free solution (MFR) and afterdischarges (ADs) evoked by brief bursts of high frequency electrical stimulation.

Autism is a spectrum disorder, meaning that it can manifest very differently in different individuals. The rodent VPA model seems to reproduce this aspect of the disorder, as alterations manifest to a different degree in each treated animal. Taking into account the results of behavioral tests and the severity of the malformations, we divided the treated animals into two groups (strongly vs weakly autistic) using principal component analysis.

Behavioral tests indicate that strongly autistic animals were able to perform surface righting reflex, negative geotaxis, auditory startle and visual placing reflex significantly later than the other VPA-treated and control rats. The results of social interaction tests indicate a stronger social defect in treated male rats.

VPA treatment showed an increase in seizure activity, suggesting an increased tendency to epilepsy. AD threshold was lower in strongly autistic group than in weakly autistic group and control slices. The length of ADs and burst length were higher in VPA-treated groups. In MFR, burst length and frequency were higher in treated groups than in control.

Tail and limb deformities in VPA-treated rats correlated with autism severity: the malformations were predictive of developmental delay, and electrophysiological data suggest increased neuronal excitability in strongly autistic rats compared with the other treated rats. Before drawing final conclusions, further analysis of the data is needed.

The present study was supported by CELSA (grant no. 874735) and Gedeon Richter Plc. Centenarial Foundation.

Poster Session 2
Novel techniques

P2.01 High-sensitivity quantification of anti-AAV neutralization from preclinical model and human sera

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Adeno-associated virus (AAV) vectors are the principal vehicle for gene therapy. In a large share of humans, immunity against AAVs can reduce or completely inhibit transgene expression, and can lead to adverse outcomes. The level of immunity can be estimated by assaying AAV neutralization level of the blood serum. Surprisingly, there is no widely adopted sensitive assay for the quantification of AAV neutralization.

We developed an *in vitro* neutralization assay, and quantified its reproducibility and sensitivity via longitudinal evaluation of neutralization in preclinical models and humans. Compared to established protocols, our method exhibits lower variability and reports stronger neutralization levels when tested on the same serum samples.

Our results may offer readout modality of neutralization with superior accuracy and reproducibility, thereby serving as a solid foundation for safety assessment of new AAV constructs.

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P2.02 Optogenetic stimulation monitored with functional imaging in large brains

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Long-term monitoring and modulation of cortical activity in large-animal models remains difficult as there is no single method that can bridge scales from single neurons to whole brain. In mice, mesoscale multiphoton imaging can report activity virtually across all cortical layers and over multiple cortical areas. In large-animals, standard mesoscale imaging options (e.g., fMRI) have limited compatibility with other modalities that could probe activity at finer spatial and temporal scales.

Here we show the results of our developments that allow monitoring activity upon natural or artificial optogenetic stimulation in deep-cortical areas in a large-animal species, cats. We evaluate the stability of our method in multiple cats for several months, in terms of data variability and overall stability of the implant.

Our results demonstrate that multimodal access to large-brains is feasible in longitudinal experiments, for close to a year. Multimodal access to neuronal activity in large brains may become an essential component in translational neuroscience studies.

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P2.03 Screening AAV delivery routes, capsids and promoters for cortex-wide functional and long-term stable access to brain function in large animal species

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In neuroscience, a central question revolves around understanding how signals relevant to behavior and cognition are processed within neural circuits, engaging thousands of neurons across multiple brain regions. Despite over a century of research, our current comprehension of brain-wide neural circuits enabling various brain functions in large-animal species (e.g., cats, primates) remains limited. In stark contrast, significant progress has been made in dissecting mouse brain function, enabled by the availability of transgenic driver and reporter lines.

We set out to emulate the quality and reproducibility of transgenic mouse reporter lines in large-animal models by establishing a long-term stable gene delivery method that achieves functional protein levels.

We quantified transduction efficiency upon screening a set of constructs in the cat brain. We identified one construct that yields brain-wide labeling upon a single injection.

We provide a precise, highly reproducible brain-wide transduction method that has not been available up to now, one that approximates the qualities attributed to transgenic reporter mouse lines. Using this method, genetically targeted dissection of both local and brain-wide functional circuits may gain broad application in large-animal models.

P2.04 Are cortical columns ubiquitous? High-resolution identification of functional domains in cat visual cortex using 3D functional ultrasound imaging

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In the visual cortex of carnivore and primate model species, elementary visual features like stimulus orientation or motion direction are organized into functional maps. It remains unclear how the layout and interrelations of cortical maps observed from surface layer activity extend into deeper layers of the cortex. Electrophysiology allows for precise temporal resolution, but it falls short in providing comprehensive spatial coverage, impeding a holistic comprehension of functional organization. On the other hand, optical imaging techniques have contributed valuable insights into cortical organization, but they are limited to < 1 mm penetration depth, which translates to the coverage of ~layer 2/3 in large brains. Here we ask whether the existence of canonical columnar functional architecture can be confirmed using high-resolution 3D activity imaging.

We performed longitudinal 3D functional ultrasound imaging from a large part of the cat visual cortex. We analyze stability and spatial clustering of 3D architecture of functional domains across the visual cortex and identify rules governing the layouts of distinct functional maps.

Comprehensive identification of the functional architecture in the visual cortex at unprecedented coverage and resolution provides an alternative perspective on the classical columnar notion of functional architecture in the cat visual cortex.

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P2.05 High-resolution 3D mapping of cat visual cortex using functional ultrasound imaging

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Unlike optical imaging methods, functional ultrasound imaging (fUSI) can record neuronal activity down to 20 mm deep in the brain, at orders of magnitude higher resolution than that of functional magnetic resonance imaging.

We developed a complete acquisition, analysis and visualization pipeline that allows efficient identification of 3D anatomical and functional layout of the brain. We evaluated mesoscale functional map quality along orthogonal acquisition slices across tens of mm in the cat visual cortex.

Our fUSI pipeline allows capturing cortical functional maps at unprecedented resolution in space and time, demonstrated via reconstructing the retinotopy in visual areas in the cat. FUSI can be readily combined with additional modalities like recording high-density electrophysiology or optogenetics, thus our method offers a versatile access to 3D canonical functional maps in large-animal species.

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P2.06 Autofluorescence in large brains: origins, elimination method and quantitative analysis

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Fluorescence is the modality of choice for highly specific readouts from biological processes, from whole organism down to the scale of single molecules. While the origins and utility of endogenous and exogenous fluorescence signals can be identified, it remains practically challenging to separate the two signal sources. Here we present a comprehensive catalogue of sources of endogenous fluorescent signals in large-animal and human brains and introduce a generic method that effectively attenuates endogenous fluorescence to allow the detection and quantification of exogenous sources of fluorescence.

We combined several autofluorescence decreasing (AFD) techniques with two- and three-step fluorescent stainings to increase the exogenous over the endogenous signal in virally injected cat and monkey brain as well as human organotypic cultures. Green fluorescent protein (GFP)-based exogenous and autofluorescent signals were identified, quantified, including validation via immunoperoxidase reaction.

Distinct sources of autofluorescence were identified, such as lipofuscin, hemosiderin puncta, red blood cells, signs of trauma, background fluorescence and neurons with bright cell body and dendrites. Fluorescent staining increased the GFP signal mainly in the cell bodies and dendrites. AFD techniques largely reduced autofluorescence, but several also vanished the axonal signal, or made sections wavy and fragile.

High-intensity, broadly dispersed autofluorescent signals make the detection of the specific fluorescent signals difficult in large animals and humans. We developed a protocol which offers a scalable toolset for accurate characterization of exogene expression patterns in large-animal and human brain samples.

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P2.07 Optimal inter-electrode distances for spike sorting in different brain regions

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Spike sorting stands out as a crucial step in extracting valuable information from extracellular multichannel electrode recordings. While considerable effort has been invested in developing new and more efficient sorting algorithms, relatively less attention has been given to the arrangement of electrodes, a factor that undeniably influences the sorting process's effectiveness. In this study, we systematically investigate the dependency of spike sorting efficiency on inter-electrode distance. We assess the performance in terms of good-quality clusters per electrode channel across different brain regions, various species, and employing two distinct spike-sorting algorithms (Kilosort 1 and 2). Contrary to the assumption that higher electrode density inherently leads to more efficient sorting, our results demonstrate otherwise. Instead, we identify optimal inter-electrode distances for linear probes in the rat neocortex, hippocampus, and thalamus, as well as for the mouse neocortex. These findings highlight the significance of species- and tissue-specific optimization of electrode designs, showing a substantial increase in the yield of high-quality units in extracellular multielectrode recordings.

The research leading to these results has received funding from the Hungarian Brain Research Program Grant (NAP2022-I-2/2022). R.F. was supported by the Bolyai János Scholarship of the Hungarian Academy of Sciences and by the ÚNKP-23-5 New National Excellence Program of the Ministry for Culture and Innovation (ÚNKP-23-5-PPKE-128). This research was also supported by the Hungarian National Research, Development, and Innovation Office, under grant number K135837 (Z.S.), the Hungarian Research Network (HUN-REN) under grant number SA-114/2021 (Z.S.).

P2.08 Cell- and layer-type specific intracortical effects of continuous infrared neural stimulation revealed by high-density laminar recordings in the rat neocortex

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Infrared (IR) neuromodulation research has consistently shown temperature to be an important neuronal state variable over the last decade. Multiple studies have described the ability to stimulate or block peripheral nerve activity with IR radiation. The promise of IR inhibition in treating neurodegenerative diseases, such as epilepsy, underscores the significance of further elucidating its biophysical mechanism.

The effects of IR neuromodulation on cortical neurons in vivo were examined in this study via high-density laminar recordings. The neocortex of anesthetized rats was exposed to pulsed and continuous infrared light (1550nm) using a photonic microtool. Over 7500 single units were recorded from 8 rats using a Neuropixels probe.

Putative principal neurons and inhibitory interneurons with suppressed or increased activity were identified, highlighting cell- and layer-specific responses. We analyzed the temporal dynamics during stimulation trials and their correlation with temperature changes. Pulsed light preferentially excited units over suppression, while continuous light tended to suppress. The temperature increases varied with frequency and were correlated with the number of responsive units. Examining alterations in the baseline firing rate across trials indicated a long-lasting effect of stimulation, maintaining the excitability of the affected neurons at an elevated level. Furthermore, analysis of individual neuron responses at varying frequencies revealed diverse patterns.

This study offers new insights into the mechanisms of infrared neuromodulation by accurately characterizing layer- and cell-type-specific responses. By analyzing thousands of neurons, our findings on neuronal sensitivity to infrared irradiation parameters may assist in optimizing future applications.

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P2.09 Improving the functionality of microscale electrodes implanted in neural tissue with upconverting nanoparticles

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In this research, the primary focus was on the development and characterization of a drug delivery system. This system integrates a micro electrocorticography array with upconverting nanoparticles (UCNP) to create a surface-modified, light-triggerable nanosystem for potential application in nerve tissue.

The methodology involves using a soft micro ECoG with a silicone rubber substrate and Pt/Ir measuring sites. The electrode surface undergoes a sequence of treatments, including exposure to oxygen plasma and the binding of azide groups. Simultaneously, the UCNP exterior, composed of rare earth materials like ytterbium and erbium, undergoes modification with an aminosilane layer and a BCN-NHS linker compound. This linker compound facilitates the attachment of the UCNPs to the electrode surface. A tetrazine-kumarin-rhodol-based dye is then applied to the surface, forming a bond with the UCNPs. This bond can be cleaved by the upconverted light, enabling controlled drug release.

Various analytical techniques, such as Fourier-transform infrared spectroscopy (FTIR), contact angle measurements, scanning electron microscopy, and electrochemical impedance spectroscopy, are employed to assess the success of the surface functionalization process and the stability of the molecular bonds.

Results indicate successful covalent bond formations, confirmed by peaks in the FTIR spectra, and the hydrophilicity/hydrophobicity changes of the electrode surface through contact angle measurements. Morphological analysis using scanning electron microscopy reveals satisfactory particle distribution even after mechanical testing. Electrochemical impedance spectroscopy suggests insignificant magnitude differences between modified and unmodified ECoGs, allowing for future electrophysiological measurements. Model drug release is evaluated using UV/Vis spectroscopy. The chosen dye exhibits fluorescence upon light-triggered cleavage of the linker bond.

Looking ahead, we plan to apply this system to in vitro rat brain slices, then progress to in vivo implantation in rats, and finally conduct long-term drug release analysis using two-photon microscopy. The ultimate objective is to transition from model dyes to real drugs, enabling the evocation and measurement of local neural responses.

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P2.10 Towards layer-by-layer infrared neuromodulation: presentation and functional characterisation of an intracortical optrode needle featured with a micromirror tip ending

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The application of infrared (IR) light irradiation as a neuromodulation technique has been proven as safe and applicable in numerous studies. Either the pulsed or the continuous wave mode of IR stimulation (INS) showed promising neural responses under various conditions in vitro and in vivo as well. One of the advantages of INS compared to the classical electrical stimulation is that INS does not induce photoelectric artefacts in electrophysiological recordings. Another advantageous property of INS is that the propagation of light can be shaped easier than in case of electrical signals. Therefore, the stimulus can be more directional, the neuromodulation impact can be localized.

In this work we present an intracortical IR optrode needle, that can be implanted into the brain tissue and performs optical stimulation and multi-site electrophysiological recording simultaneously. This silicon (Si) based microimplant has two modalities integrated into a single device: extracellular electrophysiological sensing and IR waveguiding. The needle-like Si shaft (0.19×0.17 mm) of the optrode holds 16 platinum (Pt) recording sites (900 µm2) with 100 µm inter-site distance. This multimodal probe can even be implanted to layers deeper than 4 mm in the tissue. IR waveguiding property is embedded into the Si substrate material of the same shaft. The optrode's shaft ends in a parabolic micromirror. This tip shape aims to direct the outcoupled IR light laterally towards neighbouring tissue, therefore more photons get absorbed closer to the recording sites causing the positioning of the maximum heating effect in the vicinity of Pt recording sites.

The proposed work shows the outcomes of numerous different characterisation methods to demonstrate the in vivo applicability of this optrode. Various optical investigations and thermal tests were made to calibrate the optically induced heating preceding the in vivo use. The first in vivo tests were made in the somatosensory cortex of anaesthetised rats. Our findings owing to the IR illumination in the recorded extracellular electrophysiological data are presented from various aspects.

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P2.11 A Novel 3D-Printed Micro-Drive System for Infrared Neuromodulation and Electrophysiological Recording in Freely Roaming Rodents

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This work presents a novel micro-drive system designed to facilitate long-term investigations into rodent neural activity through the combined use of infrared neuromodulation and electrophysiological recording. Our innovative approach comprises a 3D-printed micro-drive, securely attached to the rodent's skull, along with a protective head stage to ensure the integrity of the micro-drive and the attached optical electrode (optrode) during unrestricted movement of the animal.

The utilization of cost-effective 3D printing technology in our fabrication process not only highlights the affordability of our system but also demonstrates its flexibility to suit an extensive range of experimental requirements. The micro-drive interface serves as a precise tool, enabling researchers the ability to exercise fine control over the penetration depth of the optrode. This capability enables targeted neuromodulation and simultaneous electrophysiological recordings in freely moving rodents, providing a unique opportunity to explore the dynamics of neural circuits in a naturalistic context.

This micro-drive's unique modular design promotes the reusability and reimplantability of the optrode, thereby facilitating multiple experiments over an extended period of time. Several tests have been conducted to evaluate the accuracy and durability of the micro-drive, considering both physical and biological aspects. Additionally, the smoothness of the insertion mechanism has been thoroughly evaluated to ensure minimal disruption during experimental procedures.

Overall, our 3D-printed micro-drive and head stage system presents a valuable tool for researchers seeking reliable and cost-effective solutions for long-term neuroscientific studies in freely moving rodents. The combination of innovative fabrication methods and comprehensive testing ensures the efficacy and versatility of the proposed interface, opening new possibilities for exploring neural circuitry and behaviour in a dynamic and physiologically relevant context.

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P2.12 Cortical low frequency activity changes generated by continuous infrared neuromodulation recorded by intracortical optrode during anesthesia

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Infrared neuromodulation (INM) is a safe optical method used to stimulate neuronal populations by locally changing the temperature within the brain tissue. Temperature dependency was found in every type of neural activity. During anesthetized in vivo measurements, the low-frequency components are dominant. Therefore, we examined the changes in delta- and theta waves and sleep spindles during continuous infrared stimulation.

The experimental device is a sharp-tip optrode, that also holds an embedded waveguide that transmits infrared light into the cortical tissue. Along the device shaft, there are 12 electrodes linearly placed. These electrodes recorded local field potential activity along the different cortical layers. The acute in vivo measurement was performed on 8 rats.

Every experiment repeated the stimulation 5 times. To be able to compare the different spectral components of the recording with altering stimulation state (ON or OFF) the analysis was performed on one-minute-long time periods. Our study revealed that a versatile evoked response can be observed in all three frequency ranges. In this preliminary study, we present the changes in all frequency bands considering the two channel groups exhibiting two different reactions to the infrared stimulation. For instance, during optical modulation, a higher delta power was detected in the supragranular layer, while lower delta power was observed in the infragranular layers. Along with the spectral analyses, a multiunit-based state detection was performed on these data. The detected down- and up-states also changed during the stimulation. As the down states are longer during the ON periods, the up states are shorter.

Our future aim in this study is to find the temperature dependency of slow wave oscillations and to characterize the effect of the infrared neuromodulation on state lengths during sleep.

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P2.13 Effect of electrical microstimulation parameters on in vivo neuronal calcium responses in the visual cortex of anesthetized mice

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Sensory neuroprostheses aim to restore sight or hearing through electrical microstimulation via implanted neural interfaces. Although this field has made significant progress in recent years, there are still gaps in our understanding of how to precisely target and activate specific neurons or neuron populations by intracortical microstimulation. One possible solution to achieve more precise control over neuronal activity without increasing the number of electrodes could be the application of advanced stimulation patterns such as current steering and dynamic stimulation. In this study, we developed flexible three-shank probes containing twenty-one small iridium-oxide electrodes to assess the effects of advanced electrical microstimulation strategies on cortical activity obtained using in vivo two-photon calcium imaging. The fabricated probes were implanted into the visual cortex (V1) of Thy1-GCaMP6 transgenic mice anesthetized with ketamine/xylazine, and a custom-made high-channel-count neural stimulator device was used to generate electrical stimulation patterns. Here, we present preliminary results from the first experiments, where we used a two-photon laser scanning microscope (laser wavelength between 820-920 nm) to image the calcium activity in layer 2/3 of V1, adjacent to the implanted probes. Calcium imaging (raster scanning at 31 Hz) was performed through a 20x water immersion objective with a numerical aperture of 1, providing a field of view of 550 µm × 550 µm. In initial in vivo electrical stimulation experiments, we explored different stimulation parameters and observed diverse spatial and temporal activation patterns of neurons. The calcium imaging datasets we captured are utilized by multiple open source calcium analysis tools that have been recently developed. Presently, we are designing a processing and analysis pipeline that integrates suite2p and CalmAn. Following data processing steps, there is an opportunity to analyze the spatial and temporal activation patterns of neurons triggered by advanced electrical microstimulation. Our plans for the future involve exploring the influence of diverse advanced stimulation patterns on the activity of the visual cortex and identifying promising stimulation strategies that can improve the resolution of state-of-the-art visual cortical prostheses.

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P2.14 Flexible, ultra-long polymer-based neural probes for deep brain recording and stimulation

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Neural probes made from flexible and soft materials typically have a limited shank length, restricting access to deeper brain areas. In this study, we developed various designs of single-shank polyimide-based neural probes with an ultra-long implantable shank, enabling penetration depths of several centimeters. The probes, realized on 6-inch silicon wafers, are composed of multiple individual components assembled using gold-gold thermocompression bonding after wafer-level fabrication. The length of the devices assembled using three components can exceed 300 mm. The tapered shank that penetrates the brain tissue is approximately 200 µm wide and 15 µm thick, containing 32 linearly placed iridium-oxide microelectrodes with a diameter of 30 µm and center-to-center distance of either 100 µm or 150 µm. Different probe designs have been realized based on the fabrication process (single or dual metal layers sandwiched between polyimide layers) and the microelectrode layout (edge and center design). Here, we demonstrate the functionality of the developed devices based on preliminary results of in vitro and in vivo experiments. Impedance measurements performed *in vitro* in physiological saline solution showed that the functional microelectrodes had an average impedance magnitude of 200.45 ± 95.52 kΩ at 1 kHz (n=68 sites). The acute electrophysiological performance of the probes was validated in neocortical and hippocampal areas of anesthetized rats. To facilitate the insertion of the flexible shank into the brain tissue, the dura and pia mater were removed over the targeted brain area. We were able to record high quality local field potentials (including cortical slow waves and hippocampal gamma activity), as well as single- and multi-unit activity from functional microelectrodes. Spike waveforms with amplitudes greater than 100 µV were detected in several recordings, and the simultaneous activity of multiple well-isolated single units could be recorded on multiple sites. Concurrently with probe validation, we are developing methods for accurate and reliable probe implantation and a brain tissue equivalent phantom to aid in implantation tests. Our future plans include chronic implantation of the polyimide probes in small and large animal models to evaluate their long-term electrophysiological performance, as well as the brain tissue response in the vicinity of the probe shank.

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P2.15 Functional reinnervation of degenerated forelimb muscles following cervical ventral root avulsion injury: the use of a detailed gait analysis system

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Avulsion injury of the brachial plexus provokes functional disability in the affected limb. During such injuries, the continuity between the axon and the perikaryon of the motoneurons disrupts. Without therapeutic intervention the majority of the affected motoneurons face rapid degeneration leading to paralysis of muscles innervated by the affected segment. With the immediate reimplantation of the affected ventral roots a number of motoneurons can be saved resulting in reinnervation of the affected muscles. In a prior research involving avulsion injuries of the lumbar spinal segments we successfully established our detailed locomotor pattern analysis system. This system has proven to be sensitive and cost-effective tool for detecting alterations in movement patterns of the impaired hind limb. In this current study we explored the diagnostic opportunities of our system in the forelimb following cervical avulsion injuries and reimplantation.

To investigate the impact of cervical avulsion injuries, C7 avulsion and immediate reimplantation on Sprague-Dawley rats was performed. We recorded the gait patterns of the animals biweekly. A custom-made plexiglas runway with a mirror system was used to record the steps of the rats with a high-resolution and high-speed digital camera. In total, we measured nine parameters including joint angles and different distances among the participating bones. Twelve weeks after the reimplanation surgery, Fast Blue, a fluorescent retrograde tracer was applied to label the reinnervating motoneurons. Morphological analysis of the spinal cord was carried out with the use of fluorescent microscopy.

Our preliminary results confirmed the sensitivity of our system and showed a relationship between the kinetic improvement and the morphological reinnervation of the forelimb muscles.

P2.16 Optimizing Stereovision Test Combinations for Amblyopia Screening in Children: A Perceptron Model Approach

István Szabó[1], Zsófia Csizek[1], Eszter Mikó-Baráth[1,2], Anna Budai[1], András Frigyik[3], Ágota Pusztai[4], Vanda Á. Nemes[1,2], László Závori[1], Diána Fülöp[1], András Czigler[1], Kitti Szabó-Guth[1,2], David Piñero[5], Péter Buzás[1,2], Gábor Jandó[1,2]

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This study aims to determine the most effective stereovision test combination for screening amblyopia and amblyogenic risk factors in children, using various density static and dynamic random dot stereograms with or without noise.

A Perceptron model was trained with four-dimensional input data, representing scores from four tests: static random dot stereogram (SRDS) with 8% dot density, dynamic random dot stereogram (DRDS) with 1% dot density, DRDS with 0.7% dot density, and DRDS with 1% density including 0.5% uncorrelated noise. The Neural Network Toolbox was employed for optimization and cross-validation to ensure model generalizability. The study tested various combinations of input variables to evaluate their impact on the model's performance, focusing on convergence rates and Area Under the Receiver Operator Characteristic Curve (AUC) metrics. One hundred repetitions were used to test the convergence of the network and assess variability.

The study revealed that modifications to the output function and the use of the Levenberg-Marquardt training algorithm significantly accelerated the Perceptron's convergence. The Perceptron's generalization ability, assessed through AUC metrics, remained stable across both training and testing sets, indicating robustness against overfitting. Pairwise comparisons of different configurations, with Bonferroni's correction applied, showed significant differences in performance variables. The findings suggest that single test performance is inferior to combinations involving two or more tests. The best results were observed when combining static and at least one dynamic test. The noisy stereogram contributed the least to overall performance; excluding it from the four-dimensional version did not significantly deteriorate performance. However, including all four tests may enhance stability.

The study successfully identified effective combinations of stereovision tests for screening amblyogenic risk factors in children. The Perceptron model, with its effective convergence and reliable generalization ability, proved a valuable tool in determining the optimal mix of tests, emphasizing the importance of combining static and dynamic elements in stereovision assessments.

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P2.17 A multistep analysis workflow for classification of cortical LFP events

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Cortical local fields and oscillatory events can be analyzed with spectral methods (spectral analysis, time-frequency decomposition), but identification of specific features of the waveforms on a large dataset is poorly achievable with these methods. Applying multidimensional statistical methods like Principal Component Analysis improves the classification, helps discover hidden patterns and create new features. We aimed to assemble a multistep analysis workflow that can transform the oscillatory LFP activity in various cortical layers into a statistical representation. It consists of the following steps: preprocessing, extracting new features, clustering the events, and creating a topological map from the probability distribution.

We applied the workflow to cortical slow waves, theta-, and spindle oscillations recorded from juxtasomal position of pyramidal cells and interneurons in freely moving rodents. During the preprocessing step, the LFP signal was down sampled and filtered, and then LFP segments were collected according to the detected events of interest (e.g., spindle, theta cycles, delta/down state waves). New features were generated by projecting the original data into the lower dimensional space defined by the principal components. Individual LFP segments were clustered in the feature space by fitting a Self-Organizing Map (SOM) and finding the Best Matching Map Units (BMMU) for each detected event. As a final step, a 2D probability distribution (SOM Profile) was calculated for each cell based on its events' cluster assignments. To consider the topological structure of the distribution, we used the Earth Mover's Distance (EMD) as a similarity measure between different SOM profiles.

The application of the workflow on 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. We conclude that local field potentials recorded juxtacellular to individual cells have cell-type specific features. This suggests that field potentials in the network are highly compartmentalized and retain identities of cellular units in space and time, even if neuronal populations are in a silent state.

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P2.18 Three-dimensional laminar electrode array for neural recordings in human cortex allow identification of synaptically coupled neurons *in vivo*

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We pioneered functional assessment of human synaptic function in acute brain slices with multiple patch clamp recordings combined with correlated light and electron microscopy and identified distinctive features of human feed-forward synaptic networks. A landmark result of our human slice experiments was that individual neurons triggered sequences of events in the network lasting an order of magnitude longer than detected previously in other species. These event series were composed by specifically alternating glutamatergic and GABAergic postsynaptic potentials and required selective spike-to-spike coupling from pyramidal cells to GABAergic interneurons. Individual neuron activated groups of cells resembled the so-called functional assemblies which were proposed by D. Hebb as building blocks of higher order cognitive representations. In order to validate human slice results in behaving humans, we developed for clinical application a three-dimensional, multi-shank high-density electrode array for single-neuron recording in drug-resistant epilepsy patients. The minimal vertical distance of recording sites is 50 µm, and the minimal lateral spacing for electrode shanks is 1 mm. Our electrode arrays contain a scalable number of recording sites, can span all layers of the cortex, and allow quick manual surgical placement and incorporation into standard intracranial EEG grids. We successfully implanted several in-house built prototypes of the novel electrode array in combination with standard intracranial EEG grids for 7 to 11 days in five patients with epilepsy. Following the surgical removal of the arrays, we performed in vitro targeted patch clamp experiments in the neighborhood of in vivo electrode tracks with full anatomical recovery of the patched cells and having only a slight (17±8%) drop in the number of connected pairs relative to slices made from the tissue without in vivo electrode penetration. The clinical application of three-dimensional laminar microelectrode array allowed us to identify monosynaptically coupled human cortical neurons in vivo. Moreover, we confirmed the existence of powerful human pyramidal cell to interneuron spike to spike coupling and relatively weaker pyramid to pyramid interactions in vivo. We conclude that the novel three-dimensional high density electrode array is successful in identifying Hebbian sequences of firing in groups of relatively closely spaced pyramidal cells and interneurons with time scales corresponding to high frequency cortical motifs in the human cortex.

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P2.19 Acousto voltage imaging with voltage sensitive dyes and genetically encoded sensors

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Two-photon Ca^{2+} imaging is a widely used tool for studying cortical network dynamics at multiple depths, that underlie higher cognitive functions. While this technique provides a high spatial resolution, its main limitation is low temporal resolution. This may be circumvented by using electrophysiological recordings; however, it reflects the activity of a neuronal population from a confined brain region only and lacks cellular specificity. To better understand the direct communication between neurons, it is essential to monitor the activity changes even at the subcellular level with a high spatiotemporal resolution. Genetically encoded voltage indicators (GEVIs), such as JEDI-2P, are emerging tools to capture changes directly in neuronal membrane potential. Combining fast acousto-optic imaging with voltage sensors enables non-invasive monitoring of fast neuronal activity at subcellular level on the millisecond timescale. Here, we present various applications that demonstrate the power of direct voltage imaging. By using the novel acousto-optical scanning technique and JEDI-2P indicator, we will show that the activity of multiple cells can be reliably recorded with a temporal resolution of up to 50 kHz both in vitro and in vivo. Certain experimental systems, such as those involving ex vivo human tissue, are not compatible with the expression times of virally delivered GEVIs, that may be as long as several weeks. Voltage sensitive dyes (VSDs) enable ready voltage imaging through the fast staining of cell membranes, but the currently available sensors are limited by their low fluorescence enhancement and brightness. In this work, we synthetically modified the role of the lipophilic wire part of a prominent VSD (RhoVR) and we studied its action mechanism by computational methods to identify a pathway to more efficient VSDs. These novel tools working in combination can revolutionize our knowledge of neuronal computation science.

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P2.20 Time Series Classification Redesigned via Gold-Washing the Important Features: Feature Space Reduction for Ultrahigh-Dimensional, Multiclass Data, and Benchmarking on Synthetic Data

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Time series analysis traditionally involves seeking motifs and expert-defined features. This process can be automated through computer-generating hundreds of thousands of candidate features based on kinematic properties of any single or multi-channel signal then machine learning to assign class labels of interest. The processing of these ultra-high-dimensional, multi-class datasets faces two significant challenges: the scarcity of freely available datasets, whether real-life or synthetic, and the consequent scarcity of software packages capable of extracting meaningful features from such data, along with the ability to compare these software packages through standardized tests.

Feature screening software plays a crucial role in various fields by selecting a most useful small subset of the original features just as gold-washers separate gold from dirt. In neuroscience, the analysis of EEG and ECoG recordings with high spatial resolution in close or overlapping cortical regions encounters challenges of shared information between channels and the identification of patterns that forecast neural diseases. Analogous methods are required in fMRI for defining ROIs. Similarly, in biometrics, models authenticate users based on multichannel biometric data, such as the handwritten signature acquired as a time series that displays high variability.

Our contribution includes the introduction of BiometricBlender, a Python package serving as an ultra-high-dimensional, multi-class synthetic data generator. This tool allows the benchmarking of a diverse range of feature screening methods. During the data generation process, users can control the overall utility and intercorrelations of blended features, enabling the synthetic feature space to emulate key properties of real biometric datasets.

Additionally, we present a novel method, Random Forest-based Multiround Screening (RFMS), specifically designed to effectively filter out irrelevant features and identify binary and higher-order feature interactions. The proposed algorithm partitions the feature space into small subsets and executes a series of partial model builds. These partial models are then employed for tournament-based sorting and feature selection based on their importance.

To evaluate RFMS, we utilized the synthetic feature space generator, BiometricBlender. The results demonstrate that RFMS is on par with industry-standard feature screening methods, while simultaneously possessing numerous advantages over them.

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Poster Session 3
Neuroendocrinology

P3.01 Ghrelin amplifies the nicotine-induced dopamine release in the rat amygdala, bed nucleus of stria terminalis (BNST) and striatum

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Ghrelin is an orexigenic neuropeptide that is known for stimulating the release of growth hormone (GH) and appetite, but it has been also implicated in addiction to drugs, such as nicotine. Nicotine is the principal psychoactive component in tobacco that is responsible for the reward sensation produced by smoking that could be mediated by the amygdala, bed nucleus of stria terminalis (BNST) and striatum. Therefore, in our previous and present in vitro studies nicotine and ghrelin were superfused and the dopamine release was measured in the amygdala, BNST and striatum of male Wistar rats. In order to determine which receptors mediate these effects, mecamylamine, a non-selective nicotinic acetylcholine receptor (nAchR) antagonist, and GHRP-6, a selective growth hormone secretagogue receptor (GHS-R1A) antagonist, were also superfused. Nicotine increased significantly the release of dopamine, and this effect was inhibited significantly by mecamylamine. Ghrelin increased similarly or even more significantly the dopamine release than nicotine did, and this effect was inhibited significantly by GHRP-6. Moreover, when administered together, ghrelin amplified significantly the nicotine-induced release of dopamine, at least in the BNST and the striatum, and this additive effect was reversed partly by mecamylamine and partly by GHRP-6. Therefore, the present study provides a solid base of evidence for the participation of ghrelin to dopamine-signaling in the amygdala, BNST and striatum, that may contribute to nicotine addiction.

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P3.02 The effects of CRF and the urocortins on the release of noradrenaline from the locus coeruleus and the release of serotonin from raphe nuclei in rats

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Corticotropin-releasing factor (CRF) and the urocortins (UCN1, UCN2 and UCN3) are structurally related neuropeptides involved mainly in the neuroendocrine, autonomic and behavioral stress responses. They act via two distinct CRF receptors (CRF1 and CRF2) with putatively dualistic actions in the brain. The aim of the present study was to investigate the effects of CRF, Ucn1, Ucn2 and Ucn3 on the release of noradrenaline from the locus coeruleus and the release of serotonin from the raphe nuclei. For this purpose, male Wistar rats were used, their locus coeruleus and raphe nuclei were isolated and dissected, and the slices were incubated with 5 µM of tritium-labelled noradrenaline or serotonin and superfused with 100 nM of CRF, Ucn1, Ucn2 or Ucn3. The noradrenaline release in the locus coeruleus was increased significantly by CRF and influenced differently by Ucn1, Ucn2 and Ucn3. In contrast, the serotonin release from the raphe nuclei was decreased significantly by CRF, but affected differently by the urocortins. Therefore, the present study suggests the existence of two distinct CRF systems in the locus coeruleus and raphe nuclei, which probably modulate the autonomic and behavioral responses to stress in a dualistic manner through inhibition or stimulation of local release of noradrenaline and serotonin.

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P3.03 Long term saccharose consumption associated activity of limbic forebrain glucose-monitoring neurons: A pilot study in the rat

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The glucose, as primary energy source of the brain cells, is well known to play important role in the regulation of food intake. It is also known that glucose receptors are present not only in the internal organs, but in the central nervous system as well. This glucose sensing takes place via the activation of so called glucose-monitoring (GM) neurons that are considered to continuously detect the changes in the glucose concentration of the local intracerebral extracellular space or in the systemic circulation.

The main goal of the present experiments was to further investigate the involvement of these GM chemosensory neural cells in the organization of feeding and metabolism related regulatory processes. In this study, firing rate changes of the forebrain GM neurons will be examined along with prolonged low-calorie sugar beverage consumption in laboratory rats.

Extracellular neuronal activity was recorded by means of the multibarreled microelectrophoretic technique before, during and after repeated epoques of microelectrophoretic administration of D-glucose, as well as along with a 4 week-long sugar drinking treatment (0,04 g saccharose /ml).

The activity changes of altogether 283 neural cells, among them 8 GM neurons, were recorded in these experiments, and the chemosensory, as well as the glucose-insensitive units appeared to display differential responsiveness during the long-term sugar treatment. To prove the significance of these changes, however, more data, obtained in future experiments, are needed.

PTE ÁOK KA 2013/34039/1; EFOP-3.6.1-16-2016-00004; EFOP-VEKOP; TKP2.

P3.04 Inhibition of aldosterone synthesis during the stress-hyporesponsive period modifies behavior and selected parameters of the endocannabinoid system in juvenile rats

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In the developing rodent, there is a period (postnatal day (PND) 3–14), called the stress-hyporesponsive period, when pups show reduced capacity to secrete corticosterone in response to several stress stimuli (1). We have demonstrated that rat pups show increased rather than reduced response of the mineralocorticoid hormone aldosterone to several acute stress stimuli throughout this developmental period (2). These findings suggest that aldosterone is a more physiologically important stress hormone than corticosterone during development. We tested the hypothesis that inhibiting aldosterone synthesis during the stress-hyporesponsive period results in altered behavior, changes in the endocannabinoid (ECB) system, and modified neuroendocrine response to stressors in juvenile rats. Newborn Sprague-Dawley rat pups (males n=40, females n=41) were treated with aldosterone synthase inhibitor FAD286 (30 mg/kg per day, orally) or vehicle (water) from PND 3 to PND 9. To confirm the effectiveness of the treatment, blood samples and adrenal glands from 10-day-old pups were analyzed. The rest of the pups were weaned on PND21. They were behaviorally tested in an open field (PND 23) and elevated plus-maze (PND 29) and half of them were exposed to restraint stress for 120 min (PND46). FAD286 did not modify the general locomotor activity assessed in juvenile rats. Inhibition of aldosterone synthase by FAD286 resulted in altered anxiety-like behavior in a sex-dependent manner. Postnatal FAD286 treatment increased anxiety-like behavior in female rats, not male rats. In juvenile rats treated with FAD286 postnatally, the rise in corticosterone concentrations in response to restraint stress was much more pronounced than in vehicle-treated ones, regardless of sex. Moreover, FAD286-treated animals exhibited elevated corticosterone concentration even under non-stress conditions. FAD286 influenced also the parameters of the ECB system. Gene expression of the CB1 receptor was increased and FAAH expression was higher in FAD286-treated rats. Obtained results demonstrate that the lack of aldosterone in the early postnatal period as a consequence of aldosterone synthase inhibition influences anxiety-like behavior in female, but not male juvenile rats. Blockade of aldosterone synthesis during the stress-hyporesponsive period in newborn rats has consequences on parameters of the ECB system and stress hormone release later in life.

