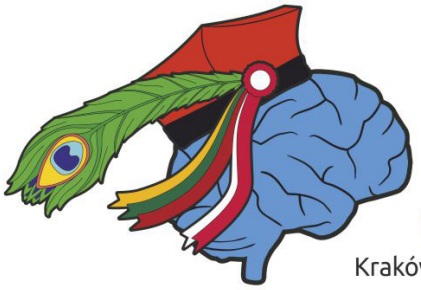

FENS
REGIONAL MEETING
Kraków, Poland, 25-27 August 2021

Virtual FENS Regional Meeting 2021
25-27 August 2021

Book of Abstracts

PTBUN Polish Neuroscience Society

Lithuanian Neuroscience Association

IBRO PERC

FENS Federation of European Neuroscience Societies

Kraków

Honorary Patronage
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Jacek Majchrowski

FENS
REGIONAL MEETING
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Organized by
the Polish Neuroscience Society and the Lithuanian Neuroscience Association

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WELCOME TO THE FENS REGIONAL MEETING 2021!

The FRM 2021 is organized jointly by the Polish Neuroscience Society (PNS) and the Lithuanian Neuroscience Association (LNA) under the auspices and with support from the Federation of the European Neuroscience Societies (FENS), and also with support from the International Brain Research Organization Pan-Europe Regional Committee (IBRO PERC). The FRM 2021 original city venue was Krakow, however, due to the COVID-19 pandemic, the Organizing Committee decided to hold the event on-line to ensure all attendees may meet safely.

The conference will present the latest developments in neuroscience research and host panel discussions on topics ranging from directions for future development to diversity issues in the academia. Traditionally, the Regional Meetings foster interactions among the researchers in the region. This year, we want to take the on-line format as an opportunity, and showcase neuroscience research in our region to the global community

Be a part of the FENS Regional Meeting 2021!

On behalf of the FRM Organizing and Scientific Committees,

Grzegorz Hess,
President of the Polish Neuroscience Society

Osvaldas Rukšėnas,
President of the Lithuanian Neuroscience Association

Contents

IBRO PERC Lecture

Modeling human development and disease in cerebral organoids

Jürgen Knoblich

IMBA - Institute of Molecular Biotechnology of the Austrian Academy of Science Vienna, Austria

The human brain is unique in size and complexity, but also the source of some of the most devastating human diseases. While many of these disorders have been successfully studied in model organisms, recent experiments have emphasized unique features that can not easily be modeled in animals. We use cerebral organoids to recapitulate those features in vitro and to test their role in human disease. Cerebral organoids derived from patients suffering from neuro-developmental disease can recapitulate the developmental defects leading to those diseases and allow us to disentangle the mechanistic complexity of disorders like Epilepsy and Autism. Our new data demonstrate that by studying those defects, we can gain unique insights into the development of the human cortex that cannot be made in rodent model organisms.

Plenary Lecture

Problematic usage of the Internet – disorders, mechanisms and interventions

Naomi Anne Fineberg

University of Hertfordshire and Hertfordshire Partnership University NHS Foundation Trust, Rosanne House, Welwyn Garden City, UK

The Internet is now all-pervasive. While its many positive uses have been demonstrated to the full during the recent COVID-19 pandemic, a proportion of the public develop Problematic Use of the Internet (PUI), an umbrella term incorporating a range of repetitive impairing behaviours including excessive and compulsive video gaming, gambling, compulsive sexual behaviour, buying, streaming, cyberchondria or social networks use. There is growing public and national health authority concern about the health and societal costs of PUI across the lifespan. Gaming Disorder is now included as a mental disorder in the newly released ICD-11. However, more research is needed into disorder definitions, validation of clinical tools, prevalence, clinical parameters, brain-based biology, socio-health--economic impact, and empirically validated intervention and policy approaches. Potential cultural differences in the magnitudes and natures of types and patterns of PUI need to be better understood, to inform optimal health policy and service development. In this lecture, I will review the landscape from the perspective of key research priorities.

Session 1: Gut, microbiota and the brain

Novel insights into microbiota-gut-brain signalling in reward and obesity

Harriet Schellekens

APC Microbiome Institute, University College Cork, Cork, Ireland

The gastrointestinal microbiota is emerging as a unique and inexhaustible source for metabolites with potential to modulate G-protein coupled receptors (GPCRs). The ghrelin receptor [growth hormone secretagogue receptor (GHSR)-1a] is a GPCR expressed throughout both the gut and the brain and plays a crucial role in maintaining energy balance, metabolism, and the central modulation of food intake, motivation, reward, and mood. Short-chain fatty acids (SCFAs), lactate, and different bacterial strains, including *Bifidobacterium* and *Lactobacillus* genera, can modulate GHSR-1a signaling. We identify, for what is to our knowledge the first time, a potent effect of microbiota-derived metabolites on GHSR-1a signaling with potential significant consequences for host metabolism and physiology.

Shaping a second brain in the bowel: a microbial perspective in the context of malnutrition

Filipe De Vadder

The Institute of Functional Genomics of Lyon, University Claude Bernard Lyon, France

Undernutrition-induced stunting is a major worldwide health issue, affecting 150 million children under 5 years of age[1]. In early life, it is associated with persistent stunting. We previously demonstrated the ability of selected probiotic strains to buffer the deleterious effect of undernutrition on juvenile growth[2]. Besides deficiencies in the central nervous system, malnourished animals also show abnormal development of the ENS. In order to selectively address how the microbial environment shapes maturation of the ENS after weaning, we used a mouse with a simplified microbiota. Overall, our findings suggest that modulating the microbial environment during malnutrition shapes the maturation of the ENS. Further studies will reveal the mechanisms underlying such phenotypes.

[1] 2020 Global Nutrition Report. https://globalnutritionreport.org/.

[2] Schwarzer, M. et al. Lactobacillus plantarum strain maintains growth of infant mice during chronic undernutrition. Science 351, 854–857 (2016).

MicroRNAs and gut microbiota signatures for vulnerability to food addiction

Elena Martín-García

Laboratory of Neuropharmacology-Neurophar, Department of Experimental and Health Sciences, Pompeu Fabra University (UPF), Barcelona, Spain

Food addiction is characterized by loss of behavioural control over food intake and may promote obesity and alter gut microbiota diversity and composition. We have investigated the involvement of microbiota content and epigenetic changes in the mechanisms underlying food addiction. We used the YFAS 2.0 score to classify extreme food addiction mouse and human subpopulations to identify gut microbiota signatures and miRNAs associated with vulnerability to this disorder. We have then functionally validated the involvement of selected miRNAs differentially expressed in addicted mice and food addict patients in specific phenotypes of this behavioural disorder. Differential miRNAs expression in the medial prefrontal cortex and gut microbiota content were revealed in addicted and non-addicted mice. Similarly, circulating miRNAs and gut microbiota content were associated in our human cohort with the differential values obtained in the YFAS 2.0 score. Close cross-talks were demonstrated between these miRNAs and microbiota changes. Interestingly, sharp similitudes were identified in the miRNAs and gut microbiota signatures in our animal and human cohorts. Finally, we used a Tough-Decoy inhibitor approach in the mouse brain to functionally validate the specific role of the most relevant miRNAs in each behavioural hallmarks of food addiction. We have identified specific changes in microbiota content and miRNAs closely involved in the biological substrate of food addiction. The elucidation of the mechanisms underlying these behavioural alterations provides new advances toward innovative biomarkers and interventions for food addiction and related disorders.

Targeting the microbiota-gut-brain axis in Alzheimer's disease

Aurelijus Burokas

Life Sciences Center, Vilnius University, Vilnius, Lithuania

A rapid population ageing has resulted in a growing number of patients with Alzheimer's disease (AD) that creates an increasing need for early diagnosis, treatment and prevention of illness. Additionally, the growing "epidemic" of diabetes and its close relationship with AD is increasingly becoming more evident that led researches to investigate how these diseases have an influence on each other. In both cases, impaired immune system that is closely related to the gut microbiota has been reported. With the growing number of facts about the effects of the microbiota on various physiological processes in the body, its exploration has been chosen not only to better understand the mechanism of AD progression, but also to use it as a biomarker in order to create a method for early diagnosis of the disease, which would enable doctors to start treatment much earlier.

Session 2: The synaptic bases of mental diseases

Short description:
During the last 11 years, researchers and psychiatrists of the Swiss National Centre Competence in Research NCCR-SYNAPSY have joined forces to study the biological basis of psychiatric disorders. By focusing on the genetic factors that determine neurodevelopmental disorders and their underlying psychopathologies, we will present preclinical and related clinical work that illustrate how the synergy of translational research is used to understand the neurodevelopmental cellular and behavioral mechanisms associated with autism and schizophrenia. By focusing on the genetic factors that determine neurodevelopmental disorders and their underlying psychopathologies, Stephan Eliez and Alan Carleton will present the results of translational projects aimed at identifying neurodevelopmental alterations responsible for the increased risk of developing psychosis in patients with 22q11 deletions as well as in a mouse model called Lgdel. Marie Schaer and Camilla Bellone will present a combination of clinical and preclinical approaches used to understand the neurodevelopmental cellular and behavioral mechanisms associated with autism and to evaluate treatment approaches informed by the pathophysiology of autism.

Neurodevelopmental markers of early transition to psychosis in 22q11 Deletion Syndrome

Stephan Eliez

Department of Psychiatry, University of Geneva, Michel-Servet Geneva, Switzerland

Maeder J, Sandini C, Zöller D, Schneider M, Bostelmann M, Pouillard V, Caroni P, Kliegel M, & Eliez S: Long-term verbal memory deficit and associated hippocampal alterations in 22q11.2 deletion syndrome. *Child Neuropsychology* 2019: 1–23. https://doi.org/10.1080/09297049.2019.1657392

Analysis of microcircuit dysfunctions in animal models of psychiatric disorders

Alan Carleton

Department of Basic Neuroscience, University of Geneva, Michel-Servet Geneva, Switzerland

Marissal T, Salazar RF, Bertollini C, Mutel S, De Roo M, Rodriguez I, Müller D, Carleton A: Restoring wild-type-like CA1 network dynamics and behavior during adulthood in a mouse model of schizophrenia. *Nat Neurosci* 2018: Oct;21(10):1412-1420. doi: 10.1038/s41593-018-0225-y. PMID: 30224804

Measuring neurodevelopmental trajectories of preschoolers with autism

Marie Scher

Department of Psychiatry, University of Geneva, Michel-Servet Geneva, Switzerland

Kojovic N*, Ben Hadid L*, Franchini M, Schaer M: Sensory Processing Issues and Their Association with Social Difficulties in Children with Autism Spectrum Disorders, *Journal of Clinical Medicine* 2019: 8:10

Neuronal mechanisms underlying social deficits in animal models of Autims Spectrum Disorders

Camilla Bellone

Department of Basic Neuroscience, University of Geneva, Michel-Servet Geneva, Switzerland

Bariselli S, Hörnberg H, Prévost-Solié C, Musardo S, Hatstatt-Burklé L, Scheiffele P, Bellone C: Role of VTA dopamine neurons and neuroligin 3 in sociability traits related to nonfamiliar conspecific interaction. *Nat Commun* 2018: Aug 9;9(1):3173. doi: 10.1038/s41467-018-05382-3.

Session 3: Perspectives of brain stimulation for memory

Individual differences in electric fields induced by transcranial electrical stimulation

Daria Antonenko

Department of Neurology, University Medicine Greifswald, Germany

Computational modeling allows accurate head reconstruction and simulation of current distributions induced by transcranial electrical stimulation (tES). These simulations unveil differences in electric fields between individuals as well as associations with several individually varying factors such as head and brain anatomy, but also with empirically assessed neurophysiological and behavioral tES effects. I will introduce the opportunities modeling approaches provide in explaining interindividual variability in tES effects on the human brain, showing recent data linking the estimated fields in each participant to the magnitude of individually induced tES effects. I will further demonstrate how the examination of individual field differences can be used for the ultimate aim to develop individualized interventions.

Causal role of cross-frequency coupling in cognitive control

Justin Riddle

Department of Psychiatry, University of North Carolina at Chapel Hill, USA

Cognitive control is the capacity to guide motor and perceptual systems towards abstract goals. High-frequency neural oscillations related to motor activity (beta; 13-30 Hz) and visual processing (gamma; >30 Hz) are known to be modulated by cognitive control signals via cross-frequency coupling with low frequency network oscillations in prefrontal cortex (delta, 2-3 Hz; and theta, 4-8 Hz). Thus, we delivered cross-frequency transcranial alternating current stimulation (CF-tACS) during performance of a task that manipulated cognitive control demands along two dimensions: abstraction of rules (action-planning) that increased delta-beta coupling and number of rules (held in memory) that increased theta-gamma coupling. We found that CF-tACS increased the targeted phase-amplitude coupling and modulated task performance of the associated cognitive control component

Personalized frequency-modulated oscillatory tDCS for memory enhancement

Jovana Bjekić

Human Neuroscience Group, Institute for Medical Research, University of Belgrade, Serbia

One of the main prerequisites for translating brain stimulation form basic research to clinical applications is reducing the individual differences in response to tDCS. This is especially notable when it comes to cognitive functions like memory. One step in that direction is to abandon the one-size-fits-all approach and move towards personalization of different aspects of the stimulation protocol. Here I will focus on personalization of the tDCS by matching the frequency of oscillations in the stimulation protocol to the peak theta-band (4-8Hz) frequency recorded in the pre-stimulation EEG. The effects of this new stimulation protocol on the associative memory performance in healthy volunteers will be presented and discussed.

Modulation of episodic memory in aging

Marco Sandrini

Department of Psychology, University of Roehampton, London, UK

What is new and exciting in Alzheimer's disease (AD) research is the idea of prevention trials, such as helping healthy people reduce their risk of developing AD (primary prevention), or delaying the progression of amnestic mild cognitive impairment (aMCI) to AD (secondary prevention). Since pharmacological interventions have failed to show efficacy in clinical trials with aMCI, non-invasive brain stimulation interventions, have received increasing attention. In this talk I will present an overview about memory formation, consolidation and modification through reconsolidation. Next, I will show the causal role of lateral PFC in episodic memory reconsolidation. I will conclude presenting some transcranial direct current stimulation (tDCS) studies showing memory enhancement in physiological and pathological aging.

Session 4: EJN Best Publication Award 2021 Lecture

Debra J. Skene Metabolomics of sleep deprivation: sex differences

Ulrich Schüller TCF4 in development and tumorigenesis of the central nervous system

Session 5: How to maximize benefit from good research practice?

Negatives are Positives — How to Publish Negative Data

Thomas Steckler

Janssen Pharmaceutica NV, Belgium

Publishing negative results seems still relatively rare in the scientific literature when compared to the publication of positive, catchy results that seem to confirm the working hypothesis, despite the fact that both positive and negative results are essential for the progress of science. In part, this has to do with the believe that negative results are more difficult to publish or of low interest. A set of criteria has recently been described by Bespalov et al. (2019) that should help scientists, reviewers and editors to publish technically sound, scientifically high-impact negative (or null) results originating from rigorously designed and executed studies. These criteria will be presented and their utility to facilitate the publication of negative data will be discussed.

Bespalov A, Steckler T, Skolnick P (2019) Be positive about negatives - recommendations for the publication of negative (or null) results. Eur Neuropsychopharmacology 29, 1312-1320.