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P3.05 Application of dehydroepiandrosterone as a neuroprotective agent for the therapy of Alzheimer's disease in a mouse model

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Alzheimer's disease (AD) is currently one of the most significant neurodegenerative diseases, and its effective treatment remains a challenge. The dehydroepiandrosterone (DHEA) is an androgen molecule, which protects in vitro against amyloid-ß (Aß) toxicity, and so might potentially improve cognitive functions. As steroid might influence wide range of processes both short (via membrane receptors) and long term (via intracellular receptors) we can expect beneficial effect already after one injection.

Six months old male 3xTg-AD mice (B6;129-Tg(APPSwe,tauP301L)1Lfa Psen1tm1Mpm/Mmjax) were treated intraperitoneally with DHEAS (a water-soluble sulphate salt of DHEA, 10 mg/10ml/kg) and compared to vehicle treatment. Behavioural tests (Y-maze and social discrimination) were performed 30 minutes after the injection, and after 24/48 hours we transcardially perfused the animals. We performed immunohistochemistry on 30 µm thick sections for acetylcholinesterase (cholinergic fibre density) and amyloid-β accumulation.

In previous studies we observed that the 3xTg-AD animals exhibited increased anxiety and cognitive disorders. While the Y-maze test did not reveal any significant effects of DHEAS on specific motor and cognitive deviations, changes in social behaviour were evident in the social discrimination test. As often seen in AD, amyloid plaques and neurofibrillary tangles appeared in the brains of these mice, as well as cholinergic fibre destruction in sensory cortex. The treatment was able to influence these morphological changes.

In conclusion, our findings support the potential of DHEAS as a protective agent for nerve cells, suggesting its usefulness as a novel therapeutic option for neurodegenerative diseases, including AD.

P3.06 Age-dependent dynamics of FOSB/ΔFOSB content in paraventricular nucleus of hypothalamus and hippocampus in a chronic stress rat model

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FOS proteins are early responding gene products and contribute to form the activator protein-1 (AP-1). The occurrence of FOS proteins (FOSB and ΔFOSB) can be induced by chronic variable mild stress (CVMS). CVMS may causes mood disorders that shows age-dependent manifestation. The amount of FOSB proteins are also associated with synaptic plasticity. The hippocampus is involved in orchestrating of stress machinery, and it shows usually decreased function/morphology/plasticity in aged periods.

Since there is no information about the age-dependency of CVMS-induced FOSB, ΔFOSB rise in the hippocampus, we aimed to semi-quantify the FOSB/ΔFOSB content of the dorsal hippocampal areas (CA1, CA2, CA3, CA4 and dentate gyrus [DG]) in the course of aging in a CVMS male rat model.

Wistar rats clustered into six age groups (2-months-old [M], 3M, 6M, 12M, 18M, 24M). Half of the groups were exposed to two weeks long CVMS. To test the stress efficacy, we measured the total body, thymus, and adrenal glands' weight. We collect plasma samples for corticosterone (CORT) measurements. On the brain samples we performed a diamino-benzidine FOSB/ΔFOSB immunolabelling on the coronal sections of the hippocampus.

The body, adrenal and thymus weight data with CORT results supports that the CVMS exposure was effective. The paraventricular nucleus of the PVN showed constantly high FOSB/ΔFOSB cell count in all CVMS groups, while in the controls we did not detect any signal. In both CA1, CA2 and CA3 regions we detected higher FOSB/ΔFOSB content in CVMS groups than in their respective controls, but only in CA1 and CA2 regions we found significant differences and only in some age periods (3M, 18M). The CA1, CA2, CA3 and DG FOSB/ΔFOSB contents decrease during ageing, while CA4 did not show any age dependent changes (at group comparison level). Spearman correlation analysis underlined the age dependent decline of FOSB/ΔFOSB content in both control groups and CVMS groups in almost all regions, but this decrease was less that we detected in subcortical areas previously.

Corresponding to our previous results on FOS, FOSB the magnitude of FOSB/ΔFOSB is also function of the age, but hippocampal FOSB exhibit late-onset decline compare to subcortical stress-associated centers. Further investigation needed to understand the age-characterized changes in the stress machinery.

P3.07 Examining the combined effect of fast acting insulin and fasting on male and female Wistar rats at the beginning of the light and dark cycles

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Diabetes mellitus is a widespread disorder and hypoglycaemia is one of the most serious complications both of the disorder itself as well as the insulin therapy. Understanding the details of mechanism is imperative to the proper management of blood sugar. The outcome of hypoglycaemic episodes might be influenced by several factors, like fasting or the time of day when the episode occurs (see e.g. Somogyi effect). Our aim was to investigate in an insulin-induced hypoglycaemia animal model the effect of these influencing factors.

We administered fast-acting insulin (Novorapid, NR, 20NE/100g/0.2ml saline) or saline intraperitoneally to adult male and female Wistar rats at the beginning of the light or dark phase (9am or 9pm) with or without 12h fasting. 60 minutes after intervention we collected trunk blood for blood glucose and – to assess stress level through rodents' primary adrenal corticosteroid -corticosterone (CORT) measurements.

NR reduced blood glucose levels in all groups ($p<0.0001$). However, the fall was the strongest after fasting, and induced CORT elevation only in these groups ($p<0.001$). The fasted animals had similar blood glucose but higher CORT levels during the dark, active period. In the non-fasted groups at the beginning of the light phase NR reduced the blood glucose levels in both male ($p<0.0001$) and female ($p<0.0001$) rats, but had no effect on CORT levels. During dark period (also non-fasted groups), female rats treated with NR had higher CORT levels than either the NR-treated females in the light period ($p< 0.01$), saline-treated females in dark period ($p< 0.001$) or NR-treated males in dark period ($p<0.001$). Additionally, saline-treated males in the dark period had lower blood glucose levels after fasting ($p<0.01$).

All in all after fasting the animals were more sensitive to the hypoglycaemic effect of insulin, inducing remarkable CORT elevation as well. There were no substantial difference between the sexes supporting that the basic mechanisms of glucose regulation are the same. Thus, for further studies fasting seems to be imperative, but the cycle of the day or the sex should not be taken into account that much.

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P3.08 Role of NUCB2 in the response to osmotic stress

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NUCB2 is a prohormone of nesfatin-1, an anorexigenic neuropeptide that also affects water intake. Nesfatin-1 is highly coexpressed with vasopressin (AVP) and oxytocin (OT) in the supraoptic nucleus (SON). Chronic osmotic challenges induce adaptive changes in AVP and OT neurons, which are manifested by plastic morphological changes in the SON. Nesfatin-1 is known to act locally in the SON through dendritic release. Therefore, we hypothesized that it may be an important molecule in the osmotic stress-induced plasticity of SON.

In our first experiment, groups of rats were given either a high-salt solution (2% NaCl, HS) to induce plastic changes in the SON or tap water (NS) *ad libitum.* A pair-fed (PF), water-drinking group was also included as control. The animals were sacrificed on day 4 of the experiment. NUCB2 and AVP mRNA levels were measured in SON by RT-PCR. In the next experiment, shRNA was used to block NUCB2 expression in SON. We had 4 four experimental groups: the HS and NS groups were further divided into NUCB2-shRNA (N-sh) treated and scrambled shRNA (Scr-sh) treated subgroups. The animals were perfusion fixed on day 7. AVP and OT expressions were analyzed in the SON by immunohistochemistry. Finally, a nesfatin KO mouse model was used to evaluate the function of nesfatin-1 in SON. OT and AVP mRNA levels were measured in naïve mice by RT-PCR. Urine osmolality was measured in response to water loading (WL) and water deprivation (WD).

Our results showed that both NUCB2 and AVP mRNA levels were increased in the HS group compared to NS and PF controls. The HS and N-sh conditions led to an increase in AVP immunoreactivity in the ventral dendritic zone of SON, with the greatest effect of the combination of the treatments. The experimental treatments reduced AVP immunoreactivity within the cells to a similar extent. OT immunoreactivity of cells was also decreased by HS, but increased by N-sh. HS induced an enlargement of AVP and OT cells, whereas N-sh treatment had no effect to this parameter. AVP mRNA levels in heterozygous (HZ) and KO mice were significantly higher than in wild-type (WT) mice. OT mRNA expression was also high in HZ animals. Naïve HZ and KO mice produced more concentrated urine than WT mice. In response to WD, urine was concentrated regardless of genotype. WL resulted in a decrease in urine osmolality, but to a lesser extent in HZ and KO animals than in WT mice.

Our data suggest an important role for NUCB2 in osmotic regulation.

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P3.09 Histological, cellular, and molecular changes induced by chronic exposure to the (neuro)endocrine disruptor tributyltin in a widely used invertebrate model species

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Contamination by tributyltin (TBT), the biocide component of antifouling paints, has historically been one of the major threats to aquatic environments due to its rapid bioaccumulation and high toxicity to a wide range of animals even at very low concentrations. Besides its well-known endocrine-disrupting effects, recent studies reported that TBT was also able to alter lipid metabolism, pointing out that more detailed studies are required to reveal the precise biological impact of TBT.

To contribute to the understanding of the multifaceted physiological changes mediated by TBT, adult (4-5-month) specimens of the widely used model animal of invertebrate neuroendocrinology, the great pond snail *(Lymnaea stagnalis),* were exposed to 100 ng/L TBT for 21 days. After the chronic exposure, the potential histological, cellular, and molecular alterations were investigated in the CNS, kidney, and hepatopancreas.

In the CNS, tin was evenly distributed in all ganglia. Most tin compounds were located in the capsule and extracellular spaces, however, some of them were also seen in perikarya. The neurons showed no remarkable morphological changes. In contrast, pathological alterations occurred in both kidney and hepatopancreas. In these organs, the main targets of tin accumulation were epithelial cells resulting in characteristic morphological changes thought to be necrosis. Using a HPLC-MS method for untargeted lipidomics, we identified hundreds of lipids in all tissues investigated. From the identified lipids, the amount of 17 lipids in the CNS and the amount of 31 lipids in both kidney and hepatopancreas changed significantly due to the chronic TBT exposure. The phospholipids changed the most in each tissue, but the given lipids were highly tissue-specific. We identified a homologous sequence in our *Lymnaea* neuronal transcriptome data to 17ß-hydroxysteroid dehydrogenase-12 (HSD17B12) enzyme which is involved lipid metabolism in vertebrates. The *in silico* analysis showed that the deduced protein was indeed a bona fide HSD17B12 candidate. Using quantitative real-time PCR, we demonstrated significant alterations in the expression of HSD17B12 in different tissues due to the chronic TBT exposure.

Overall, our findings showed that TBT could cause alterations at different biological levels and confirmed the recent idea that it could induce changes in lipid metabolism in aquatic animals. Moreover, our results suggest that HSD17B12 enzyme in molluscs, as in mammals, is likely to be involved in lipid metabolism and had a highly conserved function during the animal evolution.

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P3.10 Morphological study of the ventral tegmental area in wild type and pituitary adenylate cyclase-activating polypeptide (PACAP) knockout mice

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PACAP is a neuroprotective peptide that effectively attenuates oxidative stress, reduces inflammation, and modulates apoptotic pathways. Numerous studies showed its protective role in Parkinson's disease models. In our earlier study we compared the substantia nigra of PACAP knockout (KO) and wild type mice (WT) of different ages and did not find significant changes in the cell number of dopaminergic neurons in both groups, but in ageing PACAP KO mice we detected higher number of resting microglia. In this study we aimed to examine the ventral tegmental area (VTA), another dopaminergic region, which has important function in the regulation of social behavior.

We examined the ventral tegmental area of WT [n=5-5-5] and PACAP KO mice [n=5-4-5] aged 1.5, 4, and 8 months. In our immunohistological examination we used triple labelling, dopaminergic neurons were stained with tyrosine hydroxylase, microglia with Iba1 and we labeled the expression of specific PACAP receptor (PAC1R) in the VTA. We also categorized the activation of microglia based on morphological characteristics.

We observed a significant age-related decline in dopaminergic neurons in both genotypes, with a more pronounced decrease in KO mice compared to WT animals. Colocalization with PAC1R was sporadic. Microglia analysis showed an age-associated reduction in cell numbers in both genotypes. The number of inactive microglia decreased in KO mice with age, on the other hand, in wild time mice the number of active microglia was reduced. We also found significantly more inactive microglia in 1.5-month-old KO mice compared to age-matched WT mice.

Although earlier we did not find dopaminergic cell loss in the substantia nigra, the number of dopaminergic cells was significantly decreased with age in the VTA, which was more pronounced in PACAP KO animals. Based on these results we suggest that because of the age dependent dopaminergic cell loss of VTA, PACAP KO animals could show behavioural alterations earlier than motor symptoms. The number of microglia cells was also decreased with age in both groups, but the number of active microglia did not change in PACAP KO mice due to the lack of immunosuppressive effect of endogenous PACAP, which might also serve as a mechanism for increased dopaminergic cell death in PACAP KO animals.

P3.11 Arsenics` impact on the transcriptional expression of distinct hormone receptors and simultaneous assessment of mitochondrial dynamics and respiration rates in the hypothalamus of mice

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Arsenic (AS), historically known as the “poison of kings,” is a concerning environmental toxicant found in tap water, wells, and groundwater.

Our hypothesis is that AS exposure alters intracellular mechanisms in the hypothalamus, including estrogen receptors (ERα, ERβ), thyroid receptors (TRα, TRβ), peroxisome proliferator-activated receptor (PPARγ); receptor mRNA expression levels, and mitochondrial morphology.

To validate that in vivo, we administered AS via intraperitoneal injections in mice. We assessed the expression of ERα,β, TRα,β, PPARγ; and mRNA levels, concurrently evaluating mitochondrial respiration rates (MRR). We examined the mitochondrial ultrastructure in agouti-related protein (AgRP) and pro-opiomelanocortin (POMC) neurons, critical components of the melanocortin system regulating energy balance.

Results show that ERα,β, and TRα expression was significantly increased by AS, in all concentrations examined. In contrast, TRβ and PPARγ remained unaffected after AS injection. Arsenic-induced dose-dependent changes in state 4 mitochondrial respiration (St4). Mitochondrial morphology was affected by AS in that the 5 mg dose increased the size but decreased the number of mitochondria in AgRP while increasing the size without affecting the number of mitochondria POMC neurons. Arsenic also increased the size of the mitochondrial matrix per host cell.

Complex analysis of dose-dependent response patterns between receptor mRNA, mitochondrial morphology, and mitochondrial respiration in the hypothalamus suggests that instant AS effects on receptor mRNAs may not be directly reflected in St3-4 values, however, mitochondrial dynamics is affected, which predicts more pronounced effects in hypothalamus-regulated homeostatic processes after long-term AS exposure.

Our study suggests that As acts as an endocrine disruptor even at low doses and has immediate effects (six hours post-injection), impacting hormone receptor expression and mitochondrial function in the hypothalamus. These disruptions may have far-reaching consequences on homeostatic functions, potentially contributing to conditions such as diabetes, accelerated aging, and cognitive impairments. The findings underscore the importance of understanding the rapid effects of As on endocrine and metabolic regulation in the context of environmental exposures.

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P3.12 Acetylcholine modifies function of GnRH neuron and luteinizing hormone secretion: involvement of a frequency-dependent ACh/GABA co-transmission

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Hypophysiotropic GnRH neurons orchestrate reproduction under regulatory control of neurotransmitters. Acetylcholine (ACh) has been shown to modify reproduction centrally, however, the exact target site/s and the involved mechanisms haven't been clarified yet. To shed light on the interaction between ACh and GnRH systems, various methods were used in adult, male mice. 3DISCO immunocytochemistry revealed the innervation of cell bodies and dendrites of GnRH neurons by vesicular acetylcholine transporter (VAChT)-IR axons. The immunoelectron microscopic analysis identified cholinergic synaptic inputs to GnRH cells. Retrograde rabies virus labeling from GnRH-cre neurons explored the origin of the cholinergic afferents from the medial septum and diagonal band of Broca. Expression profiling and patch-clamp studies confirmed the expression of α4β2, α3β4 and α7 nicotine and M1-M5 muscarine ACh receptors (n- and mAChRs) in GnRH neurons. Carbachol first evoked an inward current, then decreased the frequency of mPSCs. The former action was prevented by mecamylamine, the latter one by atropine confirming the involvement of both n- and mAChRs in the regulation. ACh and carbachol also influenced the firing rate in GnRH neurons in a biphasic manner. For acceleration of firing nicotine and M1/M3 muscarine receptors, while for the inhibitory phase M2/M4 muscarine receptors were responsible. The actions of both facilitatory and inhibitory muscarine receptors were eliminated by tetrahydrolipstatin indicating the involvement of retrograde endocannabinoid signaling in the process. Optogenetic stimulation of channelrhodopsin-2 expressing cholinergic axons at 5 Hz exerted a biphasic effect on frequency of both firing and mPSCs in GnRH neurons similar to the pharmacological challenges. In a subpopulation of GnRH neurons (10%), the LED-evoked mPSCs at 0.2 Hz were abolished by picrotoxin, while at 5 Hz, in addition to picrotoxin, blockade of n- and mAChRs (by mecamylamine + atropine) was necessary for extinction of the effect. The finding reveals co-transmission of GABA with ACh in a frequency-dependent manner. In triple transgenic, Chat-Cre-Gq DREADD orchidectomized mice, clozapine-N-oxide (CNO) treatment evoked a significant, two-fold increase in both the basal and mean luteinizing hormone (LH) levels peaking at 30 minutes after ligand delivery. Collectively, the results indicate that ACh is a potent regulator of reproduction via hypophysiotropic GnRH neurons.

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P3.13 Effect of estradiol on the Transient Receptor Potential Vanilloid 1 and Ankyrin 1 receptors regulated pain responses

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Sex differences exist in chronic pain pathologies, and gonadal estradiol (E2) alters the pain sensation. The nocisensors, Transient Receptor Potential Vanilloid 1 (TRPV1) and Ankyrin 1 (TRPA1) receptors play critical role in trigger of pain. Here we examined the impact of E2 on the function of TRPV1 and TRPA1 receptors in mouse sensory neurons *in vitro* and in mice *in vivo.*

Mechanonociceptive threshold of the plantar surface of the paw of C57BL/6J male mice was significantly higher compared to female mice. This marked difference did not disappear in the mechanonociceptive pain perception of TRPV1 knockout (KO) animals. The same sex difference was observed in pain response of TRPA1 wild type (WT) and KO mice too. Both mechano- and thermonociceptive thresholds of TRPV1 WT female mice were significantly lower in proestrus compared to estrus phase. This difference was absent in TRPV1 receptor-deficient mice. In TRPA1 WT mice the differences of pain sensitization between the proestrus and estrus phase were not detectable. Furthermore, E2 potentiated the TRPV1 receptor activation-induced mechanical hyperalgesia in ovariectomized mice. E2 pretreatment abolished the capsaicin-induced TRPV1 desensitization in sensory neurons. After co-administration of E2 and tropomyosin-related kinaseA (TrkA) receptor inhibitor the sensitization was prevented.

Our study provides the first *in vivo* and *in vitro* evidence for E2-induced TRPV1 receptor upregulation, and sensitization mediated by TrkA via E2-induced genomic and non-genomic mechanisms. The sensitization and upregulation of TRPV1 receptor by E2 in sensory neurons may explain the greater pain sensitivity in female mice. We are planning to perform further experiments to clarify the different effects of E2 on TRPV1 and TRPA1 receptors.

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P3.14 Topography of the GLP-1 / GLP-1 receptor system in the spinal cord of male mice

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Glucagon-like peptide-1 receptor (GLP-1R) agonists are now commonly used to treat type 2 diabetes and obesity in developed countries. GLP-1R signaling in the spinal cord has been suggested to account for the mild tachycardia caused by GLP-1R agonists, but the neuroanatomy of the GLP-1/GLP-1R system in the spinal cord is still poorly understood. Here we applied *in situ* hybridization and immunohistochemical techniques to study the distribution of GLP-1/preproglucagon and GLP-1R transcripts and proteins in the spinal cord with special attention to the sympathetic preganglionic neurons. GLP-1R mRNA and protein were widely expressed in neurons, in almost every laminae of the gray matter. At ultrastructural level, GLP-1R-immunoreactivity was present not only in perikarya and dendrites, but also in axon varicosities. Extremely high levels of GLP-1R protein were observed in the cell body and processes of cerebrospinal fluid-contacting neurons. Only a small subset of sympathetic preganglionic neurons expressed GLP-1R. These GLP-1 receptive preganglionic neurons were localized primarily at the rostral tip of the intermediolateral cell column in the first two thoracal segments. GLP-1 fibers provided moderate to dense innervation to most of the gray matter, but only very sparsely innervated laminae I-III of the dorsal horn. This is in high contrast with the high density of GLP-1R neurons and axons in laminae II and III. We also observed a small number of preproglucagon mRNA-expressing neurons present specifically in the cervical and lumbar enlargements.

The results demonstrate that several distinct neuron populations express GLP-1R. GLP-1 fibers are positioned to regulate most of these GLP-1 receptive neuronal groups except in laminae II and III of the dorsal horn. In addition, we identified a small subset of GLP-1R-expressing sympathetic preganglionic neurons that may mediate the cardiovascular effects of GLP-1R agonist drugs.

P3.15 Cholecystokinin-induced hyperthermia is mediated by cyclooxygenase-2 in the brain

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Cholecystokinin (CCK) causes hyperthermia via CCK2 receptors in the brain. However, the underlying mechanisms of this thermoregulatory effect are largely unknown. Since some similarities were previously identified between the thermal effects of CCK and prostaglandin E2, we studied the interaction between central CCK signaling and the cyclooxygenase (COX) pathway.

In male Wistar rats, we measured body temperature responses to CCK infused into the lateral cerebral ventricle after intraperitoneal pretreatment with the nonselective COX enzyme inhibitor metamizol (120 mg/kg) or a selective COX-2 inhibitor, meloxicam, or etoricoxib (10 mg/kg for both). To discover the neuronal mechanisms, in separate experiments, we studied neuronal activation in thermoregulation nuclei with c-Fos immunohistochemistry in response to central CCK administration with or without metamizol pretreatment.

As expected, central CCK infusion induced hyperthermia. A novel finding of our study was that the hyperthermic response to CCK was significantly attenuated by metamizol. Moreover, metamizol also reversed the CCK-induced changes in the number of c-Fos-positive cells in the median preoptic area, in the dorsal hypothalamic area, and in the rostral raphe pallidus. The CCK-induced hyperthermia was also completely blocked with both selective COX-2 inhibitors studied. Finally, the central administration of the CCK2 receptor antagonist YM022 attenuated the late phases of fever induced by intravenous infusion bacterial lipopolysaccharide.

We conclude that centrally administered CCK causes hyperthermia through changes in the activity of “classical” thermoeffector pathways and that the activation of COX-2 is required for the development of this response.

NKFIH FK 138722

Poster Session 4
Systems neuroscience

P4.01 Acetylcholine redistributes synaptic efficacy in neocortical microcircuitry

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Acetylcholine (ACh) as a neuromodulator can have various effects, many of which are not yet fully understood. Here we investigated how synaptic transmission is modulated by ACh in the somatosensory cortex.

We prepared C57BL/6 mouse brain slices and bath applied ACh at three doses (1, 10 and 100 µM) as we performed whole cell recordings with multiple electrodes simultaneously. Our data was obtained from somatosensory cortex excitatory (E) and inhibitory (I) neurons which were synaptically connected. Presynaptic neurons were stimulated with trains of current pulses and the responses of the postsynaptic neurons recorded before, during and after ACh application.

Our findings show a reduction in the amplitude of excitatory postsynaptic potentials (EPSPs) under all the ACh dosages tested (1-10-100 µM). Inhibitory postsynaptic potentials (IPSPs), however, responded biphasically: under low concentrations of ACh (1-10 µM) we observed a decrement in amplitudes of IPSPs. However, the IPSP amplitudes at 100 µM of bath-applied ACh had similar amplitudes compared to the baseline, which persisted or increased during the wash out period. We hypothesize the mechanism in the distinct IPSP responses is related to two different receptor pathways: The G protein-coupled muscarinic receptor pathway being responsible for the low concentration responses (1-10 µM) while the high concentration of ACh (100 µM) activates the ligand-gated nicotinic receptors.

Our study highlights the importance of understanding distinct mechanisms of neuromodulation in the somatosensory cortex and how these may contribute to the regulation of neuronal circuits.

P4.02 An alternative cholinergic innervation of the hippocampus

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Cortical functions are highly regulated by ascending subcortical pathways, some of which originate from the basal forebrain. Cholinergic cells of the medial septum (MS) and the horizontal diagonal band (HDB) in the basal forebrain play a vital role in the regulation of attention and memory formation. While the cholinergic innervation of the hippocampus is known to originate from the MS, we discovered an additional cholinergic pathway from the HDB. Using tracing techniques combined with immunohistochemistry and electron microscopy, we found that HDB cholinergic cells mostly target hippocampal layers that are only sparsely targeted by the MS and HDB preferentially target the hilar mossy cells. Our preliminary chemogenetic behavioral experiments suggest that HDB cholinergic cells drive hippocampal novelty detection and memory formation via the mossy cells. Our results provide new insights into the regulation of memory formation and may help better understand cholinergic system-related neurodegenerative diseases.

(ÚNKP-23-2-II-DE-146)

P4.03 Investigating the role of neuromodulators in mice during associative learning with a 50% reward schedule

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Reward prediction error (RPE) is the difference between actual and expected reward. According to reinforcement learning theory, value is updated based on the RPE. The release of dopamine (DA) in the ventral striatum (VS) was shown to represent RPE during classical conditioning. However, DA release in the prefrontal cortex (PFC), an area that is crucial to value-based decision making, was much less studied. Cholinergic neurons have been shown to play an important role in associative learning, suggesting that they are involved in the processing of stimuli that predict future outcomes. It has been previously demonstrated that the release of norepinephrine (NE) follows the threat prediction error, but its relationship with RPE has not been studied yet. We aimed to investigate the role of DA, acetylcholine (ACh) and NE in associative learning and the correlation of their release with the RPE.

To address this, we trained mice (n=20) on a sound detection Pavlovian conditioning task with a 50% reward schedule that allowed examining clean representations of +RPE (rewarded trials) and -RPE (reward omission trials), while we measured DA, ACh and NE release by fiber photometry. Behavioral updating of value representations based on the outcome of the previous trial was indexed by the licking activity of mice in the anticipation of reward.

As expected, anticipatory licking during the stimulus decreased after omitted rewards but increased after rewarded trials. DA release followed a similar pattern not only in the VS but also in the PFC. Moreover, we found significant positive correlations between DA release and anticipatory lick rate (ALR) difference both in VS and PFC. In the case of ACh, we did not observe anything similar in any of the examined brain areas. No clear correlation was observed between the change in ACh release in any of the brain areas and the change in the ALR. NE release in the BLA followed a similar pattern to ACh, but it decreased after rewarded trials and increased after omitted rewards in the PFC. Furthermore, we observed a negative correlation in the PFC between NE release and ALR changes.

These results indicate that the dopaminergic system broadcasts similar RPE signals to both striatal and frontal cortical targets. In contrast to DA, NE release decreased after rewarded trials and increased following omitted rewards and it was also negatively correlated with the intensity of the animals' anticipatory behavior.

P4.04 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 such as Alzheimer's and Parkinson's diseases. While the role of neuromodulators is increasingly in the focus of the research of explicit learning, their participation in implicit learning (nonepisodic learning of complex information in an incidental manner, without awareness of what has been learned) and memory processes is poorly understood.

To examine this we have used a previously developed automated training setup in which animals have to respond to light at different locations in a sequential task in the order of the sequence. After the mice have been for one week in the setup we implanted a custom-built microdrive with eight tetrode electrodes in the prefrontal cortex and also dopamine sensor was injected into the contralateral side of the brain. The release of dopamine was monitored through fiber photometric measurements. During the task behavioral data was collected and synchronized with the neural and photometric data. After the surgery we also used a method which allowed us to precisely localize electrodes and fibers in mice in vivo by giving us high-resolution information about bone landmarks provided by micro- CT scanning.

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. Comparing the animals' behavior between sequential and randomized blocks, we found that the reaction time was lower while the accuracy was higher in the sequential blocks. We observed robust and -- on multiple separate timescales -- correlated activation during learning and the execution of the task.

Furthermore, we found that both dopamine level and a subset of the single units precisely represented the current stage of the animal in the sequence, i.e., how many more steps are required to receive reward.

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

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P4.05 Large-Scale Single Unit Recordings of the Dorsal Medial Thalamus of Naturally Behaving Mice

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The medial part of the thalamus consists of several non-sensory thalamic nuclei implicated in arousal, motivated behavior and in the homeostatic maintenance of sleep choreography. Yet, how neuronal activity in the medial thalamus is embedded in the activity of the limbic and cortical systems is poorly understood. We performed large-scale single unit recording in the dorsal part of the medial thalamus (DMT) in freely moving mouse during sleep-wake behaviors, simultaneously with population activity from the prelimbic cortex (PL) and the ventral subiculum (vS), ¬two key forebrain areas of the limbic system that show strong reciprocal connections with the DMT. We expressed Channelrhodopsin-2 in calretinin-expressing (CR) neurons of the DMT and performed single-unit opto-tagging to identify recorded neurons. CR neurons fired at a slower rate and were less “bursty” than non-CR neurons. Spontaneous self-organized activity patterns in the vS and PL during sleep or wakefulness allowed us to classify neuronal groups in DMT and identify their temporal organization in various brain states. Individual DMT neurons displayed distinct activity patterns during hippocampal ripples, theta or cortical slow wave (UP-DOWN states) activity. Ripple-related activity was dominant in the anterior DMT which is reciprocally connected to vS. Neurons in the posterior regions were suppressed during ripples. DMT neurons throughout the structure co-varied with cortical slow waves, increasing and decreasing their spiking during UP and DOWN states, respectively. However, we identified an antagonistic subgroup, which specifically increased their spiking during cortical DOWN states. These DOWN-state active neurons (DSA) were largely absent in the anterior DMT and did not show ripple-related activity increase. DSA neurons showed higher correlation with each other during both in NREM sleep and during wakefulness, indicating the presence of two antagonistic subnetworks within the dMT. Theta-modulated units were also present in our sample. During NREM sleep, DMT neurons showed prominent ultraslow periodic activity, corresponding to NREM ‘packets’ in parallel with gradual deepening of NREM (increased power in the delta and spindle band), terminating with transient increased muscle activity (‘microarousals’). Our findings reveal a yet undocumented neurophysiological heterogeneity in the DMT neurons and demonstrate how neocortical and subicular regions can exert a differential influence on them.

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P4.06 Characterisation of inhibitory and excitatory afferents of the paraventricular thalamic nucleus

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The paraventricular thalamic nucleus (PVT) plays a crucial role in regulating emotional and motivational functions by innervating various forebrain areas through its excitatory axon collaterals. Modulating PVT activity, either through activation or inhibition, leads to significant changes in the processing of fear, arousal, and stress-related signals, influencing homeostatic behaviors. Maintaining the optimal balance between excitation and inhibition is essential for PVT function. However, the sources of glutamatergic and GABAergic afferents to the PVT remain unclear.

A substantial cell population within the PVT expresses calretinin (CR). These CR+ cells constitute a major source of thalamic inputs to the prelimbic cortex, amygdala, and nucleus accumbens, exhibiting selective c-Fos expression in response to stress. Whether PVT/CR+ neurons display selectivity in their afferent connections remains unexplored. Thus, our study aimed to address three key questions: i) the origins of excitatory and inhibitory inputs to PVT, ii) the selectivity of these inputs for PVT/CR+ cells, and iii) the extent to which these different inputs converge or segregate within the PVT.

Employing retrograde and anterograde viral labeling in vGLUT2-Cre, vGAT-Cre, and vGLUT2-Cre/vGAT-Flp double transgenic mouse strains, we analyzed subcortical inputs. Additionally, Rbp4-Cre, NTSR1-Cre, and FoxP2-Cre strains were utilized to label cortical inputs. Our findings revealed that subcortical afferents to PVT are widely distributed, yet the origin of excitatory and inhibitory inputs predominantly segregates. Co-innervation by both GABAergic and glutamatergic afferents was observed uniquely from the periaqueductal gray. Furthermore, axons from different subcortical areas significantly overlapped and exhibited high selectivity for the CR+ zone in the core region of PVT.

In contrast, the majority of cortical inputs originating from layer 5 pyramidal cells selectively targeted the lateral, transient zone of PVT, which contains fewer CR+ cells. Significant cortical inputs to the core region were exclusively found in FoxP2 animals, labeling deep layer 6 cells. These results underscore the integration of excitatory and inhibitory information by PVT from distinct subcortical centers, with a predominant targeting of CR+ neurons. In summary, our data elucidate the processing of cortical and subcortical information by distinct cell populations within the PVT.

P4.07 Unravelling the role of hemokinin-1 in age-related deterioration of motor coordination and muscle strength

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Musculoskeletal problems and consequent fractures significantly reduce the quality of life in old age. A group of neuropeptides, tachykinins, have regulatory functions in the central and peripheral nervous system, and this group includes hemokinin-1 (HK-1). It is present in high concentrations in the cerebellum and reproductive organs and can be detected in bone and muscle. We investigated its role in locomotor coordination and muscle function and we were also looking for possible sex differences in 3-4-, 12- and 18-month-old C57BL/6 wild type and HK-1 deficient (Tac4KO) male and female mice.

In the static rod test, which is used to investigate locomotor coordination, mice are placed on the ends of rods of different thicknesses and the time it takes them to turn and reach the end of the rod is measured. In the grid test, the mice have to cling upside down on a metal grid. In the horizontal bar test, the animals have to grip the bars with their forelegs and climb out to the edge of the bar. These last two tests measure muscle strength.

No difference was found between the wild and gene-deficient groups in the young animals. In the ageing animals (12 and 18 months), both males and females, a significant deterioration in locomotor coordination was observed in the static bar test, which was significantly more severe in 12-month-old male Tac4 gene-deficient animals compared to respective wild types, but the opposite effect of gene deficiency was observed in 18-month-old females. A significant decline in muscle strength was also detected in older wild-type animals in both tests. In the grid test, the loss of muscle strength was significantly smaller in females compared to males, a phenomenon also observed in the horizontal bar test.

Our results suggest that HK-1 may play a complex regulatory role in locomotor coordination in old age, where important sex differences can be observed. However, HK-1 do not affect muscle strength. Therefore, elucidating the mechanism of action of HK-1 and its interactions with sex hormones may be important for drug developmental purposes.

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P4.08 Septal-preoptic connectivity and their thalamic input in suckling rats

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Mothers have to deal with a combination of sensory cues from the pups which has to be processed by numerous brain regions by forming a complex integrative neural circuit. We aimed to reveal the mainly implicated brain regions and their relation in the regulation of maternal care in a histological approach. First, we determined that the highest level of suckling-induced activation occurs in the medial preoptic area (MPOA) and the ventral part of lateral septum (LSv) in rat dams. Although, the MPOA is a well-known area which controls maternal care, little is known about the involvement of the LSv. First, we addressed to reveal the effect of cue modality from the pups on the septal activation. We used pup-deprivation method in rat mothers and confirmed that the level of activated neurons was significantly lower in those dams who received only pup-related vocal, visual and olfactory but not physical stimuli compared to suckling mothers, but still elevated when compared to totally pup-deprived mothers. As the highest activation is attributed to suckling, we addressed to reveal LSv input brain regions implicated in somatosensory processing. We used retrograde tract tracing and found that maternally-activated neurons of the posterior intralaminar thalamic nucleus (PIL), which has been shown to participate in touch-related stimuli processing among adult conspecifics, send projection to LSv neurons. Moreover, PIL neurons are known to express a suckling-induced neuropeptide, parathyroid hormone 2 (PTH2), thus we supposed PTH2 action on septal neurons. We showed that PTH2+ terminals form synaptic connection with activated LSv neurons. LSv projection of PTH2+ PIL fibers was confirmed by using distinct pathway tracing methods combined with immunohistochemistry in suckling dams. Furthermore, we defined that maternally activated septal neurons send the most prominent projection to the MPOA, which also receives PTH2+ PIL neuronal projection. Based on these, we investigated and showed that the same neurons of the PIL project to both LSv and MPOA neurons, which raises the possibility of their simultaneous regulation by PTH2 signal. We further found PTH2+ terminals in close vicinity of MPOA-projecting LSv and LSv-projecting MPOA neurons. In conclusion, these data indicate that the LSv and MPOA compose a subcircuit in maternal brain network and may transfer the stimulatory signal of the pups from PIL PTH2+ neurons thereby contributing to maternal behaviour.

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P4.09 Characteristics of place field formation in the hippocampal CA1 and CA3 regions

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Hippocampal pyramidal cells (PCs) expressing spatially tuned activity (i.e., 'place cells' exhibiting 'place fields', PF) provide spatial representations of environments to support successful navigation. Representations constantly evolve by the birth of new PFs; however, the cellular mechanisms of PF formation are elusive. Behavioral time scale synaptic plasticity (BTSP) is a novel form of synaptic plasticity described in mouse CA1PCs, which has been proposed to underlie rapid formation of PFs [1]. During BTSP induction, a previously silent PC exhibits a large regenerative Ca2+ plateau in dendrites accompanied by somatic burst firing, and this event initiates the emergence of subsequent activity near the location of the induction event. Owing to this mechanism, activity is strongest during formation and lower during subsequent visits to the PF. In addition, BTSP in CA1PCs has an asymmetric, seconds-long plasticity window that gives rise to a backward shift in PF location after the formation event. The properties and distribution of PFs formed by BTSP are still incompletely understood. It is also not well established if BTSP operates in CA3PCs, and if so, whether its characteristics are similar to those in CA1PCs.

Here we recorded the activity of PCs in the CA1 or the CA3a subfield using two-photon Ca2+ imaging in head-fixed Thy1-GCaMP6s mice navigating in two randomly alternating virtual environments. We developed an algorithm to detect and classify PFs as either newly formed or established already at the beginning of the imaging session. Inspired by recent work [2], we calculated features including formation lap gain (i.e., relatively strong activity in the formation lap) and initial shift (i.e., backward shift in PF position) that can capture the proposed properties of BTSP-formed PFs in CA1.

We show that newly formed PFs in CA1PCs exhibit higher formation gain and larger backward shift than established PFs, suggesting that BTSP plays a prominent role in the formation of spatial representations in this region. In contrast, newly formed PFs in the CA3a area do not differ from established PFs with respect to these features. The properties of new PF formation were statistically different between CA1 and CA3 PCs. Our results suggest that BTSP is either less prevalent as a mechanism underlying new PF formation in the CA3a subfield, or it manifests with different properties.

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P4.10 Parallel processing of noxious stimuli in the basolateral amygdala circuits

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The basolateral amygdala (BLA) is composed of distinct nuclei, including the lateral (LA) and basal nuclei (BA). The BLA plays a critical role in Pavlovian fear conditioning. In this robust learning paradigm, a neutral conditioned stimulus (CS) becomes associated to a biologically relevant unconditioned stimulus (US). Noxious stimuli, such as mild electrical shocks, proved to be a highly potent US and thus are widely applied in studies of fear learning. Therefore, here we aim to reveal the activity in the BLA neural networks upon delivery of the noxious stimuli. To this end, in awake head-fixed and anesthetized mice we used silicon probes to simultaneously record single-unit activity in the LA and BA during the presentation of mild electrical shocks. In addition, in anesthetized mice we juxtacellularly recorded the responses in randomly sampled neurons in the LA and BA during the administration of electrical shocks. Our data revealed that two populations of responsive principal cells (PCs) could be distinguished based on the latency of their stimulus-evoked firing. In the first population, PCs responded with a short latency to the noxious stimuli, while the stimulus related elevation of firing rates in the second group occurred with a longer latency. Both cell groups were present in both LA and BA. In further experiments we determined the latency of the noxious stimulus-evoked responses of two subpopulations of BA neurons projecting to the dorsomedial striatum (DMS) or to the medial prefrontal cortex (mPFC).We expressed channelrhodopsin-2 in these amygdalar neurons in a projection-specific manner using retrograde adeno-associated viruses. We used silicon probes to record the stimulus evoked responses of the optotagged DMS-projecting or mPFC-projecting BA neurons. Our results show that these subpopulations show markedly distinct responses to noxious stimulation.

Together, these results indicate that the LA and BA process noxious inputs simultaneously and that the shock-evoked response latencies in the BA occur in a projection-specific manner.

P4.11 fMRI monitoring of non-invasive deep brain stimulation in rats reveals anatomical shunting

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Transcranial Electrical Stimulation (TES) is a noninvasive method that can modulate neuronal activity. Its ability to limit the effects of stimulation to a target region while minimizing the currents in non-target areas is challenging (i.e., focusability), because the electric fields expected to be highest in superficial cortical regions underneath the stimulating electrodes and decreasing progressively as a function of distance from the cathode. However, experimental evidence for this assumption is lacking. Brain areas distal from the stimulation electrodes might be affected by the inhomogeneous conductivity of the brain, particularly current shunts via ventricles.

To measure the whole brain effects of TES, we combined neurostimulation with electrophysiology and BOLD fMRI in urethane anesthetized rats. We measured the TES-induced neuronal activity in the auditory cortex (i.e., below the cathode and anode), CA1 and CA3 regions of the hippocampus and thalamus in the same rat by repeated penetrations with a 4-shank Neuropixel 2.0 probe (total of 1536 sites). We acquired fMRI data with T2*-weighted single-shot GE-EPI sequence. The rat brain, excluding the cerebellum, was covered using 23 axial slices with the following parameters: TE/TR=13.4/(1500 or 2200)ms (TES or resting state fMRI), 300 repetitions and resolution = 0.23 x 0.23 x 0.8mm. 500-ms TES pulses were applied at various intensities (50, 100 and 250 μA), and frequencies (direct current pulses and 2, 4 and 8 Hz alternating current pulses). To validate our MRI data, we performed electrophysiology recording of spiking responses from the hippocampus after the fMRI experiments.

In our electrophysiology experiments, we found that electric fields did not decrease monotonically with distance from the stimulation electrodes but showed decreased field around the lateral ventricles. Single unit responses were strongest in cortex and weakest in thalamus. Our fMRI findings indicated that several brain regions, including somatosensory/motor cortices, hippocampus, and thalamus showed BOLD responses to TES. However, brain regions around the ventricles even in deep structures were also affected by TES. Our results indicate that TES can affect deeper brain regions via current shunting of the ventricles. We are also planning to further confirm these results by quantifying the c-Fos expression induced by TES.

P4.12 Noxious stimulus-responsive neurons in the ventral PAG and dorsal raphe nucleus

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The ventral periaqueductal grey (vPAG) including the ventrolateral PAG and dorsal raphe nucleus plays a critical role in controlling anxiety, fear memory formation, autonomic processes and most particularly, it is involved in descending modulation of pain processing. It has been shown that different neuron types, such as dopaminergic and serotonergic cells are part of this circuitry – besides the glutamatergic and GABAergic neurons – however their exact functions remained unclear. Additionally, malfunctioning of the circuit operation in the vPAG contributes to several neuropsychiatric disorders, the treatment of which is still a great challenge. Therefore, understanding the functional properties of neurons in this region can be critical in proposing new therapeutic approaches.

Here, we explored the single-unit activity in the vPAG in urethane-anesthetized mice in response to different types of noxious and neutral stimuli by applying two different recording approaches. First, we performed acute silicone probe recordings to determine how neurons respond to different type of stimulations. Additionally, using the juxtacellular recording technique with the same set of external stimuli and post hoc immunocytochemistry to identify the recorded cell types, we distinguished functionally different neuron types in the vPAG. Analysing the firing features and neurochemical content of the recorded neurons, we found that dopaminergic neurons can be separated into two groups based on their response latency and vasoactive intestinal polypeptide content, suggesting their different involvement in noxious stimulation processing. Further, we revealed that serotonergic neurons are heterogeneous and can be clustered into five groups based on their responses upon noxious stimulation. Based on our observations serotonergic and dopaminergic neurons were exclusively responsive to non-neutral stimuli.

Our current results show that the firing of the monoaminergic neurons in the vPAG circuitries is distinctly modified by noxious stimuli, implicating their different contribution to pain processing in this clinically important brain region.

P4.13 Investigating neuromodulatory systems by fiber photometry in different learning paradigms

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Dopamine, acetylcholine, and serotonin are neuromodulators that can dynamically change brain states and rapidly alter information processing of neurons. Neuromodulatory systems play an important role in cognitive functions, including learning and memory processes. Neuromodulatory systems can regulate overlapping cognitive processes and often show similar modes of action. In order to understand the connections between different neuromodulatory systems, it is important to examine them at the same time. Therefore, we directly compared and simultaneously monitored pairs of neuromodulation systems probing any two of the dopaminergic, cholinergic and serotonergic projection systems using fiber photometry and neurotransmitter sensors, which produce a fluorescent signal proportional to the neurotransmitter release. We trained mice on different learning paradigms, such as operant conditioning, gambling, and implicit learning, to address how neuromodulatory systems are related to associative learning processes. In the subcortical regions (ventral striatum and basolateral amygdala), we observed the release of acetylcholine and dopamine related to the reward and its prediction, while in the cortex we found an oscillatory pattern during the release of all three neuromodulators. Our cross-correlation analyzes showed that a correlated activity can be observed between acetylcholine and dopamine neuromodulator release, while a negative correlation was observed between acetylcholine and serotonin release. Our results show that the release kinetics of individual neuromodulators are different in the cortex and subcortical regions. Furthermore, we found that neuromodulators control the encoding of predictions in different learning types in a correlated manner, but in different time windows.

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P4.14 Diversity and connectivity of principal neurons in the lateral and basal nuclei of the mouse amygdala

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The basolateral amygdala is a cortical structure playing a role in various cognitive processes. In spite of many studies focusing on local information processing with the circuits of the basolateral amygdala, the features of amygdalar principal neurons remained elusive. Here, we combined neuroanatomical, electrophysiological and tracing techniques to determine the single-cell features and dendritic and axonal projections of principal neurons within the lateral (LA) and basal amygdala (BA). Using a mouse reporter line, we found that cholecystokinin (CCK) expression defined two groups of spatially segregated principal neurons both in the LA and BA. CCK+ principal neurons in the LA had small somata and short dendrites which matched to their passive and active membrane properties. CCK- principal neurons in the LA and all BA principal neurons had similarly ramified dendrites and single-cell features with some differences. Importantly, the dendritic arbors of principal neurons restricted to the sub-nuclei defined by the CCK expression, which matches some of the extra-amygdalar inputs. Axonal arborization patterns of principal neurons within the basolateral amygdala and surrounding areas showed correspondence to their soma location. For instance, BA principal neurons that projected to the medial prefrontal cortex or dorsomedial striatum were found in the medial part of the BA, revealing a significant overlap between the two neuronal populations. In contrast, those BA principal neurons that projected to this lateral part of the central nucleus located in the lateral region of the BA. Our results uncovered the diverse input and output properties of principal neurons in the LA and BA that help define the information flow within the basolateral amygdala networks.

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Poster Session 5
Cellular neuroscience

P5.01 Exploring trends in interneuronal diversity of the dorsolateral prefrontal cortex and caudate nucleus in primates

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Calretinin (CR)-immunopositive interneurons represent a major class of interneurons both in the dorsolateral prefrontal cortex (DLPFC) and the caudate nucleus (NC). The diversification of CR-immunopositive interneurons in primate brain evolution has been reported by transcriptomic and neurohistological studies (Krienen et al. 2019). Nevertheless, no comprehensive investigation has been carried out regarding the topography of CR subclasses and their functional diversity in closely related non-human primate species. Clinical relevance of the scope of our study is underlined by the fact that CR-immunopositive interneurons play a prominent role in excitatory/inhibitory imbalance observed in autism spectrum disorder and schizophrenia.

Our study aims to harness the opportunity provided by the Primate Brain Collection (Neuropsychiatry Laboratory, Semmelweis University) and provide quantitative and qualitative investigation of CR subtypes in several primate species. Our research mainly involves postmortem quantitative immunohistochemical analysis. The stained section are digitalized by a 3DHistech whole slide scanner and the labeled cells is annotated in Aperio Image Scope software.

The CR-immunopositive interneuron density of the NC was lower in the hominoid species Pongo pygmaeus and Papio hamadryas (~1500 cells/cm^2) than in the New World monkeys, for example in the Saimiri sciureus and Saguinus oedipus (more than 2000 cells/cm^2). In terms of frequency distribution of diameters, no major differences were found between the species studied.

Results represent the first stage of an initiative that aims to provide a comprehensive view on the cellular changes of the NC during primate brain evolution. As ”nothing in biology makes sense except in the light of evolution” (Dobzhansky, 1973), exploring trends in primate interneuronal diversity in the DLPFC and NC will help to understanding the patomechanisms underlying autism spectrum disorder and schizophrenia.

P5.02 Investigation of calretinin and parvalbumin immunopositive interneurons in the caudate nucleus and dorsolateral prefrontal cortex in domesticated and wild type foxes

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The underlying neurological changes corresponding with animal domestication are still a mystery yet to be solved. In this study we aim to investigate the neurohistological correlates of behavioral phenotypes between two subtypes of domesticated and wild type red foxes (Vulpes vulpes). The foxes were deliberately bred for tame vs aggressive behaviors for more than 50 generations at the Institute foar Cytology and Genetics in Novosibirsk, Russia.

We carried out qualitative and quantitative analyses on brain tissue of tame, aggressive and wild animals in search of changes in density of the calretinin and parvalbumin immunopositive interneurons in the caudate nucleus and dorsolateral prefrontal cortex. The stained section are digitalized by a ZEISS LSM900 confocal microscope and the labeled cells are annotated in Aperio Image Scope software.

So far we found a decreased density of calretinin immunopositive interneurons in the caudate nucleus, in one of the tame foxes (N=1, density=2182 neurons/cm^2) compared to the wild one (N=1, density=3256 neurons/cm2).

This work may help our understanding of cellular level alterations in neuropsychiatric diseases with marked behavioral phenotypes such as autism spectrum disorder and schizophrenia.

P5.03 Genetic approaches to target axo-axonic cells in the basolateral amygdala

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Axo-axonic cells are GABAergic interneurons that selectively innervate the axon initial segments of principal neurons in cortical structures, including the basolateral amygdala. The spiking of principal neurons can be effectively controlled by axo-axonic cells, as their axon terminals form synaptic contacts on that part of the axon initial segments where the action potentials are generated with the highest likelihood. To understand the role of these cortical region-specific GABAergic interneurons in circuit operation, their function needs to be selectively manipulated and monitored.

In this study, we used two types of genetic approaches to selectively target axo-axonic cells in the basolateral amygdala. First, we took advantage of the fact that the Ca2+ protein calbindin (Calb1) is predominantly expressed in parvalbumin (PV)-containing basket cells, but not in axo-axonic cells. Using an intersectional strategy, axo-axonic cells were labeled in offspring generated by crossing Calb1-Cre and PV-Flp mice by injecting AAV-Cre(off)-Flp(on)-ChR2-EYFP into the basolateral amygdala. Conversely, basket cells in these double transgenic mice were targeted by infecting them using AAV-Cre(on)-Flp(on)-EYFP. The specificity of this labeling strategy was confirmed by single-cell electrophysiological recording and labeling and global analysis of synaptic targets. Second, we use Vipr2-Cre mouse line to target axo-axonic cells in the basolateral amygdala. By injecting AAV-DIO-EYFP into this cortical structure, the vast majority of labeled interneurons showed axo-axonic cell morphology. As a support for this finding, most of EYFP-expressing cells lacked immunolabeling for vasoactive intestinal polypeptide (VIP), a neurochemical marker, which is present in a group of interneurons that may also express type 2 VIP receptors (VIPR2) based in RNA-seq data.

In summary, our results show that both strategies combining viral techniques with transgenic mouse models allow the targeting axo-axonic cells in the basolateral amygdala with high selectivity, opening a possibility to reveal the function of these GABAergic cells in amygdala-related operations.

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P5.04 Astrocyte: Cell for all season or simple glutamine factory? – Tricarboxylic acid cycle in astrocytes

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In the structure of central nervous system both neurons and glial cells have very important role. In the brain glial cells are mostly astrocytes, which supply the neurons with various substrates (e.g.: glutamine, lactate, glutathione precursors). The literature of the astrocyte's metabolism is quite inconsistence. The astrocytes have active glycolysis. They can donate the glucose to the neurons via two different routes: deliver directly the glucose molecule or transform throw the glycolysis to pyruvate then convert to lactate what feeds the tricarboxylic acid (TCA) cycle in the neurons. However, pyruvate's role in the anaplerosis of TCA cycle is still unclear. The various anaplerotic and cataplerotic pathways of the TCA cycle are the key mechanisms of the cell's metabolism.

Examination of the astrocyte's metabolism, including the characteristics of the TCA cycle. Take a stand on the controversial issues of the metabolic pathways of the astrocytes.

Astrocytes were isolated from new-born (P0-2) mouse brain and cultured in medium (MEM supplemented with FBS, glutamine, amphotericin B, gentamycin). TCA cycle enzyme activities were measured with spectrophotometry on lysed cells. Oxygen consumption was detected on intact and permeabilized cells with Clark-electrode. Metabolite concentration was measured with mass spectrometry.

Astrocytes have active glycolysis but also able to oxidate fatty acids and ketone bodies. The second part of the TCA cycle (molecules with 4 carbon atoms) has higher enzyme activity and substrate concentration compared to the first part of the cycle (molecules with 6 or 5 carbon atoms). The $NADP^+$-dependent isocitrate dehydrogenase (NADP+-IDH) and aconitase have significant extramitochondrial enzyme activity.

Our results suggest that pyruvate is not the main acetyl-CoA donor for the TCA cycle. Ketone body constant oxidation is not favoured in physiological condition. These outcomes may suggest that fatty acid oxidation is quite important in the astrocyte metabolism. α-ketoglutarate gets across throw mitochondrial inner membrane. In the cytosol, it assists the glutamate-glutamine cycle.

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P5.05 Dendritic spikes in human and mouse neocortical interneurons.

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Dendrites in the mammalian cortex fire a wide diversity of spikes that play a key role in behavior and perception (Larkum et al., 2022). Most of our knowledge of dendritic spikes has come from excitatory neurons, whereas little is known about dendritic spikes in interneurons (INs). Only a few studies reported that dendritic spikes occur in hippocampal INs (Katona et al., 2011, Chiovini et al., 2014, Cornford et al., 2019, Judák et al., 2022) with relatively small amplitude as compared with excitatory neurons. Surprisingly, there is no direct evidence for dendritic spikes in neocortical INs. Hence; although INs dendrites are considered as passive cables; it has recently been suggested that dendrites of prefrontal INs could express both supra- and sublinear behaviour (Tzilivaki et al., 2019). Here we investigate the excitability of dendrites in the human and the rodent neocortex in response to glutamate synaptic stimulation. We found that the dendrites of interneurons in both humans and rodents can integrate synaptic inputs nonlinearly and fire dendritic spikes. We further investigated the biophysical mechanisms of the dendritic spikes and the identity of the INs involved. Our results suggest that the dendrites of cortical interneurons have nonlinear behaviour that has the potential to increase their computational power.

P5.06 Low firing threshold shortens action potential generation delay in human fast-spiking interneurons

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In human brain, high energy-demanding neurons such as fast-spiking interneurons in the neocortex need reduced ion fluxes for action- and synaptic potentials because they exhibit increased somatic input resistance compared to rodent cells. This has to be an evolutionarily important adaptation in human neurons because it also slows down electrical reactivity of a neuron making cell soma slower in processing electrical inputs and generating action potential. Slowness is not beneficial to fast-spiking neuron computation, and therefore compensatory mechanisms have evolved in human neurons to boost their electrical rapidity. We report that fast-spiking interneurons in the human neocortex exhibit lowered action potential firing threshold, and this shortens the delay to generate action potential in response to excitation in soma. We show with anatomical analysis and demonstrate with realistic single-neuron computational model that the firing threshold is regulated through axon initial segment (AIS) length and proximity to soma whereas sodium channel localization pattern in the AIS is similar across fast-spiking cells.

P5.07 Synaptic and dendritic architecture of two types of hippocampal somatostatin interneurons

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GABAergic inhibitory neurons fundamentally shape the activity and plasticity of cortical circuits. A major subset of these neurons contains somatostatin (SOM); these cells play crucial roles in neuroplasticity, learning and memory in many brain areas including the hippocampus, and are implicated in several neuropsychiatric diseases and neurodegenerative disorders. Two main types of SOM-containing cells in area CA1 of the hippocampus are oriens-lacunosum-moleculare (OLM) cells and hippocampo-septal cells. These cell types show many similarities in their soma-dendritic architecture, but they have different axonal targets, display different activity patterns in vivo and are thought to have distinct network functions. However, a complete understanding of the functional roles of these interneurons requires a precise description of their intrinsic computational properties and their synaptic interactions. In the current study we generated, analyzed and make available several key datasets that enable a quantitative comparison of various anatomical and physiological properties of OLM and HS cells. The dataset includes detailed scanning electron microscopy-based 3-dimensional reconstructions of OLM and HS cells along with their excitatory and inhibitory synaptic inputs. Combining this core dataset with other anatomical data, patch-clamp electrophysiology and compartmental modeling, we examined the precise morphological structure, inputs, outputs, and basic physiological properties of these cells. Our results highlight key differences between OLM and HS cells, particularly regarding the density and distribution of their synaptic inputs and mitochondria. For instance, we estimated that an OLM cell receives about 8300, whereas an HS cell about 14800 synaptic inputs, about 16% of which are GABAergic. Our data and models provide insight into the possible basis of the different functionality of OLM and HS cell types and supply essential information for more detailed functional models of these neurons and the hippocampal network.

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P5.08 Optical recording of unitary synaptic connections between CA3 pyramidal cells using Voltron imaging

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Understanding the impact of synaptic connections and the rate of connectivity between individual neurons is crucial for drawing the blueprint of neuronal networks. We employ a novel voltage imaging method to map unitary synaptic connections with high efficacy between a large number of neurons. Voltron is a genetically encoded voltage indicator that is capable of detecting both action potentials and small subthreshold events, such as unitary EPSPs. We used Voltron imaging in acute slices to test the connectivity between CA3 pyramidal cells. These connections are thought to be crucial for major hippocampal memory functions; however, there are controversies in the literature about how densely CA3 pyramidal cells excite each other. Voltron was expressed sparsely in CA3 neurons using a mixture of two rAAVs expressing the Voltron protein Cre-dependently and the Cre enzyme. 4-8 weeks after the rAAV injections, we prepared acute slices that were incubated with a fluorescent dye (Janelia Fluor 549) that covalently binds to the perisomatically expressed Voltron protein. We measured the membrane voltage changes in up to hundreds of neurons using epifluorescent illumination with a CMOS camera at high speed (1 kHz) in a large field of view (375x235 micrometers). We detected spontaneous spiking activity of CA3 pyramidal at room temperature. Typically, we identified 10-40 active cells during one imaging session (2.5-4 minutes long imaging) within one field of view. If an individual cell elicited a sufficient number of spikes (>10 APs), we were able to observe subthreshold responses with sufficient signal-to-noise ratio in other cells that were also identified based on their spontaneous spiking. Thus, we correlated both the sub- and suprathreshold responses of potentially connected pairs (n = 1895 pairs). Only those pairs were accepted as connected where both response types showed clear excitatory effects (n = 85 connections). As the Voltron signal persisted after fixation and labelled the perisomatic dendrites and spines of the imaged cells, the spiking cells were anatomically verified as pyramidal cells or different types of GABAergic cells. In some of the experiments we tested the involvement of AMPA receptors in the detected pyramidal cell connections by using a specific inhibitor, NBQX and a positive modulator, cyclothiazide. Altogether we showed that Voltron imaging can reveal the synaptic connectivity of neuronal networks with high efficacy.

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P5.09 Cellular and molecular footprint of aging in a defined neuronal network encoding associative memory

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Due to the complexity of 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 models. One such model is the great pond snail *(Lymnaea stagnalis),* which has been used extensively for decades to study the functioning of the CNS with a top-down approach (behaviour-to-molecule). Its use as a versatile aging model showcased this species as a contemporary choice for modelling the behavioural, circuit, cellular, and molecular mechanisms of aging and age-related memory impairment.

We made the neuronal transcriptome assembly of *Lymnaea* and identified several evolutionarily conserved homolog sequences, such as klotho, huntingtin, presenilin, and RbAp48, to genes involved in aging, age-related memory impairment, and neurodegenerative diseases (e.g., Parkinson's disease) of vertebrates including humans, as well. 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 (12-16-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. Moreover, the expression of klotho, presenilin, and RbAp48 was visualized in the CNS of young and aged animals by in situ hybridisation. In the CGC, the expression of 143 transcripts showed an age-dependent manner. Using a novel HPLC-MS method for untargeted lipidomics, we identified 291 lipids in the whole CNS and showed that the amount of 79 lipids significantly changed during aging.

The identified cellular and molecular changes both at the system and single-cell levels during aging which may contribute to age-related memory impairment. Based on the preliminary results, the involvement of the genetic modification method can open avenues for 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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P5.10 Loss of microglial P2Y12 receptor function alters microglial morphology and contactomics

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Microglia are the main immune cells in the central nervous system that contribute to physiological and pathological brain states. Our laboratory has identified specialized contacts between microglial processes and both neuronal cell bodies and blood vessels through which microglia can shape neuronal activity and cerebral blood flow. The P2Y12 purinergic receptor (P2Y12R) is microglia-specific in the brain and plays a vital role in baseline microglial surveillance, while proper P2Y12R function is essential for the regulatory roles of microglia. In this study, we set out to investigate the effect of P2Y12R loss on microglial physiology and contact with other cell types.

The role of microglial P2Y12R was studied in wild-type mice, P2Y12R knockout mice as well as in mice injected icv with a selective P2Y12R antagonist. For morphological analysis and the contactomics of microglial cells with other cellular elements, we used confocal laser-scanning microscopy.

We found that the absence of P2Y12R altered microglial morphology both in the case of acute pharmacological inhibition and genetic deletion. The loss of function of P2Y12R also selectively changed microglial contactomics with glial cells and dedicated parts of the vasculature in the CNS. The acute and chronic lack of P2Y12R also affected microglia neuron interactions, while genetic deletion of P2Y12R also raised microglial cell numbers.

Our results indicate that P2Y12R function is essential for proper microglial actions even under physiological conditions. Disturbance of P2Y12R signaling has fundamental effects on microglial morphology and function. Since P2Y12R is only expressed by microglia in the brain potentially allowing cell-specific targeting, our results may give way to new therapeutic approaches in a broad range of neurological disorders.

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P5.11 Microglia undergo rapid phenotypic transformation in acute brain slices but remain essential for neuronal synchrony

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Acute brain slices represent a widely used model system for studying the central nervous system (CNS) from subcellular events to complex network functions. While slice preparation per se involves tissue injury, it is not known how microglia, the main immune cells of the CNS – with exceptional damage sensing capabilities – shape tissue integrity ex vivo. To this end, we have studied the mechanisms of microglial phenotype changes and contribution to neuronal network organisation and functioning in acute brain slices. Using a novel ATP-reporter mouse line and microglia reporter mice, we show that acute slice preparation induces rapid, P2Y12 receptor (P2Y12R) dependent dislocation and migration of microglia, paralleled with marked morphological transformations driven by early ATP surges and subsequent ATP flashes. Gradual depolarization of microglia is associated with the downregulation of purinergic P2Y12R and time-dependent changes of microglia-neuron interactions, paralleled by altered numbers of excitatory and inhibitory synapses. Importantly, functional microglia not only modulate synapse sprouting, but the absence of microglia or microglial P2Y12R markedly diminishes the incidence, amplitude, and frequency of sharp wave-ripple activity in hippocampal slices. Altogether, our results suggest that microglia are inherent modulators of complex neuronal networks, and their specific actions are indispensable to maintain neuronal network integrity and activity ex vivo. These findings could ignite new lines of research resulting in more advanced ex vivo methodologies and a deeper understanding of microglia-neuron interactions both in physiological and pathological conditions.

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P5.12 Role of microglial NKCC1 in inflammation and injury in the brain

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The Na+-K+-2Cl- co-transporter (NKCC1) is a member of the cation-chloride co-transporter family and mediates the uptake of Cl- in various cell types, including neurons and glial cells. Research suggests that NKCC1-mediated actions play a role in the pathophysiology of common neurological disorders such as cerebral ischemia and epilepsy. Inhibitors of NKCC1 have been shown to mitigate brain injury, exhibit antiepileptic effects, and regulate brain edema, primarily attributed to neuronal NKCC1-mediated actions. The function of NKCC1 in microglia, the main inflammatory cells of the brain, has remained unclear to date. To investigate NKCC1 function and its involvement in microglial activation, we have generated a transgenic mouse line to eliminate NKCC1 from microglia. According to our previous results, microglial NKCC1 has an effect on both baseline and reactive microglia morphology, process recruitment to the site of injury, and adaptation to changes in cellular volume in a cell-autonomous manner via regulating membrane conductance. In this study, we found that the deficiency of microglial NKCC1 leads to the priming of the NLRP3 inflammasome and increased production of interleukin-1β (IL-1β), increasing the susceptibility of microglia to exaggerated inflammatory reactions. Furthermore, in C57BL/6 mice, lipopolysaccharide (LPS)-induced cytokine levels *in vivo* were increased by intracortically administered NKCC1 blocker, bumetanide, and also in the absence of microglial NKCC1 in the brain, but not in peripheral tissues (spleen, liver). In contrast, systemic bumetanide application decreased inflammation in the brain. Additionally, in microglial NKCC1-deficient mice, the chronic systemic LPS administration markedly changed cytokine levels in the brain. Thus, NKCC1 emerges as an important player in controlling microglial inflammatory responses through which microglia modulate brain injury. The contribution of microglia to central NKCC1 actions is likely to be relevant for common neurological disorders, but further investigations are required to explain the mechanisms behind NKCC1-dependent regulation of microglial activity as well as the cell-specific effects of NKCC1 inhibitors.

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P5.13 Assessment of somatic microglia-neuron junctions throughout a lifespan

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Microglia, the resident immune cells of the brain play important roles in physiological and pathological processes, from development to aging. However, changes in microglial interactions with other cells across the lifespan remain elusive. Our lab recently discovered a novel form of direct contact between microglial processes and mature neuronal cell bodies in adult mouse and human brain, termed somatic purinergic junctions. Through these special purinergic connections, microglial processes monitor and protect neuronal functions in adulthood.

Due to the possible importance of similar interaction sites throughout development, we examined the presence, prevalence, structure and function of these somatic junctions during embryonal, postnatal and adult neurogenesis. By using advanced molecular anatomy and imaging, we show that somatic junctions are present on developing neurons from the embryonic age to adulthood. We demonstrated the specific ultrastructure of these developmental somatic junctions. Furthermore, we have shown that microglia can regulate neuronal proliferation and the development of cortical cytoarchitecture in a P2Y12 receptor-dependent manner through somatic junctions during development and in adulthood.

Previous studies have noted the importance of microglia in age-related conditions. Therefore, we examined possible changes of somatic junctions in old age. We have developed an in vivo 2-photon microscopy method that allows us to study changes of the same neuron-microglia junctions longitudinally, over months. Our results suggest that somatic junctions contribute to broad physiological and pathological processes and provide a key interactions site for microglia to monitor and regulate neuronal fate throughout the lifespan.

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P5.14 Epigenetic regulation of burn injury-induced nociception in the spinal cord

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Epigenetic mechanisms, specifically histone post-translational modifications (PTMs), are known to play an essential role in the regulation of various biological processes, including inflammation and pain. Histone H3 is a crucial protein that governs gene regulation and is also involved in burn injury. In the superficial dorsal horn of the spinal cord, spinal dynorphinergic (Pdyn) neurons contribute to the development of burn injury-induced heat hyperalgesia via histone H3.1 phosphorylation-dependent signaling. However, burn injury likely affects other histone PTMs in addition to histone H3.1 phosphorylation.

To investigate the potential involvement of other histone post-translational modifications (PTMs) in the epigenetic alterations resulting from burn injury in the mouse spinal cord, we conducted a Simple Western assay (WES). This technique allows for the efficient and reliable analysis of protein expression levels, with the added benefit of requiring minimal sample preparation. The findings revealed that each of these histone H3 PTMs was affected to some extent by burn injury, with three out of five PTMs showing significant upregulation.

To investigate the possible involvement of spinal Pdyn neurons in inflammation-associated burn injury that is linked to elevated levels of histone H3 PTMs, a double immunohistochemical approach was employed on free-floating spinal cord sections obtained from mice. The results from the double immunohistochemistry confirm the results obtained from the WES study.

The knowledge of post-translational modifications of histone H3 can offer possible targets for clinical therapy in diseases associated with pain. Therefore, it is crucial to continue ongoing research in this field to improve pain management strategies, as well as to develop new treatments for pain-related conditions.

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P5.15 Simple modeling method to investigate the Ca2+ transients in different types of cochlear supporting cells

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Supporting cells of the hearing organ, the organ of Corti is studied mostly in young and still deaf rodents. The receptors of the purinergic system are spread in the supporting cells and their roles in controlled spontaneous activity during the development has been recognized, but our knowledge about the presence and role in older mice is much sparse.

We have investigated the ATP induced Ca^{2+} transients with both experimental and modeling approaches.

ATP-evoked Ca^{2+} responses were measured in three types of supporting cells (Deiters' (DCs), Hensen's (HCs) and Claudius' cells (CCs)) in the mouse hemicochlea preparation. The evoked Ca^{2+} transients have different characteristics (in duration and amplitude) depending on the cell-type, but also has shown similarities.

We have set up a mathematical model to simulate the mechanisms of the ATP-induced responses (activate both the intracellular stores and the extracellular influx of Ca^{2+} through the ionotropic P2X receptors) separately. The model showed that DCs and CCs are more similar to each other than the HCs.

Our model was emulated reliably the Ca^{2+} transients measured by the functional imaging. The models suggest that the parameters of Ca^{2+} removing mechanism (especially in parameters of SERCA function) and the P2X receptors have the biggest differences between the cells. These new aspects of Ca^{2+} transient evolvement, provided by the model, are suitable to initiate new experiments to decipher the precise role of the intracellular Ca^{2+} regulation in DCs, HCs and CCs in the function of hearing and hearing sensitivity.

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P5.16 Characterization of galectin-1 positive subpopulation of microglia cells in aging brain

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Galectin-1 is a well-characterized immunmodulatory lectin in the peripheral immune system but its microglial expression and function is not-well understood. Through single cell data based in-silico analysis, a sub-cluster of microglial population with increased number of galectin-1 mRNA expressing cells was identified in the aged mouse brain. Similar galectin-1 expressing subcluster was visible in Alzheimer's disease inherited human brain microglial population. These aging coupled subcluster mostly differed from the other sub-clusters in immune regulatory gene expression, and these population contained the highest number of p62 expressing cells, suggesting the importance of the p62 coupled intracellular degradatory pathway in microglial cells during aging process. p62 protein is an autophagy regulator, recruits the ubiquitinylated proteins and send them into the lysosomal degradation pathway. To clarify the microglial function of galectin-1 and p62 in aging coupled biological events, rat primary cortical cultures were prepared and cultured for 21 days. During cultivation flow cytometry measured activation markers showed decreased expression level in the cortical cultures isolated microglial population suggesting a similar exhausted immunmodulatory capacity of microglial cells like the single cell mRNA data was represented. The detailed fluorescence immuncytochemical analysis showed that the galectin-1 expression coupled to the activated amoeboid like morphology of microglial cells. The extracellularly administered galectin-1 trigger the phagocytic capacity of microglial cells suggesting its autocrine function. In the long term cultured microglial cells there are higher number of p62 containing vesicles in amoeboid cells than in ramified microglial cell population. Our results nicely represents in the example of galectin-1 and p62, that long term in vitro cultivation based molecular results have similarity with in vivo neurodegenerative processes, and these similarities could be a basis to develop new therapies against aging coupled microglia based neurodegeneration.

P5.17 Altered purine, fatty acid and ester, amino acid and hormone profiles in migraineurs during the ictal and interictal periods

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Migraine is a primary headache affecting 10% of people worldwide. The pathophysiological mechanisms are still not clearly understood, and the therapy is often unsatisfactory. Therefore, disease- and headache-specific mediators and potential novel therapeutic targets need to be identified by hypothesis-free unbiased approaches using patients' plasma samples.

Here we analyzed the metabolomic changes in the plasma of patients during ictal and interictal periods in comparison with healthy controls.

Thirty six female and 1 male subjects were enrolled in this study: 24 episodic migraine patients with or without aura and 13 healthy controls. The studied groups were similar, matched in age, and anthropometric measurements such as body mass index (BMI). Samples were run using 4 different instrumental setups, with the liquid chromatographic separation of 106 metabolites, and the flow injection analysis of 524 metabolites using MxP® Quant 500 kit. For data analysis, samples with >20 % CV were filtered out, MetaboAnalyst 5.0 online available tool was used for this step. Log transformation was performed to normalize the data.

Enrichment analysis revealed pantothenate and CoA phosphatidylcholine and phosphatidylethanolamine biosynthesis, citric acid, alpha linolenic acid and linoleic acid metabolism, sphingolipid metabolism, fatty acid elongation in mitochondria, bile acid biosynthesis, carnitine synthesis between healthy subjects and patients in migraine phase. Glycolysis and pyruvate metabolism were implicated in pathway analysis.

Among ictal and interictal, enrichment analysis showed altered inositol, starch and sucrose metabolism, bile acid metabolism, steroid biosynthesis, pyrimidine metabolism, arachidonic acid metabolism, propanoate metabolism, trypthophan metabolism. Pathyway analysis for this comparison collected ascorbate and aldarate metabolism, pentose and glucoronate interconversions.

Mitochondrial mechanisms such as fatty acid elongation, citric acid metabolism might be disease-dependent and primary bile acid and arachidonic acid biosynthesis headache-dependent. These results suggest that metabolomic analysis gives valuable insight into the mechanisms of migraine.

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P5.18 Cholinergic regulation of dendritic Ca2+ spikes controls firing mode of hippocampal CA3 pyramidal neurons

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The hippocampus plays an important role in spatial navigation and contextual memory. Hippocampal pyramidal cells (PCs) frequently exhibit complex spike bursts (CSB), a firing pattern driven by regenerative dendritic Ca^{2+} plateau potentials. These events can induce rapid synaptic plasticity and initiate formation of new place fields in CA1PCs in spatially navigating mice. CSBs are also present in PCs of the CA3 area, and recent results suggest that place field-inducing plateau potentials and CSBs in CA3PCs are especially prolonged1. However, the dendritic mechanisms enabling such long-lasting events in CA3PCs are not well elucidated.