Role of Funder in Rewarding High-Quality Research

Chantelle Ferland-Beckham

Cohen Veterans Bioscience, New York, USA

Growing awareness of the difficulties of replicating published findings provides an opportunity for the entire scientific ecosystem to examine the root causes. Poor implementation of the guiding principles of the scientific method leads to challenges while attempting to use or validate data preclinical study data. Many stakeholders play a role in driving a culture shift in how high-quality research is promoted and incentivized. Funders must reward researchers who foster a culture of good scientific citizenship, including data sharing, high-quality methodology, meticulous study design, and open and transparent scientific reporting. Future funding should be prioritized for those researchers who see themselves as playing an essential role in promoting the long-term objectives of science.

Research quality from the perspective of academia

Valentina Vengeliene

Dept. of Neurobiol. and Biophysics, Institute of Biosciences, LSC, Vilnius University, Lithuania

In academia, increasing publication pressure to be competitive for grants, promotions and awards is one of the main reasons for researchers patching up botched studies and publishing unreliable results, contributing to the problem with the irreproducibility of findings. Focus on establishing high research quality may remove many of the reasons that sustain bad practices and increase the overall efficiency of the lab. From bachelor students to senior postdocs, high research rigor, will ensure reliability of the results, reduce the number of failed experiments and ensure that time spent in the lab is valuable. A quality profile will also enhance the opportunities for collaborations with the pharmaceutical industry and receiving grants from funding agencies.

High quality standards as a vehicle to attract collaboration

Malgorzata Pietraszek, Anton Bespalov

PAASP, Hauptstr. 25, Heidelberg, Germany

It is difficult to imagine modern scientific research without collaboration. Collaborative research in biomedical research contributes to boosting knowledge and speeds up development of novel therapeutic strategies. There are different models of research collaborations. However, regardless of collaboration model, success of the collaboration depends to a large extent on the quality of results generated by collaborating parties (Vaudano 2020). Thus, apart from scientific excellence, collaborators need to adhere to high quality standards. This increases confidence in the quality of generated evidence and facilitates decision making regarding selection of collaborators and building of research networks.
In this presentation, we will demonstrate why high-quality research is a prerequisite of successful research collaboration.

Vaudano, E. (2020) Research Collaborations and Quality in Research: Foes or Friends? In Bespalov, A., Michel, M.C., Steckler. T. (eds), Handb Exp Pharmacol. 257, pp. 383-398.

Session 6: Pitch your science communication

Six competitors selected from the satellite workshop (for details see: Tue, Aug 24, 2-4 PM CEST) will pitch their science communication project to a jury of experts. The best three projects in the following categories: online science communication, science communication for younger audiences and animal research for non-scientific audiences, will be rewarded with a grant to fund their project. All FRM registrants are welcome to watch the competition and vote for their favourite project.

Plenary Lecture

Biology of bedtime: cellular insights into why we sleep

Amita Sehgal

HHMI, Perelman School of Medicine, University of Pennsylvania, Philadelphia, USA

The function of sleep remains a mystery. There is universal agreement that lack of sleep impairs performance, especially cognitive ability, during waking hours and considerable evidence supports adverse effects of sleep loss on other physiological parameters as well. Thus, sleep may be regarded as important for waking function. However, what happens during sleep to facilitate wake performance and promote health? Some studies posit that replay of wake experiences is specific brain regions during sleep helps in memory consolidation, but it is likely that sleep affects fundamental physiology on a brain-wide and perhaps even body-wide level. Ongoing research seeks to address this question by investigating cellular and molecular processes impacted by sleep.

Session 7: Circadian clocks: more beyond neurons

Exploring mechanisms of microRNA rhythms in the mouse cerebral cortex

Davide de Pietri Tonelli

Neurobiology of miRNAs lab, Istituto Italiano di Tecnologia (IIT), Genova, Italy

Post-transcriptional mechanisms control daily oscillations of protein abundance in the mouse liver. Whether this occurs in brain is unknown. MicroRNAs are small noncoding RNAs with post-transcriptional regulatory capacity. Analysis, around the 24 hours, of transcriptome/miRNome indicated that ~19% of the miRNAs are circadian in cerebral cortices of adult mice. Most of oscillating miRNAs peak in the active phase (between ZT10-ZT18), therefore 'in phase' with the Per and Cry circadian repressors, and were compromised upon the deletion of Bmal1 gene in astrocytes, which impairs brain circadian rhythms, cognition and lifespan. We will discuss mechanisms of miRNA oscillations in cortical cell subpopulations and relevance of miRNA rhythms in circadian circuits.

Barca-Mayo O, Pons-Espinal M, Follert P, Armirotti A, Berdondini L, De Pietri Tonelli D. Astrocyte deletion of Bmal1 alters daily locomotor activity and cognitive functions via GABA signalling. Nat Commun. 2017 Feb 10;8:14336. doi: 10.1038/ncomms14336.

Barca-Mayo O, Boender AJ, Armirotti A, De Pietri Tonelli D.Deletion of astrocytic BMAL1 results in metabolic imbalance and shorter lifespan in mice. Glia. 2020 Jun;68(6):1131-1147. doi: 10.1002/glia.23764. Epub 2019 Dec 13.

Circadian astrocytes: Networked in cortex and cancer

Erik Herzog

Department of Biology, Washington University, St. Luis, USA

Circadian rhythms in the brain regulate sleep-wake, feeding-fasting and other daily behaviors. The cells of the suprachiasmatic nucleus play a central role in coordinating daily rhythms in the brain. This talk will explore how daily rhythms in the neurons and astrocytes of the cortex arise and synchronize to local time in health and in glioblastoma, the most common and deadly form of brain cancer.

Slat, E.A., Sponagel, J., Marpegan, L., Simon, T., Kfoury, N., Kim, A., Binz, A., Herzog, E.R., Rubin, J.B. (2017) Cell-intrinsic, Bmal1-dependent Circadian Regulation of Temozolomide Sensitivity in Glioblastoma. J Biol Rhythms. 32, 121-129.

Tso, C.F, Simon, T., Greenlaw, A.C., Puri, T., Mieda, M., Herzog, E.D. (2017) Astrocytes Regulate Daily Rhythms in the Suprachiasmatic Nucleus and Behavior. Curr Biol. 27,1-7.

How fish “sense” the light? Fish as model to study the deep brain photoreception

Cristiano Bertolucci

Department of Biosciences and Biotechnology, University of Ferrara, Ferrara, Italy

During the Cambrian explosion animal body plans evolved very rapidly and image-forming eyes and visual systems emerged. However, in the pre-Cambrian era early organisms already evolved photoreceptors that were capable of light detection to mediated simple behavioral responses as the phototaxis. For this reason, extra-retinal photoreceptors represent the most basal form of light reception. Fish represent the most fascinating model to study deep brain photoreception because they colonized all aquatic habitats characterized by different photic environments including the subterranean waters in perpetual darkness. Comparative studies on pigeon and hypogean fish species could help to shed light on key genetic and physiological mechanisms whereby animals directly or indirectly respond to light.

Calderoni, L., Rota-Stabelli, O., Frigato, E., Panziera, A., Kirchner, S., Foulkes, N. S.,Fuselli, S. (2016). Relaxed selective constraints drove functional modifications in peripheral photoreception of the cavefish Phreatichthys andruzzii and provide insight into the time of cave colonization. Heredity.

Cavallari N., Frigato E., Vallone D., Fröhlich N., Lopez Olmeda J.F., Foà A., Berti R., Sánchez Vázquez F.J., Bertolucci C., Foulkes N.S. (2011) A Blind Circadian Clock in Cavefish Reveals that Opsins Mediate Peripheral Clock Photoreception. PLoS Biology 9(9): e1001142.

Cryptochrome: the dark side of a circadian photoreceptor comes to light

Gabriella Mazzotta

Department of Biology, University of Padova, Padova, Italy

In Drosophila, Cryptochrome acts as the main circadian photoreceptor in the central pacemaker neurons and as component of the circadian repressor complex in the peripheral clocks. While the light-activation mechanism is being thoroughly studied, the nature of the transduction signaling that activate CRY in the dark remains largely unknown. We hypothesize a novel mechanism regulated by Ca2+/CaM, that could be involved in the light-independent activation of Drosophila and act in consolidating the light-response stimulation.

Mazzotta GM, Bellanda M, Minervini G, Damulewicz M, Cusumano P, Aufiero S, Stefani M, Mammi S, Costa R and Tosatto S (2018). Calmodulin enhances Cryptochrome binding to INAD in Drosophila photoreceptors. Frontiers in Molecular Neuroscience. 11:280. doi:10.3389/fnmol.2018.00280

Schlichting M, Rieger D, Cusumano P, Grebler R. Costa R, Mazzotta GM*, Helfrich-Förster C* (2018). Cryptochrome interacts with actin and enhances eye-mediated light sensitivity of the circadian clock in Drosophila melanogaster. Frontiers in Molecular Neuroscience 11:238. doi: 10.3389/fnmol.2018.00238. eCollection 2018. (* Co-corresponding Author)

Session 8: Impact of early life experience on prefrontocortical circuits

Impact of early aversive experiences on the structure, connectivity and plasticity of prefrontocortical neurons

Juan Nacher

Neurobiology Unit, Institute for Biotechnology and Biomedicine, Universitat de Valencia, Spain

The prefrontal cortex continues to develop after birth, during the infancy and the adolescence and, consequently, aversive experiences such as chronic stress can affect the final steps of its construction. These alterations lead to permanent alterations in the structure and physiology of the adult prefrontal cortex, which may contribute to the development of certain psychiatric disorders. Recent work in our laboratory has shown that different forms of chronic stress during early life have a strong impact on prefrontocortical inhibitory circuits and their plasticity, particularly on parvalbumin expressing interneurons.

Garcia-Mompo C, Curto Y, Carceller H, Gilabert-Juan J, Rodriguez-Flores E, Guirado R, Nacher J. Transl Psychiatry. Δ-9-Tetrahydrocannabinol treatment during adolescence and alterations in the inhibitory networks of the adult prefrontal cortex in mice subjected to perinatal NMDA receptor antagonist injection and to postweaning social isolation. 2020 Jun 1;10(1):177. doi: 10.1038/s41398-020-0853-3.

Carceller H, Guirado R, Ripolles-Campos E, Teruel-Marti V, Nacher J. Perineuronal Nets Regulate the Inhibitory Perisomatic Input onto Parvalbumin Interneurons and γ Activity in the Prefrontal Cortex. J Neurosci. 2020 Jun 24;40(26):5008-5018. doi: 10.1523/JNEUROSCI.0291-20.2020. Epub 2020 May 26.

Adolescent stress and disruption of prefrontal inhibitory maturation

Laurence Coutellier

Departments of Psychology and Neuroscience, The Ohio State University, Ohio, USA

During adolescence, the prefrontal cortex gains inhibitory control through the maturation of its GABAergic system. This gain of prefrontal inhibitory control contributes to the maturation of executive functions. It is agreed upon that stress during adolescence impairs these cellular and behavioral maturational processes, potentially leading to cognitive deficits as observed in schizophrenia. The molecular mechanisms driving stress-induced abnormal development of prefrontal inhibitory circuits and associated executive functions remain to be determined. Using genetic, behavioral and molecular approaches in mice, my lab has shown that the transcription factor Npas4 regulates of normative prefrontal circuits maturation during adolescence and mediates the effects of adolescent chronic stress on executive functions deficits.

Shepard R, Heslin K, Coutellier L. (2017) The transcription factor Npas4 contributes to adolescent development of prefrontal inhibitory circuits, and to cognitive and motional functions: Implications for neuropsychiatric disorders. Neurobiol Dis. 99:36-46. doi: 10.1016/j.nbd.2016.12.012.

Page CE, Alexander J, Shepard R, Coutellier L. Npas4 deficiency interacts with adolescent stress to disrupt prefrontal GABAergic maturation and adult cognitive flexibility. Genes Brain Behav. 17(6):e12459. doi: 10.1111/gbb.12459.

Child abuse associates with increased recruitment of perineuronal nets in the ventromedial prefrontal cortex: evidence for an implication of oligodendrocyte progenitor cells

Naguib Mechawar

Dept of Psychiatry, McGill University, Douglas Hospital Research Centre, Verdun (Québec), Canada

Child abuse (CA) is a strong predictor of psychopathologies and suicide, and can lastingly alter normal trajectories of brain development, in particular in areas closely linked to emotional responses such as the ventromedial prefrontal cortex (vmPFC). I will present evidence that a history of CA is specifically associated with increased recruitment and maturation of perineuronal nets (PNNs) around parvalbumin-immunoreactive interneurons in human vmPFC, and that oligodendrocyte progenitor cells (OPCs) are involved in this phenomenon. These findings suggest that early-life adversity may lead to persistent patterns of maladaptive behaviours by reducing the neuroplasticity of cortical circuits through the enhancement of developmental OPC-mediated PNN formation.

Child abuse associates with increased recruitment of perineuronal nets in the ventromedial prefrontal cortex: evidence for an implication of oligodendrocyte progenitor cells. Arnaud Tanti, Claudia Belliveau, Corina Nagy, Malosree Maitra, Fanny Denux, Kelly Perlman, Frank Chen, Refilwe Mpai, Candice Canonne, Maria Antonietta Davoli, Gustavo Turecki, Naguib Mechawar. bioRxiv 2020.10.19.345355; doi: https://doi.org/10.1101/2020.10.19.345355

Child abuse associates with an imbalance of oligodendrocyte-lineage cells in ventromedial prefrontal white matter. Tanti A, Kim JJ, Wakid M, Davoli MA, Turecki G, Mechawar N. Mol Psychiatry. 2018 Oct;23(10):2018-2028. doi: 10.1038/mp.2017.231. Epub 2017 Nov 21.

Epigenetic regulation in the prefrontal cortex: relevance to schizophrenia

Marzena Maćkowiak

Laboratory of Pharmacology and Brain Biostructure, Pharmacology Department, Maj Institute of Pharmacology, Polish Academy of Sciences, Kraków, Poland

Epigenetic mechanisms are involved in the regulation of brain development. Findings from a neurodevelopmental model of schizophrenia (MAM-E17) showed changes in a dynamic of histone H3 methylation during the rat prefrontal cortex maturation. Impairments in histone H3 methylation at lysine 4 (H3K4me3) affected expression of genes (Gad1, parvalbumin) in the adult MAM-E17 cortex. Adolescent environmental factors (enriched environment, social isolation) influenced on H3K4me3 protein level that resulted in altered gene expression in the adult MAM-E17 cortex. Thus, modifications in histone methylation during prefrontal cortex development changed trajectory of interneurons maturation, and malfunction of prefrontal cortex might be related to schizophrenia-like abnormalities observed in the MAM-E17 model.

Bator, E., Latusz, J., Wędzony, K., Maćkowiak, M. (2018). Adolescent environmental enrichment prevents the emergence of schizophrenia-like abnormalities in a neurodevelopmental model of schizophrenia. Eur Neuropsychopharmacol., 28(1), 97-108. doi: 10.1016/j.euroneuro.2017.11.013.

Session 9: Female brain as a target of sex hormones

No more stigma, it's science: Joint dynamics of menstrual cycle and brain structure

Rachel G Zsido

Emotion & neuroimaGinG (EGG) Lab, Max Planck Institute for Human Cognitive and Brain Sciences, Leipzig, Germany

Ovarian hormones are key modulators of neuroplasticity, with animal research offering robust evidence of endocrine regulation of brain morphology. Yet, we still require longitudinal human studies that investigate how subtle hormone fluctuations influence the brain. In our study, we utilized the menstrual cycle as a natural experimental set-up to model how endogenous ovarian hormone fluctuations influence hippocampal subfield volume and structural connectivity. Healthy female participants of reproductive age were invited for 6 time-points during their menstrual cycles to undergo 7-tesla ultra-high field magnetic resonance imaging and rigorous cycle monitoring. We will review our systematic protocol for accurate characterization of cycle changes as well as individual phenotyping of brain structure to identify hormone-associated neuroplasticity during the reproductive years. Given the scarcity of female data in basic and clinical neurosciences, we contribute to bridging this gap in knowledge by providing a rich and detailed new dataset which sheds light on the menstrual patterns of the female human brain.