In our previous studies, conducted in acute rat brain slices, we observed large heterogeneity among CA3PCs both in CSB prevalence2 and in the kinetics of dendritic Ca^{2+} spikes[3-4]. While many CA3PCs express long duration (~50 ms) compound Ca^{2+} spikes, a group of cells exhibits unusually short (few ms long) Ca^{2+} spikes, which do not support sustained CSB firing. This raises the question whether specific conditions are required to gate the ability of these CA3PCs to fire prolonged plateaus.

Dendritic integrative functions are influenced by neuromodulation, including the cholinergic system that orchestrates memory processes. Acetylcholine (ACh) affects various ion channels including those mediating and shaping Ca^{2+} spikes in CA3PCs. This led us to investigate how dendritic Ca^{2+} spikes are regulated by cholinergic activity in CA3PCs.

The cholinergic agonist carbachol (2 µM) robustly prolonged pharmacologically isolated Ca^{2+} spikes, transforming short Ca^{2+} spikes into long-lasting forms. Carbachol also facilitated long-duration CSB firing in response to current injection or synaptic stimulation. On the other hand, nicotinic and muscarinic ACh receptor blockade did not affect Ca^{2+} spikes, indicating the heterogeneity of spikes is not due to variable cholinergic tone in the slice. Optogenetic stimulation of cholinergic axons in ChAT-Cre/Ai32transgenic mice increased CSB rate and duration, indicating that endogenous cholinergic activity can control Ca^{2+} spikes in CA3PCs.

We propose that cholinergic neuromodulation can gate the ability of CA3PCs with short-duration Ca2+ spikes to generate sustained plateau potentials, providing a state-dependent dendritic mechanism potentially contributing to memory encoding and retrieval.

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P5.19 Examination of viral PEPH3 peptide-functionalized nanoparticles on a culture model of the blood-brain barrier

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Nanoparticles (NPs) are promising new tools to increase the transfer of drugs across the blood-brain barrier (BBB) to the CNS. The vesicular NPs with appropriate ligands they are suitable for targeted drug delivery across the BBB. The aim of this study was to investigate the PepH3 peptide, isolated from the capsid protein of Dengue virus as a targeting ligand of NPs to elevate the cargo penetration across the BBB.

In our experiments, we prepared PepH3-targeted NPs loaded with the Texas-Red bovine serum albumin (TR-BSA) or single-domain antibody (sdAb) against amyloid beta peptide as cargo. The physico-chemical properties of NPs, such as particle size, polydispersity index and surface charge were measured by dynamic light scattering. The encapsulation efficiency was detected by spectrofluorimeter or western blot. The effect of PepH3-targeted NPs on the viability of primary rat brain endothelial cells was monitored by impedance measurement. The cellular uptake and co-localization of NPs cargo with endoplasmic reticulum (ER), golgi apparatus and lysosomes were visualized by confocal microscope. We quantified the cellular internalization, mechanisms of cellular uptake, also the penetration of NPs across the culture model of the BBB with spectrofluorimeter.

The mean diameter of untargeted and N-PepH3 particles was between 98-207 nm, respectively. The NPs have slightly negative surface charge and relatively narrow size distribution. The encapsulation efficiency of non-targeted and PepH3-targeted NPs with TR-BSA cargo were 32 and 24 %, in the case of sdAb loaded NPs were 93 and 68 %. PepH3 as a targeting ligand successfully elevated the cellular internalization of TR-BSA cargo in each time point, compared to the non-targeted group. The uptake of TR-BSA cargo was energy- and surface charge dependent process, also that was partially mediated by endocytosis. Furthermore, bigger colocalized area of NPs cargo was determined with ER and the second highest colocalization level was detected with Golgi, and limited amount of cargo could be colocalized with lysosomes. The transfer of TR-BSA loaded nanovesicles across the BBB model were approximately 3% in both NPs group, although PepH3-targeted nanovesicles with sdAb cargo had significantly higher penetration across the BBB model compared to non-targeted NPs after 24 hours incubations.

Our results proved that PepH3 is a good candidate to be used as a peptide for targeted brain delivery of therapeutic biomolecules.

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P5.20 Investigating α7 Nicotinic Acetylcholine Receptors in Human Induced Pluripotent Stem Cell-Derived Dentate Gyrus Granule Cells: A Molecular and Pharmacological Approach

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Nicotinic acetylcholine receptors (nAChRs) are widely distributed throughout the mammalian central nervous system (CNS) and play a crucial role in various presynaptic and postsynaptic neuronal activities by mediating endogenous cholinergic transmission. These receptors are pentameric structures composed of six α and three β subunits, with the α7 nAChRs being homopentameric structures containing five α7 subunits. The α7 nAChRs are of particular interest due to their selective localization in the central nervous system and their unique physiological and pharmacological properties. Extensive pharmacological research is focused on this receptor population as a potential target for developing cognitive-enhancing agents. Additionally, α7 nAChRs are present in human induced pluripotent stem cell (hiPSC)-derived dentate gyrus granule cells, serving as an in vitro model of hippocampal neurogenesis. This model system provides an opportunity to investigate the properties and function of α7 nAChRs in human neurons.

In our study, we generated dentate gyrus granule cells from induced pluripotent stem cells following the protocol established by Yu et al (2014) and validated the obtained cells through MAP2 and Prox1 staining, as well as functional assays. We demonstrated the expression and localization of α7 nAChR on neuronal cells using quantitative polymerase chain reaction (qPCR) and immunofluorescence staining. Furthermore, we employed Ca-imaging to monitor intracellular calcium transients in cells following treatment with choline (a selective agonist), PNU-120596 (an allosteric modulator), and methyllaconitine (MLA, an α7 nAChR antagonist).

Our results indicate successful generation of MAP2 Prox1 positive dentate gyrus granule cells from induced pluripotent stem cells and the presence of α7 nAChR, as confirmed by qPCR and immunostaining. Functional activity of the cells was verified using Ca-imaging, demonstrating that cells responded to both choline and PNU-120596 treatment by increasing calcium transients, which could be abolished by the addition of MLA.

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P5.21 Antidepressant drugs modify the permeability of blood-brain barrier by altering membrane trafficking

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The selective serotonin reuptake inhibitor class of represent promising candidates for drug repurposing. Fluvoxamine, an antidepressant belonging to this class of drugs modulated the endocytic membrane trafficking in non-neuronal cell types (Glebov, 2021). However, the mechanisms underlying their mode of action remain unclear. Based on these observations, our aim was to investigate the effects of fluvoxamine and sertraline on the endocytic pathways on a human culture model of the blood brain barrier. Real-time impedance measurements showed no cytotoxic effect of fluvoxamine or sertraline on human brain endothelial cells in the therapeutic concentration range of 30 nM to 1 µM. Fluvoxamine in 80 nM concentration increased the internalization of lucifer yellow (442 Da), a small hydrophilic dye and a marker of fluid phase endocytosis at the 4-hour time-point but not that of a larger endogenous protein, galectin-1 (15 kDa), which is internalized by a receptor-mediated mechanism. We also observed a two-fold higher uptake of lucifer yellow compared to galectin-1. Interestingly, sertraline at the same 80 nM concentration had no effect on the uptake of these two molecules. Next we investigated permeability: fluvoxamine treatment (80 nM) increased the permeability of galectin-1 and the passive paracellular marker molecule 4 kDa FITC-dextran across the co-culture model of the blood-brain barrier at the 1- and 2-hour time-points compared to the control groups. Furthermore, fluvoxamine treatment at 80 and 400 nM concentrations for 1 hour increased the labeling for EEA1 positive early endosomal compartment and LAMP1 positive late endosomes/lysosomes in human brain endothelial cells. In conclusion, we demonstrated that fluvoxamine, but not sertraline, is able to increase the permeability of a human culture model of the blood-brain barrier by modifying the endocytotic pathways.

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P5.22 Fear learning and aversive stimuli differentially change excitatory synaptic transmission in perisomatic inhibitory cells of the basal amygdala

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Inhibitory circuits in the basal amygdala (BA) have been shown to play a crucial role in associative fear learning. How the excitatory synaptic inputs received by BA GABAergic interneurons are influenced by memory formation, a network parameter that may contribute to learning processes, is still largely unknown. Here, we investigated the features of excitatory synaptic transmission received by the three types of perisomatic inhibitory interneurons upon cue-dependent fear conditioning and aversive stimulus and tone presentations without association. Acute slices were prepared from transgenic mice: one group received tone presentation only (conditioned stimulus, CS group), the second group was challenged by mild electrical shocks unpaired with the CS (unsigned unconditioned stimulus, unsigned US group) and the third group was presented with the CS paired with the US (signed US group). We found that excitatory synaptic inputs (miniature excitatory postsynaptic currents, mEPSCs) recorded in distinct interneuron types in the BA showed plastic changes with different patterns. Parvalbumin (PV) basket cells in the unsigned US and signed US group received mEPSCs with reduced amplitude and rate in comparison to the only CS group. Coupling the US and CS in the signed US group caused a slight increase in the amplitude of the events in comparison to the unsigned US group, where the association of CS and US does not take place. Excitatory synaptic inputs onto cholecystokinin (CCK) basket cells showed a markedly different change from PV basket cells in these behavioral paradigms: only the decay time was significantly faster in the unsigned US group compared to the only CS group, whereas the amplitude of mEPSCs increased in the signed US group compared to the only CS group. Excitatory synaptic inputs received by PV axo-axonic cells showed the least difference in the three behavioral paradigm: the only significant change was that the rate of mEPSCs increased in the signed US group when compared to the only CS group. These results collectively show that associative learning and aversive stimuli unpaired with CS cause different changes in excitatory synaptic transmission in BA perisomatic interneuron types, supporting the hypothesis that they play distinct roles in the BA network operations upon pain information processing and fear memory formation.

P5.23 Dendritic imaging of voltage and calcium signals during visual learning paradigm

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Although dendritic signaling strongly influences sensory processing and learning, the connection between dendritic integration and somatic output is still not fully understood. In this project, we aim to map the origins of dendritic action potentials, bursts and subthreshold events that occur during different aspects of visual learning.

We used 3D two-photon laser scanning microscopy with acousto-optic deflectors to simultaneously record jRGECO1a (calcium) and JEDI-2p (voltage) signals in the apical dendrites of layer 5 pyramidal cells—from the trunk to the distal tuft—in the primary visual cortex of mice during visual discrimination. We examined mice with controlled water access participating in various visual discrimination tasks, in which half of the trials were randomly rewarded with a drop of water. The stimuli consisted of drifting gratings with different orientations and directions to allow the investigation of dendritic events related to visual stimulation. The dendrites were imaged using 3D lines and chessboard patterns utilizing the drift-based 3D scanning technology, which allows us to quickly drift the scanning field in three dimensions while continuously recording fluorescence data and eliminates the need to maintain the same scanning position throughout the measurement.

So far, we have found that various types of voltage signals appear along the entire dendrite during visual stimulation, and that they seem to be analogous to the calcium signals. This opens up the possibility of determining the origin of the signals detected by the calcium and voltage sensors. Additionally, we have recorded dendritic activity during the training of the animals, during which they had to discriminate between rewarded and non-rewarded visual stimuli. In these measurements, we were able to detect dendritic events related to distinct aspects of the behavioral protocol, such as reward or error signals. Our goal from here is the detailed analysis and characterization of the composition and propagation of these voltage signals.

P5.24 Rapamycin and analogue macrolide immunosuppressants activate the cold sensor TRPM8 ion channel

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The Transient Receptor Potential Melastatin 8 (TRPM8) is a cold sensitive ion channel expressed by a small subset of the somatosensory neurons in the dorsal root and trigeminal ganglia (DRGs and TGs). It plays a key role in the detection of external temperature and its agonists, like menthol or icilin, evoke marked cold sensation. In this study we report that rapamycin, a macrolid immunosuppressant, also activates TRPM8 by binding to novel amino acid residues not involved in menthol binding.

We demonstrated in patch clamp and intracellular Ca^{2+} imaging experiments that Rapamycin effectively activates the human recombinant TRPM8 in HEK cells as well as its native variant in mouse somatosensory neurons from DRGs and TGs. Rapamycin also potentiated the cold induced TRPM8 responses, but other thermosensitive TRP channels (TRPV1, TRPA1, TRPM3) were not activated by the macrolid. The TRPM8 activation was found independent of the mammalian target of rapamycin (mTOR), as rapamycin activated TRPM8 in excised membrane patches, as well. Furthermore, advanced saturation transfer triple difference NMR (STTD NMR) spectroscopy revealed direct binding of rapamycin to TRPM8. Docking simulations based on the recently published high resolution TRPM8 models revealed possible rapamycin binding sites on TRPM8 independent of menthol binding pocket. Site directed mutagenesis based on the docking models identified amino acids selectively involved in rapamycin binding, whereas menthol insensitive mutants were activated by rapamycin. Investigating several analogs of rapamycin (rapalogs), we identified a key motif of the molecule essential to activate TRPM8. Without this motif, rapalogs acted as very weak partial agonists and competitively inhibited the activation of TRPM8 by rapamycin but did not affect menthol-induced activation, further confirming that the macrolide binding site differs from the menthol binding pocket.

Our findings describe TRPM8 as a new molecular target for rapamycin, and provide new insights into the mechanisms of its activation by agonistic ligands. These results may assist in the development of novel therapies targeting TRPM8.

P5.25 Effects of contrast agents used in medical imaging on the blood-brain barrier

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The use of contrast media (CM) during cerebrovascular surgeries has rapidly increased. During neurointervention computer tomography revealed extravasation of the CM across the blood-brain barrier (BBB) to extracellular spaces of the brain parenchyma. The purpose of this study was to investigate the effect of CM on BBB integrity, since the impact of CM extravasation and the related side effects still remain controversial.

We established an *in vitro* BBB co-culture model using rat primary endothelial cells, astrocytes and pericytes. Two types of CM were tested: iodixanol, an isoosmolar agent and iopamidol, a hyperosmolar agent. To evaluate the effect of CM on BBB functions a short term exposure (30 min) to both contrast agents was measured and the recovery of the cellular monolayers after treatment was tested. To measure cellular impedance across brain endothelial monolayers the real time cell electronic sensing method was used. Barrier integrity was evaluated using the primary triple co-culture BBB model by measuring transendothelial electrical resistance (TEER), permeability for fluorescent tracers and by analyzing tight junction morphology. In all experiments mannitol, an effective agent used for the delivery of therapeutics for central nervous system malignancies was tested at the same osmolarity as the isoosmolar and hyperosmolar CM.

Short term direct CM exposure caused a significant drop in the impedance of the brain endothelial monolayer without the possibility of recovery and causing a long-term damage of the cells. 1% and 10% treatment concentrations also decreased the barrier integrity, but these groups recovered to the level of the control after 24 hours. Junctional morphology, resistance and permeability studies also showed a disturbance in barrier integrity.

Extravasation of CM across the BBB raises awareness to the negative impact of neurointervention and endovascular therapy. It also shows that the clinical outcome for patients with acute ischemic stroke depends on the approach how CMs are used in everyday practice.

P5.26 HCN channels at the cell soma ensure the rapid electrical reactivity of fast-spiking interneurons in human neocortex

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Accumulating evidence indicates that there are substantial species differences in the properties of mammalian neurons, yet theories on circuit activity and information processing in the human brain are based heavily on results obtained from rodents and other experimental animals. This knowledge gap may be particularly important for understanding the neocortex, the brain area responsible for the most complex neuronal operations and showing the greatest evolutionary divergence. Here, we examined differences in the electrophysiological properties of human and mouse fast-spiking GABAergic basket cells, among the most abundant inhibitory interneurons in cortex. Analyses of membrane potential responses to current input, pharmacologically isolated somatic leak currents, isolated soma outside-out patch recordings, and immunohistochemical staining revealed that human neocortical basket cells abundantly express hyperpolarization-activated cyclic nucleotide-gated cation (HCN) channel isoforms HCN1 and HCN2 at the cell soma membrane, whereas these channels are sparse at the rodent basket cell soma membrane. Antagonist experiments showed that HCN channels in human neurons contribute to the resting membrane potential and cell excitability at the cell soma, accelerate somatic membrane potential kinetics, and shorten the lag between excitatory postsynaptic potentials and action potential generation. These effects are important because the soma of human fast-spiking neurons without HCN channels exhibit low persistent ion leak and slow membrane potential kinetics, compared with mouse fast-spiking neurons. HCN channels speed up human cell membrane potential kinetics and help attain an input–output rate close to that of rodent cells. Computational modeling demonstrated that HCN channel activity at the human fast-spiking cell soma membrane is sufficient to accelerate the input–output function as observed in cell recordings. Thus, human and mouse fast-spiking neurons exhibit functionally significant differences in ion channel composition at the cell soma membrane to set the speed and fidelity of their input–output function. These HCN channels ensure fast electrical reactivity of fast-spiking cells in human neocortex.

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P5.27 Anticonvulsant effect of the nonsteroidal anti-inflammatory drug meclofenamate via TRPM4 inhibition

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TRPM4 is a Ca^{2+}-activated non-selective cation channel regulating diverse physiological function of excitable cells. Previously we showed that TRPM4 is present and functionally active in hilar mossy cells. Furthermore, it contributes to mossy cells death following status epilepticus and therefore modulates seizure susceptibility.

Recently the non-steroidal anti-inflammatory drug meclofenamate has been identified as a potent TRPM4 blocker. Here we demonstrate that in vivo application of meclofenamate before the induction of status epilepticus reduces the frequency and duration of seizures. Furthermore, we showed that mossy cell loss is reduced in the ventral hippocampus upon meclofenamate treatment following status epilepticus. Finally, we detected *TRPM4* expression in human mossy cells.

This data indicates that pharmacological blocking of TRPM4 may serve as an effective antiepileptic strategy.

P5.28 Expression of connexins in the human epileptic neocortex

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Gap junctions (GJ) have a role in shaping both physiological and pathological synchronies. A relation between GJs and epileptiform activity has been found in in vitro and in vivo animal studies. GJ blockers decreased epileptiform activity suggesting that GJ communication has a synchronizing effect during seizure-like activity. Immunohistochemical studies examining neuronal and glial connexins in human epileptic neocortex are sparse.

Here we apply two commercially available antibodies against CX-36 (Invitrogen) and CX-43 (Abcam), the main neuronal and astroglial connexins, respectively. We aim to compare expression patterns in non-epileptic- and in the reorganized epileptic human neocortex.

We examined the postoperative neocortical tissues of epileptic patients (n=4) suffering from therapy resistant focal epilepsy. As controls we used the postoperative brain tissue of patients with brain tumor but without epilepsy (n=5). Immunostaining against CX-36 and CX-43 were done on sixty µm thin neocortical sections. The staining intensity was compared in non-epileptic and epileptic human tissue by measuring mean gray value on photomicrographs of the sections. The values were normalized to the values of unstained sections.

CX-36-immunostaining gave a very faint granular labelling in the neuropil of the neocortex. CX-43-staining displayed a strong granular labelling in the neuropil, and a characteristic staining surrounding blood vessels. In case of CX-36 staining, in the non-epileptic samples the mean gray values were 21% and 25% on average of the values of non-stained tissue in the supragranular and infragranular layers, respectively. In the epileptic samples the mean gray values were 41% and 46%. on average of the values of non-stained tissue in the supragranular and infragranular layers, respectively. In the case of CX-43 staining, these values were 33% and 28% on average in the non-epileptic samples and 52% and 49%. on average in the epileptic samples.

Our results may refer to a decreased expression of both neuronal and glial connexins in the epileptic human neocortex. This may be a compensatory mechanism in epilepsy to reduce synchronization. In the future we plan to increase our sample size and to correlate our findings with electrophysiological data of the same samples to be able to draw solid conclusions.

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P5.29 Electrophysiological and immunohistochemical comparison of the dentate gyrus projecting principal cells in the lateral and medial entorhinal cortex

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The entorhinal cortex (EC) is the primary input and output structure of the hippocampus forming a network hub in the cortico-hippocampal circuits. The medial (MEC) and lateral entorhinal cortex (LEC) convey spatial (grid cell) and contextual (odor, social) information to the hippocampus, respectively. This difference is largely attributed to the diverge inputs from other brain areas converging to these two subregions of the entorhinal cortex. However, the entorhinal cortex not only transmits information but also processes it. In order to understand the computational power in the two areas, we systematically compared the intrinsic electrophysiological properties of the dentate gyrus projecting stellate (MEC) and fan (LEC) cells in layerII.

We used in vitro whole-cell patch-clamp electrophysiology to compare their basic firing properties, action potentials and passive membrane characteristics. After electrophysiological recordings, we verified the biocytin-filled cells with specific immunohistochemical markers, reelin and WFS1.

We found that fan cells differed from stellate cells in many aspects. Stellate cells showed faster time constant and larger h-currents. During depolarization a spike-doublet appeared in stellate but not in fan cells at the beginning of the firing, which was consistently present during every sweep even at higher amplitude depolarization currents. When we used CsCl-containing intracellular solution, the doublets were absent in both stellate and fan cells. Cs+ acts as a voltage-gated potassium channels blocker, therefore, we assume potassium currents are involved in spike-doublet generation in stellate cells.

In order to shed light on the potential differences in voltage-sensitive ion channel expressions of these neurons, we will perform patch-seq single cell transcriptomic experiments and immunohistochemically verify the differences in expression levels.

Poster Session 6
Cognitive neuroscience

P6.01 Age-dependent changes in the activity of basal forebrain cholinergic neurons in ChAT-Cre and in the 3xTg Alzheimer-model animals

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By releasing acetylcholine (ACh), the basal forebrain cholinergic neurons (BFCNs) and their widespread projections to the cortical mantle play a key role in the control of cognitive functions. During ageing and in Alzheimer's disease (AD) the BFCNs undergo several physiological and morphological changes, such as degeneration of dendrites, axons and synapsis; nonetheless, the connection between cholinergic activity and the age-related neurodegeneration is still not completely clear. To address this, we examined the changes in the activity of BFCNs in different age groups of mice and the 3xTg AD-model animals.

We used head fixed mice during an auditory cued Pavlovian conditioning task. First, we optogenetically inhibited the cholinergic cells of the horizontal band of Broca (HDB) during the presentation of conditioned stimuli (CS). Second, we electrophysiologically recorded cholinergic cells of the HDB. Lastly, we simultaneously measured ACh release in the basolateral nucleus of the amygdala (BLA) and in the medial Prefrontal Cortex (mPFC) using dual fiber photometry.

We found that optogenetic inhibition of the cholinergic neurons in the HDB during the CS presentation caused learning impairments.

Our electrophysiology and photometry results suggest that BFCNs respond with an increase of activity to the reward-predicting CS and to the unconditioned stimuli (US) but not the punishment-predicting CS. In old mice and in AD-model animals ACh levels in the BLA and in the mPFC showed weaker to no response to the reward-predicting CS.

According to our results, ACh release from BFCNs is required during the acquisition of the CS-US association during Pavlovian conditioning. We found that during ageing and in the 3xTG AD-model animals, both the activity pattern and the ACh release of the BFCNs changed.

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P6.02 Placebo and nocebo effects in proprioceptive accuracy

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Changes in performance caused by positive and negative expectations (i.e., placebo and nocebo responses) were found to play an important role in many aspects of motor performance, such as maximal strength, endurance and sprint performance. In other cases, only the perceived, but not real performance was affected. The possible impact on proprioceptive accuracy, an important aspect of motor functions was not investigated to date. The goal of this study was to test this effect and the assumed impact of psychological factors: dispositional optimism and anxiety. 78 undergraduate university students were assigned into three experimental groups. The placebo group (n=26) received positive, the nocebo group (n=26) negative, and the control group (n=26) neutral instruction about the effect of a sham subliminal electric stimulation. Proprioceptive accuracy was measured with active and passive versions of the joint position reproduction task (i.e. participants had to reproduce elbow joint angles with active or passive motion of the arm). Mean of the absolute error scores were used as performance index. Accuracy was measured before and after the intervention. Expected and perceived change in performance were also assessed with visual analogue scales. Change in state anxiety and optimism were used as control variables (covariates). We used mixed analyses of variance models with one within subject factor (time) and one between subject factor (group) to test the hypotheses. The results indicated that the experimental manipulation did not affect actual proprioceptive accuracy ($p>0.05$), but it had an effect on expected and perceived performance ($p<0.05$) in both versions (passive and active) of the test. Adding the covariates (change in state anxiety and optimism) to the models did not influence the results. To explore the association between subjective and objective aspects of performance, we conducted correlation analysis. No significant association was found between actual and perceived change in performance in the active test, and only a weak correlation was found in the passive test. Expected performance did not predict actual performance but predicted perceived performance in both versions of the task. The results suggests that only subjective aspects of proprioceptive accuracy are susceptible to placebo and nocebo interventions.

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P6.03 Proprioceptive accuracy cannot be generalized across modalities

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Empirical evidence indicates that accurate perception of the state of different parts of the motor system (dubbed proprioceptive accuracy) shows substantial individual differences. Studies including multiple joints or multiple parts of the motor system are rarely conducted thus not much is known about the generalizability of proprioceptive accuracy. In this laboratory study, accuracy of perception of the actual angle of the knee and elbow joints (joint position reproduction task) and tension of the flexor muscles of the upper arm (weight discrimination task) was measured in 87 young individuals. Beyond objective performance, expected and perceived performance were also assessed for each task. Frequentist and Bayesian correlation analysis indicated that proprioceptive accuracy values with respect to different parts of the motor system are largely unrelated. No dominant-subdominant differences for actual performance were found; however, participants' performance with respect to the dominant and subdominant limb was associated in many cases. Finally, participants' perceived performance were unrelated to their actual performance; it was consistently predicted by expected performance for all three proprioceptive modalities. In summary, no generalized proprioceptive accuracy exists. Moreover, as actual performance in a proprioceptive accuracy test cannot be accurately perceived, expectation substantially impacts perception, as proposed by the predictive processing framework.

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P6.04 Shared neural signals for the processing of facial expressions.

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Recent studies using electroencephalogram (EEG)-based multivariate classification analyses have revealed shared neural signals associated with face familiarity [1, 2]. However, it remains untested whether such signals exist that capture other crucial facial properties. Here, we conducted an experimental investigation focusing on facial expressions, as these attributes represent salient features within facial stimuli and have previously been shown to be decodable from EEG signals at an individual level [3]. In this investigation, our primary objectives were to replicate these prior findings and, additionally, to extend our analysis to encompass cross-participant examinations.

Participants made two-alternative forced choice decisions about facial expressions (neutral, happy, angry, sad) present on photographs of 8 identities (4 male, 4 female), with each image presented 12 times, for 1 second, in a randomized order. 64 channel EEG was recorded, segmented between -200 and 1200 ms, and down sampled to 200 Hz. Time-resolved image × image representational dissimilarity matrices (RDMs) were constructed within-participant by split-half pairwise classification, and cross-participant by pairwise leave-one-subject-out classification, using linear discriminant analysis classifiers. These within- and cross-participant empirical RDMs were compared to stimulus identity, sex, and facial expression model RDMs.

Our preliminary results (n=16) revealed similar neural dynamics both in time and magnitude for within- and cross-participant sex and identity model correlations. In the case of the facial expression model, however, only an initial, ca. 150 ms effect was observed for the cross-participant RSA, while the within-participant RSA yielded an extended effect between 110 and 450 ms.

These results show that while the neural codes for certain facial characteristics, such as sex and identity, are shared across participants, the processing of emotional expressions are more idiosyncratic. It remains to be explored what contributes to these individual differences, as such, this result lays the groundwork for future studies exploring developmental, neurodivergent, and clinical populations.

P6.05 Shared neural codes of encoding and retrieval.

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Our previous investigations using cross-experiment multivariate pattern analysis (MVPA) found shared neural signals for memory recall, observed across various stimulus types and experimental paradigms, as well as participants [1, 2]. This study extends this research by considering not only the recall phase of the experiment, but including the study phase as well, to investigate the neural dynamics of visual stimulus processing for encoding, recall, and correct rejection in an old/new paradigm, using electroencephalography (EEG).

In multiple blocks, participants (n = 22) were experimentally familiarized with images of items drawn from different categories (faces, bodies, houses, toys, animals, vehicles, produce, furniture) about which they subsequently made old/new decisions. The images were presented for 1 second, and randomly assigned to be part of the study or novel set for each participant. 64 channel EEG was recorded, segmented between -200 and 1200 ms, and downsampled to 200 Hz. Time-resolved study-category/old-category/new-category representational dissimilarity matrices (RDMs) were constructed cross-participant by pairwise leave-one-subject-out classification, using linear discriminant analysis classifiers. Their correlations with stimulus category, memory condition, memory phase and seen/unseen predictor RDMs were calculated.

The cross-participant representational similarity analysis (RSA) revealed an effect of stimulus category, peaking between 100 and 200 ms. All memory-related contrasts yielded significant effects starting at ca, 300 ms, with the largest effects observed for the seen/unseen (study & new vs. old) model, followed by memory condition (study vs. old vs. new) and the memory phase (study vs. old & new) model. This was further corroborated by contrasting old/new, encoding/old, and encoding/new model correlations, where the encoding/old model yielded the highest effect, starting around 250 ms.

Here, we examined the shared neural representational dynamics of memory processes of encoding and recall, across participants and visual stimulus types. We found that both stimulus category and memory conditions can be identified using cross-participant RSA, with the strongest effects observed for encoding and correctly recalled items.

P6.06 The long-term impact of early life events on GABA and Glx levels in the human brain: A magnetic resonance spectroscopy study

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Early life events experienced during childhood shape the development of the brain and have long-term consequences on cognitive and emotional functions. Here, we studied the influence of a "positive" early life experience, i.e. musical training and a "negative" early life experience, i.e. children raised by institutionalized/foster care. Small, but significant metabolic changes in the brain can be measured using modern magnetic resonance (MR) spectroscopy methods. Our aim was to compare γ-aminobutyric acid (GABA) and glutamine/glutamate (Glx) concentrations in the anterior cingulate cortex of young adults who had either musical training or were raised by institutionalised/foster care.

We recruited twenty-five subjects (11 males, 27 ± 4.4 years) with musical training backgrounds and 23 subjects raised by institutionalized/foster care (11 males, 20 ± 1.6 years). Adverse childhood experiences were assessed with the Childhood Trauma Questionnaires (CTQ), and the current psychosocial well-being of the subjects were evaluated with the Beck Depression Inventory (BDI) and State-Trait Anxiety Inventory (STAI) questionnaires. Measurements of GABA and Glx levels were carried out with a Siemens 3T Prisma fit MR scanner, and the quantitative evaluation was performed with Gannet 3.3.2. GABA Analysis Toolkit and SPSS 29 statistical software.

In the institutionalized/foster care group, the average Glx/Creatine ratios were slightly higher (Glx/Cr 0.084 ± 0.012; GABA/Cr 0.0982 ± 0.011) compared to subjects with musical training background (Glx/Cr 0,069 ± 0.01; GABA/Cr 0.0975 ± 0.009). We observed a 0.72% surplus in GABA/Cr and 22.24% in Glx/Cr values of the institutionalized/foster care group. However, we could not detect any correlation between brain metabolite levels and the results of the psychological tests (CTQ, BDI, STAI).

Our present preliminary data suggests that early life events do not alter significantly the major excitatory and inhibitory neurotransmitter levels in the cingulate cortex of young adults.

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P6.07 The prediction of mental fatigue based on eye-tracking and psychological data: a machine learning study

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Mental fatigue is a result of prolonged cognitive performance that is associated with decreased cognitive performance and an increased feeling of subjective fatigue. Since fatigue is highly relevant for human safety, the machine-learning based prediction of fatigue has become important in recent years.

The aim of the current study was to train classification and regression models to differentiate between fatigued and non-fatigued states and to predict the level of fatigue caused by a prolonged cognitive task, respectively.

Thirty participants performed a prolonged and cognitively demanding mouse-pointing task, while eye-movements and pupil sizes were monitored via eye-tracking. For classification, eye-tracking and psychophysical variables were calculated for the first 5-min of the task (i.e. labelled as the "non-fatigue" state) and for the last 5-min of the task (i.e. labelled as the "fatigue" state). For regression, demographic, eye-tracking, psychophysical and psychological variables were used to predict the level of fatigue caused by the task. Both classification and regression models were trained using 5-fold cross-validation.

The best classification accuracy was achieved by the random forest classifier (AUC = 0.675 [CI95% = 0.646 – 0.704], precision = 0.636 [CI95% = 0.608 – 0.665]). Among the regression models, elastic net regression showed the best predictive performance (R2 = 0.608 [CI95% = 0.578 – 0.639], RMSE = 15.025 [CI95% = 14.318 – 15.732]). Based on permutation importance, the best predictors of post-task fatigue were the left and right pupil sizes, fixation instability and sleep duration prior to the experiment.

The classification algorithms trained on eye-tracking and psychophysical data performed poorer compared to previous studies that trained the algorithms on brain or heart activity data. However, the regression-based prediction of fatigue was successful indicating that eye-tracking variables might explain individual differences in mental fatigue caused by prolonged task performance.

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P6.08 Neuropeptide QRFP enhances memory in passive avoidance paradigm

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The incidence of dementia is on the rise worldwide with nearly ten million new cases occurring every year. To address this problem, active research of numerous neuropeptides, including the RFamide peptides and their properties on cognition, was initiated. Previously we have demonstrated that pyroglutamylated RFamide peptide (QRFP) enhances consolidation of spatial memory in rats. This effect was related (at least in part) to neuropeptide Y (NPY) mechanism. The aim of the present study was to examine the effects of QRFP on aversive memory.

Administration of two doses of QRFP (200 ng and 400 ng) was investigated in a step-through passive avoidance paradigm. NPY Y1 receptor antagonist BIBP3226 was applied to elucidate whether it can prevent effects of QRFP. All the drugs were applied directly into the lateral hypothalamic area (LHA), the primary location of QRFP synthesis within the CNS.

QRFP administered in 200 ng dose significantly increased the step-through latency in passive avoidance response, while a higher dose was revealed to be ineffective. The antagonist itself and combined Antagonist + QRFP treatments led to elongated step-through latency as well. The results remained significant one week after the first testing. These data suggest that neuropeptide QRFP facilitates passive avoidance learning, but this phenomenon cannot be linked to the NPY system.

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P6.09 The effect of the applied visual stimuli with different level of complexity on audiovisual equivalence learning

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The Rutgers Acquired Equivalence Test (RAET) is a learning task, where the subjects learn visual stimuli pairs, and after the learning, they have to retrieve and generalize the previously learned associations. We developed three audiovisual equivalence learning tests with the same structure, in which the antecedents were the same four different distinguishable sounds, but the consequents differed in complexity and semantic meanings. In the SoundFace test the consequents were four drawn faces, in the SoundFish four different colored fish (all same in size and shape), and in the SoundPolygon test blank geometric shapes. In the present study we compared the psychophysical performances of 52 healthy volunteers between the three audiovisual tests. We asked whether there is any difference between the performances when visual stimuli differ in complexity and semantic meanings. In all parts (learning, retrieval and generalization) of the tests, the performance was significantly better in the SoundFace test compared to the other two, but there was no significant difference between the SoundFish and SoundPolygon. Our results suggest that the color information alone could not significantly enhance the effectiveness of the associative learning. However, the verbalizability of individual features of the visual stimuli seem to determine primarily the performances in associative equivalence learning.

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P6.10 Comparing the performances of adult migraine patients and healthy controls in audiovisual associative learning

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Acquired equivalence learning is a type of associative learning where the subject learns that two or more stimuli are equivalent if they have the same consequences. Earlier, we compared the performances of migraine patients and a healthy control group in a visual learning paradigm, and we found that the patients performed significantly worse in the acquisition and most robust in the generalization parts of the task. In this study, we asked whether the same phenomena can be observed in an audiovisual equivalence learning paradigm with the same structure as the original visual test.

The participants had to form associations between an auditory antecedent and a visual consequent stimuli. The antecedents were four distinct sounds (human voice, engine starting, meowing cat and a guitar strumming), and the consequents were cartoon faces (man, woman, boy and girl).

We analyzed the data of 44 adult migraine patients and 44 healthy controls matched by age, gender, and level of education. In contrast to the visual study, the migraine patients performed significantly better in the acquisition phase of the audiovisual test. However, there were no significant differences in the retrieval and the generalization parts of the test phase between the two groups, except for reaction times in the generalization.

Based on these results, we suggest a probable overcompensating effect of multisensory information processing in migraine patients that could lead to better performances in equivalence learning and balance the profound functional alterations of migraine patients in the generalization functions.

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P6.11 Altered multisensory integration in pediatric migraine without aura

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Alterations of visual processing in migraine are well known. It seems to be that multisensory information processing is altered in migraine, too, but this aspects of migraine are strongly underexplored, especially regarding pediatric migraine.

In the present study performances in visual and audiovisual associative equivalence learning of the pediatric patients with migraine without aura (aged 7-17.5 years) were compared to those of age- and sex-matched controls.

The application of audiovisual stimuli significantly facilitated associative pair learning in healthy children and adolescents, but not in pediatric migraine patients. Although the performances were not significant worse in pediatric migraineurs compared those of the controls but the multisensory gain is significantly reduced in these patients.

The results of this study suggest that multisensory integration is altered not only in adult migraineurs but this dysfunction can be already observed in pediatric migraine patients, too.

P6.12 Adverse childhood experiences, psychosocial well-being and cognitive development among young adults raised by institutionalized/orphanage care.

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Children raised by institutionalized/orphanage care are exposed to various adverse childhood experiences (ACEs) affecting their emotional and neuro-cognitive development. Here, we studied the long-term impact of ACEs on psychological well-being, cognitive functioning and mentalization skills of young adults raised by institutionalized/orphanage care.

A total of 55 young adults participated in this study. 35 participants were raised by institutionalized/orphanage care and 20 participants were in the control group. To assess the severity of ACEs, we used the Hungarian version of the Childhood Trauma Questionnaire (CTQ). Mentalization skills were measured with the Reading the Mind in the Eyes Test (RMET) while social reasoning was evaluated with the Faux pas test. To assess the level of intelligence, we used 4 types of tasks, from the Wechsler Adult Intelligence Scale (WAIS). We also used two tests from the Cambride Neuropsychological Testing Automated Battery (CANTAB) to measure cognitive functions: the Paried Associates Learning (PAL), and Spatial Working Memory (SWM).

As expected, numerous differences were found between the groups. The incidence of ACEs, both physical and emotional abuse and neglect was more typical in individuals raised by institutionalized/orphanage care. In addition to that, such individuals also performed significantly worse in some parts of the CANTAB test, mentalization tests and the IQ test.

We found correlations between the CTQ scales and the reaction time and performance of the mentalization tests in the institutionalized/orphanage group. Further correlation was found in the institutionalized/orphanage group between SWM and physical abuse. In the control group, we detected correlation between SWM and physical neglect and the mean of the CTQ. Regarding the PAL test, correlation was found between PAL and sexual abuse in subjects raised by institutionalized/orphanage care.

We conclude that ACEs have a negative influence on the cognitive development and individuals with a history of childhood abuse show impaired performance in specific cognitive tests. Our findings provide further evidence that negative childhood experiences have detrimental impact on neurocognitive development and highlight the importance of adequate childcare on healthy development.

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P6.13 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.

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P6.14 Navigating the depths of crossed and uncrossed binocular disparities in visual short-term memory

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Successful interaction with our environment requires depth perception as well as temporary storage of this information. The exploration of visual perceptual memory has encompassed various stimulus attributes, including spatial frequency, colour and contrast, with previous studies unveiling specific time courses and a reliance on stimulus parameters. This study investigates visual perceptual memory for binocular depth, utilizing Dynamic Random Dot Stereograms (DRDS) featuring near or far disparities.

Within a delayed match-to-sample paradigm, we employed four distinct reference disparities (17.5', 28.8' either crossed or uncrossed) at two contrast levels (20%, 80%), spanning interstimulus intervals (ISI) of up to 4 seconds. Test stimuli represented a spectrum of equally spaced values centred around the reference disparity of the ongoing trial. Additionally, we explored the impact of a masking stimulus during memory retention. The Point of Subjective Equality (PSE) indicated the remembered disparity value.

The precision of responses exhibited superior performance for smaller reference disparities (+/- 17.5') compared to larger ones (+/- 28'), and it decayed as a function of ISI. The PSE demonstrated a consistent shift with longer ISIs, irrespective of the magnitude of the initial disparities, converging gradually toward the range of 20-23' and deviating away from the reference disparity. Notably, the influence of masking stimuli on the PSE was more marked when their disparities diverged from the reference value.

The findings from our study indicate that the retention of absolute disparity in memory exhibits imprecision, converging towards a central value. This central value could potentially signify an optimal point within low-level perceptual memory. Alternatively, it may be associated with perceptual averaging whereby the visual system computationally derives a statistical summary of the presented disparities over time. The latter mechanism would aid in the computation of relative disparity in a dynamically changing environment.

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P6.15 Positive valence regulated by pontine inhibitory cells: behavioral evidence

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The lateral habenula (LHb) regulates behavioral flexibility that is essential for effective decision making and can facilitate aversive or rewarding behavior as a function of its excitatory/inhibitory inputs. However, despite its abundant connections with hindbrain areas, the role of brainstem inputs is still unclear. Using viral tract tracing and optogenetic behavioral experiments in transgenic mice, we investigated a previously unrecognized population of gamma-aminobutyric acid (GABA)-ergic cells in the pons and their role in reward and fear encoding behavior. We found that stimulation of these GABAergic cells induced both acute and conditioned place preference in behavioral experiments, suggesting its potential role in reward behavior. Conversely, inhibition of these pontine neurons induced acute and conditioned place avoidance, suggesting that their baseline activity may be necessary to maintain motivation during spatial exploration. In addition, optogenetic inhibition of these cells during contextual reward learning prevented reward memory formation. We also found that these neurons have a potent anxiolytic effect, which is normally essential for proper fear encoding, preventing mice from overgeneralization. Our results suggest that a novel GABAergic pontine nucleus plays a major role in the processing of positive valence, reward-seeking and normal fear behavior, and may have a role in anxiety or mood disorders.

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P6.16 Measurement of GABA and Glx levels in the archicortex of subjects who had febrile seizures using MEGA-PRESS in vivo magnetic resonance spectroscopy

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Gamma-Aminobutyric acid (GABA) and glutamate + glutamine (Glx) are main neurotransmitters of the human brain and their concentrations can be measured in vivo using MEGA-PRESS magnetic resonance spectroscopy. Since this method is rather challenging, the first aim of this study was to test the reliability of GABA and Glx measurements in different cortical areas of healthy subjects. Secondly, we compared GABA and Glx levels in the hippocampi of subjects who had neonatal febrile seizures, a condition which is often associated with the later development of epilepsy.

For the reliability analysis, we carried out in total of 60 MEGA-PRESS measurements involving 10 healthy subjects, who were re-tested with a 1-week interval between the sessions using an identical protocol. Afterwards, we measured GABA and Glx levels in the hippocampi of 19 adults with and without febrile seizures. Measurements were carried out using a 3T Siemens Prisma Fit MR scanner and results were evaluated using Gannet 3.1 and SPSS software.

Measurement of GABA concentrations in subjects with febrile seizures revealed small, i.e. 2% difference of GABA/water concentrations and 1% difference for GABA/Cr levels when compared to subjects without febrile seizures. The largest difference between groups was in the right hippocampal GABA/water ratio, which was more than 9%. However, Glx levels were higher in subjects with febrile seizures. The Glx/water ratio difference was 18% and the Glx/Cr ratio was 14%. Largest difference was detected in the left hippocampi where the difference in Glx/water ratio was 18%.

Here, we demonstrate that GABA and Glx levels are reproducible, however, numerous factors influence the efficacy of such measurements. We found small differences in GABA levels between the subjects with or without febrile seizures, suggesting that febrile seizures may produce lasting effects.

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P6.17 Expected stimuli modulate early, low-frequency cortical activity

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Statistical learning is a cognitive process through which environmental regularities and co-occurrences can be acquired based on the transitional probabilities (TP) between stimuli. This learning facilitates sensory processing and reduces reaction time since certain stimuli with high TP become expected. Cortical changes have been investigated regarding the phenomena, including the early, low-frequency oscillations. It has been observed that alpha waves (8-12 Hz) reduce in the first 400 ms of presentation in case of expected stimuli compared to unexpected ones with exceptionally low TP. This study aimed to find similar differences between expected and neutral stimuli of average TP.

For this, we measured the EEG activity of 48 participants while they were exposed to a visual stimulus sequence, where they had to indicate the appearance of target stimuli. Unbeknownst to the volunteers, we manipulated the TP of the pictures, by having certain stimuli always follow each other in the sequence, creating stimulus pairs with a TP of 1. This manipulation created three conditions: the leading image (P1), the expected trailing image (P2), and control images (S), which had no statistical relationship above chance with other stimuli. After the EEG session, participants performed a familiarity test, where they had to indicate whether the presented stimulus pair was familiar or not based on the previously seen sequence. The answers to the familiarity test were converted into A' values according to Grier's formula. The EEG data was preprocessed then spectral analysis was performed using Morlet wavelets.

The behavioral results indicated that participants acquired the stimulus pairs above chance, which was tested using one-sample t-test (mean±SD=0.59±0.2, $t(47)=3.27$, $p<0.001$). We tested the difference in the 8-12 Hz activity in the first 400 ms after stimulus presentation using permutation-based statistics with cluster correction. The test yielded significant results and clusters emerged between conditions P1 and P2 (tsum=934.51, $p=0.011$) and conditions S and P2 (tsum=235.18, $p=0.027$). In both cases expected stimuli elicited lower alpha power.

We managed to detect the previously seen alpha activity changes. Additionally, this low-frequency modulation was observed between expected and neutral stimuli. Our results are in line with the literature, and it is further proof that high transitional probabilities modulate perception expectations at an early stage of stimulus presentation.

P6.18 Major remodelling of procedural training induced NREM sleep spindle clustering in adolescence

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We investigate the clustering of sleep spindles in a human developmental context, employing two procedural learning tasks widely acknowledged as inducing local plasticity in distinct cortical regions, thereby seeking to elucidate the neurophysiological underpinnings of memory consolidation during critical developmental stages.

Leveraging the high spatiotemporal resolution afforded by HD-EEG, sixty participants, aged 12, 16, and 20 years, underwent polysomnographic recording over an adaptation and two consecutive nights. Interposed between the latter, three sessions of contour integration and a sequential finger-tapping task were carried out, followed by a retest in the subsequent morning. Building upon our prior research describing developmental trajectories of relevant sleep spindle parameters (Bocskai et al., 2023) and their topographical relocation during adolescence (Gombos et al., 2022), we asked whether spindle clustering also goes through a major remodelling between childhood and adulthood.

We aggregated the 122 EEG channels and task- and spindle modality-specific derivations and investigated the differences in the spindle clustering parameters of the two nights. Our analysis differentiated between slow (9-12Hz) and fast (12-16 Hz) sleep spindle frequencies manifesting spontaneously during non-rapid eye movement of full-night sleep. In alignment with contemporary literature (Antony et al., 2018; Boutin et al., 2023), we quantified Spindle Trains to ascertain spindle clustering — spindles occurring with inter-spindle intervals not exceeding six seconds. We introduced the Spindle Local Density (SLD) metric to assess low-frequency clustering of spindles, which evaluates spindle frequency over a 60-second spindle-centered sliding window. Please see our posters by Gerván et al, and Berencsi et al. for the analysis of spindle clustering in the task-relevant cortical networks as a function of behavioural improvement in the perceptual and motor tasks.

We found a significant elevation of fast spindle clustering within the scalp, specifically the occipital, temporal, and frontal-polar-frontal areas, as a result of training. The results for SLD, inter-train interval, and the ratio of spindles in ‘trains’ became more robust with age. These findings suggest that in parallel to the topographical relocation of sleep spindles in adolescence, spindle clustering also goes through major alterations before the emergence of the adult pattern.

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P6.19 Daytime performance determines subsequent NREM sleep spindle clustering in a motor learning task

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It has recently been proposed that the clustering of sleep spindles into trains is critical for efficient reactivation and consolidation of motor memories in humans (Boutin & Doyon, 2020; Boutin et al., 2023). Relying on HD-EEG, here we aim to obtain a more detailed view of spindle clustering in task-relevant cortical networks as a function of behavioral improvement in a motor task.

As it is also detailed in the abstract by Gombos et al., we invited a developmental cohort (between 12 and 20 years of age) to spend an adaptation, and two consecutive nights under polysomnographic recording of full-night sleep. At least three weeks preceding the experimental sessions, and also three times between the two analyzed sleep recordings, participants practiced in a four-element sequential finger tapping task. There was a retest session after the third night. Although this training schedule is relatively short, we assumed that we can still tap into network-specific procedural memory consolidation due to the pre-recording motor learning session that potentially helped participants to acquire the cognitively demanding part of the task, reducing cognitive load in later sessions. Since motor performance of 16- and 20-year-olds was very similar, we collapsed these two age-groups for further analysis (n=40, female=20).

Regions of interest in the HD-EEG recordings included parietal and frontal somatomotor regions, and frontal-prefrontal areas. Performance rate in the motor task (correct taps/s) was calculated as a combined measure of speed and accuracy. We then analyzed the correlation between daytime and overnight motor improvement and the difference between spindle organization characteristics preceding and following motor training.

While overnight improvement was not associated with clustering, daytime motor improvement was linked to sleep spindle organization. The number of fast and slow spindle trains increased with decreased train intervals and decreased number of isolated spindles in somatomotor and frontal-prefrontal localizations bilaterally as a function of daytime improvement. This pattern suggests that within the short training regime there was still a significant engagement of cognitive resources explaining the lack of area-specific changes associated with offline learning. On the other hand, the strong association between daytime learning and subsequent bilateral spindle clustering suggests the role of sleep spindles in the stabilization of task-related memories.

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P6.20 Learning-dependent occipital NREM sleep-spindle clustering following extensive training in a visual perceptual task

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Sleep spindles have been implicated to contribute to brain plasticity and the consolidation of procedural memories. More recently, research by Boutin and colleagues (Boutin & Doyon, 2020; Boutin et al., 2023) suggests that the clustering of spindles into groups, referred to as trains, plays a critical role in motor learning. However, the association of clustered sleep spindles with perceptual learning has not yet been investigated.

In this study, we aimed to explore how spindle clustering contributes to visual perceptual memory consolidation by applying a contour integration task in humans. Perceptual learning in contour integration specifically addresses the long-range horizontal connections in the primary visual cortex (Kovacs & Julesz, 1993; Angelucci et al., 2002) and together with its documented sleep-dependent (Gerván et al., 2007) manner, this paradigm offers a well-established investigation of learning-dependent changes in electroencephalographic activity during sleep.

As it is also detailed in the abstract by Gombos et al., we invited a developmental cohort (between 12 and 20 years of age) to spend an adaptation, and two consecutive nights under polysomnographic recording of full-night sleep. At least three weeks preceding the experimental sessions, and also three times between the two analyzed sleep recordings, participants practiced in the contour integration task. There was a retest session after the third night. Since perceptual performance of 16- and 20-year-olds was very similar, we collapsed these two age-groups for further analysis (n=39, female=20). Based on contour integration performance improvement between the first and last sessions, we divided the participants into two distinct groups: non-learners (n=17) and learners (n=22).

To explore the connection between memory consolidation and sleep spindles, we compared the operating characteristics of aggregated occipital channels before and after training. We found an elevated level of spindle clustering by the second night in the 'learner' group as it is reflected in the significant change in the ratio of fast spindles organized into trains, an increase in the number of fast spindle trains, and a decrease in the inter-spindle-interval within trains. No such pattern of clustering was found in the 'non-learner' group. These findings suggest that the clustering of sleep spindles into trains may support the efficient reactivation and consolidation of perceptual memories.

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P6.21 Unlocking the Growth Spurt of the Brain: The Transient Tale of Adolescent Cognitive Abilities

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Adolescence represents a critical period not only for biological and social but also for brain and cognitive development. We demonstrated earlier that biological age has a selective effect on cognitive abilities in adolescence (Kovacs et al, 2022). Here we investigate the potential long-term effects of objectively measured pubertal maturity on cognitive abilities.

Using ultrasonic bone age assessment to estimate biological age, participants (117 females, 11-15 y, one-year-wide age bins) were categorized into decelerated, average, and accelerated pubertal status groups at Time 1 (T1), based on the difference between their bone age and chronological age. Cognitive abilities were assessed applying WISC-IV subscales. At T1, bone maturation determined performance in Working Memory and Processing Speed within the same age-bins, with better performance at higher maturity levels. Testing the long-term effects on a subset of 60 participants (17-19 y, mean age=18.41, (SD=0.62)) of the same cohort at T2 we had an opportunity to examine whether faster maturation leads to long-term overdevelopment or whether the benefits of more speedy maturation are transient. It also allowed us to estimate whether individual variability in puberty onset time may alter developmental trajectories spanning into adulthood. At T2, there were no significant differences in cognitive performance among the maturity groups. The trajectories of the decelerated and average groups aligned with that of the accelerated group, suggesting that the early advantage observed at T1 is a transient developmental event. We argue that the short-term developmental differences, even if transient, are still essential to discuss since they could temporarily place the child outside the typical range and cause heightened stress levels.

Our research group presents another poster at this conference entitled *"Navigating Pubertal Goldilocks: The Optimal Pace for Hierarchical Brain Organization"* by Szakács et al., indicating that cortical entropy production of the same teenage participants follows a different pattern than the one observed with respect to cognitive development. We interpret these findings as an indication for the presence of both transient developmental changes and potentially long-term deviations from normative development related to the speed of pubertal maturity.

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P6.22 Navigating Pubertal Goldilocks: The Optimal Pace for Hierarchical Brain Organization

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The human brain undergoes a series of transformations during adolescence, a process that has been linked to the pubertal increase of gonadal steroids. However, the underlying mechanisms of pubertal hormonal changes and the reorganization of the brain during adolescence are not clear yet. Uncertainty around the impact of atypical pubertal timelines arises from ineffective maturity measures and difficulty dissociating chronological age (CA) from pubertal maturity. We recently introduced ultrasonic bone age (BA) assessment as a reliable indicator of physical maturity to resolve this ambiguity. We combined this method with a measurement of entropy production, an index of the level of hierarchical organization in the brain, to explore whether it changes as a function of physical maturation. In thermodynamics and systems biology, asymmetry and established directionality of flow in the state space is linked to the level of hierarchy in the system. This is based on the second law of thermodynamics, stating that a system will go from order to disorder over time. Quantifying asymmetry through entropy production captures the level of functional hierarchical organization by analyzing the temporal dynamics of brain signals.

Entropy production calculations were performed on 87 eyes-closed resting-state EEG recordings in the alpha frequency range, collected from 61 adolescent females in three non-overlapping developmental stages (decelerated, average and accelerated) in two chronological age groups, and 26 emerging adult females. We parsed EEG recordings into 12 phase-based connectivity patterns, and we studied the sequence in which they occurred, specifically focusing on the asymmetrical nature of their transitions.

To dissociate the effects of CA and BA, we analyzed the data through various grouping approaches forming age groups, maturity groups, and maturity groups within two age groups. While entropy production marginally increased with age, it was significantly higher in the average maturity group compared to both the decelerated and accelerated groups. The same result was found among maturity groups of one age bracket. The results indicate an advantage of on-time maturation with respect to hierarchical brain organization within our neurotypical cohort. Significant deviations towards accelerated or delayed maturational speeds might amplify these disparities, increasing neurodiversity, and potentially leading developmental trajectories into the clinical spectrum.

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P6.23 Multi-night EEG reveals positive association between sleep efficiency and hippocampal subfield volumes in healthy aging

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Age-related atrophy of the human hippocampus and the enthorinal cortex starts accelerating at around age 60. Due to the contributions of these regions to many cognitive functions seamlessly used in everyday life, this can heavily impact the lives of elderly people. The hippocampus is not a unitary structure and mechanisms of its age-related decline appear to differentially affect its subfields. Human and animal studies have suggested that altered sleep is associated with hippocampal atrophy. Yet, we know little about subfield specific effects of altered sleep in healthy aging and their effect on cognition. Here, in a sample of 118 older adults (Mage = 63.25 years), we examined the association between highly reliable hippocampal subfield volumetry, sleep measures derived from multi-night recordings of portable electroencephalography and episodic memory. Objective sleep efficiency – but not self-report measures of sleep – was associated with entorhinal cortex volume when controlling for age. Age-related differences in subfield volumes were associated with objective sleep efficiency, but not with self-report measures of sleep. Moreover, older adults characterized by a common multivariate pattern of subfield volumes that contributed to positive sleep– subfield volume associations, showed lower rates of forgetting. Our results showcase the benefit of objective sleep measures in identifying potential contributors of age-related differences in brain-behavior couplings.

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P6.24 Daily associations of sleep, dreaming and emotions with cognitive errors in the elderly

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Suboptimal sleep quality (SQ) and emotional disturbances are potential precursors of cognitive ageing, and nightmares specifically seem to have a distinctive role in that. Cognitive performance can be measured with objective tests, but the perception of one's cognitive decline is also a risk factor for neurodegenerative disorders. The aim of the present study is to capture the links between sleep, emotions and cognition with prospective measurements in naturalistic contexts.

72 elder individuals participated in a one-week-long home-based study, which started with an intelligence test; then, over the course of seven days, participants reported on their dreams, SQ, emotions, and subjective cognitive errors daily. Additionally, a portable electroencephalographic headband was used to obtain objective sleep indicators. Principal component analyses were performed on the rated emotions and the electroencephalographic markers. After calculating the within-person weekly mean values of the variables, we carried out linear regression to uncover the associations between nightmares, dream and morning emotions, subjective and objective SQ and subjective cognitive errors with age, gender and the intelligence quotient as covariates.

Results showed that even though, due to the low frequency of nightmares in our sample, these dysphoric dreams did not predict a higher rate of subjective cognitive errors, both negative dream emotions (even when negative mood was controlled for) and negative morning affect (regardless of dream recall) did so. As for SQ, higher subjective sleep fragmentation was associated with more subjective cognitive errors, but neither the hyperarousal nor the sleep continuity components of objective SQ were significant predictors of subjective cognitive errors. In each case, the intelligence quotient was a significant covariate, implying that the self-assessment of cognitive functioning seems to be a valid proxy of cognitive performance.

In conclusion, negative affective states in the morning, which can be the result of not only poor SQ, but also bad dreams, are linked to the perception of decreased cognitive abilities, even if the individual did not have a nightmare episode. Furthermore, subjective SQ is a stronger predictor of subjective cognitive errors than the physiological parameters of sleep. Our findings based on daily data with high ecological validity indicate that improving SQ and emotion regulation might lead to a reduced risk of cognitive ageing.

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P6.25 Assessment of various dynamic random dot stimuli for the electrophysiological examination of binocularity – towards a standardized clinical protocol

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Binocular vision and stereopsis are visual functions, whose absence can be indicative for several neuro-ophthalmological syndromes. Our goal is to develop a robust stimulus and study design for the objective clinical determination of stereopsis based on steady-state visually evoked potentials (ssVEPs).

It is widely accepted that the most suitable types of stimuli for the selective examination of stereopsis are dynamic random dot stereograms (DRDS) and correlograms (DRDC). By using DRDC or DRDS, numerous different binocular stimuli can be generated, such as spatial surfaces or changes in the perception of depth over time. Viewed with one eye, these stimuli appear as dynamically refreshing random dots, but with both eyes, they evoke the perception of stereoscopic depth, provided the observer has intact stereopsis. In this study, we used the widely accessible and cost-effective anaglyph technique to stimulate the left and right eyes separately.

We aimed to select the most reliable stimulus that elicits a statistically significant, reproducible ssVEP in as many participants as possible. For determining significance, we used the T2circ statistic at $p<0.01$.

Altogether, 24 participants aged 34.3±9.2 years with intact stereopsis were enrolled in the study. The ssVEPs were recorded from standard electrode positions O1, Oz, O2, P7, P3, Pz, P4, P8, FP1, FP2. The duration of the recording was at least 90 s for each stimulus.

The following parameters were varied resulting in 19 different stimuli:

- type of stimulus: DRDS or DRDC
- depth-encoded spatial pattern: full-field, checkerboard, central square
- temporal frequency of change in depth pattern: 0.9375 - 3.75 Hz
- presentation mode: onset or reversal
- size of random dots: 5.6', 7.5', 9.4'
- refresh rate of random dots: 60 Hz or 30 Hz.

The three most effective stimuli were all DRDCs (refresh rate 60 Hz, dot size 7.5') with variations of depth pattern or frequency of depth pattern change and evoked significant response in 85%, 83% and 77% of cases. The most reliable response was evoked by the DRDC full-field pattern stimulus. Control measurements where one of the eyes was covered resulted in no significant response. Interestingly, the perceptual salience of depth reported by the participants was not a reliable predictor of ssVEP response. In summary, our current results suggest that coherent ssVEP responses evoked by dynamic random dot correlograms can be used as an objective marker of stereopsis.

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P6.26 Application of the self-ordered spatial search paradigm for the investigation of primate working memory

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The self-ordered spatial search (SOSS) task is a non-verbal touchscreen paradigm probing visual spatial working memory (SWM) that can be applied in non-human primate research in a preclinical translational experimental setting. We examined the performance of the animals in the task on control baseline days and after a transient cognitive impairment induced by the muscarinic acetylcholine-receptor antagonist scopolamine.

Fourteen adult male rhesus monkeys performed the SOSS task in 1-hour sessions with 300-500 trials each. In every trial the animals were shown 4-8 identical cyan colored squares on a touchscreen, and they had to touch each square in an arbitrary order once, and only once. Each touch was followed by a 0.5-2s long delay period after which all squares reappeared at the same location. Besides accuracy, we analysed continuous (a stimulus is touched twice in a row) and recurrent (a stimulus is touched again, but not directly after the previous touch) perseverative error rates (CPE and RPE, respectively). This distinction is important because while CPEs can be caused by simple motor perseveration, RPEs are more likely to involve failures of memory processes. The distribution and ratio of the errors throughout the trials can also give us information about the animals' task solving strategies and limitations of memory capacity.

Increasing the set size resulted in selectively increased proportion of the RPEs that presumes higher levels of SWM involvement. Even though scopolamine treatment dose-dependently deteriorated performance, it was not selective to any of the errors. The close-to-zero amount of CPEs in all choices and the higher RPE rate in the later phase of the choice sequence suggest an "n-back" capacity of working memory in this task.

Our present version of the SOSS task design involving different set sizes appears to be a sensitive assessment of behavioural correlates of SWM processes. The observed transient amnestic effects of muscarinic antagonist agent scopolamine proved the applicability of the SOSS task for preclinical cognitive research. Associating the observed effects to specific working memory processes and further optimizing the SOSS task will make it suitable for efficacy assessment of novel cognitive enhancer pharmaceutical drug candidates.

P6.27 Association between nucleus accumbens and slow wave sleep in the Hungarian Longitudinal Study of Healthy Brain Aging

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Aging is characterized by a gradual deterioration of brain structure and sleep quality, and the degree of deterioration is implicated in many psychiatric and neurodegenerative diseases. However, the relationship between age-related changes in sleep and brain structure remains poorly understood. Slow wave sleep (SWS), the deepest non-rapid eye movement sleep stage, appears to be particularly vulnerable, as it declines linearly throughout adult life. Since previous research in animal models revealed that nucleus accumbens (NAc) might play an important role in controlling slow wave sleep, here we aimed to investigate the association between subcortical volumes with an emphasis on the NAc region and SWS in cognitively unimpaired older adults (N=69). Structural MRI measurements were performed on a Magnetom Prisma 3T MRI scanner. Subcortical volumes were derived from FreeSurfer and volume deviation scores were calculated using normative modeling. Sleep was recorded longitudinally in all participants at home up to 7 nights using a four-channel portable EEG device (Dreem 2) and sleep stages were annotated automatically. Both MRI and sleep data were recorded twice, 1.5 years apart as part of the Hungarian Longitudinal Study of Healthy Brain Aging. Regression modeling revealed a positive association between SWS duration and NAc volume as well as a negative association between SWS and caudate nucleus (CN) volume. These results suggest that NAc is involved in SWS regulation in humans, which is consistent with previous experimental research in rodents. Furthermore, our findings also revealed an association between CN and the duration of SWS, suggesting that both the ventral and dorsal striatum may play an important role in regulation of sleep and its impairment during aging.

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P6.28 Positive valence regulated by pontine inhibitory cells: anatomical evidence

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The lateral habenula (LHb) is a regulatory centre for negative experiences and effective decision-making via integrating sensory and experience-related information to regulate various motivational and cognitive processes. Regulation of the excitation and inhibition of its neurons should be well balanced. The LHb has abundant connections with hindbrain areas, but the role of its brainstem inputs is still unclear. Using viral tracing experiments in transgenic mice, we infected all inhibitory inputs of the LHb and found a previously unrecognized gamma-aminobutyric acid (GABA)-ergic cell population in the pons. We found that this cell population is the largest pure GABAergic input of the LHb. We performed mapping experiments to define the dimensions of this nulceus by visualizing its borders with known markers for neurons in pontine nuclei. To investigate the pathway, we found that this pontine nucleus dominantly innervates the medial and posterior part of the LHb. Furthermore, using intersectional viral labeling strategy we found that axonal fibers in the LHb are only positive for vesicular GABA transporter (vGAT) but not for other major transporters or transmitters. Using cell type-specific retrograde rabies virus experiments, we found that this nucleus receives inputs from the pontine, midbrain and cortical areas. Our results suggest that this novel GABAergic pontine nucleus may play a major role in the processing of negative experiences as well as in reward-seeking behaviors, which may ultimately play a role in the development of anxiety-related disorders.

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P6.29 Long-term effects of memantine and two alpha7 nicotinic acetylcholine receptor compounds on novel object recognition memory of aged rats

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Aging has been demonstrated to be the main risk factor for the emergence of neurocognitive disorders (NCDs) that are characterised with progressive neuronal dysfunction and deterioration of cognitive abilities. Currently, there is no proper cure for NCDs yet, thus the discovery of new avenues for the treatment of NCDs would be crucial in the field. As we previously demonstrated, naturally aged rats show marked age-related cognitive impairment and certain pathological aspects of human NCDs, thus they are relevant model for testing pharmacological effects of new treatment strategies.

Our aim was to evaluate the pro-cognitive effect of the non-competitive NMDA receptor antagonist memantine and two different α7 nicotinic acetylcholine receptor (α7-nAChR) agents (the orthosteric agonist PHA-543613 and a novel positive allosteric modulator, called CompoundX) using a sub-chronic (two-week long) treatment regimen in aged rats. The long-term declarative memory of the animals was measured with the novel object recognition paradigm (NOR). Memantine was applied in the following doses: 0.03 mg/kg, 0,3 mg/kg, 3.0 mg/kg. PHA-543613 was administered in the doses of 0.03 mg/kg, 0.1 mg/kg and 2.0 mg/kg and CompoundX was administered in the doses of 0.1 mg/kg, 0.3 mg/kg and 3.0 mg/kg.

Results showed that memantine at 0.03 mg/kg and 3.0 mg/kg doses improved discrimination performance of aged rats during the treatment period, and their effects were maintained even after the end of treatments. However aged animals who received memantine at 0.3 mg/kg dose showed poor memory performance. PHA-543613 at 0.1 and 2.0 mg/kg dose improved discrimination performance during but not after the treatment period, while PHA-543613 at the lowest 0.03 mg/kg dose had no effects neither during nor after the treatment period. CompoundX at 0.3 and 3.0 mg/kg dose decreased age-related cognitive decline during the treatment period, however at 0.1 mg/kg dose no effects were observed.

To sum up, permanent improvement can be achieved with the applied sub-chronic pharmacological treatments against age-related cognitive impairment. Our results can contribute to the development of new and effective therapeutic strategies for human NCDs.

P6.30 Effects of mental fatigue on stability and flexibility of visually guided movements

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Cognitive functions mediated by prefrontal activity and limited in capacity seem to be particularly sensitive to fatigue caused by prolonged task performance. Such cognitive functions include movement planning and movement execution. Results from a previous study suggested that the preparation (i.e. initiation) phase of visually guided pointing movements tends to be impaired with increasing fatigue induced by Time-on-Task. However, no research has been addressed to fatigue sensitivity of movements in terms of movement stability and flexibility. Therefore, in two experiments (N_1 = 26, N_2 = 27), we investigated the stability and flexibility of movements in visually guided movement tasks. Flexibility was considered the ability to adapt to unexpected changes during a movement trial and to modify the initiated track of movements. Stability was considered the ability to maintain the performance level at the presence of distractors. In the first experiment, we examined the stability of movement with a mouse-tracking version of the Eriksen flanker task. An arrowhead centered on the screen and flanked by distractors (congruent or incongruent) indicated the direction of the target stimulus participants needed to point with the cursor. The stability of movement under fatigue was assessed based on participants' ability to inhibit incongruent distractor information with increasing Time-on-Task. In the second experiment we examined the flexibility of movement along with trial conditions where the spatial position of the target stimulus changed unexpectedly after its appearance (change-trials). The difference between change-trials and non-change trials were examined with increasing Time-on-Task. Variables of movement preparation, movement execution, and subjective fatigue were recorded. Gaze position recording was also assessed to control fixation. In both experiments, the results indicated a clear detrimental effect of Time-on-Task on movement initialization suggesting that enhanced level of fatigue is manifested in slow movement preparation. Nevertheless, both stability and flexibility of movements remained unchanged with increasing Time-on-Task induced fatigue.

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P6.31 Decomposing object-location working memory capacity in humans and non-human primates

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List memory paradigms have excellent prognostic value in diagnosing age-related memory disorders, but the standard verbal tests are not translatable to animal research. While item recognition memory has been thoroughly investigated in match-to-sample tasks, associative forms of memory have not been studied yet from a cross-species, translational perspective. Here we compare rhesus macaques and human volunteers with respect to performance and cognitive strategies in the Paired Associates Learning (PAL) visual object-location list memory task.

Two versions of the PAL task, one aimed at measuring the memory set size effect, another measuring the effect of choice location set size were used in monkeys and humans. Nine (previously trained) rhesus macaques performed the PAL task with 3–6 memoranda from a pool of 800 schematic visual stimuli. For comparison, we tested 51 task-naïve human volunteers using a smaller subset (n=138) of the large stimulus pool. Performance levels of macaques (80-95%) matched or even exceeded the range of human volunteers' performance. We adapted the double-high-threshold discrete capacity model to object-location memory, including a capacity and an attentional engagement parameter. This attention+capacity model provided a good fit for performance and captured the set size effects surprisingly well in the PAL object-location short-term memory task in humans, suggesting estimated memory capacities for 3-7 item-location associations and a strong attentional bottleneck. For the macaques, set size effects were weak or absent and the model validated in humans did not fit the data. Preliminary results show that among the two species, memory set size effects were generally stronger in the case of human volunteers, but choice location set size effects were stronger in the macaques.

Although rhesus macaques performed on par with humans, the fact that the model which was suitable for explaining the human data failed in the case of monkeys suggests that mnemonic strategies of monkeys differed from those of humans. Interestingly, increasing the number of locations seemed to have a stronger effect on macaques, possibly because this aspect of the task was not emphasized during the extensive task training of the animals. Modeling commonalities and dissociations in human and macaque object-location working memory holds promise for optimizing task paradigms and analytic pipelines for translational efficiency.

P6.32 Modulation and investigation of cortical excitability with non-invasive transcranial magnetic stimulation and electroencephalography in awake non-human primates

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Age-related neurocognitive disorders (NCD), beyond functional cognitive decline, are well-characterised by the disruption in the balance of excitatory and inhibitory (E/I) cortical networks. Thus, utilising non-invasive transcranial magnetic stimulation (TMS) and electroencephalography (EEG) methods which are similarly applicable in both patients and in translationally relevant preclinical animal models, we established baseline cortical excitability in rhesus macaques, followed by measurements under diazepam to intentionally modulate the E/I balance.

First, we trained the animals to perform a simple eye-fixation task, during which we recorded scalp-EEG from 27 electrodes by a telemetric amplifier system (min 20 sessions). In parallel, we measured individual motor thresholds (MT) by stimulating over the hand area of the primary motor cortex (assisted by neuronavigation) and quantifying the evoked motor responses using electromyography. MT – indicative of excitability threshold – was measured with 2.12% within-subject SD and 0.865 Intraclass Correlation Coefficient, suggesting a good reliability of MT measurements. To further characterise baseline cortical excitability, we recorded two consecutive input-output (I/O) curves with multiple stimulation intensities ranging from 50-150% of MT, semi-randomly ordered with 8 single-pulses at each intensity.

Then, we systemically introduced diazepam (GABAA PAM) – that is known to shift E/I balance – in 3 doses: 0.1, 0.3 and 1mg/kg. In scalp-EEG, a marked increase in low-frequency (alpha-beta) oscillatory power and a decrease in high-frequency (gamma) power with a strong frontal focus was observed indicating a shift of the E/I balance towards inhibition. In the TMS protocol, the first I/O curve (10 min post-administration) shifted to the right, showing a similarly pronounced decrease in excitability. Both the main effects of the stimulation levels ($F_{1,382}=84.12$, $p<0.001$) and the treatment ($F_{3,382}=28.00$, $p<0.001$) were statistically significant, with no interaction between the two factors ($F_{3,382}=1.11$, $p=0.344$).

In summary, combining scalp-EEG and single-pulse TMS offers a complementary and reliable preclinical research method for investigating cortical baseline excitability, as well as for detecting changes in E/I balance. Thus, the research provides the potential for deeper understanding of cortical excitability in a translationally relevant manner with the future goal of development of better treatment options in NCDs.

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P6.33 Fronto-temporal interactions in associative recall explored by multielectrode recordings

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Object-based visual working memory relies critically on the fronto-temporal cortical network. Investigating interactions within this network is crucial for comprehending the neural mechanisms of goal-directed recall in memory. A delayed color recall task, wherein colors were associated with achromatic images of scenes and geometric drawings, was employed to examine interactions between the temporal and prefrontal cortex through high-density electrocorticography (ECoG) in two macaque monkeys. Regions with similar functionality were identified by clustering the time-varying signal of electrodes in the ECoG arrays over the prefrontal and temporal cortices. The clustering results aligned with the dorso-ventral and rostro-caudal functional segregation of cortical areas. Analysis of task-specific sub-regional activities revealed a dynamically changing pattern throughout the task in both the temporal and prefrontal cortices. Granger causality analysis in various frequency bands explored the relationship of sub-regional activities within and between the two cortical regions. Mnemonic activities showed higher interregional causal relationships between regions than within them, specifically in the theta and alpha bands, while these differences largely diminished at higher frequencies. Similar observations were noted during the cue period. Phase-Amplitude Coupling (PAC) analysis is underway to gain a deeper understanding of the oscillatory dynamics of fronto-temporal interactions during goal-directed recall in memory.

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P6.34 Training of a simple touch-screen-based continuous performance task in cynomolgus macaques

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Continuous performance test (CPT) is a neuropsychological test used to assess motivation and sustained attention. The basic notion of the task is to reinforce the agent when it responds to a visual stimulus on touchscreen presented at various positions.