Dynamic causal modelling of the menstrual cycle: predicting ovulation from brain connectivity

Esmeralda Hidalgo-Lopez

Centre for Cognitive Neuroscience and Psychology Department, Faculty of Natural Sciences, University of Salzburg, Austria

Specific brain connectivity patterns characterize each phase of the menstrual cycle in healthy women, related to their endogenous hormonal milieu. Dynamic causal modelling and parametric empirical Bayes were performed in a triple model consisting of the default mode, salience and executive central networks during resting state. Effective connectivity within and between these three core networks will be detailed for menses (low progesterone and estradiol); pre-ovulatory phase (peak estradiol, low progesterone), and the mid-luteal phase (high progesterone and estradiol). Remarkably, the specific cycle phase in which a woman was in could be predicted by the connections that showed the strongest changes.

Progesterone antagonism beneficial for premenstrual dysphoric disorder

Erika Comasco

Department of Neuroscience, Science for Life Laboratory, Uppsala University, Sweden

Premenstrual dysphoric disorder (PMDD) is a psychiatric condition characterized by late luteal phase affective, cognitive, and physical symptoms, causing significant distress in about 3-5% of women of reproductive age. Progesterone is posited to be implicated in the symptomatology, thus we tested the efficacy of a selective progesterone modulator (SPRM) for PMDD. In a multicentre, double-blind, placebo-controlled clinical trial, we demonstrated that half of the women receiving the treatment improved completely, while the corresponding proportion of women receiving placebo was 21 per cent. Furthermore, SPRM treatment was associated with enhanced reactivity in the dorsal anterior cingulate cortex and dorsomedial prefrontal cortex during aggressive response to provocation stimuli, as assessed by functional magnetic resonance imaging. The mechanism of action of the study drug provides insights into the potential molecular mechanisms underlying this psychiatric disorder and its treatment, suggesting a beneficial effect of progesterone receptor antagonism on top-down emotion regulation.

Investigating effects of oral contraceptives on women's intra and intersexual social behaviours and their neural correlates

Ann-Christin Kimmig

Department of Psychiatry and Psychotherapy & IMPRS of Cognitive and Systems Neuroscience, University of Tübingen, Germany

Worldwide millions of women use oral contraceptives (OC). Evidence is accumulating that oral contraceptives may alter a range of socio-emotional processes. We were interested whether this holds also for (intrasexual) empathy and (inter) sexual approach and avoidance behaviour. Thus, we investigated women taking combined oral contraceptives (n = 37) and two groups of naturally cycling women (early follicular: n = 37, and periovulatory: n = 29) using the Tuebinger Empathy Test (TET) and an erotic approach avoidance task (AAT) during fMRI. Next to the results of the cross-sectional analysis, an outlook on a longitudinal comparison of women who stop taking OCs will be given.

Plenary Lecture

Why should a neuroscientist be interested in social interaction?

Riitta Hari

Aalto University, Espoo, Finland

Our brains are tuned to social interaction that shapes us throughout the lifetime. Social interaction is complex but feels simple. During natural interaction, such as conversation, people spontaneously align and adapt their actions. Social touch and laughter modulate the release of endogenous opioids, likely supporting bonding between individuals. Since social interaction is the property of autonomous dyads rather than of single individuals, one cannot study social interaction without the presence ofboth partners. We thus need experimental settings for two-person neuroscience where the brains of two participants are scanned simultaneous. The most intriguing question concerns the primacy of social interaction: Is it the default that enables social cognition and mutual understanding rather than a property emerging from lower-level brain functions?

FENS Presidential Lecture

Neuronal reward mechanisms

Wolfram Schultz

Department of Physiology, Development & Neuroscience, University of Cambridge, UK

Rewards are involved in learning, approach behaviour, economic choice and positive emotions. We use neurophysiological and behavioural methods in experimental designs based on animal learning theory and economic decision theory. We explore reward processing by dopamine neurones. Dopamine neurones carry a two-component reward prediction error signal that reflects the physical impact and the value of rewards, respectively. The reward signal codes formal economic utility and is influenced by risk. Slower components of the same neurones signal motor activation. The understanding is that the different reward centers in the brain need to cooperate for individuals to make optimal choices and maximise reward intake.

Session 1: Learning by reinforcement

Cognitive switches and sensory learning using value backpropagation

Abhishek Banerjee

Adaptive Decisions Lab, Faculty of Medical Sciences, Newcastle University, UK

Animals adapt their behaviour in response to variable changes in reward reinforcement. Value-based decision-making involves multiple cognitive maps across distributed brain areas. It is less clear which brain regions are essential and how changes in neural responses flexibly re-map guiding adaptive behaviour. In this talk, I will highlight behavioural-neural interactions between frontal and sensory circuits that implement flexible decision-making. I will present further evidence how some of these functions are disrupted in autism spectrum disorders, arguing for a new conceptual framework based on computational psychiatry to understand cognitive pathophysiology in neurological disorders.

Banerjee A, Parente G, Teutsch J, Lewis C, Voigt FF and Helmchen F (2020) Value-guided remapping of sensory cortex by lateral orbitofrontal cortex. Nature 585:245-250.

Banerjee A, Rikhye RV, Breton-Provencher V, Tang X, Li C, Li K, Runyan C, Fu Z, Jaenisch R, and Sur M (2016) Jointly reduced inhibition and excitation underlies circuit-wide changes in cortical processing in Rett Syndrome. PNAS 113(46):E7287-E7296.

Reinforcement signals broadcast by neuromodulatory systems during associative learning

Balázs Hangya

Lendület Laboratory of Systems Neuroscience, Department of Cellular and Network Neurobiology, Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest, Hungary

Neuromodulatory systems have traditionally been associated with signalling rewards and punishments. Specifically, midbrain dopaminergic neurons were shown to transmit reward prediction errors. However, prediction error signalling has been demonstrated in multiple neuromodulatory and other circuits and the division of labour across these areas during associative learning remained unclear. I will present data shedding new light on learning-related reinforcement signaling in the basal forebrain cholinergic and midbrain dopaminergic systems. I will also demonstrate how these signals might change through aging and in the course of neurodegenerative disease.

Laszlovszky T, Schlingloff D, Freund TF, Gulyás A, Kepecs A, Hangya B (2020) Distinct synchronization, cortical coupling and behavioural function of two basal forebrain cholinergic neuron types. Nat Neurosci, 23:992-1003.

Sturgill JF, Hegedus P, Li SJ, Chevy Q, Siebels A, Jing M, Li Y, Hangya B, Kepecs A (2020) Basalforebrain-derived acetylcholine encodes valence-free reinforcement prediction error. bioRxiv 2020.02.17.953141; doi: https://doi.org/10.1101/2020.02.17.953141

The ubiquity of model-based reward prediction in the dopamine system

Angela Langdon

Niv Lab, Princeton Neuroscience Institute, Princeton University, Washington, USA

Phasic activity in dopamine neurons has long been identified as a neural correlate of reward prediction error signals in the brain. Recent findings suggest dopamine prediction error signals reflect more dimensions of an expected outcome than scalar reward value. These features imply a richer learning process in the brain than what is typically assumed in current AI learning models, suggesting instead that neural reward predictions are embedded in learned expectations about the structure of the task environment—a form of 'model-based' reinforcement learning. Using these results, I will highlight a number of intriguing and perhaps surprising implications for the algorithmic understanding of learning in both health and disease.

Langdon A.J., Sharpe M., Schoenbaum G., Niv Y. (2018). Model-based predictions for dopamine. Current Opinion in Neurobiology 49:1-7.

Takahashi Y*., Langdon A.J*., Niv Y., Schoenbaum G. (2016). Temporal specificity of reward prediction errors signaled by putative dopamine neurons in rat VTA depends on ventral striatum. Neuron 91(1):182-193. (*Equal contribution)

Heterogeneity of cholinergic activities during visual discrimination learning

Yang Yang

School of Life Science and Technology, ShanghaiTech University, Pudong, China

Acetylcholine is known to play a key role in learning and memory. However, it's unclear how cholinergic neurons in the basal forebrain respond to salient events such as sensory stimuli, rewards and punishment, during the course of reinforcement learning. We trained mice to perform a visual discrimination task, and recorded the cholinergic neuronal activities during the learning process. Both the temporal properties and the strength of the cholinergic activities change alongside behavioral performance, and cholinergic neurons show heterogeneous responses to visual stimuli and behavioral outcomes, supporting the notion that cholinergic neurons play diverse roles in learning.

Yang, Y.*, Liu, D-q*, Huang, W, Deng, J, Zuo, Y., Poo MM. (2016). Selective synaptic remodeling of amygdalocortical connections associated with fear memory. Nature Neuroscience, 19(10):1348-55 (*Equal contribution)

Yang, Y. and Zador, AM. (2012). Differences in sensitivity to neural timing among cortical areas. Journal of Neuroscience, 32(43):15142-7.

Session 2: The neural architecture of visual awareness

The neural architecture of visual awareness

Kristian Sandberg

Center of Functionally Integrative Neuroscience, Aarhus University, Denmark

Many previous studies have examined various aspects of the neural architecture of visual consciousness using MRI, e.g. studies of grey matter volume, white matter integrity, or GABA, but often in samples that are smaller than power analyses would suggest to be sufficient. Here, I present the framework of our large-scale experimentation in COST Action 18106 where each study relates a range of MRI-based indices of neural architecture to behavioural consciousness data in samples of typically minimum 200 participants. I present preliminary data from the Action as well as already published findings from precursors of the Action1,2, and I discuss the relevance to theories of consciousness.

Sandberg, K. et al. Improved estimates for the role of grey matter volume and GABA in bistable perception. Cortex 83, 292–305 (2016).

Song, C., Sandberg, K., Møller Andersen, L., Udby Blicher, J. & Rees, G. Human occipital and parietal GABA selectively influence visual perception of orientation and size. The Journal of Neuroscience 3945–16 (2017) doi:10.1523/JNEUROSCI.3945-16.2017.

Electrophysiological correlates of first and second-order consciousness

Nathan Faivre

Laboratoire de Psychologie et Neurocognition (LPNC), Université Grenoble Alpes, Grenoble, France

Perceptual consciousness encompasses two interrelated phenomena: the subjective experience associated with a sensory event, and the reflexive monitoring of the corresponding percept which involves a second-order representation of a stimulus. The study of first and second-order consciousness is now based on empirical yet mostly distinct grounds. A way to simultaneously characterize first and second-order consciousness is to ask volunteers to detect stimuli presented around the threshold for detectability, and to provide subsequent confidence ratings about the likelihood that they correctly detected the stimulus. Based on this paradigm, I will present a series of behavioral, electrophysiological, and modeling results documenting the commonalities and specificities of the mechanisms involved in first and second-order conscious processes.

What constitutes an optimal brain architecture? The role of sensory processing versus sleep

Chen Song

Brain Complexity and Consciousness Lab, Cardiff University Brain Research Imaging Centre, Cardiff University, UK

Structure shapes function. Understanding what kinds of brain structure are optimal for cognitive function is fundamental to neuroscience research as well as to the design of artificial intelligence. Traditionally, large brain volumes, large number of neurons and strong neural connections are considered beneficial. In this talk, I will present our experimental and theoretical work that challenges the conventional wisdom. We show that a functionally optimal visual cortex is constituted of a large cortical surface area, but a small cortical thickness and weak lateral connections. This architecture enables neurons in visual cortex to have high selectivity, short latency, and human participants to excel in visual tasks. To achieve the optimal brain structure, we show that sleep plays an essential role. Sleep facilitates the overnight improvement in cognitive function through homeostatic weakening of neural connections and thinning of cortex, at both a cellular and a system level. The overnight brain structural changes in turn enhance the cost-efficacy of brain activity. I will conclude by discussing how a balanced view of brain structure and function requires the consideration of sleep-wake cycle, and how the contrast between awake and sleeping brain activity may be key to our intelligence.

Modulation of auditory steady-state responses by the fluctuations in the state of consciousness

Marek Binder

Institute of Psychology, Jagiellonian University, Krakow, Poland

Auditory-steady state responses (ASSRs) are defined as the frequency-domain EEG activity elicited by the periodic acoustic stimulation. These oscillations reflect not only the integrity of the auditory system within the cortex and the subcortical regions, but they also appear to be sensitive to the fluctuations in the state of consciousness. During this talk I will present the results of our recent research on sleep and disorders of consciousness and how they influence the ASSR, and describe our experiments aimed to identify the mechanisms underlying this sensitivity.

Session 3: Microglia and neuronal death in brain diseases

Microglia and their contribution to the pathomechanism of Alzheimer's disease

Grzegorz A. Czapski / Joanna B. Strosznajder

Department of Cellular Signalling, Mossakowski Medical Research Centre Polish Academy of Sciences, Warsaw, Poland

The growing body of evidence highlights the significance of inflammatory processes in the pathomechanism of Alzheimer's disease (AD). Depending on age, disease stage, and other environmental conditions, neuroinflammatory processes may be beneficial, promoting neuroprotection and neuroregeneration, or can result in neuronal damage. Microglia, resident immune cells in the brain are equipped with a set of membrane receptors and detect changes in the local environment trying to maintain brain homeostasis. However, prolonged activation of microglia in AD may evoke the sustained release of inflammatory mediators and reactive oxygen species, leading to neuroinflammation which may promote amyloid-beta (Aβ) peptide accumulation and proteinopathy, changes in gene transcription, epigenetic regulation, formation of the self-propagating alterations, and neuronal death.

The novel research tools revealed the heterogeneity of the microglial population and the complexity of microglial function/dysfunction. The transcriptome-wide analysis of human microglia indicated the age-related transcriptome changes. It was found that expression of the specific set of genes was altered in AD, was increased during aging, and was affected by APOE2 haplotype. Microglia may adopt different activation states (phenotypes) to handle specific tasks in brain tissue. Because of this phenotypic diversity, targeting microglia in AD is challenging and it is necessary to understand the specific function of each type and its contribution to the pathomechanism of AD to create the way for a new therapeutic approach.

Cieślik M, Czapski GA, Wójtowicz S, Wieczorek I, Wencel PL, Strosznajder RP, Jaber V, Lukiw WJ, Strosznajder JB (2020) Alterations of Transcription of Genes Coding Anti-oxidative and Mitochondria-Related Proteins in Amyloid β Toxicity: Relevance to Alzheimer's Disease. Mol. Neurobiol., 57, 1374- 1388.

Czapski GA, Zhao Y, Lukiw WJ, Strosznajder JB (2020) Acute Systemic Inflammatory Response Alters Transcription Profile of Genes Related to Immune Response and Ca(2+) Homeostasis in Hippocampus; Relevance to Neurodegenerative Disorders. Int. J. Mol. Sci., 21.

Microglial phagocytosis of live neurons and synapses in neurodegeneration

Guy C. Brown

Department of Biochemistry, University of Cambridge, Tennis Court Road, Cambridge, UK

Excessive microglial phagocytosis of live synapses and neurons may contribute to neurodegeneration. We find that the exposure of cell-surface sialic acid and galactose are key regulators of this phagocytosis. Activated microglia release a sialidase that desialylates microglia and neurons, activating phagocytic residues on microglia, and enabling opsonins to bind to neurons. However, microglial engulfment of neurons requires UDP release from neurons activating the P2Y6 receptor on neurons. Knockout of the P2Y6 receptor in mice prevents microglial phagocytosis of live neurons, as well as neuronal and memory loss in mouse models of neuroinflammation, amyloidosis and tauopathy.