In this study, in a preclinical translational research environment, we used a simple touch-screen-based CPT paradigm to train naïve young adult macaques to use the touchscreen and build knowledge that is generalizable towards understanding more complex cognitive paradigms in the future. Our primary aim was to train the monkeys to be able to routinely utilize the touchscreen and understand the underlying basic task contingencies.

Eight male and four female cynomolgus macaques had been trained for 6 months for one session per weekday in large cubical boxes equipped with a Monkey CANTAB (Cambridge Neuropsychological Test Automated Battery), a touch screen cognitive testing device specially designed for non-human primates. All subjects started with the same settings, and then, we gradually adjusted task parameters towards target values while monitoring individual motivation, performance, and distribution of effective working time during the sessions. The most important parameters were: session length, stimulus size, stimulus duration. We observed how many training days it took the animals to reach the final task complexity and tested different settings to find which can help to develop the necessary skills and performance at a certain difficulty level. We also constantly monitored the animals' body weight and adjusted their additional food intake in the home environment to reach and maintain a desirable motivational level in the task.

By the 14th training day, all animals reached the 30-min session length, from this time they continued with individual differences. On the 76th training day, 8 (75%) animals reached the 55-min session length. By the 70th training day, 7 (56%) animals had reached the smallest stimulus size, 3 of them worked with adequate motivation and performance, so from the 86th training day we continued to improve their precision and generalization by changing the stimulus shape.

Based on our experience, in the present testing environment, cynomolgus macaques may need at least 80 training days to learn the CPT task. Daily monitoring and individualized adjustments are necessary for the continuous development. As CPT is an essential first step to master more complex cognitive tests, the presently described training process will be useful for optimizing the initial touchscreen training regimes of naïve animals in the future.

P6.35 Investigation of the role of hemokinin-1 in murine memory decline

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The tachykinin hemokinin-1 (HK-1) is expressed in several brain regions such as frontal cortex, hippocampus, amygdala both is mice and humans and its role in pain transmission and mood regulation is documented. In contrast, little is known about its involvement in learning and memory functions. Only one study demonstrated stimulatory action of HK-1 on cholinergic neurons. Therefore, we investigated the role of HK-1 in learning and memory functions in aging (in 18 months old animals), and after the induction of memory loss by the muscarinic antagonist scopolamine in 3-5-month-old male and female Tac4 gene-deleted ($Tac4^{-/-}$) mice compared to C57Bl/6 wildtypes (WT).

Different memory functions were assessed with Y and radial arm mazes (YM and RAM) as well as novel object recognition test (NOR). During the 5-min-long experimental periods spontaneous alternations and arm entries in YM, reference and working memory errors in RAM and discrimination as well as recognition indices in NOR were determined in untreated young and old as well as 24 hours after intraperitoneal injection of 1 mg/kg scopolamine compared to the saline.

In the YM test, male WT animals showed a higher alternation index than females, and scopolamine reduced this parameter in males but not in females. This decrease in alternation was also observed in the aged male WT animals, but no changes were observed in females neither by treatment nor by aging. In RAM, female $Tac4^{-/-}$ mice (treated with both saline and scopolamine) showed worse memory with more errors than their WT counterparts, but this difference was not detected in males. Interestingly, old male $Tac4^{-/-}$ mice have found less rewards in this test compared to the WTs. Scopolamine treatment did not significantly affect RAM parameters in either group. Scopolamine did not cause significant changes in the NOR test in WT animals, but after treatment, $Tac4^{-/-}$ animals of both sexes showed a reduced recognition index compared to WT animals. The memory decline seen in $Tac4^{-/-}$ mice also occurred in older animals compared to young ones.

We provide here the first dta for the involvement of HK-1 in memory consolidation. Investigating its mechanisms of action can help to understand the modulatory roles of HK-1 in cognitive functions.

Hungarian Research Network (Chronic Pain Research Group), Pécs, National Brain Research Program 3.0, National Research, Development and Innovation Office - OTKA K138046 and OTKA FK137951, TKP2021-EGA-16, TKP2021-EGA-13, János Bolyai Research Scholarship of the Hungarian Academy of Sciences (BO/00592/19/5), Project no. RRF-2.3.1-21-2022-00015 has been implemented with the support provided by the European Union.

P6.36 A pilot study using DREADD technology to develop a novel model of cognitive impairment

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Designer Receptors Exclusively Activated by Designer Drugs (DREADD) is a novel chemogenetic technology where genes of modified human receptors without any endogenous ligands are expressed. These receptors can be reversibly activated by specific actuators, small molecules selectively binding to their DREADDs and not to any naturally occurring receptors. Thus, DREADD is a powerful tool that can be widely applied in basic research and preclinical drug development.

The primary aim of our pilot experiments were to investigate the changes in performance of rats during multiple behavioral experiments as a result of silencing the targeted brain areas using DREADDs. Our long-term goal is to develop a novel translational model representing the pathophysiology of cognitive decline in humans.

We stereotaxically injected 500 nl of adeno-associated virus vector serotype 5 (AAV5) carrying the gene of the modified human M4D(Gi) cholinergic receptor into either the hippocampus (HC), the anterior cingulate cortex (ACC) or the infralimbic cortex (IL) in rats. After recovery, the training of rats started for the psychomotor vigilance task (PVT) in the Coulbourn Habitest operant conditioning system. In PVT, parameters informing about general alertness (arousal) and sustained attention were measured 30 min after the administration of 3 different doses of DCZ or VEH s.c. based on a Latin square design along with 6 non-operated control animals. For the assessment of explicit memory, we performed novel object recognition (NOR) tests 30 min after DCZ administration and calculated discrimination index (DI) based on exploration time of the novel and the old object. Spatial learning skills were tested using the Morris water maze (MWM) task 30 min after injecting high doses (1.0 mg/bwkg) of DCZ in a between-subject design, measuring escape latency on 3 training days and the time spent in the goal quadrant (Q) during the probe trial.

In the IL group, increased reaction time was detected in the PVT, whereas HC operated animals showed significantly prolonged motor response, and a decreased number of missed trials. In the NOR tests, the HC targeted animals could recognize the novel object only if they did not receive DCZ treatment. The MWM test showed significantly increased escape latency in the ACC group compared to the three other groups, indicating impaired spatial learning.

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Poster Session 7
Stem cells and development

P7.01 Dynamics of neuropeptide expression in the pre- and early postnatal period of life in the 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 a number of neuropeptides, including 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 show the peptidergic neurochemical phenotype as mirrored by expression *Cart* and *Ucn1* mRNAs and the immunopositivity for CART and UCN1 peptides. According to a database we anticipated that *Cart*/CART will be produced in the intrauterine life 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) 14.5th and E16.5 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* and *Ucn1* mRNA as well as CART and UCN1 peptide content was assessed by RNAscope *in situ* hybridization combined with 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 E14.5 and E16.5 cells were localized to the mesodiencephalic basal plate, while 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 neuropeptide with full co-localization.

In utero developing peptidergic EWcp neurons can be identified by their Cart mRNA and peptide content, while the use of *Ucn1*/UCN1 for this purpose is not optimal because it appears in the second postnatal week. Further research will determine the neurochemical characteristics of the developing EWcp cells.

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P7.02 Disruption of semaphorin signalization facilitates proliferation in neuroprogenitor cells and increased apoptosis in early postmitotic neurons in the spinal cord of chicken embryos

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The differentiation of post-mitotic neurons from neuroprogenitor cells is a distinctive process that occurs shortly after neurulation. Following their exit from the cell cycle, spinal cord neurons migrate away from the ventricular zone and populate a highly organized layered structure. Semaphorins play a crucial role in neuronal differentiation, particularly in axon pathfinding. Although semaphorin receptors are expressed in the ventricular zone of the developing spinal cord, their role in the neuronal cell cycle remains unclear. However, our recent study has shed some light on the potential involvement of secreted semaphorins and their receptors in this process. We utilized a dominant-negative approach to disrupt the signaling pathway of secreted semaphorins by targeting the main receptors of semaphorins, neuropilin 1 and 2. Plasmids were introduced into the spinal cords of chicken embryos, which coded for the dominant-negative neuropilin receptors, and GFP for labeling with in-ovo electroporation. The samples were then analyzed with histology and RNAseq, as well as at the protein level by simple western assay (WES). The findings showed that cells expressing either the dominant-negative neuropilin 1 or 2 remained in the ventricular zone compared to control GFP-expressing spinal cords. After conducting BrdU and immunohistochemistry against cell cycle markers, the researchers discovered that, in the case of neuropilin 2, most of the labeled cells were post-mitotic but failed to develop or withdraw migratory processes in the presumptive dorsal horn and underwent apoptosis. Interestingly, they also registered an increased expression of proliferation marker PCNA and vimentin. Downregulating neuropilin 1 resulted in a similar phenotype, but the researchers found an increased expression of NeuN and MAP2, indicating their hasty maturation. The results of this study provide valuable insights into the role of secreted semaphorins and their receptors in neuronal differentiation.

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P7.03 Regulatory factors of neurite generation of human stem cell-derived neural progenitor cells

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Neural progenitor cells (NPCs) differentiated from human pluripotent stem cells are a natural choice for studying neural development and neuronal regeneration after injury. During differentiation NPCs undergo cell polarization, which is initiated by protrusion of several small projections, so-called neurites. This process requires rapid actin remodeling, which is modulated by numerous intrinsic and environmental factors. Our goal was identifying the regulatory mechanisms controlling the formation of neurites in human stem cell-derived NPCs.

Enhanced green fluorescent protein (GFP) was stably expressed in NPCs previously differentiated from human embryonic or induced pluripotent stem cell lines. Neurite outgrowth of GFP-expressing NPCs was studied under normal and injury-related conditions using a high-content screening system. We found that extracellular matrix components strongly influenced the rate of neurite formation but inhibitors of the non-muscle myosin II and the upstream regulatory kinase, ROCK1 were able to override the inhibitory effect of a restrictive environment. We also investigated the modulatory effect of microglial cells on neurite formation of NPCs. We observed that NPCs' differentiation and proliferation was distinctively influenced by microglia depending on their activation state, i.e., resting, stimulated with pro- or anti-inflammatory agents.

Our results help better understanding of human neural development and regeneration at the progenitor level, thus providing opportunities for development of novel regenerative therapeutic interventions.

Poster Session 8
Behaviour

P8.01 A distinct population of neurons in the posterior thalamic nuclear group projects to subcortical regions to shape motor action and behavior

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The thalamus is a critical relay center towards the neocortex. Nuclei in its posterior division typically process higher order sensory information and integrate inputs from different sensory organs.

We recently identified a group of neurons which express the calcium-sensor protein secretagogin in the posterior thalamic nucleus. We aimed to explore the projection pattern of this specific neuronal pool and their role in sensory processing and behavior.

Neuronal projections were traced anterogradely by using adeno-associated viral vectors carrying the mCherry transgene in 12-16 weeks old Scgn-Cre mice and imaging was performed after standard immunohistochemistry on a ZEISS 780 CLSM platform. For behavioral testing, AAV particles carrying Cre-dependent DREADD expression systems for neuronal activation (hM3Dq) were injected into the posterior thalamic nucleus, and CNO-pretreated animals were exposed to behavioral tests, including open field, tail suspension and elevated plus maze tests.

Secretagogin-containing neurons of the posterior thalamic nucleus do not show cortical projection. Instead, they project onto the ventral pallidum, zona incerta, ventral anterior/lateral thalamic nuclei, the pontine gigantocellular nuclei, midbrain periaqueductal grey and the locus coeruleus. Virus-mediated stimulation of posterior thalamic secretagogin-neurons decrease the time spent in the open arm and in the open field during elevated plus maze test and open field test, respectively. Tail suspension test brought no difference in animal behavior after secretagogin-cells activation.

We identified a neuronal population in the multimodal thalamic division which lack cortical projection. Instead, these neurons robustly project on subcortical fields involved in motor control. Their activity shape behavior-dependent motor actions.

P8.02 Investigating the Impact of Neuroinflammation on Long-Term Neuropsychiatric Consequences of Perinatal Asphyxia

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Perinatal asphyxia (PA) poses a significant threat to neonatal well-being and is linked to enduring cognitive impairments and neurodevelopmental disorders. Despite its prevalence, the underlying mechanisms of persistent brain injury resulting from asphyxia remain elusive, hindering targeted therapeutic interventions during the critical early period of neuroplasticity. In this study, we employed a translational rodent model of PA to explore neuropsychiatric outcomes and the associated neurobiological cascades, with a specific focus on microglia as key neuroinflammatory mediators and potential intervention targets.

Male Wistar rat pups were exposed to an asphyxia-inducing gas mixture (4% O2, 20% CO2) for 15 minutes under normothermic conditions at the age of 7 days. Long-term behavioural assessments encompassing motor, emotional, and cognitive domains were conducted from infancy to adulthood. Immunohistochemical analyses targeting brain regions implicated in observed deficits was performed to unravel the role of PA-induced neuroinflammation in the neuropsychiatric alterations. Additionally, we explored the impact of the anti-inflammatory agent interleukin-1 receptor antagonist (IL-1RA) administered early in the post-PA period on acute microglial morphology, long-term behavior, and histology.

PA led to heightened anxiety, notable motor impulsivity and cognitive deficits, particularly in operant learning attention and spatial memory indicating predominantly prefrontal cortex-dependent phenotypical deficits. These behavioral changes were accompanied by a sustained modification of the excitatory/inhibitory balance in the affected brain region. Analysis of microglial morphological subtypes in the acute post-asphyxia period revealed alterations in the prefrontal cortex. Administration of IL-1RA significantly mitigated cognitive deficits in adulthood and influenced short- and long-term histological changes.

Our findings underscore the significant prefrontal cortex-dependent behavioral consequences of PA, characterized by enduring excitatory/inhibitory dysregulation following acute neuroinflammatory changes. The study suggests that a systemically administered anti-inflammatory approach using IL-1RA may offer a promising treatment avenue for the long-lasting cognitive deficits manifesting due to asphyxia.

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P8.03 Thalamocortical circuits in motor learning

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The thalamus has been classically seen as the final relay station of sensory information towards the cortex. Recent knowledge however suggests that it is actively involved in cortical functions. All cortical areas receive thalamic input, which carries not only sensory information but is essential for maintaining cortical function. The role of thalamocortical circuits has been demonstrated in many cortical computations.

Thalamic inputs are divided into driver and modulator types. The basis for this division is their effects on relay cells. These inputs differ in many properties, such as size, site of origin, electrophysiological properties and electron microscopic structure. Many thalamic nuclei receive driver inputs from layer 5 pyramidal cells of the cortex (L5) and modulator inputs from L6. The properties and effects, of drivers with cortical origin have only been investigated in sensory areas.

The influence of frontal cortical areas on the thalamus, which plays a central role in the preparation and learning of goal directed movements, is still poorly understood. In our study, we investigated the morphology and behavioral impact of L5 driver inputs from the secondary motor cortex (M2) in the ventromedial nucleus (VM).

To investigate the anatomy of the pathway, we injected GFP-containing virus into RBP4-cre mice (L5-specific strain), in the M2 and primary sensory cortical area (S1) and measured the maximum cross-sectional area of the boutons in the VM and posterior nucleus area on confocal images. Boutons originating from the M2 were significantly smaller, compared to those of S1 origin.

To investigate the effect of the pathway on behavior, we injected ArchT-containing virus into the M2 region of RBP4-cre mice and axon terminals were inhibited in the VM region. The effect of L5 inhibition in VM was investigated in open field, in place aversion test, and during locomotion training on a horizontal wheel. Inhibition of the pathway did not affect the animals' movement in open field, didn't provoke place aversion or influenced the average speed on the wheel.

These data show that M2 L5-VM corticothalamic pathway is morphologically distinct from those in sensory areas. Precise function of the unique L5 corticothalamic pathway remains to be determined.

P8.04 Genomic insights into the effects of social isolation on the medial prefrontal cortex in male rats

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Social isolation has profound effects on mental and physical health that aggravate depression in humans and cause negative behavioral changes in other social species. The medial prefrontal cortex (mPFC) is critically involved in the control of social behaviors in mammals, still, the genomic background of social isolation in the mPFC remains unexplored. Therefore, we measured the effect of social isolation on gene expression alteration in the mPFC using the RNA sequencing method (RNA-seq) in male rats housed in pairs or alone for 10 days. To characterize the behavioral effects of social isolation, the social, anxiety-, and depression-like behaviors of the animals were measured using three-chamber, elevated plus maze, and forced swim tests, respectively. The sociability of isolated animals decreased markedly, and they also showed elevated anxiety-like behavior without change in their depression-like activity. We observed differential expression of 46 genes between the isolated and paired groups, some with known or predicted social functions. Based on the KEGG pathway analysis, differentially expressed genes play a key role in neuroactive ligand-receptor interaction, particularly in the dopaminergic system, and in addiction. The decreased levels of five altered genes were validated by RT-qPCR. Among them, the serotonin receptor 2c (5HTR2C) serves as a central modulator of neurotransmission and participates in various physiological and behavioral processes. However, the results first suggest its involvement in the behavioral changes following social isolation. The findings regarding alterations in the dopaminergic system of the mPFC also advance our understanding of the consequences of social isolation at the molecular level.

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P8.05 Intermale aggression is inhibited by posterior intralaminar thalamic neurons

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We have established that the posterior intralaminar thalamic nucleus (PIL) receives ascending input from the somatosensory system and that projection from the PIL to the preoptic area of the hypothalamus increases social grooming between adult female rats. It remained open if PIL neurons promote any other type of social touch between conspecifics. Therefore, in the present study, we focused on the role of PIL neurons, and the PIL-preoptic pathway in intermale aggressive behavior.

For chemogenetic manipulation of PIL neurons, we injected adeno-associated virus into the nucleus, which expressed excitatory and inhibitory DREADDs fused with mCherry. In a separate experiment, we selectively tagged socially c-Fos-activated neurons in the PIL with DREADDs. To induce aggression, the animals were separated for 2 months. On the first day of the experiments, a vehicle was injected followed by aggressive behavioral test 1.5 hours later. An unfamiliar intruder was placed in the subject animal's cage resulting in an aggressive response. On the second day, the same test was repeated starting 1.5 hours after clozapine-N-oxide (CNO) injection to activate the DREADDs. Chemogenetic stimulation decreased aggression and increased duration of positive valance contacts while inhibition of the PIL neurons resulted in an increase in aggression and a decreased duration of positive valance contacts.

To establish the activity of PIL neurons and their neuronal targets during aggressive behavior, we measured the number of c-Fos-ir cells. While the PIL and its target brain regions showed elevated c-Fos activation following aggression, the inhibition of PIL neurons during aggressive behavior decreased c-Fos expression in the PIL and in one of its target brain areas, the medial preoptic area (MPOA). In turn, CNO injection into animals previously injected with an AAV encoding the stimulatory DREADD resulted in an elevated c-Fos expression in the PIL and the MPOA in the absence of social interactions. Therefore, we also investigated the PIL-MPOA pathway by injecting a stimulatory DREADD-expressing virus into the PIL and local CNO injection into the MPOA via intracerebral cannulas in order to activate the fiber terminals in the MPOA originating from the PIL. The activation of the pathway decreased aggression and increased positive valance contacts.

Based on these results we suggest that PIL neurons reduce intermale aggressive behavior possibly by their projections to the medial preoptic area.

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P8.06 Skor2 expressing GABAergic neurons in the periaqueductal gray and mesencephalic reticular formation may be involved in the regulation of REM sleep

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REM-on neurons in the brainstem are inhibited by the GABAergic REM-of neurons in the ventrolateral periaqueductal gray (vlPAG) and the adjacent dorsomedial mesencephalic reticular formation (dMRF). In the above mentioned brain regions, a subpopulation of GABA-ergic cells with similar locations to those of the REM-of neurons express Skor2. Skor2 is a transcription factor which is involved in the differentiation of GABAergic neurons. We wanted to clarify whether the GABAergic REM-of neurons in the dMRF/vlPAG belong, at least in part, to the Skor2 expressing cells.

Male Han-Wistar rats (n=6) were deprived of REM sleep (REMS) on small platforms surrounded by water for 72 h using the water tank (inverted flowerpot) method. Animals on large platforms (n=5) or in dry cage (n=6) served as controls. At the end of the REM sleep deprivation/sham deprivation, the animals were sacrificed by intraperitoneal administration of 400 mg/kg chloral hydrate, perfused with phosphate buffered saline and 4% paraformaldehyde and the brains were removed. Sections covering the dMRF/vlPAG area were collected and stained for the expression of c-Fos and Skor2. The co-expression of c-Fos and Skor2 in the dMRF/vlPAG was analysed in the REMS deprived rats compared to that in the nondeprived control animals.

In the dMRF/vlPAG, the proportions of the c-Fos positive neurons from the Skor2 expressing cells were significantly higher in the REMS deprived rats than in the in the control animals (control rats in dry cage: 14.5%, control rats on large platforms: 23.3%, REMS deprived rats: 40.8%).

The enhancement of c-Fos expression by REMS deprivation indicates that Skor2 expressing GABAergic neurons in the dMRF/vlPAG may be involved in the regulation of REMS.

P8.07 Medial prefrontal cortical neurons projecting to the preoptic area and the thalamus differently affect social behaviours of rats

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The medial prefrontal cortex (mPFC) plays a crucial role in the control of social behaviour. Dysfunction in this area may contribute to neuropsychiatric disorders, such as autism spectrum disorder or schizophrenia. mPFC neurons project to different subcortical areas, exerting influence from the mPFC. We determined the projection pattern of two types of projection neurons, and examined their role in social behaviour using chemogenetics.

Stimulatory and inhibitory designer receptors were expressed in the mPFC neurons projecting to the medial preoptic area (MPOA), and also in mPFC neurons expressing the calcium/calmodulin-dependent protein kinase II (CaMKII) using viral gene transfer. The mPFC neurons projecting to the MPOA gave rise to collaterals to several subcortical areas, such as the accumbens nucleus, ventral pallidum, lateral septum, paraventricular hypothalamic nucleus, ventromedial hypothalamic nucleus, and medial amygdala but not to the thalamus. In contrast, the CaMKII neurons of the mPFC projected to the paratenial, mediodorsal, submedius, and reticular thalamic nuclei but not to the MPOA and other targets of MPOA projecting neurons.

When stimulating the mPFC neurons projecting to the MPOA, reduced sociability was measured in the three-chamber test. In turn, the direct social interactions between freely moving animals remained unchanged. Stimulation of mPFC CaMKII-containing cells resulted in reduced time spent with conspecifics in the three-chamber test and a decrease in the duration of several elements of social interactions between freely moving rats.

The chemogenetic manipulation of the examined mPFC projection neurons exerted an effect on the sociability of the animals indicating their role in regulating social behaviours. The differences in the effects of manipulation of the two types of mPFC projection neurons suggest their involvement in the regulation of social behaviours in different ways.

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P8.08 The Lateral Septum and its multifaceted role in anxiety

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The Lateral Septum (LS) plays an important role in controlling emotional states such as anxiety, aggression, and controls social behaviors, presumably in a sex-dependent manner. Despite the relatively sizeable amount of experimental data supporting these LS functions, the mechanistic understanding of how LS regulates these processes is hindered by a series of contradictory results. Although the LS is thought to contain exclusively GABAergic cells, we found that a fraction of LS cells expresses cholinergic neuronal markers. In this study, we try to understand the function of this specific neuronal population named LS cholinergic cells (LSCNs), using LSCN-specific viral expression of optogenetic actuators combined with behavioral testing by using paradigms such as the Open Field Test, the Elevated Plus Maze and the Light-dark Box to assess anxiety levels, and the fox odour test combined with c-Fos staining to explore neuronal activation following exposure to an inherently aversive stimulus.

Our preliminary results show that stimulation of the LSCNs has an anxiogenic effect on observed behaviour independent of sex. The fox odour test revealed activation in LS, however, with no overlap with LSCNs.

Since traditional fiber optogenetics obstruct the usability of other paradigms, such as the Elevated Plus Maze or sociability tests, due to physical constraints on the animals exerted by optic cables, we started to adopt wireless optogenetics to overcome this limitation. In the future, we aim to expand our investigations with an array of techniques, including optogenetic inhibition, fiber photometry, electrophysiology, and anatomy in order to better understand this critical yet enigmatic structure.

P8.09 Effect of intraamygdaloid oxytocin on social interaction in valproate-induced autism model

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Autism spectrum condition is a pervasive neurodevelopmental disorder that affects 1 in 36 children. One of the core symptoms is impaired social interaction. Since proper treatment has not been found yet, an investigation of the exact pathophysiology of autism is essential. The valproate (VPA)-induced rat model can be an appropriate way to study autism. In the present study, we investigated the effect of the intraamygdaloid oxytocin (OT) on sham and intrauterine VPA-treated rats' social interaction using three-compartment social interaction test. Male Wistar rats were subjected to VPA prenatally and showed impaired social interaction, however, when OT was injected bilaterally into the central nucleus of the amygdala, the time spent in the social zone increased and reached the level of sham-control rodents'. OT receptor antagonist blocked this effect of the OT but in itself did not significantly influence the behavior of the rats. Our results show that intraamygdaloid OT can significantly increase the time spent in the social zone in VPA-induced autism model and the effect of OT is receptor-specific.

P8.10 Cognitive and emotional behavioral testing of experimental animals for quantification of effects of intervention

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Behavioral testing of experimental animals has been plagued by questions about its reproducibility and the interpretation of the results. However, it is a fact that the brain does not produce something on its own, but rather performs its functions through interaction with its outer environment, in terms not only perception but also executive function. In terms of this point, behavioral testing should be a tool for a wholistic phenotyping system at the individual level, not but a specific restricted function of the brain. We have developed multiple behavioral test methods using touch-screen operant apparatus and IntelliCage apparatus. A common feature of the two analysis systems is that experimental animals are rewarded by showing the correct behavioral sequence based on spatial cues, and the difference is that touchscreen apparatus requires a very small distance to move, while IntelliCage apparatus requires a distance of several hundred steps. By using these systems, we have found the identical cognitive score of animals in an executive functioning testing. In addition, we have found some emotional behavioral abnormality in multiple animal models including developmental disorders. By using these two apparatuses, effects of antibiotic or probiotic treatment on cognition were examined. Antibiotic or probiotic treated mice showed lowered or improved performances, respective, in the onset of shift to reversal contingency. Taken together, long-term treatment with probiotics was found to improve cognition in middle-aged mice, indicating that probiotic treatment might contribute to prevention of age-related cognitive decline. These behavioral tests were found to be useful for studying the higher brain functions of experimental animals.

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P8.11 The role of prolactin-releasing peptide (PrRP) in the development of depressive-like symptoms in rats

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A growing number of nutritional, metabolic and psychological disorders are associated with stress. Several research results confirm that the RFamide peptides - especially the prolactin-releasing peptide (PrRP) - can play a role in the regulation of stress responses. Based on the close relationship between stress and depression, it can be hypothesized that PrRP also plays a role in depression. In the present experiments importance of PrRP was examined in the development of depression-like symptoms. PrRP was identified as an endogenous ligand of the GPR10 receptor, whose antagonist is not available. However, PrRP binds with high affinity to the NPFF2 receptor subtype.

Fifteen minutes forced swimming test (FST) was used to induce depression-like symptoms in male Wistar rats. On the next day the animals' behaviour was analysed during a 6-min FST, and based upon the time spent in immobility resilient (low level) and vulnerable (high level) group was identified beside a control (non-swimming) group. At the end of the experiments frozen brain tissues were collected and mRNA levels were evaluated by rtPCR. We concentrated on five in the brain areas: two noradrenergic nucleus groups of the medulla oblongata (A1 and A2), amygdala, as well as hypothalamic nuclei (paraventricular (PVN), dorsomedial (DMN), ventromedial (VMN)). In naive animals RNAscope technique was combined with immunohistochemical staining to characterize the PrRP-containing cells.

The rtPCR analysis revealed a reduction in the expression of the NPFFR2, the non-specific PrRP receptor on the VMN region of vulnerable animals in comparison to controls, while resilient has similar levels than controls. In accordance with the literature the strongest PrRP mRNA expression was found in the A1 region by RNAscope technique. However, we could simultaneously detect PrRP mRNA and protein in the VMN as well, with lower levels in the PVN.

Our results support that PrRP and their receptors might play an important role in the regulation of stress and thereby influence the development of stress-related psychological symptoms, primarily depression. However, different brain areas may have different roles, therefore further examinations are necessary to reveal the exact mechanisms about the role of PrRP in development of depression.

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P8.12 Glucose transporter 2 positive cells in the medial prefrontal cortex: unravelling their impact on posttraumatic stress disorder

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Posttraumatic stress disorder (PTSD) is a psychopathological condition induced by a traumatic event, such as physical or sexual assault or a natural disaster. It is known that neurons rely heavily on glucose as a primary energy source and energy demand of the brain increases in life situations. The median prefrontal cortex (m-PFC) is an important brain area regulating fear response, Moreover, it contains glucose sensing glucose transporter 2 (GLUT2) containing neurones. We hypothesized that manipulating these mPFC-GLUT2 cells by a chemo genetic technique will influence the behavioural outcome. GLUT2-Cre transgene mice was used and designer receptor exclusively activated by designer drug (DREADD) sequence was injected into the m-PFC by the help of an adeno associated viral vector. Two weeks after vector injection foot shock trauma was applied. As previously post-trauma sucrose consumption for 24h was showed to be protective we combined manipulation of the glucose sensitive cells (i.e. injection of the DREADD ligand clozapine-N-oxide right after trauma) with 16% sucrose drinking. The acute stress disorder (ASD) was studied 24h, while PTSD-like freezing 14-day after the trauma. Immunohistochemistry against red fluorescent protein confirmed the correct hits in the PFC region. The mice drunk more from sucrose than from water measured for 24h after trauma. However, the body weight increase was the same in all groups throughout the experiment. In general, females seemed to be more affected than males. In contrast to our expectation the effect of stimulation of the GLUT2 positive cells of the PFC and post-trauma sucrose drinking did not show synergistic effect on freezing behaviour studied both in a context as well as in cue dependent way. Our research draw attention to the importance of energy supply in the development of psychiatric disorder, especially in the decision-making m-PFC area.

P8.13 Prefrontal BDNF hyposignaling in somatostatin interneurons induces active coping in mice

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Coping with stressful challenges is regulated by conserved brain mechanisms. Although coping is a complex cognitive-emotional process, stress coping styles along the passive-active spectrum can be considered as a major dimension. Excessive passive coping is a core symptom in depression and related affective disorders, which needs further mechanistic understanding. Significant evidence shows alterations in prefrontal networks under these conditions, including somatostatin (SST) interneurons and neurotrophic (BDNF) signaling, however, causal pathogenetic mechanisms are not clarified.

In the present study, we aimed to explore the impact of BDNF hyposignaling in SST interneurons on stress coping. We used a developmental model, i.e. SST neuron-specific knockout of the BDNF receptor (tyrosine receptor kinase B-TrkB) by crossing sst-ires-cre and $TrkB^{flox/flox}$ transgenic mouse lines (SST-TrkB-CKO). First, we phenotyped SST-TrkB-CKO mice in a detailed behavioral test battery. We observed significantly increased active coping in three coping tests without major affective or cognitive alterations. Next, we mapped whole brain activity changes during the Tail suspension coping test using immunolabeling of neuronal activity marker c-Fos and SST by means of automated brain atlas alignment and quantification method. We found markedly increased cortical activity in several brain regions including the amygdala, hippocampus and sensory systems associated with reduced SST expression. We identified crucial brain regions, which could drive alterations in coping behavior by multiple criteria (i.e. altered activity, altered SST activity, correlation with coping behavior). Prefrontal cortex, retrosplenial cortex, entorhinal and agranular insular cortices emerged as major candidates. In the place, we applied local TrkB knock-down in the medial prefrontal cortex using shRNA silencing method, which could recapitulate our whole brain SST-TrkB-CKO findings, i.e. enhanced active coping.

Our findings suggest that BDNF-related hyposignaling in SST interneurons significantly shapes cortical network function resulting in altered coping behavior. Latter shift in coping is significantly mediated by altered prefrontal function that is in line with clinical observations in depressive disorders.

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P8.14 Trauma-induced alterations in spatial memory performance predicts long-term contextual fear and generalization in a rodent model of Posttraumatic Stress Disorder (PTSD)

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Experiencing traumatic stress results in posttraumatic stress disorder (PTSD) in vulnerable subpopulations (~10-20%) of trauma-exposed individuals. The core symptoms of PTSD are fear generalization and extinction deficits, progressively developing after trauma exposure. Prevention and early interventions require the identification of neuro-behavioral markers with significant prediction for vulnerability to develop symptoms. In the present study, we characterized spatial learning and memory recall performance of adult Long Evans rats and C57BL/6J mice in the Morris Water Maze prior to the trauma (single series of uncontrollable footshock) and 24 h after exposure. We identified vulnerable and resilient subpopulations based on their long-term fear generalization, i.e. high and low freezing responses in a safe context 28 days after trauma exposure (upper and lower 25-25%, respectively). We found significant performance decline in the vulnerable subpopulation of rats compared to the resilient subpopulation between pre- and post-trauma. In contrast to rats, spatial memory performance and its trauma-induced alteration predicted only contextual fear abilities in mice, but it was not related to fear generalization to safe contexts. Our study suggests that pre-trauma contextual/spatial learning abilities may predict vulnerability to develop excessive and generalized fear, potentially via hippocampus-dependent mechanisms, which needs further explorations.

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P8.15 Social Behavior in the Absence of Uncoupling Protein 2 (UCP2)

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Uncoupling proteins (UCP) act as transporters in the mitochondrial inner membrane, regulating the discharge of the proton gradient generated by the respiratory chain for functions such as thermogenesis, redox balance maintenance, and reduction of reactive oxygen species. Within this family, UCP2 emerges as a multifaceted player in the central nervous system, influencing processes like cellular stress, cell proliferation, and neuroprotection. As UCP2 is co-expressed with oxytocin and vasopressin, the well-known social hormones, UCP2 could have a role in these processes as well.

Our aim was to explore the role of UCP2 in social behaviour comparing UCP2 knockout (KO) and wild-type (WT) rats using social discrimination test. As possible background mechanism, we examined the differences in the vasopressinergic and oxytocinergic system in the brain using immunohistochemical and PCR methods.

Our discoveries illuminate the potential impact of UCP2 on molding social behavior. Additionally, we scrutinized the viability of the UCP2 knockout rats as a possible model for therapeutic interventions aimed at this protein in neuropsychiatric disorders marked by diverse social disorders. This research could provide valuable insights for animal modelling of such diseases and the development of potential therapeutic strategies.

P8.16 Calbindin neurons of the lateral septum control maternal behaviour

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The lateral septum (LS) is a forebrain area that has a role in forming prosocial behavioural elements such as maternal care. LS is supposed to have a role in maternal behaviour based on lesion studies, however, the responsible cellular networks are not known yet. Therefore, we aimed to characterize maternally activated septal neurons and their function in maternal regulation. First, we established that all of the pup-induced c-Fos+ septal neurons are inhibitory GABAergic neurons. A population of these GABAerg neurons in the ventral subdivision of the LS (LSv) contain calbindin (Cb+). The number of c-Fos-activated Cb+ neurons was markedly higher in mothers following pup exposure than pup deprivation. We demonstrated by viral based, cell type-specific anterograde tracing that Cb+ LSv neurons send massive projection to the medial preoptic area (MPOA) a centre for regulating maternal behaviour and confirmed this projection by retrograde tracing. To establish how maternal input arrives at the Cb+ LSv neurons, we first established that these neurons contain receptors of the maternally induced neuropeptide, parathyroid hormone 2 (PTH2) using Cb immunolabelling in reporter mice expressing ZsGreen in PTH2 receptor-expressing neurons. These receptors are likely activated by PTH2 released from the PTH2 terminals we also identified around Cb+ neurons in the LSv. Moreover, a synaptic connection was established between PTH2+ fibres and maternally activated inhibitory neurons in the LSv using electron microscopy. These PTH2+ terminals might arrive from the PTH2+ neurons located in the posterior intralaminar thalamic nucleus (PIL), which all show c-Fos-activation in mothers following pup exposure compared to pup-deprived controls. Functional investigation of LS Cb+ neurons in Cb-Cre mice demonstrated that the inhibition of these neurons reduced the time spent with the licking of the pups, but caused no other behavioural effects. Therefore, we conclude that the activation of the inhibitory Cb+ neurons is required for the pup licking behaviour, which may be executed by their projection to the MPOA neurons. Finally, this activation of CB+ LSv neurons is executed via the excitatory PTH2+ input from maternally activated PIL neurons.

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P8.17 Endocannabinoid biomarkers of vulnerability to trauma-induced generalized fear responses

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Traumatic experiences result in the development of posttraumatic stress disorder (PTSD), a severe psychiatric condition, in 10-30% of trauma-exposed individuals. While the neurobiological basis of vulnerability to develop the disorder following trauma are poorly understood, human clinical studies suggest that differences in endocannabinoid (eCB) signaling are potentially linked to vulnerability. Employing a rat model of PTSD, we characterized distinct resilient and vulnerable subpopulations based on trauma-induced long-lasting generalized fear response, a core symptom of PTSD. In these groups, we assessed i.) eCB levels by mass spectrometry and ii.) eCB system-related gene expression variations and iii.) neuronal activity markers by real-time quantitative PCR in the circuitry relevant in trauma-induced changes. Furthermore, employing supervised and semi-supervised machine learning based statistical analytical models, we assessed gene expression patterns with the most robust predictive power regarding PTSD vulnerability. According to our findings, electric footshocks induced generalized fear responses with sufficient variability to characterize distinct resilient and vulnerable subpopulations. Mass spectrometry assessments showed higher prelimbic and lower ventral hippocampal 2-arachidonoyl-glycerol (2-AG, one of the main eCBs) levels in resilient compared to vulnerable subjects. Furthermore, ventral hippocampal 2-AG content positively correlated with the strength of fear generalization. Vulnerability was associated with marked neuronal hypoactivity in the fear circuitry and altered expression patterns of a number of eCB-related genes. Supervised and semi-supervised machine learning-based statistical approaches highlighted that hippocampal gene expression patterns have strong predictive power regarding vulnerability. Taken together, we have pointed out that eCB changes in the hippocampus are crucial in the basis of vulnerability, as 2-AG levels in this region showed marked correlations with the strength of generalized fear and hypoactivity and eCB-related gene expression patters in this region had great predictive power of PTSD vulnerability. Our results describe a novel neuropharmacological marker which outlines vulnerability to develop PTSD following trauma-exposure and may contribute to the identification of clinically relevant subpopulations.

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P8.18 Effect of D1-like dopamine receptor agonist on the hedonic evaluation in the prefrontal cortex

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Dysfunction of the reward system is a common symptom of many mental illnesses including depression, schizophrenia or eating disorders. Anhedonia refers to the reduced ability to experience or respond to pleasure in social, physical or eating context. Human studies have suggested that the severity of anhedonia is related to the dysfunction of the prefrontal cortex (PFC) with a presumed role of dopamine.

The aim of our experiments was to examine the involvement of dopamine in the hedonic evaluation in male Wistar rats. D1 dopamine receptor agonist SKF 38393 in 1 µg/0.4 µl or 5 µg/0.4 µl dose was microinjected into the medial PFC through an implanted cannula. To investigate the ability of hedonic evaluation of rats after administration of SKF 38393, taste reactivity test was used. Positive (ingestive) and negative (rejective) facial responses were scored during intraoral infusion of 30% sucrose solution into the mouth. The potential influence of D1 dopaminergic receptor agonist on the motor function or anxiety was examined in open field and in elevated plus maze test.

Our results indicate that stimulation of dopamine D1-like receptors of medial PFC dose-dependently increases the hedonic value of 30% sucrose solution, which was primarily manifested in the reduction of rejective responses. Administration of SKF 38393 did not affect the motor activity of the animals in the open field test. The results of elevated plus maze test show that activation of D1 dopaminergic receptor has a weak anxiolytic effect, since the treated animals spent less time on the end of the enclosed arm and spent more time in the central of the maze. Based on our results, we can conclude that stimulation of D1-like dopaminergic receptors in the medial PFC affects the hedonic value of sugar, without affecting the motor system.

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P8.19 The role of substance P, senktide, and neurokinin receptors of the rat globus pallidus in learning in the Morris water maze test

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The tachykinin substance P (SP) has long been implicated in learning and memory processes after its peripheral or central administrations. SP has the highest affinity for neurokinin 1 (NK1) receptors, but it can also act on NK2 and NK3 receptors. Activation of all these tachykinin receptor types was shown to influence learning and memory. SP immunoreactive elements, as well as NK1 and NK3 receptors, have been found in the different parts of the basal ganglia (BG). The pivotal role of BG has been proven in the regulation of learning mechanisms, and as an important structure of the BG, the globus pallidus (GP) might also be involved in these processes. Indeed, learning deficits were demonstrated following electrolytic or excitotoxic lesions of the GP in different tasks. In our previous experiment, SP microinjected into the GP improved learning in the passive avoidance paradigm. Based on experimental findings, the role of the BG in spatial learning was also suggested.

The present study, therefore, aimed to examine the effects of SP administered into the GP on spatial learning in the Morris water maze test (MWM). We examined the involvement of NK1 and NK3 receptors in the mediation of SP effects, as well. Male Wistar rats were microinjected with 10 ng or 100 ng SP, or vehicle in 0.4 microliters. Results showed that a post-trial injection of 10 ng SP significantly improved performance over controls, while administration of 100 ng did not influence learning. To examine the possible role of NK receptors in mediating SP effects, high-affinity non-peptide antagonists were applied. Prior treatment with the NK1 receptor antagonist WIN51708 could not inhibit the promnestic effect of SP, while the NK3 receptor antagonist SR142801 blocked learning-facilitating effects. Furthermore, the involvement of pallidal NK3 receptors in spatial learning was supported by the application of a specific NK3 receptor agonist. Senktide was applied in 6 ng or 60 ng dose, controls received vehicle solution. The lower dose showed a similar learning-improving effect to 10 ng SP, the higher dose was ineffective.

Our results show that 1) SP in the GP facilitates learning in the MWM in a dose-dependent manner; 2) NK3 but not NK1 receptors are involved in the mediation of this effect; 3) activation of NK3 receptors in the GP by senktide has also improving effect on learning. Our results are the first to demonstrate that activation of pallidal NK3 but not NK1 receptors improves spatial learning.

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P8.20 Astrocytic synchronization governs slow wave activity and memory consolidation

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Oscillatory brain activity is widely recognized as an exclusively neuronal phenomena. However, a growing body of evidence indicates that astrocytes are involved and may even initiate both physiological (e.g. slow wave activity) and pathophysiological (e.g. epilepsy) oscillations. By exploring various molecular interactions between neuronal and astrocyte networks, we have previously shown that inhibition of astrocytic synchronization by the blockade of astrocytic gap junctions suppresses slow wave activity (SWA) in rats in vivo (Szabó et al. 2017) and inhibits epileptiform activity in acute hippocampal slices (Vincze et al., 2019), suggesting a causal role of astrocytes in neuronal synchronization. In this study we investigated the role of astrocytes in 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 connexin 43 (Cx43) antibody in adult rats. First, we explored whether manipulation of astrocytic synchronization induces changes in neuronal SWA. By electrophysiological measurements in freely moving rats, we have shown that TMA increased time spent in SWA in animals characterized by low SWA appearance during control measurements, while the Cx43 antibody decreased time spent in SWA, demonstrating that astrocytic synchronization directly influences neuronal oscillations. To explore the activity of individual networked astrocytes during SWA, we applied high-frequency 2-photon imaging of both astrocytes and neurons in anesthetized animals. The results show that synchronization of both cell types at frequencies characteristic of SWA (0.5 –2 Hz) is strongly diminished following gap junction blockade. Since SWA is known to be heavily involved in memory formation, we investigated whether manipulation of astrocytic synchronization by activation or inhibition of gap junctions may influence memory performance in the novel object recognition (NOR) memory test. We demonstrated that the working memory of rats can be enhanced by TMA, specifically in animals with weaker baseline memory performance. Treatment with the Cx43 antibody, in contrast, has been found to cause long-lasting 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.

P8.21 Cognitive profiling of Tac4 Gene Knockout: Mice

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In this study, we investigated the impact of Tachykinin 4 (Tac4) gene knockout on leaning/memory performance, while also exploring gender differences in the cognitive tests. Tac4 encodes hemokinin 1, a neuropeptide crucial for neurotransmission and involved in sensory perception, stress and pain processing 1,2,3.

We used 33 C57Bl/6 mice, comprising 8 wild-type (WT) male mice, 8 WT female mice, 8 Tac4 knockout (KO) female mice, and 9 KO male mice. The animals were tested in five memory tests, including fear conditioning (fear memory), place recognition (visual memory), spontaneous alternation (working memory), Barnes maze (navigation memory) and five-choice serial reaction time test (5-CSRTT, attention).

Results indicated no significant differences in spontaneous alternation between the groups. In place recognition, the discrimination index (DI) of WT female mice was notably lower (DI~0) compared to other groups (DI>0.22). Fear conditioning revealed that KO male mice exhibited weaker fear memory than the other groups. In the Barnes-maze, performance of the four groups did not differ with the exception of the first day when WT male mice outperformed other groups in terms of escape latency accuracy. The learning speed in the 5_CSRTT task was the following: wt males >> KO males > wt females ~ KO females.

In conclusion, although wild type male mice produced the most balanced performance in all the paradigms, we did not find a genotype-dependent characteristic cross-task cognitive alteration. Interpretation of the observed deficit of wild type females and KO males in the place recognition and fear conditioning assays, respectively, requires further investigation.

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P8.22 Effects of affective touch on autonomic nervous system and affective state

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While tactile stimulation (TS) is transmitted by the thick, myelinated Aβ axons from the skin to the primary somatosensory cortex (S1), in the case of affective touch (AT), a special form of tactile stimuli, double pathway has recently been revealed. AT is a weak (0.1-0.2 g), slow (1-10 cm/s) stroking that hits the hairy skin surface. Exteroceptive information on physical aspects of AT (location, strength) is projected to S1, but the affective aspect of AT (pleasantness) is recepted by the skin's ancient *type C low threshold mechanoreceptors* (C-LTMs), and transmitted by thin, non-myelinated, slow-conducting CT axons to the posterior insula and the orbitofrontal cortex. This pathway also carries other interoceptive information (e.g. visceral stimuli, pain). Based on its neural background and social aspects, AT is considered a stress-reducing social homeostatic safety signal. The aim of our experiment was to compare the physiological effects of TS and AT on the autonomic nervous system and on subjective responses.

After a 5-minute relaxation period, 30 undergraduate university students went through 3 types of experimental manipulations in random order: focus of attention on the forearm (control condition), rhythmic stimulation of a standard area of the forearm with a brush (TS condition), and stroking in the same place (AT condition), each for 3-3 minutes. Physiological reaction (heart rate – HR; heart rate variability – HRV; respiration rate – RR; electrodermal activity – EDA) were measured throughout the experiment. Valency (pleasantness) and arousal (intensity) of skin perception, and change of positive and negative affect were assessed before and after conditions, as well. We used repeated analyzes of variance models with within subject factor of 'condition' to test the hypotheses. The results indicated that the experimental manipulation affected the HR, RR, and the valence and arousal of skin perception ($p<0.001$ in each case), but it had no effect on HRV, EDA, and affective state of participants. Both TS and AT increased RR and decreased HR compared to the control condition, but there was no difference in TS and AT effects on parasympathetic activation. However, AT caused a significantly more pleasant and intense skin perception than TS. Despite the different neuroanatomical pathways, our results suggest no difference in response of the vegetative nervous system, nor in the effect on affective state induced by TS and AT.

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P8.23 The rat psychomotor vigilance task and its validation as a translational tool for testing potential drug candidates for age-related cognitive deficits

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The psychomotor vigilance task (PVT) is frequently used for the measurement of sustained attention and vigilance in humans. The PVT performance declines with aging and is sensitive to age-related cognitive disorders. Furthermore, the PVT has a high translational potential as it can be conducted very similarly in different species. Here, we adapted the PVT for the application in rats, and we validated it for translational pharmacological investigations by comparing the performance of young and aged rats and measuring the effects of approved anti-dementia drugs.

Ten young (<1 years old) and 12 aged (>2.5 years old) Long Evans rats were trained for the PVT in an operant conditioning environment. Aged rats were treated with different doses of donepezil (0.01-1.0 mg/kg) and memantine (0.1-1.0 mg/kg) in separate within-subject experiments, and the effects of the drugs were tested in the PVT.

Aged rats showed a tendency to worse training performance as they needed more time to learn the protocol of the PVT. Furthermore, aged rats showed markedly longer reaction time compared to young animals, while aged rats also performed less correct trials and more missed trials (when the trial was not initiated at all). A low dose of donepezil (0.03 mg/kg) significantly improved the decision phase of the reaction time of aged rats, while high doses of donepezil slowed down the responses of aged rats and further increased the number of missed trials. Memantine did not affect the reaction time of aged rats, however, it increased the number of correctly performed trials by decreasing the number of missed trials and omissions (when the rat initiated the trial but failed to respond to the cue stimulus).

In summary, the present results showed that donepezil improves attention and vigilance in low doses, while memantine increases motivation (less missed trials) and precision of responding (less omissions) of aged rats. Our results confirm that the rat PVT is a useful tool for the investigation of age-related cognitive deficits and for the preclinical testing of potential drug candidates.

P8.24 Enhanced place learning and cognitive flexibility in mother mice revealed by IntelliCage

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The transition to motherhood is a profound biological event that induces significant behavioral and cognitive changes in females across mammalian species. While maternal behaviors directly linked to offspring care have been extensively studied, executive functions remain relatively understudied in both rodent models and in human mothers despite their likely importance for maternal responsiveness and overall parenting quality. Therefore, this work addresses cognitive adaptations that indirectly contribute to successful rearing of offspring. Rodents provide a valuable model for investigating these changes, using hippocampus-dependent tasks that assess spatial learning and memory. This study also aims maternal alterations in medial-prefrontal cortex-dependent executive functions, particularly in cognitive flexibility.

Radiofrequency transponders were implanted into female mice for individual recognition, allowing for a detailed analysis of behavioral patterns in a fully automated monitoring system called IntelliCage. The experimental groups included mothers, pregnant mice, and control females.

The results reveal that mother mice exhibit superior performance in both place learning and cognitive flexibility compared to both pregnant and control females. Mothers demonstrated heightened proficiency in correctly navigating the monitored space and displayed increased adaptability to changes in this testing environment.

The findings suggest that the demands of motherhood may contribute to an enhancement in spatial learning and cognitive flexibility in female mice. This study contributes to our understanding of cognitive adaptations related to reproductive experiences, however, the neural mechanisms underlying the observed improvements require further investigation.

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P8.25 A novel approach to reveal trait anxiety and its molecular correlates through summary measures of multiple transient states

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The reliability and validity of preclinical anxiety testing is essential for translating animal research into clinical use. However, the most commonly used anxiety tests lack inter-test correlations and have repeatability issues that need to be clarified. Translational animal research should be able to capture stable individual traits to aid the development of personalised medicine. However, with the current approaches that typically involve using one type of test one time, it is only possible to measure transient states of animals that are heavily influenced by the experimental conditions. Here, we propose a validated, optimised test battery which can reliably capture trait anxiety in rats and mice of both sexes. Instead of developing novel tests, we combined widely-used tests (elevated plus-maze, open field and light-dark test) to understand their repeatability issues and low inter-test correlations, and provide instantly applicable adjustments for better predictive validity. We repeated these tests three times to capture multiple anxious states, which we combined together to generate summary measures (SuMs). Using correlations and machine learning, we found that our approach resolves between-test correlation issues of anxiety tests and provides better predictions for subsequent outcomes under anxiogenic conditions or fear conditioning. Moreover, SuMs were more sensitive to detect anxiety differences in an etiological model of social isolation. Finally, we tested our sampling method's efficiency in discovering anxiety-related molecular pathways through RNA sequencing of the medial prefrontal cortex. We identified four times more molecular correlates of anxiety using SuMs, which pointed out functional gene clusters that had not emerged in association with single testing. Furthermore, we also found that 50% of the most robust molecular findings were also found to be correlated with anxiety in the amygdala. Overall, temporally stable SuMs are necessary to capture trait anxiety in rodents, providing better predictions for potential therapeutic targets.

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P8.26 Effects of GLP-1 receptor agonist exenatide on palatability-driven food intake in nonhuman primates in a novel two-session operant behavioural paradigm

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Food intake behaviour is modulated by homeostatic and hedonic (reward-related) neuronal pathways. The homeostatic pathway is responsible for maintaining balance after energy stores are depleted, while the hedonic pathway modulates feeding behaviour based on the reward value of food. Glucagon-like peptide 1 (GLP-1) is an important neuropeptide signal of energy balance and satiety and has a major role in modulating healthy and maladaptive food consumption behaviour.

Using a novel operant food intake (FI) paradigm, we investigated reward-based feeding regulation of young adult male rhesus macaques (n=5) in two experimental sessions per day: a 2-h long Session 1 (S1) and a 1-h long S2 separated by a 1-h interval. We used the less palatable banana flavoured (b) and the more palatable very berry flavoured (v) nutritionally complete pellets, offered in all combinations with the two daily feeding sessions (S1/S2: b/b, b/v, v/b, v/v). Following the selection of an effective dose of the GLP-1 agonist exenatide (EX), we investigated the acute effects of peripheral administration of this drug.

Without treatment, the animals consumed very similar amounts of the two types of pellets in S1: the palatability of the currently consumed meal in S1 had only a weak influence on FI. Rather, it was the food offered later in S2 that strongly and primarily determined consumption in S1: the animals ate significantly less in S1 when S2 meal was highly palatable, compared to experimental conditions with low-palatability S2 meals. After acute treatment with 1 µg/kg EX 1 h before the start of S1, FI decreased significantly to the same very low level for all four conditions in S1, but in S2 we did not observe any decrease of FI compared to the control conditions. Based on this, we hypothesize that the satiety state that the animals carried over to S2 was unaffected by EX both from the homeostatic and hedonic aspects. Thus, administration of the 1 µg/kg dose of EX demonstrated its potential for disentangling different (physiological and motivational) aspects of FI behaviour.

The present experiments suggest that our novel nonhuman primate operant FI paradigm, apart from providing insight in the mechanisms behind physiological and hedonic aspects of FI behaviour and together with more abstract planning determined by future goals and drive states in mind, will also be suitable for preclinical drug development research in a realistic feeding regime where palatability and not nutritional value (energy content) is the primary determining factor in food consumption.

P8.27 Activation of the social decision-making and social-stress network in valproate-treated, autism-model mice

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The Autism Spectrum Disorder (ASD) is a pervasive neurodevelopmental condition that has a lifelong impact, with a significantly higher prevalence among males than females. Regardless of severity, ASD is clinically characterized by impairments in social behavior, particularly manifesting as difficulties in non-sexual social situations. Exposure to specific agents, such as valproic acid (VPA) during pregnancy, has been linked to ASD. In the present experiment, we utilized the VPA-mouse model of ASD, exploring neuronal activation in the nuclei of the social decision-making network (SDMN) and nuclei that may exert a regulatory effect on the SDMN in juvenile male mice across various social settings.

The effectiveness of VPA treatment was validated through a three-chamber sociability test, a widely accepted method for measuring the ASD-like behavioral phenotype. c-Fos immunohistochemistry was conducted on both control and VPA-treated individuals to capture snapshots of the momentary activity of cells during two types of social situations: mice either cohabited with familiar companions or were separated for one day and then reinstated to familiar cagemates.

Distinctive differences in certain brain regions of the SDMN were observed based on social situations and embryonic treatments. For instance, the nucleus accumbens (NAcc) and ventral tegmental area (VTA), pivotal in the mesolimbic reward system, exhibited increased activity after reinstatement in VPA-treated mice, contrasting with control individuals. Analyzing the activity patterns of the nuclei enabled us to model the functional network regulating behaviors in different social environments.

Our findings support the notion that VPA-treated animals possess a more widespread connectivity network than control animals. However, the nuclei of the SDMN exhibit less dominance than those related to regulating social stress. Hub analysis further supports the idea that the SDMN has a lower priority in the network regulating social behavior in VPA-treated animals. Exposure to valproic acid most likely disrupts the SDMN and, therefore, affects the social interactions from early postnatal development, further hindering the acquisition of normal social behavior.

P8.28 The mitochondrial uncoupling protein 2 (ucp2) is involved in cold sensation and hyperalgesia in the chronic neuropathy rat model

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Uncoupling proteins (UCPs) are located in the mitochondrial inner membrane, which translocate protons (H^+) from the intermembrane space to the matrix, therefore reduce membrane potential and uncouple ATP synthesis from mitochondrial respiration. From the five members of this family, UCP2 is mainly expressed in the heart but has also been described in primary sensory neurons. Inhibition of mitochondrial ROS production by UCP2 attenuates the upregulation/activation of several signaling pathways leading to cellular senescence and inflammation, which occur as common mechanisms in a variety of diseases. However, there are no data on its involvement in pain sensation, transmission and sensitization. Therefore, we investigated the role of UCP2 on heat- and mechanosensitivity in a rat neuropathy model.

Traumatic mononeuropathy on the right limb was induced by tight ligation of 1/3 of the sciatic nerve in female and male UCP2 knockout ($UCP2^{-/-}$) and UCP2 wildtype ($UCP2^{+/+}$) rats (12-15 weeks, 250-300 g) after determining the baseline thresholds of the hindpaws. Touch sensitivity was measured by dynamic plantar aesthesiometry, pressure by analgesimetry (Randall-Sellito test), thermonociception on the increasing temperature hot plate, and cold sensitivity by the withdrawal latency from ice cold water 7 and 14 days after the surgery.

Baseline mechanical thresholds were significantly lower in female $UCP2^{+/+}$ rats compared to their male counterparts; this pattern was similarly in $UCP2^{-/-}$ females compared to $UCP2^{-/-}$ males. However, $UCP2^{-/-}$ exhibited a significantly higher cold threshold than $UCP2^{+/+}$ in male rats. Seven days post-surgery, cold sensitivity was significantly lower in female $UCP2^{-/-}$ compared to their $UCP2^{+/+}$ counterparts.

On day 14 post-surgery, $UCP2^{-/-}$ males demonstrated reduced cold sensitivity compared to $UCP2^{+/+}$ males, while mechanical thresholds were significantly lower in $UCP2^{+/+}$ compared to $UCP2^{-/-}$ rats of both sexes. Heat sensitivity and pressure threshold values did not changed significantly.

The elevated mechanical and cold sensitivity thresholds observed in both male and female $UCP2^{-/-}$ rats imply UCP2's role in cold and mechanical sensation, along with neuropathic cold hyperalgesia. Furthermore, the lower mechanical threshold in females compared to males, irrespective of UCP2 status (in both $UCP2^{-/-}$ and $UCP2^{+/+}$), suggests a sex-related difference in pain sensitivity.

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P8.29 The effect of embryonic valproic acid treatment on the sociability of zebra finches

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Autism spectrum disorder (ASD) is a human neurodevelopmental disorder associated with impaired communication and social behaviors. Valproic acid (VPA) is an antiepileptic and mood-stabilizing drug that, when administered during certain stages of pregnancy, might result in ASD-like symptoms in newborns. VPA has been successfully applied in previous animal models of autism, such as domestic chicks and rodents. However, we argue that zebra finches (Taeniopygia guttata) provide a more useful animal model for studying certain aspects of ASD than widely used standard laboratory rodent species. Zebra finches are highly social, gregarious animals with ample social interactions and complex acoustic communication, similar to humans.

In this study, we applied VPA to zebra finch embryos for the first time to induce ASD-like phenotypes in adults. We investigated the effects of various VPA doses and treatment regimens on the hatching and survival of the finches. After the birds reached adulthood, we also measured the sociability of control and VPA-treated adults in adapted versions of the standard three-chamber test using single, same-sex birds, as well as groups, as social stimuli. Depending on the social stimuli, the control and VPA groups showed large variations in their sociability and showed much less clear differences than those found in rodents. However, control birds tended to prefer the proximity of their conspecifics and larger groups more than VPA-treated birds. The social behavior of the VPA group seemed to be affected by embryonic VPA treatment, similar to findings in rodent models and autistic human patients.

Poster Session 9
Modelling

P9.01 Determining the phase of oscillations at epileptic deep sources based on surface EEG measurements

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Our objective is to determine the accurate phases of oscillations at deep epileptic sources based on surface EEG measurements. To achieve this goal, we conducted experiments by replaying recorded seizures on deep brain electrodes inserted into human cadavers at various positions. Simultaneously, EEG signals were recorded on the skull using a 32-channel subcutaneous electrode system, enabling the measurement of phase relations between deep and surface electric potential recordings.

Two distinct methods, the lead-field projection method and the Gabor-Nelson method, were employed to infer deep activity from surface measurements. Both approaches operate under the assumption that the measured signals were generated by a localized deep current source dipole. The lead-field projection method assumes knowledge of the deep source's location, necessitating the solution of the forward model by calculating the lead-fields of unit amplitude dipoles at the known position. This forward solution requires MRI of the head and segmentation of different tissues based on the image.

In contrast, the Gabor-Nelson method does not assume prior knowledge of the deep source's position and only requires information about the electrode positions on the skull, eliminating the need for an MRI image and tissue segmentation.

Comparison of the signals replayed at different intracranial sources to the corresponding reconstructed dipole activity revealed that while the lead-field projection method reconstructed deep sources with slightly greater precision, the Gabor-Nelson method also yielded appropriate results. Our findings provide valuable insights into the comparative efficacy of these methods for accurately determining the phases of deep epileptic sources based on surface EEG measurements.

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P9.02 Efficient recall in CA3 requires dynamic modulation of external versus recurrent inputs

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Attractor networks are fundamental to our understanding of how neuronal networks can store and retrieve information by denoising inputs, learning episodic memories or computing via cell assemblies. Attractors correspond to local minima in the energy function associated with the neural states. Past work has been focused on how to efficiently store patterns in a recurrent circuit or what internal neuronal dynamics is required to recall them. Although external, feed-forward inputs are also needed to move between different attractors or improve the recall dynamics, how they interact with the recurrent processing remained less well understood. Here we studied the recall of memories stored in a fully connected, binary Hopfield network trained using the covariance rule. Extending previous works formulating recall as probabilistic inference, we derived the recall dynamics that is optimal for pattern completion in the presence of feed-forward inputs. The optimal feed-forward gain was small compared to the recurrent gain, but could still significantly improve recall performance. However, the feed-forward gain optimal for pattern completion could not initiate recall as the impact of external inputs was not sufficiently strong to overcome the energy barrier between different attractors. We showed by systematically varying the feed-forward gain, that a single, fixed gain parameter allows both recall initiation and accurate, near-optimal pattern completion only when both the number of stored memories and the input noise are low imposing a fundamental limitation for recall. We show that this limitation can be overcome by allowing the gain to be dynamically changing during recall. We suggest that this can be achieved by phase dependent modulation of the input gain or dendritic excitability. We speculate that segregation of feed-forward inputs to separate dendritic domain in hippocampal CA3 pyramidal neurons and their newly described dendritic calcium spike types might be involved in implementing these computations.

We are grateful to the Hungarian Research Network for their generous support.

P9.03 Structural aspects of somatostatin and TT-232 binding to SST4

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Somatostatin exerts its effects via binding to G-protein-coupled somatostatin receptors of which the fourth subtype (SST4) is a particularly important receptor mediating analgesic, anti-inflammatory, and anti-depressant effects without endocrine actions. Thus, SST4 agonists are promising drug candidates. The design of metabolically stable, highly potent and selective analogues necessitates the knowledge of atomic resolution binding mechanism of somatostatin to SST4. In the present study, the binding mechanism of somatostatin was elucidated in the explicit water molecular dynamics calculations, and key binding modes (external, intermediate, and internal) were distinguished. The most important residues on both receptor and somatostatin sides were identified. The calculated structures show good agreement with available experimental results and indicate that somatostatin binding is realized via prerequisite binding modes and an induced fit mechanism. TT-232 is a cyclic heptapeptide somatostatin analogue showing great affinity to SST4 and SST1. Docking calculations revealed that TT-232 exhibits a similar binding pattern to somatostatin and its calculated interaction energy is similar to or stronger than that of several selective SST4 agonist reference compounds. The identified binding modes and the corresponding key residues provide useful information for future drug design targeting SST4.

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P9.04 Repeated diagnostic ultrasound exposure increases structural complexity of CA1 neurons

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The development of neurons is regulated by several spatiotemporally changing factors, which are crucial to give the ability for neurons to form functional networks. Although early developmental stages of the neurons may be influenced by external physical stimuli, the mid-and long-term effects of these external influencing factors are still not investigated in depth.

Using an animal model, this study focuses on the morphological changes of the hippocampus that may occur as a consequence of fetal ultrasound examination.

We selectively labeled CA1 neurons of the hippocampus with *in utero* electroporation to analyze their morphological features.

US exposure significantly changed several morphological properties of the basal dendrites and one significant difference in case of the apical dendritic tree. Significant elevation was observed also in the spine density at the basal dendrites, with several changes related to the individual spine morphology.

Our results suggest that US-derived changes in the dendritic trees of CA1 pyramidal cells may lead to an increased dendritic input.

P9.05 Development of a Human Induced Pluripotent Stem Cell-Derived Cerebellar Organoid Model

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The cerebellum is important for processing motor and sensory information. Due to its continued development during the post-natal period, it is vulnerable to a number of pathological processes, for example to different type of ataxias. Since the development of the human cerebellum is completely different from that of the mouse cerebellum, it is essential to use human model systems to study both developmental and neurodegenerative processes.

We have previously developed a human induced pluripotent stem cell (hiPSC)-derived cerebellar organoid system, containingthe disease relevant cell types in a tissue-like organization. However, Calbindin-positive Purkinje neurons seen on day 50 of the organoid differentiation do not show mature Purkinje morphology.

Increasing the duration of cerebellar organoid culture times can lead to the generation of Purkinje neurons with morphology and functions similar to postnatal cells. From the point of view of disease modeling, this provides a more relevant test system for the development of therapeutic interventions than our currently available cerebellar organoid model.

Therefore we increased the culture times of the cerebellar organoids from 50 days to 120 days to develop a reproducible differentiation protocol for the generation of a hiPSC-derived organoid model of the cerebellum containing mature Purkinje neurons. From day 75 to day 120, the organoids were maintained in two different conditions, where one of the mediums has been supplemented with glucose. The cell types found in the organoids as well as the morphology of Purkinje neurons on days 75 and 120 of the differentiation were characterized by immunocytochemical methods.

Overall, it can be concluded that we have created an organoid model of the human cerebellum, which contains mature Purkinje neurons, so in the future, we can use this improved protocol to study neurodegenerative diseases.

I am deeply grateful to the ÚNKP (23-3-I.) for their generous support, which has been instrumental in both the progress of my research and the creation of the poster presentation. Their funding has significantly contributed to the success of my work, and I appreciate the opportunities it has provided.

P9.06 The effect of head motion on brain age prediction using deep convolutional neural networks

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Deep learning can be used effectively to predict participants' age from brain magnetic resonance imaging (MRI) data, and a growing body of evidence suggests that the difference between predicted and chronological age—referred to as brain-predicted age difference (brain-PAD)—is related to various neurological and neuropsychiatric disease states. A crucial aspect of the applicability of brain-PAD as a biomarker of individual brain health is whether and how brain-predicted age is affected by MR image artifacts commonly encountered in clinical settings. To investigate this issue, we trained and validated two different 3D convolutional neural network architectures (CNNs) from scratch and tested the models on a separate dataset consisting of motion-free and motion-corrupted T1-weighted MRI scans from the same participants, the quality of which were rated by neuroradiologists from a clinical diagnostic point of view. Our results revealed a systematic increase in brain-PAD with worsening image quality for both models. This effect was also observed for images that were deemed usable from a clinical perspective, with brains appearing older in medium than in good quality images. These findings were also supported by significant associations found between the brain-PAD and standard image quality metrics indicating larger brain-PAD for lower-quality images. Our results demonstrate a spurious effect of advanced brain aging as a result of head motion and underline the importance of controlling for image quality when using brain-predicted age based on structural neuroimaging data as a proxy measure for brain health.

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P9.07 Gap junction opening is attributed to the redox state of cysteines

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Astrocytic gap junction channels (GJC) form a network, that allows rapid progression of Ca2+ signals (Szabó et al., 2017). This signal transduction propagates through Cx43 GJC channels, that are formed by two connexon hemichannels (HCs). HCs are coupled by H-bonds farther from the membrane. HC subunits themselves are connected by three disulphide bonds per chain. These disulphide bonds are able to open depending on the redox environment. Astrocytic GJCs play a vital role in synchronized neuronal activities, e.g. slow wave sleep and epilepsy (Szabó et al., 2017, Kékesi et al., 2015). Understanding the effect of gap junctions in these processes is currently limited by the lack of GJC-specific inhibitors. Since GJCs have no endogenous ligands, the first step in inhibitor design is to understand the molecular details of GJC formation.

To understand GJC formation from HCs, we modelled two Cx43 HCs based on the cryo-EM structure of Cx31.3 HC (Lee et al., 2020,) and positioned them face-to-face according to the arrangement observed in Cx26 GJC (Cx43 HC-HC), embedded it in explicit membrane and subsequently performed molecular dynamics (MD). Gradually decreasing their distance, the HC-HC docking process could be followed, while *in silico* opening of the disulphide bonds allowed us to imitate changes in the Cys redox state. We found, that 1, appearance of trans GJ stabilization centers (SCs) during 100ns MD is indicative of HC-HC docking, 2, original trans-GJ SC pattern re-emerges after Cx43 HC-HC distance was set to 3 Å, allowing simulation of HC-HC docking 3, trans-GJ SCs appear near disulphide forming Cys residues, 4, opening of Cys disulphide bonds abolishes trans-GJ SCs, 5, the solo HC model, however, is consistent with open Cys disulfide bonds. Furthermore, we analyzed the correlation between Cys redox state and the functional open/close state of HC and GJC using the novel experimental Cx43 HC and GJC structures (Qi et al., 2023). We found that 6, the primary open GJC channel state is attributed to the closed S-S state, while 7, the functionally closed channel state is in accordance with the open S-S configuration.

We showed that disulfide bonds – although located farther from the gap – have an important effect on GJC formation. These observations also provide mechanistic clues that relate gap structure and HC docking through extracellular Cys residues and enhances our understanding of trans-GJ interactions.

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P9.08 Mapping of prerequisite agonist binding modes on the TRPA1 receptor

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Transient receptor potential ankyrin 1 (TRPA1) is a transmembrane receptor coupled to a calcium ion channel. TRPA1 is a polymodal nocisensor, that can be activated by thermal, mechanical stimuli and a wide range of chemically damaging molecules including small volatile environmental toxicants and endogenous algogenic lipids. After such compounds activate the receptor, its channel part opens up by the widening of its central pore to allow calcium influx into the cytoplasm, that consequentially induces signal transduction pathways.

Recent experimental determination of structures of apo and holo forms of TRPA1 opened the ways towards computational binding studies of prerequisite and orthosteric electrophilic agonists. These binding modes of agonists are investigated applying molecular docking and molecular dynamics calculations, suitable for orthosteric covalent and prerequisite binding studies. In the apo form, prerequisite binding modes were detected, and their contribution to the conformational change of the binding site was shown in atomic detail for the first time. Furthermore, prerequisite binding properties were identified, that can forecast the possibility of a strong covalent bond formation. Together, these findings might contribute to the overall understanding of the activation of the TRPA1 receptor, and generally the novel prerequisite design strategy of drugs for other targets as well.

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P9.09 Exploring the firing dynamics and integrative properties of human interneurons by computational modeling

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Recent progress in research on adult human neurons revealed sophisticated biophysical and molecular mechanisms that regulate their signal transfer and information processing capabilities. Differences between the biophysical and morphological properties of human and rodent cortical neurons indicate complex regulatory mechanisms that optimize the speed and information capacity of human neurons in spite of their larger size. As an example, the localization and composition of the axon initial segment (AIS) play a strong role in the regulation of synaptic integration in fast spiking inhibitory neurons of the human cortex. It is also known that the AIS is subject of activity-dependent plasticity that can contribute to the overall excitability of the neurons and how they fire in response to ongoing synaptic activation. In the present study we aimed at developing a 3-compartmental computational model of human parvalbumin-expressing neurons based on patch clamp recordings on acutely prepared brain slices from neurosurgical samples. Next, we investigated the firing responses of the model under various stimulus paradigms such as static current steps, single EPSPs applied on the proximal dendrite and synaptic bombardment. By manipulating the length and localization of the AIS in the model we explored how such morphological parameters regulated the activity of the model cells. Our results hint to excitability regulating mechanisms more intricate than previously suggested. The length of the AIS is positively correlated with the intrinsic excitability of the model, however, moving it in the proximal direction toward the somatic compartment causes slight drop in the firing output. Our results suggest that the AIS localisation and length are major determinants of the excitability and spike timing of human cortical interneurons.

P9.10 Deciphering Neuronal Dynamics: Unraveling Active, Passive, and Capacitive Current Components

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Measuring the spatio-temporal distribution of synaptic currents at the single-neuron level remains an ongoing challenge, awaiting a comprehensive solution. While it is relatively straightforward to calculate the density of the net membrane current, known as the Current Source Density (CSD), from extracellular potentials measured by multichannel electrode systems, distinguishing between its two primary components poses a more challenging task. The CSD consists of the transmembrane (ohmic) current and the capacitive current. The transmembrane current encompasses synaptic, active, and passive channel currents, while the capacitive current corresponds to charge accumulation on the membrane, typically manifesting as counter currents accompanying a primary sink or source in the CSD distribution. Given their often opposing signs, these two components tend to cancel each other, resulting in the extracellularly observable net membrane current (the CSD) representing only a fraction of the total membrane currents.

Our recently developed membrane potential integration method offers a solution by enabling the inference of membrane potential and the differentiation between the two CSD membrane current components—ohmic and capacitive—utilizing parallel multichannel extra and intracellular recordings. Applying this method to multiple paired recordings has unveiled the contribution ratio of transmembrane and capacitive currents, shedding light on the ratio between extracellularly observable and concealed currents. Furthermore, since passive current is proportionate to the membrane potential, the membrane potential integration method allows for a finer division of the transmembrane current, estimating the contributions of passive and active currents to the net membrane current.

Our parallel extracellular and intracellular measurements, complemented by morphological reconstruction and the membrane potential integration method, provide a more detailed understanding of membrane potential dynamics and the input-output transformation executed by neurons.

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P9.11 Characterization of a novel human cell-based microfluidic blood-brain barrier & midbrain organoid co-culture model

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Blood-brain barrier (BBB) forms a dynamic interface between the blood and the central nervous system (CNS). The BBB provides oxygen and nutrients for the parenchyma and protects the brain from harmful insults. But these protective mechanisms also restrict the entry of pharmaceutical drugs into the brain limiting the treatment of CNS diseases. Cell culture models are essential to investigate cerebral drug delivery. Microfluidic chip devices allow the more complex and physiological modelling of the BBB, enable the co-culture of multiple human cell types paving the road to decrease the need for animal experiments. The latest trend in the pharmaceutical drug testing is the use of human brain spheroids and organoids derived from human induced pluripotent stem cells (iPSC). Our aim was to create and optimize a new, dynamic BBB-organoid cell culture model by the co-culture of human endothelial cells, brain pericytes and human midbrain organoids.