Allendorf DH, Puigdellívol M, Brown GC. (2020). Activated microglia desialylate their surface, stimulating complement receptor 3-mediated phagocytosis of neurons. Glia, 68, 989–998.

Vilalta A, Brown GC (2018) Neurophagy, the phagocytosis of live neurons and synapses by glia, contributes to brain development and disease. FEBS J., 285, 3566-75.

Extracellular tau-induced microglia-mediated neuronal death

Vilmante Borutaite

Neuroscience Institute, Lithuanian University of Health Sciences, Kaunas, Lithuania

Structural and compartmental changes of tau are being thought to be involved in various tauopaties, including frontotemporal dementia, Pick's disease, etc. Recently it has been shown that extracellularly secreted tau can be more toxic than intraneuronal but the mechanisms of this type of tau-induced neurotoxicity are unclear. In this talk, I'll compare neurotoxic effects of various isoforms of extracellular tau and give some insights into molecular pathways how extracellular tau interacts with microglia causing neuronal loss.

Pampuscenko K, Morkuniene R, Krasauskas L, Smirnovas V, Tomita T, Borutaite V (2020). Distinct Neurotoxic Effects of Extracellular Tau Species in Primary Neuronal-Glial Cultures. Mol Neurobiol. (in press). doi: 10.1007/s12035-020-02150-7.

Pampuscenko K, Morkuniene R, Sneideris T, Smirnovas V, Budvytyte R, Valincius G, Brown GC, Borutaite V. (2020) Extracellular tau induces microglial phagocytosis of living neurons in cell cultures. J Neurochem. 154, 316-329. doi: 10.1111/jnc.14940.

Session 4: How a society journal handles your paper. The peer review process and beyond

How a society journal handles your paper. The peer review process and beyond

Juan Lerma

Editor-in-Chief of Neuroscience; Instituto de Neurociencias CSIC-UMH, Spain

John Foxe

Editor-in-Chief of European Journal of Neuroscience; University of Rochester Medical Center, School of Medicine and Dentistry, Rochester, USA

The most important skill a scientist needs, after the skills needed to execute a study, is the ability to report their scientific endeavours in the written form. Indeed, there is no point in conducting research if one cannot articulate new scientific knowledge. The aim of this workshop, presented by the editors of two international neuroscience journals, is to inform on what happens to a paper once the 'submit' button is pressed. We will discuss what editors consider when deciding whether to review a paper, what we expect from reviewers, the importance of contributing to the peer review process, and ethical and reproducibility issues around publishing scientific papers. In particular, we intend

1) To show precisely what happens to a paper once submitted to a journal. After years of pains-taking work and months of writing, what actually happens when you finally submit your paper. We will explain what happens in the office and what the editors do.
2) To clearly explain what we look for in a good paper, what makes a paper worthy of going into the peer--review process and, by extrapolation, what do we consider a 'bad' paper or bad aspect of a paper.
3) To stress how the peer-review process is one of the bed-rocks of the scientific method and how the peer review process works. What we expect reviewers to do and how we as editors, deal with reviews.
4) To emphasize the importance of ethical issue and issues of reproducibility in the publishing process, as well as the problem of plagiarism. Finally, our aim is to demystify the role of editors. We are scientists ourselves, just as members of the audience, who want and need to publish our work.

Session 5: Special interest event by the CHET committee: Career pathways in neuroscience and training opportunities

This Special Interest Event is dedicated for students and early career scientists participating at FENS Regional Meeting 2021 (FRM2021) interested to interact with inspiring representatives from business, publishing, industry and public sectors, all sharing a neuroscience background. Our speakers will reveal how their neuroscience background successfully contributed to their career-paths

Session 6: Promoting continuity of international collaboration in animal neuroscience research

Moderated by Anna Mitchell (FENS Committee on Animals in Research, Oxford Neuroscience), this debate will aim to examine the benefits of international scientific collaboration and identify current challenges and opportunities including 3Rs strategies that are relevant for researchers working with animal models. The event will feature talks from Kirk Leech (European Animal Research Association) and Frances Wiseman (UK Dementia Research Institute) and will be followed by a live Q&A session.

Plenary Lecture

Epigenomics in psychiatric disease: the circadian perspective

Arturas Petronis

Centre for Addiction and Mental Health, and University of Toronto, Toronto Ontario, Canada and Institute of Biotechnology, Life Sciences Center, Vilnius University, Vilnius, Lithuania

In addition to genetics and environment, disease phenotypes are shaped by epigenetic modifications. Progress in uncovering the epigenetic basis of disease, however, depends on how well we understand the fundamental principles of epigenomic regulation. Our group has recently discovered that epigenetically modified cytosines oscillate in a circadian (or diurnal) fashion. I will provide a series of reasons indicating that malfunction of circadian regulation, one of the oldest and nearly universal adaptive mechanism, can help understanding a number of clinical and molecular findings in psychiatric diseases. Since circadian and epigenetic parameters can be modified by diet, lifestyle, and medications, therapeutic interventions rectifying circadian aberrations may be used to reduce disease risk, or at least delay its age of onset.

Session 7: Animal models of drug abuse - towards new neuronal mechanisms and high translational value

Relapse after voluntary abstinence: Behavior and circuit mechanisms

David Reiner and Yavin Shaham

Behavioral Neuroscience Branch, IRP-NIDA, NIH, USA

Relapse to drug use during abstinence is a defining feature of addiction. In humans, abstinence is often self-imposed or voluntary, and in many cases occurs because of the availability of nondrug alternative rewards that are chosen over the drug. We have recently developed rat models of relapse after voluntary abstinence, achieved by providing mutually exclusive choices between the self-administered drug and nondrug rewards. In this lecture, we will provide an overview of one of these models in which we have used palatable food as the alternative nondrug reward and discuss recent circuit findings from studies using this model.

Reiner DJ, Fredriksson I, Bossert JM, Shaham Y (2019) Relapse to opioid seeking in rat models: behavior, pharmacology, and circuits. Neuropsychopharmacology 44:465-477.

Fredriksson I, Venniro M, Reiner DJ, Chow JJ, Bossert JM, Shaham Y (2021) Animal models of drug relapse and craving after voluntary abstinence: a review. Pharmacological Review 73:1050-1083.

Biobehavioural basis of the flexible inflexibility that characterises maladaptive drug-seeking at relapse

David Belin

Behavioural and Clinical Neuroscience Institute, University of Cambridge, Cambridge, UK

The inflexible pursuit of drug-seeking and tendency to relapse that characterize addiction have been associated with the recruitment of the dorsolateral striatum-dependent habit system. However, the mechanisms by which the resulting maladaptive drug-seeking habits contribute to relapse have not been elucidated. During this presentation I will show that a long history of cocaine-seeking invigorated by response-produced drug-paired cues specifically results, at a time it is mediated by the habit system, in aberrant drug-seeking at relapse. This exacerbated relapse is underpinned by a transient engagement of the dorsomedial striatum-dependent goal-directed system promoted by the inability to enact seeking habits during abstinence, but not the lack of the drug. These results shed light on the psychological and neural basis of relapse.

Amygdalar silent synapses in appetitive learning and addiction

Anna Beroun

Laboratory of Neuronal Plasticity, Nencki-EMBL Center of Excellence for Neural Plasticity and Brain Disorders, BRAINCITY, Warsaw, Poland

Silent synapses are excitatory connections that possess one of the two main type of glutamate receptors – NMDA receptors, while AMPA receptors are either absent. They do not participate in the basal synaptic transmission, hence the term "silent". Yet they can be easily recruited in LTP processes, such as learning, where they acquire AMPA receptors, and become fully functional contacts that strengthen the excitatory connection. In my talk, will describe the phenomenon of silent synapses induction in cocaine addiction models and present our latest research on the function of silent synapses in amygdala in appetitive learning and the development of alcohol addiction.

Stefaniuk, M., Beroun, A., Lebitko, T., Markina, O., Leski, S., Meyza, K., Grzywacz, A., Samochowiec, J., Samochowiec, A., Radwanska, K. & Kaczmarek, L. (2017) Matrix metalloproteinase-9 and synaptic plasticity in the central amygdala in control of alcohol-seeking behavior. Biol Psychiatry, 81, 907-917.

ARC in the amygdala prevents compulsive alcohol seeking

Roberto Pagano

Laboratory of Molecular Basis of Behavior, Nencki Institute of Experimental Biology, Warsaw, Poland

Alcohol use disorder is a chronic psychiatric disorder characterized by the compulsion to seek and consume alcohol. This maladaptive behavior is driven by cellular and molecular adaptations that are still poorly understood. Using advanced tools, such as RNA sequencing, local genomic manipulation with the CRISPR/Cas9 system in vivo and behavioral analysis of the mice in IntelliCages, we looked for molecular markers that regulate compulsive alcohol drinking. We discovered that ARC protein expression in the amygdala during alcohol withdrawal prevents compulsive response to alcohol-predicting cues.

Session 8: Brain imaging in neuropsychiatric disorders: innovation and translation

fMRI amygdala neurofeedback for Major Depressive Disorder

Kym Young

Department of Psychiatry, University of Pittsburgh, Pittsburgh, USA

Patients with major depressive disorder (MDD) show hypoactive amygdala responses to positive stimuli, including positive autobiographical memories (Young et al., 2016). By providing real-time fMRI neurofeedback regarding amygdala activity, patients with MDD are able to increase their amygdala response during positive memory recall. This results in significant symptom improvement. Furthermore, training in one direction supports adaptive control of amygdala activity that can be altered in both directions, with increased amygdala responses to positive stimuli and decreased responses to negative/stressful stimuli following training. Dr. Young will present the results of several ongoing randomized clinical trials examining this intervention in patients with MDD.

Young, K.D., Siegle, G.J., Bodurka, J., & Drevets, W.C. (2016) Amygdala activity during autobiographical memory recall in depressed and vulnerable individuals: Association with symptom severity and autobiographical overgenerality. Am. J. Psychiatry, 173, 78–89.

Structural and functional imaging biomarkers of Bipolar Disorder and Schizophrenia

João Valente Duarte

CIBIT, ICNAS, University of Coimbra, Coimbra, Portugal

Differential diagnosis between schizophrenia (SCZ) and bipolar disorder (BPD) is often challenging, especially in early phases, namely when BPD patients present psychotic symptoms. I will present the results of a direct comparison of neuroimaging-derived structural and functional discriminative features of SCZ and BPD. We found opposite changes in gyrification and neural responses underlying social cognitive dysfunction in a core social brain hub subserving theory of mind functions. The joint analysis of different morphometric features and functional features provides a promising strategy for differential diagnosis of BPD and SCZ and may also represent an anatomical target for differentiated neural modulation, that can ameliorate impaired social cognition in two archetypal mental disorders.

Madeira, N., Duarte, J.V., Martins, R., Costa, G.N., Macedo, A., & Castelo-Branco, M. (2020) Morphometry and gyrification in bipolar disorder and schizophrenia: A comparative MRI study. NeuroImage Clin., 26.

Neurofind: Using deep learning to identify abnormal brain structural patterns in neuropsychiatric disorders at the individual level

Sandra Vieira

King's College London (KCL), London, UK

In the last decade, many machine learning approaches have been put forward to address the growing demand for translational psychiatric research. Deep learning has shown promise across several disciplines, including in neurologic and psychiatric disorders (Vieira et al., 2017). This talk will introduce Neurofind, a tool to detect brain-based disorders at the individual level based on a normative model of brain morphology, developed using a deep learning approach known as auto- encoders and ~20,000 anatomical brain scans of healthy controls.

Vieira, S., Pinaya, W.H., & Mechelli, A. (2017) Using deep learning to investigate the neuroimaging correlates of psychiatric and neurological disorders: Methods and applications. Neurosci. Biobehav. Rev., 74, 58–75.

Automated analysis of free speech as a marker of neuroimaging findings in individuals with Obsessive-Compulsive Disorder

Pedro Morgado

ICVS, School of Medicine, University of Minho, Braga, Portugal

No diagnostic biomarkers are available for obsessive-compulsive disorder (OCD). We aimed at identifying how automated speech graph analysis could be related to neuroimaging structural and functional alterations. OCD patients and HC presented significant differences in speech, bot semantically and in terms of the connectedness of speech. While the resting-state networks (RSN presented no between groups significant differences, speech graph attributes and semantic similarit to symptomatic terms found different correlations in various RSN when comparing both groups Speech analysis is a new useful tool for improving OCD comprehension, related to specifi neuroimaging alterations attributed to the disorder, with potential to develop diagnostic biomarker for OCD.

Session 9: Cortical Interneurons: from birth to networks

Developmental trajectories of cortical inhibitory neurons

Lynette Lim

VIB-KU Leuven Center for Brain and Disease Research, Leuven, Belgium

The focus of the introductory talk will be on mechanisms controlling interneuron (cIN) migration and axon targeting. We will provide evidence that different classes of cINs use distinct routes of migration to reach the cortex, that define the IN-subtype specific axonal pattern and function. We suggest that migration and axon targeting programmes are coupled to optimize the assembly of inhibitory circuits in the cerebral cortex.

Lim, L., Mi, D., Llorca, A., Marín, O. (2018) Development and Functional Diversification of Cortical Interneurons. Neuron Oct 24;100(2):294-313. doi: 10.1016/j.neuron.2018.10.009.

Lim, L., Pakan, JMP, Selten, MM., Marques-Smith, A., Llorca, A., Bae, SE., Rochefort, NL., Marín, O. (2018) Optimization of interneuron function by direct coupling of cell migration and axonal targeting. Nat Neurosci. 21(7):920-931. doi: 10.1038/s41593-018-0162-9.

Mechanisms controlling the postnatal development of cortical interneurons

Myrto Denaxa

BSRC Alexander Fleming, Institute for Fundamental Biomedical Research, Vari, Greece

The place of origin determines certain aspects of cortical interneuron (cIN) fate, but it is only when they reach their target lamina and form contacts with the local circuitry that their number is defined and their mature properties are established. Recent evidence suggest that network activity and activity-dependent genetic programs are implicated in these late stages of cIN development. In this talk we will present work that addresses mechanisms controlling the development of distinct-IN subtypes during this critical time window of the first two postnatal weeks.

Denaxa, M.*, Neves, G., Burrone, J., Pachnis, V. (2018). Homeostatic control of interneuron apoptosis during cortical development. J *Exp Neurosc*. 5(12):1-3 *corresponding author https://pubmed.ncbi.nlm.nih.gov/30013387/

Denaxa M* ^, Neves G*^, Rabinowitz A, Kemlo S, Liodis P, Burrone J*, Pachnis V*. (2018) Modulation of apoptosis controls inhibitory interneuron number in the cortex. *Cell Reports* 12(7):1710-1721 *corresponding authors ^ equal contribution https://pubmed.ncbi.nlm.nih.gov/29444425/

The integration of upper layer cortical interneurons into the cortical circuits

Theo Karayannis

Brain Research Institute, University of Zurich, Zurich, Switzerland

Upper layer interneurons are involved in modulating barrel cortex activity and perception during active whisking. We will discuss our work on the identification of structural and functional connectivity motifs that allow these interneurons to respond to distinct sensory stimuli during development.

Kastli R, Vighagen R, van der Bourg A, Argunsah AÖ, Iqbal A, Voigt FF, Kirschenbaum D, Aguzzi A, Helmchen F, Karayannis T. (2020) Developmental divergence of sensory stimulus representation in cortical interneurons. Nat Commun. 11(1):5729. doi: 10.1038/s41467-020-19427-z.