For modelling the BBB, a co-culture of human stem cell derived endothelial cells and brain pericytes was used (Cecchelli et al., 2014). Human midbrain organoids were differentiated from iPSCs from healthy people (WT) and Parkinson's disease patients (PD) (Nickels et al., 2020). The cellular composition of the midbrain organoids was characterized by immunostaining for glial and neuronal cells. The barrier integrity of the BBB chip model was investigated in the presence of midbrain organoids by the measurement of impedance and permeability for fluorescent markers. The morphology of brain endothelial cells was examined by immunostaining for tight junction proteins. Targeted nanoparticles carrying a fluorescent cargo were introduced to the system and their passage across the brain endothelial monolayer to the brain organoids was followed. All culture aspects were optimized and tested in static cell culture conditions as well using cell culture inserts.

During our study we successfully optimized the culture conditions of the human BBB model with midbrain organoids. This complex organ-on-a-chip system can be a valuable tool for pharmaceutical testing, pathology modelling and for toxicological studies.

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Poster Session 10
Repair and regeneration

P10.01 Fucoidan as a potential therapeutic agent for enhancing motoneuron survival and regeneration following ventral root avulsion and reimplantation

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The majority of motoneurons perish due to avulsion injuries. Fucoidan, a sulfated polysaccharide present in brown algae, is recognized for its anti-inflammatory properties and its capacity to regulate selectins. Studies suggest that selectins may have a role in the onset of central nervous system disorders. Our research endeavors to explore whether fucoidan-induced inhibition of selectins can improve the survival and regeneration of damaged motoneurons following ventral root avulsion and reimplantation.

In our study, we removed the left lumbar 4 (L4) ventral root and reattached it on the side. After the injury, we injected fucoidan into the abdominal cavity once a day for a week at a dosage of either 50 or 100 mg/kg of body weight. The control group of animals only underwent the L4 ventral root removal and reattachment. After one week, we assessed the response of microglia/macrophages in the L4 segment using Iba-1 and CD68 immunohistochemistry. Additionally, we analyzed the changes in gene expression of various inflammatory factors (IL1B, IL6, TNFA, CCL3, NLRP3, NLRC4) using qPCR. For the groups with long-term survival (up to three months), we cut the ventral branch of the L4 spinal nerve and marked the proximal end with Fast Blue, a fluorescent dye, to identify the reinnervating motoneurons. We assessed functional reinnervation by analyzing movement patterns.

The administration of fucoidan resulted in a significant improvement in the survival and reinnervation potential of injured motoneurons, ultimately leading to the functional reinnervation of previously denervated hind limb muscles. Additionally, the expression of CD68 in the affected ventral horn decreased following fucoidan treatment. Moreover, the gene expression levels of various inflammatory factors were reduced with fucoidan administration. These findings suggest that fucoidan's ability to target selectins may present a unique therapeutic approach for addressing neuroinflammation and promoting the survival and functional recovery of motoneurons after avulsion injury.

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P10.02 Neuroectodermal stem cells improve the functional and morphological outcome after chronic spinal cord injury via various mechanisms

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Spinal cord contusion injury leads to severe tissue loss and subsequent deficit of motor, sensory and vegetative functions below the lesion site. In this study we investigated whether transplantation of neuroectodermal stem cells into the injured rat spinal cord is able to induce significant morphological and functional improvement in a chronic spinal cord injury model.

Mouse embryonic clonal neuroectodermal stem cells (NE-TR-4C) were grafted intraspinally five weeks after a thoracic spinal cord contusion injury performed in SD rats. Control animals underwent contusion injury without stem cell transplantation. Functional tests (BBB test, video-based locomotor pattern analysis) and detailed morphological analysis were performed to evaluate the effects of grafted cells in different time points.

Grafted animals showed significantly better functional recovery compared with control animals. Morphologically, the contusion cavity was significantly smaller, and the amount of spared tissue was significantly higher in grafted animals than in controls. Retrograde tracing studies showed a statistically significant increase in the number of FB-labelled neurons rostral (spinal cord segments, raphe nuclei, somatomotor cortex) to the injury.

The extent of functional improvement was related to the number of inhibitory factors (GFAP, CS-56) around the cavity and microglial reactions in the injured segment. Five days after transplantation the majority of grafted cells appeared to survive, formed clusters and a small proportion of the cells differentiated into neurons and astrocytes. Ten days after grafting the majority of the grafted cells appeared as nonviable fragments in microglia/macrophage cells.

These data suggest that grafted neuroectodermal stem cells are able to induce morphological and functional recovery after chronic spinal cord contusion injury despite the limited survival of transplanted cells.

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P10.03 Delayed intraspinal delivery of mRNAs encoding a combination of cytokines and GDNF promotes morphological and functional recovery following spinal cord injury

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Spinal cord injury results in irreversible tissue damage followed by limited recovery of function. Our earlier study has shown that a cocktail with recombinant human (h) interleukin-10 (hIL-10), interleukin-6 (hIL-6), macrophage inflammatory protein 1-alpha (hMIP-1-alpha) and glial cell line-derived neurotrophic factor (hGDNF) loaded via osmotic pump is able to induce neuroprotection and functional recovery following spinal cord contusion in a rat model.

Based on these results intraspinal delivery of mRNA-LNPs encoding a combination of cytokines (hIL-6, hIL-10, hMIP-1-alpha) and hGDNF was applied at the level of thoracic 10 vertebra 7 days after contusion injury.

The functional analysis showed that the treatment group of therapeutic proteins enhanced the coordinated movement relative to controls. The administration of hGDNF, hIL-10, hIL-6 and hMIP-1 alpha resulted in significantly smaller lesion area at the epicentre of the injury and rescued significantly greater amount of tissue. Analysis of supra and propriospinal connections with the retrograde tracer Fast Blue indicated that the treatment with therapeutic proteins enhanced the number of connections between the segments caudal to the lesion and various cranial parts of the CNS. Astrocytes, microglial cells and neurons also expressed hGDNF, hIL-10, hIL-6 and hMIP-1 alpha proteins after mRNA LNP injection up to 5 days in the injured spinal cord.

These results demonstrate that the delayed treatment with mRNA-LNPs which encode a combination of therapeutic proteins is able to induce morphological and functional improvement after spinal cord contusion. The mixture of hGDNF, hIL-10, hIL-6 and hMIP-1 alpha mRNA LNP provides a simple and controllable new therapeutic approach that is less-invasive than other treatments and does not integrate into the genome.

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P10.04 Defining drivers of central nervous system regeneration: a saga from earthworms

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Over the past century, the scientific community has postulated that the process of tissue regeneration is reliant on the nervous system, evidence to prove this notion has, to date, been sporadic and only indirect. In this study we utilized *Eisenia andrei,* an earthworm which is characterized by remarkable regenerative capacity, to explore the histological, cytological, metallomic and molecular genetic basis of central nervous system regeneration. In *E. andrei,* the extirpation of the cerebral ganglion resulted in an anterograde transport from neurons of the suboesophageal ganglion to the transected circumpharyngeal connectives. This, in turn, triggered the formation of an anatomical scaffold consisting of dorsal neural fibres, to which a large number of small basophil cells (progenitor cells) attached. Cell proliferation and differentiation were observed in the progenitor cells, from which both neurons and glial cells originated. Whole genome RNAseq of the first four ventral nerve cord ganglia revealed transcripts which were up- and downregulated during regeneration. Signalling pathways were identified which are thought to be linked to neuronal development, including axogenesis, axon guidance, synapse formation etc. Numerous other transcripts may indirectly influence the formation of the renewal of the cerebral ganglion (e.g. genes linked to cell communication, migration and adhesion, angio- and vasculogenesis, extracellular matrix disassembly and organization). Besides, the distribution pattern of physiological metals (Ca, Fe and Zn) was determined in the original and the regenerated cerebral ganglion. An unexpected finding was that the iron concentration was depleted (by one fifth) in the regenerating brain tissues until the 7th postoperative week. Taken together we uncover tantalizing insights into the mechanisms that underpin the striking capacity of neuronal regeneration in earthworms.

This work was supported by the Biotechnology and Biological Sciences Research Council (BBSRC) funded London Interdisciplinary Doctoral Programme (LIDo), the National Brain Project (#NAP2022-I-10/2022) and the Hungarian Scientific Research Fund (#138039).

Poster Session 11
Other

P11.01 Maternal activation and projections of distinct neurochemically identified neurons in the posterior intralaminar thalamic nucleus of mice

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Tuberoinfundibular peptide of 39 residues (TIP39) containing neurons in the posterior intralaminar thalamic nucleus (PIL) were shown to be activated by pups in mother rats and suggested to affect maternal behaviour via their projection to the medial preoptic area (MPOA). Therefore, we hypothesized that TIP39 neurons as well as other types of neurons in the PIL may also be activated in mother mice by pup exposure. We found that calbindin-positive neurons delineate the boundaries of the PIL: they are abundant in the PIL but absent in the adjacent nuclei.

In contrast to previous data from rats, calbindin and TIP39 were located in distinct cell populations in the PIL of mice as shown by double immunolabelling. In turn, labelling of TIP39 and calbindin in reporter mice containing ZsGreen in GABAergic neurons expressing vesicular GABA transporter (VGAT), neither TIP39 nor calbindin neurons in the PIL are GABAergic demonstrating that there are three separate cell populations in the PIL: GABAergic, TIP39- and calbindin-expressing neurons.

Projection maps of these neurons established by TIP39 immunolabelling, and injection of adeno-associated virus expressing mCherry in Cre-dependent manner into calbindin-Cre and VGAT-Cre mice revealed different projections of the 3 neuron population of the PIL.For example TIP39-, calbindin-, GABAergic fibers of PIL origin were present in high, moderate and low density in the MPOA, respectively.

Neuronal activation in mice dams in response to pup exposure was investigated by the c-Fos technique. All TIP39 neurons showed c-Fos activation following direct contact with pups but not if pups were reunited with the mothers without the possibility of direct contact. In contrast, only a portion of calbindin-containing neurons showed c-Fos activation by pup exposure, either with or without direct contact. GABAergic neurons, however, were not activated by the pups.

The data suggest that calbindin- and TIP39-expressing neurons represent distinct glutamatergic neuron populations in the PIL, which are probably differently involved in the regulation of maternal behaviour.

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P11.02 Identification And Characterization Of The Binding Sites Of Organic Polysulfides On Trpa1 Receptor For Targeted Drug Design

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Inorganic polysulfides are endogenous analgesic and anti-inflammatory agents that exert their effects through the activation of TRPA1 by the release of somatostatin. However, inorganic polysulfides are not suitable as drugs due to their excessive instability. Therefore, we have turned our attention towards organic polysulfides, which have similar effects but are much more stable than inorganic polysulfides. We aimed to better understand the mechanism of action of garlic-derived organic polysulfides (dimethyl trisulfide, diallyl disulfide and diallyl trisulfide) by identifying their binding sites on the TRPA1 receptor. To this end, we created mutants that are insensitive to the organic polysulfides but retain their other functions, such as the effects of non-electrophilic agonists (e.g., carvacrol, thymol, menthol, cinnamaldehyde) and antagonists (e.g., HC-030031 and A-967079) that bind to different binding sites. First, the effects of mutations were investigated by computer modelling using molecular docking. Subsequently, mutant TRPA1 variants were generated by PCR-based site-directed mutagenesis and their sensitivity to organic polysulfides was tested in three different functional assays: calcium-sensitive fluorescence flow cytometry, radioactive calcium-45 liquid scintillation counting and whole-cell patch-clamp technique. It was found that C621, C641 and C665, which form the binding site for electrophilic agonists, also form the binding site for organic polysulfides, but only their combined triple mutation could establish complete insensitivity to organic polysulfides in the TRPA1 receptor.

National Brain Research Program 3.0 (NAP 3.0); RRF-2.3.1-21-2022-00015 National Laboratory for Drug Research and Development; TKP2021-EGA-16 National Research, Development and Innovation Fund of Hungary.

P11.03 Connections of the human and the rat prefrontal cortex

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The prefrontal cortex (PFC) is the most anterior part of the brain, located in front of the premotor cortex. During evolution, this brain area reached its largest extent in human, regarding not only the gray but also the white matter. In the background of this expansion its crucial regulating roles stand. In human, based on their functions, five prefrontal areas can be differentiated: the dorsolateral (dlPFC), ventrolateral, dorsomedial, ventromedial PFC and the orbitofrontal cortex (OFC). Different functions go hand in hand with different connections thus the white matter that seems to be so homologue differs in a great extent between PFC areas. For example, the superior longitudinal system (SLS) connects the frontal lobe with the parietal, occipital and temporal cortices. The information of the intraparietal sulcus is collected from the visual areas and from the tactile region of the primer somatosensory cortex and carried by SLS to certain parts of the frontal cortex, e.g., to the dlPFC and ventral premotor area. Similar intercortical connections can be found in the rat, as the perirhinal and posterior parietal cortex functionally interact to mediate crossmodal object representations and these areas are in connection with the orbital cortex.

The OFC is an evolutionally conserved area, controls emotion, motivation and social behavior. The orbital cortex, which represents the PFC in the rat, is a relatively large, extended area immediately above the olfactory bulb, it has extensive connections with the cingulate (the so called “infralimbic”, “prelimbic” and “cingulate” cortices) and limbic areas. Through the cingulate areas it is connected with the olfactory lobe, the medial parietal cortex (in human the precuneus) while via the limbic areas it has tight bilateral connection with the amygdala and the hippocampus. Accordingly, several pathways, which are not so important in the human brain, are pronounced in the rat, like the ventral amygdalofugal path, the stria terminalis and stria medullaris. The latter has particular importance because of its role in the reward mechanism. Additionally, the medial forebrain bundle, like in human, is an important pathway in the rat orbital cortex carrying the fibers of the ascending reticular activating system.

The detailed knowledge of white matter in different species helps us to understand the functional connections of the PFC.

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P11.04 The impact of hypoxia and hyperoxia on the number of compacted neurons and brain activity

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Oxygen plays an essential role in the efficient maintenance of the metabolic processes of mammalian cells and the normal functioning of all organs. Among these, the brain should also be highlighted, although it only accounts for 2% of the human body's mass, but it consumes 20-25% of the total resting metabolic rate. Hippocampal regions are highly sensitive to the effects of non-physiological oxygen levels, and impairment of the hippocampal network may be related to memory and learning disorders. The aim of our study was to investigate the changes in neuronal structures and activity in the hippocampus under hypoxic and hyperoxic conditions. Adult rats were exposed to mild hypoxia (16% O_2), mild hyperoxia (30% O_2), severe hyperoxia (100% O_2) or normoxia (21% O_2) for 1 hour at normal ambient pressure. The histopathological alterations were detected with Gallyas silver impregnation. Multichannel silicon probe was used to record network oscillations from hippocampal layers under anesthesia. The administration of 16% O2 led to an increase in the number of compacted (dark) neurons. Similarly, hyperoxia induced formation of dark neurons in the hippocampus at both 30% and 100% O_2 concentrations. These compacted neurons were present in all layers and regions of the hippocampus. In the case of brain activity, a significant increase in the delta frequency range was observed at 16% O_2, while the frequency decreased significantly in hyperoxic conditions. In conclusion, short-term hypoxia and hyperoxia may cause remarkable morphological changes in hippocampal neurons and directly affect hippocampal EEG states.

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P11.05 The association of neurodevelopmental maturity with nonshivering thermogenesis: a neonatal rat model

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Mature neonates and, most of all, preterm infants have limited ability to maintain their core body temperature compared to adults. As their primary mechanism of cold defense, brown adipose tissue develops most intensely shortly before birth and plays a key role in thermoregulation. We aimed to investigate the association between neurodevelopmental maturity and core temperature maintenance by using a rat model.

We used a neurodevelopmental reflex testing protocol to study the difference in the maturity level of 2- and 7-day-old Wistar rats. Each age group was exposed to a thermoneutral and a cold environment. Temperature was measured at different locations on the animals' body surface using thermocouples. At the end of the experiments, interscapular brown adipose tissue samples were collected for RT-qPCR measurement of uncoupling protein 1 (UCP1) expression as an indicator of nonshivering thermogenesis.

A significant difference was found between the two age groups in all tested reflexes: the 2-day-old group received significantly ($p < 0.05$) lower scores than the older group in most of the tests. During cold exposure, the interscapular area (i.e., the site of substantial brown adipose tissue accumulation) had higher temperature than the brain in both age groups, but the temperature difference between the measured areas was less pronounced in the 2-day-old group. UCP1 expression was markedly higher in the 7-day-old group after cold exposure compared to thermoneutral conditions, while the 2-day-old group showed only a smaller elevation in UCP1 expression.

The difference in the level of maturity and UCP1 expression between the age groups highlights the vulnerability of the neonatal animals to cold, especially in the early days of their postnatal life. Considering the similarities in neurodevelopmental maturity between our younger and older rats compared with human preterm and full-term neonates, respectively, our findings may advance the understanding and prevention of neonatal hypothermia in clinical settings.

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P11.06 Analgesic effects of cyclodextrin derivatives via modulation of Transient Receptor Potential Ankyrin 1 ion channel function

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The non-selective cation channel Transient Receptor Potential Ankyrin 1 (TRPA1) is highly expressed on nociceptive sensory nerve terminals and primary sensory neurons, where it is involved in pain integration and inflammation. TRPA1 activation is facilitated by cholesterol- and sphingolipid-rich lipid microdomains (lipid rafts) located in the membrane. Previous experiments have demonstrated that cyclodextrin (CD) derivatives forming a complex with cholesterol thus depleting it from membrane raft regions can reduce receptor activation, thereby exerting analgesic effect. Our aim is to further investigate the effect of these lipid-protein hydrophobic interactions on TRPA1 activation and to identify CD derivatives as analgesic and anti-inflammatory agents with novel mechanisms of action.

In our experiments, we compared three different CD derivatives selected on the basis of our previous results: random methylated β-cyclodextrin (RAMEB), (2-hydroxypropyl)-β-cyclodextrin (HPBCD) and sulfobutylether-β-cyclodextrin (SBECD). *In vitro* cholesterol depletion of CD derivatives was detected by fluorescence microscopy after Filipin III staining in Chinese hamster ovary cell line. We investigated the analgesic effect of CD pretreatment in the mouse model of formalin-induced acute inflammatory pain. Nocifensive behavior was measured in two phases: in the first phase (0-5 min) direct activation of free sensory nerve endings was observed, while in the second phase (20-45 min) pain evoked by the release of inflammatory mediators was observed. The cholesterol depleting effect of intraplantar CD treatment was measured by colorimetry of mouse plantar skin using Abcam Cholesterol Assay kit.

Filipin III fluorescence staining showed that 3 mM RAMEB, 10 mM HPBCD or 10 mM SBECD treatment significantly reduced the cholesterol content of the CHO cell membrane. CD pretreatment (3 mM RAMEB, 10 mM HPBCD and 10 mM SBECD) reduced the duration of nocifensive behavior during the second phase of formalin-induced acute inflammatory pain. Intraplantar CD derivative treatment reduced the total cholesterol content in the plantar skin of mice compared to the cholesterol content measured in control animals treated with physiological saline revealed by Abcam Cholesterol Assay kit.

According to our results we conclude that RAMEB, HPBCD and SBECD are able to deplete cholesterol from CHO cells applied *in vitro* and from the plantar skin of mice after intraplantar injection in vivo, also exerting analgesic effect in case of TRPA1-mediated acute pain. These results suggest, that the applied CD derivatives may be potential new compounds for peripheral analgesia with a novel mechanism of action.

P11.07 Aging-related shifts in the activity of hypothalamic neuropeptide Y

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Middle-aged obesity and later occurring aging anorexia with muscle loss (sarcopenia) of old people present a public health burden. As several mammals also show these trends, regulatory alterations may also be assumed in the background. Previously, we demonstrated that age-associated shifts in the catabolic effects of neuropeptides of the hypothalamus–adipose tissue axis (leptin and melanocortins) may contribute to both trends. We aimed to clarify, whether neuropeptide Y (NPY), the main anabolic target of leptin in the hypothalamus, shows an opposite pattern during aging and thereby contribute to both trends.

The orexigenic and hypometabolic effects of intracerebroventricularly administered NPY were investigated in five age groups of male Wistar rats from young to old. We measured hyperphagia upon single NPY injections. We also recorded changes in food intake, body weight, heart rate, body temperature, locomotor activity during a 7-day NPY infusion in a biotelemetric system. The endogenous NPY activity at mRNA and protein levels in the hypothalamic arcuate nucleus was analyzed in similar age groups using RNAscope combined with immunohistochemistry.

Both the anabolic efficacy of NPY leading to weight gain and its immunoreactivity increased in middle-aged animals preceding the peak of adiposity observed in aging rats. Later on, these parameters decreased preceding the appearance of anorexia and weight loss in old rats. These shifts may contribute to the development of both age-related obesity and aging anorexia with sarcopenia.

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P11.08 Antagonizing the CX3CR1 fractalkine receptor reduces chronic inflammatory arthritic pain in a mouse model

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The fractalkine chemokine receptor 1 (CX3CR1) is primarily expressed on monocytes/macrophages, T cells, osteoclast precursors and microglial cells. It was described to mediate inflammatory mechanisms both in the periphery and the central nervous system. Although the role of neuroinflammation has been described in some pain conditions, little is known about the role of CX3CR1 in chronic joint pain. Therefore, we investigated the involvement of CX3CR1 in the chronic adjuvant-induced mouse arthritis model.

Chronic arthritis was induced by complete Freund's adjuvant (CFA) in C57BL6/J mice. Mechanonociception was determined by aesthesiometry, thermonociception by constant temperature hot plate, paw volume by plethysmometry, neutrophil myeloperoxidase (MPO) activity by luminescence, plasma extravasation by fluorescence in vivo imaging, and histopathological alterations by semiquantitative scoring. The small molecule CX3CR1 antagonist AZD8797 (2x1 mg/kg/day, i.p.) or its vehicle was injected every day during the 21-day experimental period.

Approximately 20-40% mechanical hyperalgesia, 60% latency decrease in nocifensive behaviors on the hot plate, 80% paw edema, increased neutrophil MPO activity and plasma extravasation, and histopathological damage (mononuclear cell infiltration, synovial hyperplasia, cartilage destruction) were detected in vehicle-treated CFA-injected mice. AZD8797 treatment reduced mechanical hyperalgesia with moderate/large effect sizes between days 3 and 15 ($g>0.5$), thermal hypersensitivity with large effect size on days 15 and 21 ($g > 0.8$), but no differences were found in any inflammatory or tissue damage parameters compared to vehicle-treated controls.

CX3CR1 activation mediates chronic arthritic pain, mainly independently of the peripheral inflammatory processes. Therefore, its inhibition offers promising novel analgesic perspectives.

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P11.09 Chemosensor TRPA1 covalent ligand modifies T lymphocyte activation in vitro

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Transient Receptor Potential Ankyrin 1 (TRPA1) is a non-selective cation channel involved in sensation and sensitization to a plethora of inhaled, touched or orally consumed irritating cysteine-reactive agents and also endogenous mediators of oxidative stress such as nitric oxide, hydrogen peroxide, and inflammatory signals. TRPA1 has been reported to influence neuroinflammation accompanied by feedback mechanisms, macrophage and also lymphocyte function, but its complex role is still controversial in immune cells.

We reported earlier detectable, but orders of magnitude lower level of mRNA in monocytes and lymphocytes than in sensory neurons by qRT-PCR analyses of cells originated from primary and secondary lymhoid organs of mice. Our present goals were to (a) further elucidate the expression of Trpa1 mRNA in mononuclear cells by single cell RNA scope in situ hybridization and (b) to test the potential role of TRPA1 in peripheral lymphocyte activation.

RNA scope in situ hybridization confirmed that low-copy *Trpa1* transcripts were detectable in $CD14^+$ and $CD4^+$ leukocytes isolated from peritoneal cavity of mice.

The role of endogenous TRPA1 in the activation of lymphocytes was studied in vitro. We analyzed the effect of a potent selective small molecule TRPA1 agonist, JT010 on the TcR-mediated activation of T lymphocytes and the IgG-dependent activation of B lymphocytes. JT010 administration did not stimulate significant changes in intracellular Ca^{2+} level of these cells. However, a concentration-dependent significant inhibitory effect of JT010 could be observed on TcR-induced Ca^{2+} signal of peripheral T lymphocytes and $CD4^+$ T lymphocytes, while JT010 neither modified peritoneal B cell activation nor ionophore ionomycin stimulated elevation of intracellular Ca^{2+} level.

Though TRPA1 proved not to be a key regulator of TcR-stimulated calcium signaling in our earlier studies in TRPA1 KO mice, its function negatively modulated T lymphocyte but not B lymphocyte activation. Our results indicate that modulation of TRPA1 receptor/channel by an agonist/agent may lead a more complex, cell type-, localization- (environment-, stage-) specific effect on immune cell activation, then solely influencing elevation of intracellular Ca^{2+} level by opening the Ca^{2+}/cationic channel.

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P11.10 RNA degradation in human brain tissue samples depends on multiple factors

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Recent developments in RNA sequencing increased the use of human brain tissue samples in understanding transcriptional alterations in neurological and psychiatric diseases. The human brain tissue samples typically cannot be processed immediately for RNA isolation. Rather, the dissection of the brain is usually carried out hours after the death is confirmed. Subsequently, the brains are stored frozen or in fixative before dissection of RNA. Even the rare surgical samples cannot be processed on the site and need to be transported to a laboratory for RNA isolation. RNA is not stable as it can be degraded by RNases as well as its chemical decomposition can happen. These processes depend on the microenvironment around the RNA, which may not be the same for the different samples. Therefore, in the present study, we aimed to examine RNA quality in different human brain samples stored in the Human Brain Tissue Bank of the Semmelweis University. RNA quality was assessed by measuring the RNA integrity number (RIN) following an RNA purification protocol combining the Trizol method and the columnar purification steps. RNA quality varied depending on the brain, which the samples were dissected from. The position of the brain tissue sample within the brain had less effect on the RNA quality. Interestingly, once the RNA quality was good in a particular brain, it remained stable for several hours and showed only limited degradation with postmortem time (2 to 10 hours at room temperature). In contrast, neurosurgical samples (brain tissue removed from the brain during neurosurgery), which have a small size, showed fast degradation if stored at room temperature for 1 min to 3 hours. In addition to these factors, the RNA quality also showed some dependency on the types of disease the patient suffered from. In contrast, sex and age dependence was not found. These data suggest that the brains have to be tested for RNA quality and dissections for transcriptomics have to be performed only from selected brains with high RIN numbers. In turn, a good quality RNA (better than RIN number 7) can be obtained from many brains stored at -80 °C if properly processed.

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P11.11 The MAO-B inhibitor neuroprotective agent selegiline reduces the viability of prostate cancer cell lines and enhances the effects of anti-androgen and cytostatic agents: potential for drug repurposing

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Prostate adenocarcinoma (PAC) has the second highest age-standardized incidence mortality among men worldwide. Antiandrogens including the receptor antagonist enzalutamide and docetaxel-based chemotherapy are the bases of the treatment for advanced PAC. Monoamine-oxidases (MAO) are mitochondrial enzymes with two isotypes that catalyze the oxidative deamination of monoamine neurotransmitters and exogenous amines from alimentary sources. Higher expression and activity of MAO-A has been shown in the tumorous prostate, which positively correlates with progression and therapy resistance. MAO-B expression has also been shown in PAC cell lines, but little is known about its role. Therefore, we investigated the effect of the irreversible MAO-B inhibitor selegiline used as a neuroprotective agent on the viability of PAC cell lines.

We tested the effects of selegiline on the viability of the androgen-sensitive slowly proliferating 22Rv1 and androgen-insensitive rapidly proliferating PC3 cell lines measured by the CellTiter-Glo adenosine-triphosphate (ATP)-detecting kit. The effects were compared to the irreversible selective MAO-A inhibitor clorgyline and the non-selective blocker phenelzine, which is under phase II. clinical investigation for the treatment of metastatic PAC. Selegiline was also tested in combination with enzalutamide and docetaxel.

Selegiline (10 mM) abolished cell viability in both cell lines, 1 mM exerted a significant viability reduction, twofold in the androgen-responsive cell line. Clorgyline and phenelzine (100 µM) treatment caused significant viability decrease, phenelzine showing quadruple reduction in 22Rv1. Docetaxel (1 µM) showed significant viability results and combined with 750 and 1000 µM selegiline significantly greater enhanced effects were observed. The viability decreasing effect of 10 and 50 µM enzalutamide was potentiated by the combination of selegiline, which was significantly greater in comparison with the effects of 750-1000 and 500-1000 µM selegiline, respectively.

Therefore, the MAO-B inhibitor selegiline is suggested for drug repurposing in the treatment of both androgen-sensitive and castration-resistant PAC even for combination with the conventional hormonal and chemotherapy. Selegiline is currently tested in a clinical trial to directly prove the efficacy in humans as an add-on therapy (ClinicalTri-als.gov Identifier: NCT04586543).

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P11.12 Lipid raft disruption influences membrane fluidity in CHO cells and decreases activation of Transient Receptor Potential Melastatin 8 ion channel in in vivo mouse model

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Transient Receptor Potential (TRP) cation channels as the Vanilloid 1 and Ankyrin 1 (TRPV1 and TRPA1) and „melastatin" TRP receptor TRPM8 an TRPM3 are playing important role in pain sensation. The are expressed in subgroups of primary sensory neurons. TRP channels are proven to be embedded in lipid raft regions of the plasma membrane, that are rich in cholesterol, sphingolipids, and gangliosides. Their integrity can be broken by cholesterol depletion with methyl-β-cyclodextrin (MCD) and with our own carboxamido-steroid compound (C1) or by sphingomyelin depletion with sphingomyelinase (SMase) and myriocin (Myr). We previously described that lipid raft disruptors inhibit the activation of TRPV1, TRPA1 *in vitro,* and we also demonstrated analgesic effect *in vivo* via TRPV1/TRPA1. We aimed to test the effect of these lipid raft disruptors on the activation of the TRPM3 and TRPM8 ion channel in vitro and *in vivo,* and their effects on membrane fluidity using fluorescence spectroscopy.

For the membrane fluidity studies, native CHO cells were treated with lipid raft disruptors, then they were incubated with 40 µM Laurdan and the decay curves of the Laurdan time-lapse emission spectrum between 410-540 nm were recorded. The microviscosity and the parameters characteristic of Laurdan and its environment were determined, from which we can deduce the membrane fluidity. The role of plasma membrane microdomains of lipid rafts was analysed on isolated trigeminal (TG) neurons by measuring agonists-induced Ca^{2+}-transients with ratiometric technique. In *in vivo* experiments, mice were pretreated intraplantarly with MCD, SMase, Myr and C1 before the TRPM8 agonist icilin or the TRPM3 agonist pregnenolon-sulphate (PS)-CIM-0216 combination injection into the hindpaw of animals and we measured the duration of the pain reaction (raising, licking, chewing, shaking). It has been revealed that intracellular Ca^{2+} enhancement evoked by icilin (TRPM8) was inhibited after SMase, MCD, Myr and C1 incubation, but the response to PS (TRPM3) only decreased after C1 treatment. The duration of the icilin-induced acute pain reaction was significantly reduced by SMase, but the other compounds were not effective. The duration of the PS-CIM-0216-induced pain reaction was unaltered. MCD increased, while SMase and Myr decreased the membrane fluidity. We suggest that the hydrophobic interactions between the TRP channel and lipid raft interfaces modulate the opening properties of these channels and therefore, targeting this interaction might be a promising tool for drug developmental purposes.

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P11.13 Association between visual impairment, metabolic factors, and hip fractures in elderly patients

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In elderly patients experiencing hip fractures due to falls, the risk of recurrent fractures and mortality is high. Therefore, emphasizing prevention alongside treatment is pivotal. Causes that increase the likelihood of falls, such as diminishing vision, dizziness, or underlying metabolic causes, all contribute to the occurrence of hip fractures.

Our research aimed to investigate specific visual (impaired visual acuity, decreased or missing stereopsis) and cardiometabolic risk factors associated with low-impact injuries of the hip region.

A total of 135 individuals aged 60 and above (mean = 75.12; SD = 8.19 years) participated in our study, and were divided into control (low risk for hip fracture and visual impairment) and study groups (patients treated for hip fractures at the University of Pécs, Clinical Center, Department of Traumatology and Hand Surgery). Stereovision and visual acuity were measured using the EuvisionTab application, employing static and dynamic random dot stereograms (SRDS and DRDS) for stereovision and Landolt C stimuli for visual acuity. Cardiometabolic risk factors such as diabetes mellitus, hypertension and cardiac arrhythmias were determined by means of patient history and documentation.

After age matching, monocular visual acuity and stereo vision between the two groups were compared. The study group consistently performed worse in all tests (paired T- and Wilcoxon tests). Odds ratios (OR) were calculated to examine the correlation between various risk factors and hip fractures. No association was found for metabolic risk factors (OR $\leq$1.51; p $\geq$0.2124), however, there was a consistently significant correlation between vision-related risk factors and hip fractures (OR $\geq$3.5326; p $\leq$0.0152).

Our research did not find a link between cardiovascular risk and falls, which may be due to the diagnostic limitations and socioeconomic differences between the examined two patient groups. On the other hand, a detailed examination of visual impairment revealed a significant association, indicating that reduced vision increases the likelihood of hip fractures due to falls. It can be assumed that by performing visual assessments and appropriate refractive correction or surgical treatment if needed may lead to the prevention of some fractures within the elderly population.

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P11.14 Cariprazine exerts antinociceptive actions in mice

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Cariprazine (CAR) belonging to the atypical antipsychotics is used successfully in the treatment of schizophrenia. It has been studied in a number of in vivo behavioural (e.g. cognitive) models, but little is known about its potential effects on pain. In order to elucidate the role of this compound in pain, we tested CAR in three in vivo pain models in male NMRI mice.

The effects of CAR were investigated in the widely used and reliable mouse model of chronic traumatic mononeuropathy, the partial ligation of the sciatic nerve (Seltzer) model, where changes in touch sensitivity were monitored by dynamic plantar esthesiometry (DPA). In the formalin test (lifting, shaking and licking of the hindpaw following subcutaneous injection of 2.5% formalin) and the writhing test (abdominal contractions and characteristic writhing movements following intraperitoneal injection of 0.6% acetic acid), the acute nocifensive behaviour of the animals was investigated, in two phases in the former case and three in the latter. Oral (p.o) CAR treatment (0.5 mg/kg) was administered one and two hours before the measurements, while the control groups were treated with Tween80 (2.5%) p.o at the same time points.

The 30-40% drop of mechanonociceptive threshold due to nerve ligation was almost abolished after CAR treatment, but persisted in the control groups on day 7. Following formalin administration, there was no difference between groups in the first (0-5 min, early) phase, but significantly reduced duration of paw liftings and shakings could be registered in the second (20-45 min, late) phase in CAR-treated animals, especially in the two-hour-pretreatment group. In the writhing test, the number of writhing movements of CAR-treated animals was reduced in all time intervals (0-5, 5-20, 20-30 min) compared to Tween80-treated animals, but a significant difference was only observed in the second and third phases, in the two-hour pre-treated groups.

Our results show that CAR treatment significantly reduced pain in the three investigated models, and the two-hour pretreatment was more effective in all cases. These results support the analgesic role of CAR, suggesting its potential application in new indications.

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P11.15 A novel positive modulator of alpha7 nAChR enhances cholinergic neurotransmission and promotes intracellular calcium responses in hippocampal interneurons

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Homomeric alpha7 nicotinic acetylcholine receptors (nAChRs) are expressed in the central nervous system in cognition-relevant areas including the prefrontal cortex and the hippocampus. Numerous studies indicate the potential of nAChR ligands to improve cognitive functions. Despite the extensive efforts in the nAChR field, effective treatments remain an unmet medical need. The observed suboptimal efficacy of various alpha7 nAChR-selective agonists and partial agonists in clinical trials may be partially attributed to the desensitization-driven nAChR loss of function. Therefore, we have developed novel alpha7 nAChR selective positive modulator compounds.

Modulators were selected by their effect on intracellular Ca^{2+} elevation of a selective alpha7 nAChR agonist using plate-reader based fluorometry and on the kinetics of current evoked by choline using patch clamp in recombinant cells expressing human alpha7 nAChR. Intracellular Ca^{2+} concentration changes were measured simultaneously by multiphoton imaging in interneurons from rat hippocampal slices.

Our compound (RGH-560) elicited a significant increase in both the potency and the efficacy of choline to evoke responses showing decelerating effect on current decay of choline induced current, up to 100 nM without agonist effect. From 1 µM it evoked an inward current. RGH-560 significantly enhanced choline-evoked intracellular Ca^{2+} responses in the dendrites of hippocampal interneurons. Moreover, it promoted the action potential firing of the interneurons evoked by choline. Spontaneous neuronal activity was also elevated showing network level effect of the compound. All these effects could be blocked by the alpha7 nAChR selective antagonist MLA.

The compound was shown to be effective in vivo. RGH-560 produced a significant reversal of a scopolamine-induced cognitive deficit in the mouse place recognition test at 10 mg/kg p.o. and proved to be effective in delay-induced natural forgetting (novel object recognition) test in rats from 3 mg/kg p.o.

Our in vitro data uncover unique and promising properties of this novel positive modulator of alpha7 nAChR. Furthermore, RGH-560 displays in vivo efficacy in animal models of cognition, validating the targeted molecular mechanism of action. Further development of these compounds may provide an efficient strategy for treatment of cognitive disorders.

Registered participants

List of registered participants was prepared according to data available on 15 January, 2024.
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