The emergence and plasticity of axo-axonic synapses at the axon initial segment

Juan Burrone

King's College, Strand, London, UK

The activity-dependent rules that govern the wiring of GABAergic interneurons are not well understood. Chandelier cells (ChCs) are a type of GABAergic interneuron that control pyramidal cell output through axo-axonic synapses that target the axon initial segment. Increases in the activity of either pyramidal cells or individual ChCs during a critical temporal window result in a reversible decrease in axo-axonic connections. We will discuss work supporting the hypothesis that the direction of ChC synaptic plasticity follows homeostatic rules that depend on the polarity of axo-axonic synapses.

Pan-Vazquez A, Wefelmeyer W, Gonzalez Sabater V, Neves G, Burrone J. (2020) Activity-Dependent Plasticity of Axo-axonic Synapses at the Axon Initial Segment. Neuron 106(2):265-276.e6. doi: 10.1016/j.neuron.2020.01.037.
Wefelmeyer W, Cattaert D, Burrone J. Activity-dependent mismatch between axo-axonic synapses and the axon initial segment controls neuronal output. Proc Natl Acad Sci U S A. 2015 Aug 4;112(31):9757-62.

LNA Presidential Lecture

Repurposing of CRISPR-Cas immunity for targeted genome editing

Virginijus Šikšnys

Institute of Biotechnology, Life Sciences Center, Vilnius University

Type II CRISPR-Cas systems transformed biological research by providing tools that enable robust genome modification in living organisms. In prokaryotes the RNA-guided Cas9 DNA endonuclease of type II CRISPR-Cas system provides immunity against invading phages by cleaving viral DNA. By altering guide RNA sequence Cas9 nuclease can be reprogrammed to target any desired site in the genome and repurposed as a versatile genome editing tool for engineering biology. In my talk I will discuss how basic research in the mechanisms of bacterial immunity springboarded development of one of the most important technologies in the last decade, and highlight future challenges and perspectives.

PNS Presidential Lecture

Stem Cells in the Adult Brain: Regulation and Diversity

Fiona Doetsch

Biozentrum, University of Basel, Switzerland

Neural stem cells reside in the adult mammalian brain. The ventricular-subventricular zone (V-SVZ) gives rise to olfactory bulb neurons, as well as small numbers of glia throughout life. Adult V-SVZ neural stem cells dynamically integrate intrinsic and extrinsic signals to either maintain the quiescent state or to become activated to divide and generate progeny. I will present our recent findings highlighting adult neural stem cell heterogeneity, including the identification of novel gliogenic domains and cell types, and the key roles of physiological state and long-range signals in the regulation of regionally distinct pools of adult neural stem cells.

Session 1: Cortico-hippocampal interactions mediating memory

The entorhinal cortex as integrative gatekeeper of the hippocampal memory system

Menno Witter

Kavli Institute for Systems Neuroscience, Faculty of Medicine and Health Sciences, NTNU, Trondheim, Norway

Traditionally, the entorhinal cortex is envisioned as a main cortical input-output hub for the hippocampal formation, mediating the communication between at least two functionally different cortical networks and the hippocampus. This view however does not provide much insight into the potential functional relevance of local entorhinal neuronal circuits. In my talk I will focus on recent findings relating cortical and subcortical connectivity to specific components of these local circuits, and how these local circuits are intrinsically linked, likely contributing to a complex control of hippocampal input/output channels.

Doan TP, Lagartos-Donate MJ, Nilssen ES, Ohara S, Witter MP (2019) Convergent Projections from Perirhinal and Postrhinal Cortices Suggest a Multisensory Nature of Lateral, but Not Medial, Entorhinal Cortex. Cell Rep, 29:617-627.e7

Nilssen ES, Doan TP, Nigro MJ, Ohara S, Witter MP (2019) Neurons and networks in the entorhinal cortex: A reappraisal of the lateral and medial entorhinal subdivisions mediating parallel cortical pathways. Hippocampus, 29:1238-1254

Rethinking prefrontal-hippocampal interactions in memory

Eleanor Maguire

Wellcome Centre for Human Neuroimaging, University College London, UK

Our past experiences are captured in autobiographical memories that provide our sense of self and the continuity in our lives. Two interconnected brain regions are particularly implicated in supporting these memories, the ventromedial prefrontal cortex and the hippocampus. However, a precise understanding of the neural mechanisms involved is currently lacking. In this talk I will describe neuropsychological and neuroimaging work that provides a fresh perspective on how the dynamic interplay between the hippocampus and ventromedial prefrontal cortex might support our seamless re-experiencing of the past.

Barry DN, Maguire EA (2019) Remote memory and the hippocampus – a constructive critique. Trends in Cognitive Sciences, 23: 128-142.

McCormick C, Ciaramelli E, De Luca F, Maguire EA (2018) Comparing and contrasting the cognitive effects of hippocampal and ventromedial prefrontal cortex damage: a review of human lesion studies. Neuroscience, 374: 295-318.

Complementary memory processing across the retrosplenial cortex and hippocampus

Seralynne Vann

School of Psychology, Cardiff University, Cardiff, UK

Visuo-spatial processing is a key component of episodic memory, enabling us to form spatial representations of the locations in which events occur as well and a mechanism enabling us to navigate to - and within - these spatial locations. While the hippocampus and retrosplenial cortex both have long-standing links to spatial processing and memory, what is less clear is how they interact to support these functions. In this talk, I will present data derived from a number of complementary approaches showing how these two regions interact, and how the relative contributions of the retrosplenial cortex change over time, to provide long-term representations necessary for mnemonic function.

Milczarek MM, Vann SD (2020) The retrosplenial cortex and long-term spatial memory: from the cell to the network. Current Opinion in Behavioral Sciences, 32: 50-56.
Milczarek, M.M., Vann, S.D. and Sengpiel, F (2018) Spatial memory engram in the mouse retrosplenial cortex. Current Biology, 28: 1975-1980.e6

Convergence of hippocampal and retrosplenial input in spatial memory formation

Rafał Czajkowski

Laboratory of Spatial Memory, Nencki Institute of Experimental Biology, Warsaw, Poland,

After five decades of research dedicated to cementing the central role of hippocampus in spatial memory, cortical regions became the centre of attention in the XXI century. One of the key structures that draws growing interest is the retrosplenial cortex. In this talk I will present evidence suggesting a complementary role of retrosplenial cortex and hippocampus in the process of memory formation. Retrosplenial area undergoes experience-dependent plasticity during spatial learning, a process manifested by patterned and cell-specific expression of immediate early genes. It sends projection to deep layers of medial entorhinal cortex where it converges with the hippocampal output and gets integrated into the spatial processing circuit.

Czajkowski R, Zglinicki B, Rejmak E, Konopka W. (2020) Strategy-Specific Patterns of Arc Expression in the Retrosplenial Cortex and Hippocampus during T-Maze Learning in Rats. Brain Sci, 10:854.
Mitchell A, Czajkowski R, Zhang N, Jeffery K, Nelson AJD. (2018) Retrosplenial cortex and its role in spatial cognition. Brain Neurosci Adv, 2:1-33.

Session 2: Causes and consequences of mitochondrial dysfunction in brain diseases

Pharmacological modulation of mitochondria: are we there yet?

Aiste Jekabsone

Institute of Pharmaceutical Technologies, Lithuanian University of Health Sciences, Kaunas, Lithuania

Mitochondria have a key role in determining brain cell fate - function, life and death decision, differentiation, proliferation, inflammation, carcinogenesis. Therefore, pharmacological modulation of mitochondrial function has a great therapeutic potential. However, the ways of mitochondrial modulation are studied not enough to efficiently prevent mitochondrial failure after stroke, during neurodegeneration or in cancer development. In my contribution, I first give an overview of the mitochondrial OxPhos pathway as a pharmacological target and then report on our recent findings about the efficiency of mitochondria modulation during brain ischaemia, cancer and neuroinflammation.

Skemiene, K., Rekuviene, E., Jekabsone, A., Cizas, P., Morkuniene, R., & Borutaite, V. (2020) Comparison of Effects of Metformin, Phenformin, and Inhibitors of Mitochondrial Complex I on Mitochondrial Permeability Transition and Ischemic Brain Injury. Biomolecules, 10.
Majiene, D., Kuseliauskyte, J., Stimbirys, A., & Jekabsone, A. (2019) Comparison of the Effect of Native 1,4-Naphthoquinones Plumbagin, Menadione, and Lawsone on Viability, Redox Status, and Mitochondrial Functions of C6 Glioblastoma Cells. Nutrients, 11.

In vitro and in vivo monitoring of functional mitochondria-ER contact sites

Tito Cali

Department of Biomedical Sciences, University of Padova, Padova, Italy

In the last decades, a concept of membrane contact sites (MCSs) has evolved. MCS between any known intercellular organelle, including the endoplasmic reticulum (ER) and mitochondria have been documented. How these membrane platforms mediate and regulate cell homeostasis remains obscure, due to the lack of tools to image inter-organelle proximity under physiological and pathological conditions and in living organisms. I have designed modular, split-GFP based contact site sensors (SPLICS) engineered to fluoresce when juxtapositions between organelles occur over a range of distances. This set of valuable genetic tools will help to uncover the mechanisms of organelle contact sites formation/stabilization and their nature in health and disease.

Vallese, F., Catoni, C., Cieri, D., Barazzuol, L., Ramirez, O., Calore, V., Bonora, M., Giamogante, F., Pinton, P., Brini, M., & Calì, T. (2020) An expanded palette of improved SPLICS reporters detects multiple organelle contacts in vitro and in vivo. Nat Commun, 11, 6069.
Cieri, D., Vicario, M., Giacomello, M., Vallese, F., Filadi, R., Wagner, T., Pozzan, T., Pizzo, P., Scorrano, L., Brini, M., & Calì, T. (2018) SPLICS: a split green fluorescent protein-based contact site sensor for narrow and wide heterotypic organelle juxtaposition. Cell Death Differ., 25, 1131–1145.

Mitochondria as a target of the gut-brain hypothesis for sporadic Parkinson's disease (sPD)

Sandra Morais Cardoso

Faculty of Medicine, Center for Neuroscience and Cell Biology of the University of Coimbra, Coimbra, Portugal

Evidence suggests that sPD can start in the gut and then progress to the brain via the vagus nerve. We found that the bacterial neurotoxin β-N-methylamino-L-alanine (BMAA)s increase intestinal inflammation and compromise intestinal barrier integrity. BMAA, in the brain, specifically targets the mitochondria, leading to exposure of cardiolipin, a lipid that induces the activation of innate immunity. This effect primes the loss of dopaminergic neurons in the midbrain, which culminates in motor function deficits. Finally, we observed that the expression and aggregation of aSyn showed a rostral progression from the gut, passing through the dorsal motor nucleus of the vagus, to the midbrain of mice.

Silva, D.F., Candeias, E., Esteves, A.R., Magalhães, J.D., Ferreira, I.L., Nunes-Costa, D., Rego, A.C., Empadinhas, N., & Cardoso, S.M. (2020) Microbial BMAA elicits mitochondrial dysfunction, innate immunity activation, and Alzheimer's disease features in cortical neurons. J Neuroinflammation, 17, 332.
Cardoso, S.M. & Empadinhas, N. (2018) The Microbiome-Mitochondria Dance in Prodromal Parkinson's Disease. Front Physiol, 9, 471.

Searching for the cause of mitochondria-ER dysfunction in Alzheimer's disease astrocytes

Dmitry Lim

Department of Pharmaceutical Sciences, Università del Piemonte Orientale, Novara, Italy

In Alzheimer's disease, cellular pathology is characterized by deregulated calcium signaling, mitochondrial dysfunction, and dysproteostasis. In astrocytes, principal homeostatic cells in the CNS, cause--effect relationship between these processes we poorly understood. We report that in astrocytes from 3xTg-AD mice bioenergetics is impaired, endoplasmic reticulum (ER) is overloaded with Ca2+ while Ca2+ uptake by mitochondria upon ATP stimulation is reduced. This is accompanied by shortening of mitochondria-ER distance, activated ER-stress/UPR and impairment of protein synthesis. We suggest that the mitochondria-ER interaction changes, deregulation of astrocytic bioenergetics, Ca2+ homeostasis and proteostasis may interact, creating a pathogenic loop compromising homeostatic and defensive functions of astroglial cells predisposing neurons to dysfunction.

Dematteis, G., Vydmantaitė, G., Ruffinatti, F.A., Chahin, M., Farruggio, S., Barberis, E., Ferrari, E., Marengo, E., Distasi, C., Morkūnienė, R., Genazzani, A.A., Grilli, M., Grossini, E., Corazzari, M., Manfredi, M., Lim, D., Jekabsone, A., & Tapella, L. (2020) Proteomic analysis links alterations of bioenergetics, mitochondria-ER interactions and proteostasis in hippocampal astrocytes from 3xTg- AD mice. Cell Death Dis, 11, 645.
Rocchio, F., Tapella, L., Manfredi, M., Chisari, M., Ronco, F., Ruffinatti, F.A., Conte, E., Canonico, P.L., Sortino, M.A., Grilli, M., Marengo, E., Genazzani, A.A., & Lim, D. (2019) Gene expression, proteome and calcium signaling alterations in immortalized hippocampal astrocytes from an Alzheimer's disease mouse model. Cell Death Dis, 10, 24.

Session 3: Advances in the treatment of alcohol use disorder

Targeting Acetylcholine Muscarinic Receptors to Treat AUD

Leigh Walker

Florey Department of Neuroscience and Mental Health, University of Melbourne, Australia

Alcohol is the leading cause of death globally of people aged between 15-49 years. Despite this enormous socioeconomic burden, therapeutic treatment options for AUDs are limited and ineffective at a population level. A major hurdle in the development of new treatments is a lack of translation between preclinical animal models and efficacy in human subjects. We have recently begun to bridge this gap by concurrently examining human and rodent post-mortem samples. In this talk I will discuss our translationally relevant preclinical model of alcohol induced cholinergic dysfunction, and present recent findings from our laboratory highlighting the potential of muscarinic receptor modulation to reduce alcohol consumption and seeking.

Suppressing effect of KK-92A, a new positive allosteric modulator of the GABAB receptor, on different alcohol-motivated behaviors in alcohol-preferring rats

Giancarlo Colombo

Neuroscience Institute, Section of Cagliari, National Research Council of Italy, Monserrato, Italy

Treatment with the novel positive allosteric modulator (PAM) of the GABAB receptor, KK- 92A, has been reported to suppress several nicotine-motivated behaviors in rats [1]. The present study investigated whether the anti-addictive properties of KK-92A extend to alcohol. Acute treatment with non-sedative doses of KK-92A resulted in a dose-related suppression of (i) operant oral alcohol self- administration under fixed and progressive ratio schedules of reinforcement and (ii) cue-induced reinstatement of alcohol seeking in selectively bred Sardinian alcohol-preferring rats. These data add KK-92A to the list of GABAB PAMs being effective in suppressing alcohol-motivated behaviors in rodent models of AUD [2].

[1] Li, X., Sturchler, E., Kaczanowska, K., Cameron, M., Finn, M.G., Griffin, P., McDonald, P., Markou, A. (2017). KK-92A, a novel GABAB receptor positive allosteric modulator, attenuates nicotine self-administration and cue--induced nicotine seeking in rats. Psychopharmacology, 234, 1633-1644. doi:10.1007/s00213-017-4594-9.
[2] Maccioni, P., Colombo, G. (2019). Potential of GABAB receptor positive allosteric modulators in the treatment of alcohol use disorder. CNS Drugs, 33,107-123. doi: 10.1007/s40263-018-0596-3.

A Holistic Approach to Treatment of AUD

Boris Tabakoff

Skaggs School of Pharmacy & Pharmaceutical Sciences, University of Colorado Anschutz Medical Campus, Aurora CO, USA

We designed and synthesized a new chemical entity (NCE) that would address targets thought to be important in the development of AUD. Receptor screening studies demonstrated an affinity of the compound for GABA-A receptors with little affinity for any of the other 50 receptors/ion channels/transporters. Electrophysiologic studies and mutational analysis demonstrated that our compound was a subunit selective positive allosteric agonist (PAM) at GABA-A receptors and acted through a novel binding site located at the ECD interface of α+/β- subunits [1]. The compound when given orally or i.p. could significantly reduce abstinence-induced alcohol consumption by alcohol dependent rats, but was found not to cross the blood/brain barrier. Further work indicated that the target of our drug may be the GABA receptors in the enteric nervous system and on immune system macrophages. Studies on the pharmacokinetics and toxicology of our compound are nearing completion.

[1] Borghese, C. M., M. Herman, L. D. Snell, K. J. Lawrence, H. Y. Lee, D. S. Backos, L. A. Vanderlinden, R. A. Harris, M. Roberto, P. L. Hoffman and B. Tabakoff (2017). "Novel Molecule Exhibiting Selective Affinity for GABA(A) Receptor Subtypes." Sci Rep 7(1): 6230. doi: 10.1038/s41598-017-05966-x

Glycinergic agonists for treatment of AUD

Bo Söderpalm

Addiction Biology Unit, Section of Psychiatry and Neurochemistry, Institute of Neuroscience and Physiology, Sahlgrenska Academy at the University of Gothenburg, Göteborg, Sweden

We have identified strychnine-sensitive glycine receptors as key targets for ethanol in its dopamine releasing effect in the nucleus accumbens [1]. Local glycine perfusion or systemic administration of glycine uptake inhibitors enhances basal dopamine levels, prevents further dopamine release by ethanol and reduces ethanol intake in rats [1]. The response to glycinergic treatments appears, however, variable and further development of glycine uptake inhibitors for this indication is hindered by patent. We currently examine other means to activate glycine receptors by e.g. systemic administration of glycine and glycine analogues and to overcome response variability by exploring the possibility to interfere with mechanisms related to glycine receptor desensitization. We also explore if glycine agonists can be used as add-ons to other potential alcohol medications.

[1] Soderpalm, B., Lidö HH, Ericson M. (2017). The Glycine Receptor-A Functionally Important Primary Brain Target of Ethanol. Alcohol Clin Exp Res, 41, 1816-1830. doi: 10.1111/acer.13483

Sesssion 4: Prelude to the 2023 World IBRO Congress

Relating synapse nanoscale organization and function

Daniel Choquet

Centre National de la Recherche Scientifique, Interdisciplinary Institute for Neuroscience, Bordeaux, France

Neurotransmitter receptors are organized in front of transmitter release sites to allow fast and efficient neurotransmission. In the recent years, using largely superesolution imaging and electron microscopy, it has been found that receptors are exquisitely organized at the nanoscale in the postsynaptic density. Furthermore, it has been suggested that this organization has a strong impact on the efficacy of synaptic transmission. In addition, neurotransmitter nanoscale organization is extremely dynamic and regulated during synaptic plasticity. We will present our recent work on the co- organization of different glutamate receptors at excitatory synapses and its impact on various types of synaptic plasticity.

Groc, L., and Choquet, D. (2020). Linking glutamate receptor movements and synapse function. Science 368
Penn, A.C., Zhang, C.L., Georges, F., Royer, L., Breillat, C., Hosy, E., Petersen, J.D., Humeau, Y., and Choquet, D. (2017). Hippocampal LTP and contextual learning require surface diffusion of AMPA receptors. *Nature* 549, 384-388.

The thalamus that speaks to the cortex: spontaneous activity in sensory systems development and plasticity

Guillermina López-Bendito

Instituto de Neurociencias de Alicante (CSCI-UMH), Alicante, Spain

This is related to the cellular and molecular mechanisms involved in the development of axonal connections in the brain. In particular, this talk will focus on the principles underlying thalamocortical axonal wiring, maintenance and ultimately the rewiring of connections. The development of the thalamocortical wiring requires a precise topographical sorting of its connections. Each thalamic nucleus receives specific sensory information from the environment and projects topographically to its corresponding cortical. A second level of organization is achieved within each area, where thalamocortical connections display an intra-areal topographical organization, allowing the generation of accurate spatial representations within each cortical area. Therefore, the level of organization and specificity of the thalamocortical projections is much more complex than other projection systems in the CNS.

Antón-Bolaños N, Sempere-Ferràndez A, Guillamón-Vivancos T, Martini FJ, Pérez-Saiz L, Gezelius H, Filipchuk A, Valdeolmillos M, López-Bendito G (2019) Prenatal activity from thalamic neurons governs the emergence of functional cortical maps in mice. *Science* 364:987–990.
Antón-Bolaños N, Espinosa A, López-Bendito G (2018) Developmental interactions between thalamus and cortex: a true love reciprocal story. *Current Op. Neurobiology* 52:33–41.

Neural mechanisms of social reward

Robert C. Malenka

Nancy Pritzker Laboratory, Dept. of Psychiatry & Behavioral Sciences, Stanford. USA

Prosocial drugs, such as ecstasy, are drugs primarily used for recreational purposes. Because of its prosocial effects, such drugs, e.g. MDMA, are currently being evaluated for the treatment of psychiatric disorders. Unfortunately, their rewarding addictive properties hinder the therapeutic use. In this talk, the mechanisms mediating the effects of prosocial drugs, such as MDMA, will be discussed. Recent data from rodents, indicate that the prosocial and the rewarding effects are mediated by independent mechanisms. The prosocial effects are mediated by activation of the serotoninergic system, whereas the rewarding effect requires the activation of the dopaminergic signaling, paving the way for the development of more specific therapeutic intervention with less side effects for treating, for instance, dysfunction of prosocial behaviours such as those associated to autism.

Heifets BD, Salgado JS, Taylor MD, Hoerbelt P, Cardozo Pinto DF, Steinberg EE, Walsh JJ, Sze JY, Malenka RC (2019) Distinct neural mechanisms for the prosocial and rewarding properties of MDMA. *Sci Transl Med* 11:eaaw6435.
Walsh JJ, Christoffel DJ, Heifets BD, Ben-Dor GA, Selimbeyoglu A, Hung LW, Deisseroth K, Malenka RC (2018) 5-HT release in nucleus accumbens rescues social deficits in mouse autism model. *Nature* 65:1–594.

Knowledge representation in the human brain

Yanchao Bi

IDG/McGovern Institute for Brain Research, Beijing Normal University, Beijing

Human brain stores tremendous amount of knowledge about this world, which is the foundation of language, thought, and reasoning. What's the neural codes of knowledge representation? Is the knowledge "roses are red" simply the memory trace of perceiving the color of roses, stored in the brain circuits within color-sensitive neurons? I will present some work from my lab that addresses this issue using cognitive, neuroimaging, and neuropsychological methods with healthy subjects, individuals with sensory deprivation (blind) or with brain damage. The findings point to a highly distributed system incorporating two different types of information coding – one based on sensory experiences and one based on language.

Wang X, Men W, Gao J, Caramazza A, Bi Y (2020) Two Forms of Knowledge Representation in the Human Brain. *Neuron* 107:383–393.
Wang X, Wang B, Bi Y (2019) Close yet independent: Dissociation of social from valence and abstract semantic dimensions in the left anterior temporal lobe. *Hum Brain Mapp* 40:4759–4776.

Session 5: The Shared European Brain Research Agenda (SEBRA)

SEBRA is framed within the European Brain Research Area project, an EU-funded catalysing initiative for brain research stakeholders to streamline and better coordinate brain research across Europe. It brings together 4 key players: EBC, ERA-NET NEURON, JPND and HBP. The aim of SEBRA is to identify research opportunities and research and innovation gaps to be addressed in the field and to provide recommendations on future areas for brain research in Europe.

Session 6: ERC session: Poland and the Baltics under Horizon Europe

We will discuss the new framework program, Horizon Europe, with special focus on issues related to the successful participation of Poland and the Baltic Countries. We will elaborate on some topics such as the high-risk high-gain, ground-breaking nature of a proposal; mobility, independence and publication requirements, eligibility rules in general and in special situations such as maternity or parental leaves; and finally we will share with the audience some tips and tricks for ERC applications.

Brain Prize Lecture

The genetics and epigenetics of Rett syndrome

Adrian Bird

University of Edinburgh, United Kingdom

This lecture will discuss our current understanding of the molecular basis of the profound neurological disorder Rett syndrome, which is caused by mutations in the gene encoding the methylated DNA binding protein, MeCP2. Cytosine in DNA can be modified post-synthetically and this affects local protein-DNA interactions. MeCP2 specifically binds to methylated sites in the genome, potentially allowing it to interpret this “epigenetic” mark. Several clinical disorders are caused by MECP2 mutations, including the profound neurological disorder Rett syndrome. The severe Rett-like phenotype in micecan be reversed, raising the possibility that the human disorder iscurable. Evidence will be presented that the root cause of Rett syndrome is failure of the primary function of MeCP2, which is to restraingene expression in a DNA methylation-dependent manner. MeCP2 targets non-CG as well as CG-specific methylation. Experiments that evaluate the relative importance of these two modes of DNA binding will be presented.

Plenary Lecture

Brainstem control of fear memories

Gábor Nyiri

Institute of Experimental Medicine, Budapest, Hungary

Encoding, recalling and, if necessary, efficient forgetting the memory of negative experience is essential for survival. Malfunctions of these memory processes can lead to mental health issues, cognitive deficits or dementia. Our recent discoveries suggest that key interconnected cell populations in the brainstem play a previously unrecognized yet crucial role in these processes. Nucleus incertus inhibitory cells seem to be crucial for encoding hippocampal fear memories, while the newly discovered excitatory neurons of the median raphe seem to be fundamental in encoding fear memories.

Session 7: Sex differences in the prefrontal cortex

Sexual dimorphic effects of restraint stress in the prefrontal cortex

Kyriaki Sidiropoulou

University of Crete and IMBB-FORTH, Greece

Stress, a major regulator and precipitating factor of cognitive and emotional disorders, differentially manifests between males and females. Here, I will discuss our recent work showing the effects of 2hours restraint stress (RS) in anxiety, recency memory and long-term potentiation (LTP) in the prefrontal cortex (PFC) of male and female mice. We find that female mice only exhibited increased anxiety levels, while only male mice showed deficits in the recency memory. Furthermore, LTP studied in acute PFC slices was significantly reduced in male, but not female, mice following RS. Signaling via corticosterone receptors likely mediate the RS effect on recency memory and LTP in males.

Chalkiadaki K, Velli A, Kyriazidis E, Stavroulaki V, Vouvoutsis V, Chatzaki E, Aivaliotis M, Sidiropoulou K. (2019) Development of the MAM model of schizophrenia in mice: Sex similarities and differences of hippocampal and prefrontal cortical function. *Neuropharmacology*. 2019 Jan;144:193-207.

Sex-specific developmental effects of early life adversity on corticolimbic connectivity and behavior

Heather Brenhouse

Northeastern University, Boston, USA

Exposure to childhood maltreatment often leads to disruptions in emotion regulation and cognition, leading to anxiety, depression, and impulsivity disorders. These processes rely on corticolimbic circuitry, which continues to develop through adolescence. Importantly, males and females experience consequences of early life adversity differently, and on different developmental trajectories, with females displaying behavioral sequelae earlier than males. I will discuss our work in a rat model, characterizing the extent to which early life adversity differentially alters the maturation of amgydala-prefrontal cortex (PFC) connectivity and PFC excitatory/inhibitory balance in males and females. The findings will be related to how early life experience influences emotion regulation circuitry, with evidence that males and females might require distinct strategies for intervention.

Honeycutt J.A., Demaestri C., Peterzell S., Silveri M.M., Cai X, Kulkarni P, Cunningham MG, Ferris CF, Brenhouse HC (2020) Altered corticolimbic connectivity reveals sex-specific adolescent outcomes in a rat model of early life adversity. *Elife*. 2020 Jan 20;9:e52651.

Sex differences in fear discrimination and associated prefrontal cortex function

Carl Stevenson

University of Nottingham, UK

I will present our recent research on sex differences in fear discrimination and associated prefrontal cortex function. We have found that whether females and males discriminate or generalize between different auditory cues predicting threat or safety depended on the extent of discrimination training received. Females, but not males, discriminated after limited training. However, females generalized while males discriminated after extended training. We also found that discrimination in males was associated with differences in cue-induced prefrontal cortex activation, which was absent with generalization in females. These results will be discussed in terms of their broader adaptive significance and potential clinical relevance, given that anxiety related-disorders are more prevalent in women, characterized by overgeneralization of fear, and associated with prefrontal cortical dysfunction.

Day, H.L., Suwansawang, S., Halliday, D.M. & Stevenson, C.W. (2020) Sex differences in auditory fear discrimination are associated with altered medial prefrontal cortex function. *Sci. Rep.* 10, 6300.
Day, H.L., Reed, M.M. & Stevenson, C.W. (2016) Sex differences in discriminating between cues predicting threat and safety. Neurobiol. *Learn. Mem*. 133, 196-203.

Different prefrontal oxytocin role in social-induced facilitation of extinction in pubertal males and females

Mouna Maroun

University of Haifa, Israel

We previously reported that in the adult animal extinction in pairs resulted in enhanced extinction of fear, showing that social presence can reduce previously acquired fear responses. This facilitation by social presence is dependent on prefrontal cortex Oxytocin (OT). Recently, we started to address possible differences between juvenile and adult animals while also focusing on differences between pre-pubertal males and females. Our recent results show substantial differences in the response profile between pre-pubertal males and females. The results show that social presence accelerates extinction in males and females. Yet, we show differential and opposing effects of an OT receptors antagonist in both sexes. Whereas, prefrontal OT is not engaged in extinction in juvenile males, it is critical in females. We further report differences in the levels of prefrontal OT between males and females that may explain the differences in OT action. These results suggest that even during the juvenility period, critical mechanisms are differently involved in the regulation of fear in males and females.

Maroun M., Sarussi-Elyahu A., Yaseen A., Hatoum O.A., Kritman M. (2020) Sex-dimorphic role of prefrontal oxytocin receptors in social-induced facilitation of extinction in juvenile rats. *Transl Psychiatry*. 2020 Oct 19;10(1):356.

Session 8: Nicotinic effects on brain physiology and cognitive processes

Cellular and synaptic mechanisms of nicotinic actions on human cortical circuits

Huib Mansvelder

Department Integrative Neurophysiology, Center for Neurogenomics and Cognitive Research, Vrije Universiteit Amsterdam, Amsterdam, The Netherlands

Most of what we know about cellular mechanisms of nicotinic modulation of cortical processing comes from research on rodent brain. Little is known about whether principles of cholinergic modulation translate to human cortical circuits. In this talk I will discuss how activation of nicotinic acetylcholine receptors alter short and long-term processing by neocortical circuits in human brain in a layer specific manner.

Obermayer J, Heistek TS, Kerkhofs A, Goriounova NA, Kroon T, Baayen JC, Idema S, Testa-Silva G, Couey JJ, Mansvelder HD. (2018) Lateral inhibition by Martinotti interneurons is facilitated by cholinergic inputs in human and mouse neocortex. *Nature Commun*. 2018 Oct 5;9(1):4101. doi: 10.1038/s41467-018-06628-w.
Poorthuis RB, Muhammad K, Wang M, Verhoog MB, Junek S, Wrana A, Mansvelder HD, Letzkus JJ. (2018) Rapid Neuromodulation of Layer 1 Interneurons in Human Neocortex. *Cell Reports*. 2018 Apr 24;23(4):951-958. doi: 10.1016/j.celrep.2018.03.111.

Nicotinic impact on neuroplasticity and memory formation in humans

Michael A. Nitsche

Department of Psychology and Neurosciences, Leibniz Research Centre for Working Environment and Human Factors at TU Dortmund, Dortmund, Germany

Recent research in healthy humans has shown that nicotine has a relevant, but complex impact on cortical excitability, neuroplasticity, and associated cognitive functions, including working and long term memory. This talk will deliver mechanistic insights in these effects, and propose a model on how nicotine affects respective functions, including the effect of nicotine on brain physiology and cognition in smokers and non-smokers.

Grundey J, Barlay J, Batsikadze G, Kuo MF, Paulus W, Nitsche M. Nicotine modulates human brain plasticity via calcium-dependent mechanisms. J Physiol. 2018 Nov;596(22):5429-5441.
Grundey J, Amu R, Batsikadze G, Paulus W, Nitsche MA. Diverging effects of nicotine on motor learning performance: Improvement in deprived smokers and attenuation in non-smokers. Addict Behav. 2017 Nov;74:90-97.

Nicotinic modulation of attention in the human brain

Christiane Thiel

Department of Psychology, University of Oldenburg, Oldenburg, Germany

The talk will give an overview of pharmacological fMRI studies that used nicotine to modulate visuospatial attention and cognitive control in healthy nonsmokers. Nicotine mainly impacts on visuospatial orienting and response speed. Neural signatures are a reduction of BOLD activity in several brain regions, including parietal and frontal cortices and overall changes in brain network topology enabling faster information transfer. I will further point out that there is interindividual variability with respect to the effects of nicotine and that variations in dopaminergic neurotransmission may play an important role.

Ahrens, S. & Thiel, C.M. (2020) Effects of Nicotine on Task Switching and Distraction in Non-smokers. An fMRI Study. Neuroscience, 444, 43-53.
Giessing, C., Thiel, C.M., Alexander-Bloch, A.F., Patel, A.X. & Bullmore, E.T. (2013) Human brain functional network changes associated with enhanced and impaired attentional task performance. J Neurosci, 33, 5903-5914.

Nicotinic impact on neuroplasticity in patients with schizophrenia

Alkomiet Hasan

Department of Psychiatry, Psychotherapy, and Psychosomatic, University of Augsburg, Augsburg, Germany

The association between impairments in the cholinergic system, plasticity dysfunction and schizophrenia is evident from theoretical and preclinical studies. However, limited evidence from human research is available. The talk will present findings from several experiments with and without manipulation of nAchR in patients with schizophrenia focussing on human motor-cortical plasticity and excitability.

de Miquel C, Pross B, Papazova I, Güler D, Hasan A (2020) The two-way relationship between nicotine and cortical activity: a systematic review of neurobiological and treatment aspects. *Eur Arch Psychiatry Clin Neurosci.* 2020 Jun.
Strube W, Bunse T, Nitsche MA, Wobrock T, Aborowa R, Misewitsch K, Herrmann M, Falkai P, Hasan A. Smoking restores impaired LTD-like plasticity in schizophrenia: a transcranial direct current stimulation study. N*europsychopharmacology.* 2015 Mar;40(4):822-30

Session 9: Facets of computational models of synaptic plasticity

Modelling CaMKII dynamics in dendritic spines

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The direction of synaptic plasticity relies on the balance between kinase and phosphatase action in the post-synaptic density. Specifically, the kinase CaMKII plays an important role. CaMKII regulation is intricate and involves binding to Calcium-bound calmodulin, (auto-)phosphorylation, competition with phosphatases, and possibly spatial positioning within the dendritic spine. Over the past years, we and others have developed rule-based computational models of CaMKII to understand the nuances of its regulation, and explore how it affects synaptic plasticity. On top of improving our understanding of synaptic plasticity, these models also showcase the power of rule-based modelling of biological systems (Stefan et al., 2012, Pharris et al., 2019).

Stefan, M.I. , Marshall, D.P., & Le Novère, N. (2012). Structural Analysis and Stochastic Modelling Suggest a Mechanism for Calmodulin Trapping by CaMKII. *PloS One* 7 (1), e29406
Pharris, M.C., Patel, N.M., VanDyk, T.G., Bartol, T.M., Sejnowski, T.J, Kennedy, M.B., Stefan, M.I., & Kinzer-Ursem, T.L. (2019). A multi-state model of the CaMKII dodecamer suggests a role for calmodulin in maintenance of autophosphorylation. *PLoS Computational Biology* 15 (12), e1006941

Molecular control of NMDAR-dependent gabaergic plasticity

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Activity-dependent synaptic plasticity is one of the mechanisms underlying learning and memory. Plasticity of an excitatory synapse on a dendritic branch may be accompanied by plasticity of inhibitory synapses on that branch (Lu et al., 2000). We used a spatial reaction-diffusion model of signaling pathways together with an compartmental model of a CA1 neuron with calcium dynamics to investigate how stimulation of a single excitatory synapse influences inhibitory transmission of the branch. We evaluated how stimulation of one spine changes the acitivity of kinases and phosphatases depending on the distance from the stimulated spine in consequence influencing phosphorylation of the gabaergic receptor, and predict changes of strengths of inhibitory synapses on the branch.

Lu, Y. M., Mansuy, I. M., Kandel, E. R., & Roder, J. (2000). Calcineurin-mediated LTD of GABAergic inhibition underlies the increased excitability of CA1 neurons associated with LTP. *Neuron,* 26(1), 197–205. https://doi.org/10.1016/s0896-6273(00)81150-2

Calcium control of synaptic plasticity

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The ability of neurons to respond differentially to specific temporal and spatial patterns of stimulation underlies the storage of memory and information in neural circuits. Synaptic plasticity is one mechanism underlying memory storage, but ex vivo plasticity experiments use periodic and noise-free stimulation patterns. To investigate synaptic plasticity in vivo, we created a data-driven, multi-compartmental model of a striatal spiny projection neuron with sophisticated calcium dynamics and used experimentally recorded spike trains as synaptic stimulation. We evaluate how the direction and magnitude of synaptic plasticity are controlled by spatial synaptic interactions and trial-to-trial variability (Prager et al., 2020). These results will enable derivation of spike based, spatial plasticity rules for large scale networks of simplified neurons.

Prager, E.M., Dorman, D., Hobel, Z., Malgady, J., Blackwell, K.T., & Plotkin, J.L. (2020) Dopamine oppositely modulates state transitions in striosome and matrix direct pathway striatal spiny neurons. *Neuron, in press*

SpaRCe: Improved Learning of Reservoir Computing Systems through Sparse Representation

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„Sparse" neural networks, in which relatively few neurons or connections are active, are common in both machine learning and neuroscience. Whereas in machine learning, „sparsity'' is related to a penalty term that leads to some connecting weights becoming small or zero, in biological brains, sparsity is often created when high spiking thresholds prevent neuronal activity. Here we introduce sparsity into a reservoir computing network via neuron-specific learnable thresholds of activity, allowing neurons with low thresholds to contribute to decision-making but suppressing information from neurons with high thresholds. This approach, which we term ``SpaRCe", optimises the sparsity level of the reservoir without affecting the reservoir dynamics. The read-out weights and the thresholds are learned by an on-line gradient rule that minimises an error function on the outputs of the network. Threshold learning occurs by the balance of two opposing forces: reducing inter-neuronal correlations in the reservoir by deactivating redundant neurons, while increasing the activity of neurons participating in correct decisions. We test SpaRCe on classification problems and find that threshold learning improves performance compared to standard reservoir computing. SpaRCe alleviates the problem of catastrophic

forgetting, a problem most evident in standard echo state networks and recurrent neural networks in general, due to increasing the number of task-specialised neurons that are included in the network decisions.

Manneschi. L., Lin, A. C., Vasilaki, E.. SpaRCe: Improved Learning of Reservoir Computing Systems through Sparse Representations (IEEE Transactions in Neural Networks and Learning Systems, accepted). https://arxiv.org/abs/1912.08124

Poster session

The role of heme oxygenase in the regulation of apoptosis and autophagy in the brain of *Drosophila melanogaster*

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Heme oxygenase (HO) is one of the cyto-protective enzymes that can mitigate effects of oxidative stress. In the present study, we found that the *ho* gene mRNA level cycles in the brain of *Drosophila melanogaster* with two minima at the beginning of the day (ZT1) and the night (ZT13). This rhythm is generated by a circadian clock as it was not observed in the clock mutant per^{01}. In older flies (20 days old) the rhythm was vanished, however, 15 days of curcumin feeding restored this rhythm. Curcumin also increased mRNA level of *ho* at all time point studied. Furthermore, the increase of *ho* expression by curcumin was accompanied by higher expression of the apoptotic gene *hid* in the brain, but only at night (ZT16 and ZT20). The overexpression of *ho* in all neurons, induced higher mRNA levels of *hid* and the autophagy gene *atg5* at ZT16. In addition, our data showed that HO plays a key role in the protection against stress in time-dependent manner. Flies exposed to paraquat had higher *ho* expression in the brain, but only at specific time of the day, at ZT1 and ZT13. Our data suggest that the expression of *ho* in the fly's brain is regulated by the circadian clock, and it changes with age, exposure to stress and presence of endogenous antioxidants. HO protects the brain by stimulating apoptosis and autophagy under oxidative stress.

[1] Damulewicz M, Loboda A, Jozkowicz A, Dulak J, Pyza E (2017) Interactions between the circadian clock and heme oxygenase in the retina of *Drosophila melanogaster. Mol. Neurobio.* 54: 4953-4962.

[2] Damulewicz M, Świątek M, Łoboda A, Dulak J, Bilska B, Przewlocki R, Pyza E (2019) Daily regulation of phototransduction, circadian clock, DNA repair, and immune gene expression by heme oxygenase in the retina of *Drosophila. Genes (Basel)* 10(1): 1-20.

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The authors declare no conflict of interests.

Differences in resting-state functional connectivity between patients with Compulsive Sexual Behavior Disorder and healthy individuals

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Compulsive Sexual Behavior Disorder (CSBD) is characterised by inability to control urges to engage in various sexual activities, which causes significant distress. Brain mechanisms underlying this disorder are still poorly understood.

The aim of this study was to compare resting-state functional connectivity between patients with CSBD and healthy individuals.

The fMRI data from 52 patients with CSBD (mean age: 34.67, sd: 8.373) and 29 healthy controls (HC) (mean age: 34.93, sd: 8.831) were acquired in an MRI scanner during a 12-minute resting-state session (TR = 2000 ms, TE = 28 ms, slice thickness = 3 mm). All subjects were heterosexual males. Data pre-processing and denoising were performed using CONN functional connectivity toolbox. Resting-state functional connectivity differences between CSBD and HC group were assessed using ROI-to-ROI analysis.

Increased resting-state functional connectivity between left frontal orbital cortex (FOrb) and left insular cortex (IC) (tmax = 2.13, p-unc = 0.0366, p-FDR = 0.5685) was observed in individuals with CSBD compared to HC. CSBD patients also had increased resting-state functional connectivity between left inferior frontal gyrus (IFG) and bilateral supplementary motor cortex (SMA) (tmax = 2.20, p-unc = 0.0305, p-FDR = 0.8366 for the left SMA; tmax = 2.70, p-unc = 0.0086, p-FDR = 0.1706 for the right SMA).

To conclude, our results show altered resting-state functional connectivity in individuals with CSBD. Difference in functional connectivity between IFG and SMA was also observed by Seok & Sohn [1], however it was task-related and decreased between right IFG and right SMA among CSBD group.

[1] Seok JW, Sohn JH (2020) Response inhibition during processing of sexual stimuli in males with problematic hypersexual behavior. *J Behav Addict* 9(1): 1-12.

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Auditory closed-loop stimulation during sleep in mice modulates spatial preference

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In humans, auditory closed-loop stimulation (ACLS) in phase with the endogenous slow oscillation (SO) enhanced sleep-dependent memory consolidation[1], SOs, and thalamocortical spindles[2]. The present study aims to adapt ACLS to mice to investigate underlying mechanisms. During sleep, within the retention period between the sample and the test phase of a spatial task, local field potentials were recorded in the cingulate cortex and dorsal hippocampus by wire arrays. Upon online detection of the hyperpolarization (negative SO half-wave) in the cortex stimulation was delivered at one of four phases, in separate sessions: close to the SO downstate (DS), at the down-to-up transition (D2US), at the up-state (UpS) and during the up-to-down transition (U2DS). In a fifth session, stimulation was omitted (Sham). Mice performed above chance levels for ACLS delivered at D2US and UpS whereas performance for DS and U2DS failed to surpass chance level (one-sample t-test, D2US: p=0.005, UpS: p=0.0051, DS: p= 0.5356, U2DS: p=0.2275, sham: p=0.1489; n=6), as previously suggested by our model[3]. Importantly, performance at the UpS was higher than at the U2DS (paired t-test, UpS *vs*. U2DS: p=0.0201). ACLS entrained SOs, sleep spindle, and ripple activity as measured by RMS (root-mean-square) signals. Preliminary analyses reveal a longer suppression of hippocampal sharp-wave ripple RMS after ACLS in U2DS than UpS. In the DS there is a tendency toward decreased modulation of spindles by SO compared to Sham. Together, our ACLS protocol modulates the retention of spatial memory in mice, despite preliminary results indicating only a moderate impact on electrophysiological activity.

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[2] Krugliakova E, Volk C, Jaramillo V, Sousouri G, Huber R (2020) Changes in cross-frequency coupling following closed-loop auditory stimulation in non-rapid eye movement sleep. *Sci Rep* 10, 10628.

[3] Wei Y, Krishnan GP, Marshall L, Martinetz T, Bazhenov M (2020) Stimulation Augments Spike Sequence Replay and Memory Consolidation during Slow-Wave Sleep. *J. Neurosci*, 40(4): 811-824.

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The authors declare that there is no conflict of interest.

Intracellular chloride heterogeneity in different neuronal subpopulations

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Chloride levels inside neurons are crucial in determining neuronal excitability. In particular, the intracellular chloride concentration, $[Cl^-]_i$, dictates the efficacy of fast GABAergic synaptic inhibition, strongly affecting network excitability. However, little is known about the 'chloride profile' - the cell-to-cell variation in chloride levels across a population of neurons - in different brain areas, nor how it differs between cell classes.

The recent development of ClopHensor [1], a novel genetically encoded chloride indicator, and its optimization for *in vivo* imaging deep within tissue, using 2-photon microscopy [2], has made such measurements possible for the first time. The expression of the sensor, however, was achieved only using embryonic in utero electroporation, limiting the investigation only to the pyramidal cell population [2].

We made two improvements to facilitate the use of ClopHensor: 1) changed the linker sequence between the two fluorescent elements, to improve the sensor stability; 2) packaged the floxed construct into an AAV vector for cell specific gene delivery. We calibrated the new ClopHensor construct, using ionophore-permeabilized HEK cells bathed in buffered solutions with variable pH (6.8-7.6) and Cl- (0-50mM).

We were then able to derive population statistics of pH and $[Cl^-]_i$ in three cell classes of cortical neuron, *in vivo*, using 2-photon microscopy: pyramidal cells, and somatostatin-positive and parvalbumin-positive interneurons. Our results show class-specific difference in the $[Cl^-]_i$ distributions, and also heterogeneity in the $[Cl^-]_i$ across subpopulations of neurons of the same class (interneurons or pyramidal cells), suggesting that intracellular chloride in these neuronal classes is regulated independently.

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[2] G Sulis Sato s, et al., (2017) Simultaneous two-photon imaging of intracellular chloride concentration and pH in mouse pyramidal neurons *in vivo Proc Natl Acad Sci U S A*, 114(41):E8770-E8779.

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Establishment of a Sheep Model for Hind Limb Peripheral Nerve Injury: Common Peroneal Nerve

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Peripheral Nerve Injuries occur frequently in the modern world, affecting both humans and animals. Despite the efforts and advances made in recent years, it has not yet been possible to establish an effective therapy that promotes the complete and effective regeneration of the injured peripheral nerve and consequent functional recovery [1]. In recent years several therapeutic alternatives have been developed and explored in order to achieve an ideal peripheral nerve regeneration after injury. These new approaches include the use of cell therapies with mesenchymal stem cells and biomaterials in the form of nerve guide conduits.

The sheep model has recently been considered a good animal model for Peripheral Nerve Injury studies. Besides being a species with great availability, easy to maintain and to manipulate, the sheep also presents peripheral nerves with anatomical and physiological characteristics identical to those of humans [2]. In addition, the common peroneal nerve has been insufficiently explored as an injured nerve for further regeneration studies in this species, and access and injury induction protocols have not yet been validated [3].

In this work, a surgical protocol for access to the common peroneal nerve in the sheep model was established, allowing the induction of injuries and application of therapeutic options. Additionally, a neurological assessment protocol was created that allows to follow the functional evolution of the intervened animals over time. The results obtained are the starting point for the widespread use of this species in future works.

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Conflict of Interests: The authors declare that there are no conflicts of interest regarding the presentation of this poster.

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Distinct and Sequential Functions of PRC2 in Radial Glia Lineage Progression

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Radial glial progenitor cells (RGPs) generate different populations of neocortical projection neurons, glia and adult neural stem cells. We recently uncovered sequential temporal transcriptional fingerprints in RGPs correlating with their lineage progression during cortical neurogenesis, and RGP proliferation behavior appears to be controlled by PRC2-mediated H3K27me3. Yet, the mechanism how PRC2 instructs RGP lineage progression *in vivo* remains elusive. Here we utilized Mosaic Analysis with Double Markers (MADM) to genetically dissect the cell-autonomous function of the PRC2 core component *Eed*. Our genetic loss-of-function approaches show that global tissue loss of *Eed* results in precocious depletion of RGPs and strong microcephaly. However, we reveal that *Eed* does not regulate RGP behavior and neuron output in a cell-autonomous manner at single cell level. Furthermore, we discover a novel cell-autonomous *Eed* function which is essential for cortical astrogliogenesis. On the transcriptional level, absence of PRC2 activity from astrocytes correlates with downregulation of genes implicated in proliferation and synapse formation. Accordingly, cell-autonomous loss of PRC2 function results in reduced proliferation rates, impaired surface expansion and reduced complexity of mutant astrocytes. Altogether, our data reveal distinct sequential requirements of *Eed* and thus PRC2-mediated H3K27me3 in RGP lineage progression during cortical development and an essential role in cortical astrocyte production and maturation.

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Genetic and environmental factors influence psychostimulant-induced behaviors in *Drosophila melanogaster*

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Drosophila is a model organism used for the study of genetic basis of addiction. Forward genetic approach requires rigorous control over environmental influences so that the genetic effect can be distinct. However, behavior is the result of the complex interaction between genetic and environmental factors. Here we present how do we measure and manipulate genetic and environmental factors in order to get a more complete picture of psychostimulant's effect on behavior and neuronal functioning.

We have developed two behavioral tests: FlyBong and FlyCafe. Using FlyBong we measure the motor-activating effects of volatilized psychostimulants, and we show that flies develop locomotor sensitization to volatilized cocaine (vCOC) [1] and methamphetamine (vMETH)[2]. FlyCafe measures rewarding properties by quantifying voluntary self-administration of food laced with psychostimulants, and we

show that flies preferentially self-administer food containing COC and METH. Both methods enable quantification of behavioral parameters in individual flies, and provide insight into individual variation. FlyBong and FlyCafe share common neuronal basis, and exposure to vMETH changes preferential consumption of METH food and *vice versa* [2]. Furthermore, there is a common genetic basis exemplified by the null mutant for the gene *period*, which does not develop locomotor sensitization, nor preferential administration of METH. Preferential self-administration of psychostimulants is further modified by rearing and mating conditions.

FlyBong and FlyCafe precisely measure individual behavior and gene-environment interactions. Quantification of psychostimulant-induced behaviors combined with genetic manipulations and their genetic homology with vertebrates shows that flies will continue to be a valuable tool for the understanding of human addiction.

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[2] Rigo F, Filosevic A, Petrovic M, Jovic K, Andretic Waldowski R. Locomotor sensitization modulates voluntary self-administration of methamphetamine in *Drosophila melanogaster*. *Addict Biol*. 2021;26(3):e12963.

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Correlation of task performance and ERP-microstate conflict-related differences are specific for Simon and flanker effects

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We recorded high-density EEG while 41 subjects performed the modified Multi-Source Interference Task (MSIT) with separate and combined flanker and Simon interference conditions [1]. To investigate underlying mental processes we applied ERP-microstate (MS) and beamformer source analyses. We also calculated between-subject correlations of task related differences in intensity and duration of subsequent MSs and their relation to task performance [2].

The same processes were involved in both conditions but the interactions between early and later processing stages were different. In flanker interference the prolongation of early P3-MS6 compared to no-conflict condition, correlated with an increased error rate, as well as lower intensity and shorter duration of the sustained potential (SP-MS7). MS6 represented activation of intraparietal sulci - the dorsal attention network (DAN), while MS7 encompassed: frontal eye fields (DAN), anterior cingulate cortex (ACC) and left motor cortex. Lower intensity and shorter duration of MS7 was in turn related to delayed RTs.

In Simon interference, the prolongation of N2-MS4, reflecting the last stages of visual processing in higher visual areas, was correlated with decreased duration and intensity of SP-MS7 and SP-MS8, which did not correlate with performance. In Simon task, slower responses were associated with decreased intensity of late P3/N450-MS5, localized on posterior cingulate cortex (PCC), previously reported in context of Stroop task [3].

To conclude, in flanker tasks the attentional processing of the stimulus is crucial for late stage processing and task performance while in Simon task behaviour is related to the activation of cingulate cortex and conflict resolution skills.

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The effects of protracted treatment with positive and negative allosteric modulators of alpha5 GABAA receptors on anxiety in Alzheimer's disease mouse model

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The causal treatment for Alzheimer's disease (AD) has yet to be discovered. Positive and negative allosteric modulators of alpha5 GABAA receptors (PAM and NAM, respectively) have emerged as ligands that could alleviate cognitive impairments, but with potential influence on anxiety. The alpha2 and alpha3 GABAA receptors are involved in anxiety regulation. Hence, we assessed effects of MP-III-022 and PWZ-029 as PAM and NAM, respectively, on anxiety and GABRA2/3 mRNA expression in 5xFAD. After 10-day-treatment with PWZ-029, MP-III-022 or solvent, 6-month-old male and female transgenic and non-transgenic (Tg-m, Tg-f; Ntg-m, Ntg-f) 5xFAD mice underwent elevated plus maze and open field. The GABRA2/3 mRNA expression in prefrontal cortex (PFC) and hippocampus (HC) were measured with qPCR.

Tg-m-control and Tg-f-control showed tendency to decrease peripheral distance (%pd) compared to Ntg-control. MP-III-022 in Tg-f increased %pd compared to Tg-f-control. PWZ-029 and MP-III-022 decreased %pd in Ntg-m compared to Ntg-m-control.

PWZ-029 tended to increase percent of open arm time in Tg-m compared to Tg-m-control. MP-III-022 increased closed arm entries in Ntg-f compared to Ntg-f-control.
GABRA2 were downregulated and GABRA3 were upregulated in HC in Tg-f-control compared to Ntg-f--control. After PWZ-029 and MP-III-022 in Tg-f HC, GABRA2 and GABRA3 were increased and decreased, respectively, compared to Tg-f-control. PWZ-029 had increased GABRA2 in Ntg-f HC compared to Ntg-f-control. GABRA2 were higher in PFC in Tg-m after PWZ-029 compared to Tg-m- control.
PAM could be related to increased anxiety in females and NAM to reduced anxiety in males, and even potentially to disinhibition, but further studies are needed.

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Tissue changes in spinal cord after spinal trauma and early applied OF stimulation

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The main intention of our study was to analyze morphological and protein changes in spinal cord after Th9 compression (SCI) and immediate long lasting stimulation with a weak oscillating field (OF) using implanted miniature stimulator (OFS). Stimulator delivered oscillating current (50μA) across the injured spinal cord by means of two Ir/Pt electrodes inserted into epidural space cranially and caudally to the injury site. This experiment consists of 3 groups of experimental animals (Wistar rats): animals with Th9 spinal compression (SCI); SCI animals with active stimulator (SCI+OFS) and SCI animals with inactive stimulator (SCI+nOFS). To examine regenerative capacity of spinal tissue after OF stimulation we performed morphometric analysis of spinal tissue integrity and fluorescent and protein analysis of neurofilaments (NF-l), newly sprouted axons (GAP-43), myelin (MBP) and glial cells (APC, GFAP). Our results indicate increased density and protein level of GAP43 and NF-l in SCI+OFS animals compared to nonstimulated animals. Analysis of MBP and oligodendrocytes (APC) showed also better myelin regeneration in group with active OF stimulation compared to SCI and SCI+nOFS groups. During 8 weeks survival period, we also monitored behavior of animals using neurological BBB score, open-field and hot-plate test. Significant improvements in recovery of sensory and motor functions in stimulated compared to nonstimulated animals were seen after 4 weeks post-SCI. According to our results we suspect that early applied OF stimulation could provide a better environment for recovery of injured spinal tissue by supporting regenerative processes.

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Long-lasting facilitation of proprioceptive Ia excitation on spinal motoneurons by anodal trans-spinal direct current polarization in SOD1 G93A mouse model of Amyotrophic Lateral Sclerosis

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Despite years of intensive research, Amyotrophic Lateral Sclerosis (ALS), remains a major challenge to both scientists and clinicians working on its management. We have recently shown that intrinsic excitability and the excitatory drive provided to spinal motoneurons (MNs) from descending and peripheral inputs are both decreased in presymptomatic SOD1 G93A mouse model of ALS. Importantly, restoring MNs excitation levels by activating the cAMP/PKA pathway with chemogenetics or by direct injection of sp-cAMP into the recorded neuron, restores the deranged postsynaptic structures, and ameliorates the disease burden. Here we provide direct evidence, that similar effects can be obtained, by applying anodal trans-spinal direct current stimulation (tsDCS) to pre-symptomatic SOD1 G93A animals. 30μA, anodal tsDCS facilitated submaximal monosynaptic Ia EPSPs amplitudes recorded from triceps sure spinal motoneuron of SOD1 G93A mice, by 43±32%, n=11 during 15 minutes of the current application. Surprisingly, this facilitation was seen even though, the cell membrane potential was strongly depolarized during polarization. Even more profound 78±48%, n=11 facilitation was seen when recordings of the same motoneurons were continued for 15 min after the end polarization. In addition, the maximal EPSPs amplitudes, which are independent of Ia afferent excitability were also facilitated by 27%, n=41, during 1h following tsDCS application. Importantly, both acute and long-term EPSPs alterations were not linked to changes in any of the cells' passive membrane properties, nor to the Ia afferent excitability. This data shows that anodal tsDCS can be used as a therapeutic tool for ALS management.

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5-HT7 receptor increases excitability of inhibitory interneurons but not projection neurons in the mouse dorsal raphe nucleus

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The 5-HT_7 receptor (5-HT_7R) is one of several serotonin receptor subtypes expressed in the dorsal raphe nucleus (DRN). Activation of the 5-HT_7R is known to increase the intrinsic excitability of pyramidal hippocampal cells, however, its effects on DRN neurons are not fully understood. This study aimed at examining the effects of 5-HT_7R activation on the excitability of different types of neurons and their synaptic inputs in the DRN of male C57BL/6J mice. Whole-cell recordings were carried out from DRN

projection neurons and local inhibitory interneurons. To activate the 5-HT_7R, 5-CT was applied in the presence of WAY 100635 to block the 5-HT_{1A} receptor. Spontaneous excitatory (sEPSCs) and inhibitory (sIPSCs) postsynaptic currents were recorded from putative 5-HT cells. Immunohistochemical analysis of DRN slices stained with anti-TPH (the marker for 5-HT neurons) and anti-5-HT_7R antibodies revealed the presence of 5-HT_7Rs in TPH+ as well as in TPH- neurons. Current clamp recordings did not reveal any changes in the relationship between injected current and spiking frequency of putative 5-HT cells after 5-HT_7R activation. In contrast, activation of the 5-HT_7R resulted in an increase in the excitability of inhibitory interneurons. Voltage clamp recordings revealed that 5-HT_7Rs modulate DRN synaptic activity by increasing the frequency of sIPSCs and decreasing the frequency of sEPSCs recorded from projection neurons. These results indicate that both DRN projection cells and non-5-HT neurons express 5-HT_7Rs but 5-HT_7R-activated intracellular signaling pathways and their targets are cell type-dependent.

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The timing and reciprocity of motor-cognitive dual-task interference: insights from electrophysiology and pursuit tracking

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In everyday task situations, perception, attention and decision processes must occur concurrently with the coordination of the body's balance and motor response. Although the mental effort associated with the latter is not often apparent, dual-task studies have found interactions between cognitive and motor tasks that suggest demands on common information-processing resources. Concurrent cognitive load is a known factor in falls risk in old age, so the importance of motor-cognitive interaction is well recognised. However, the specifics of which information-processing operations within cognitive tasks interfere with continuous sensorimotor coordination are not well understood. Here, we use electrophysiology to study the detailed chronometry of a visual oddball task comprised of perception, attention and executive functions performed concurrently with a visuomanual tracking task requiring continuous speed and direction control. Using ERP magnitudes and EEG time-frequency characteristics, we identify the timings of motor-to-cognitive and cognitive-to-motor interference. At low attentional load, the tracking task attentuates attentional resourcing without itself accumulating errors in the same time-frame. Tracking errors occur only during executive function (updating) after target detection. Only when attentional load is high do tracking errors also accumulate in the same time-frame. These results demonstrate that motor--cognitive interference is a multi-component process with asynchronous and asymmetric interference patterns that change with the dual-task workload characteristics.

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A new AAV vector carrying Glyt1 promoter for tracing glycinergic neurons

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Balancing excitation and inhibition is the key to maintain healthy vision and brain physiology. One of the systems responsible for inhibition in the brain and the eye is glycinergic system and glycinergic interneurons. The direct role in visual and information processing of these cells is not well understood. Therefore, mapping the glycinergic cells connections between different parts of the visual system is a major challenge in neuroscience research. The adeno-associated virus (AAV) tracing method, besides their capacity limits, becomes second main tool in delivering various cargos into specific cells. What more, recently the AAV is used as a helper virus in neuronal tracing studies along with the modified Rabies virus to trace connections into specific cell types [1, 2]. In this project we aim to isolate a glycine transporter 1 and 2 promoters (mGlyt1, hGlyt2, respectively) and use it to trace glycinergic cells in the brain and the retina of rodents.

Preliminary tests in HeLa and HEK293T cell lines showed expression of YFP fluorescent marker under mGlyt1 promoter only in HeLa cell line, that has active glycinergic transporter 1 expression, but not in HEK293 cell line. The AAV- hGlyt2 produced fluorescent protein in both cell lines.

Summing up we have developed the AAV vector that can specifically produce proteins of interests in glycinergic inhibitory cells both in the retina and in the brain of rodents. We also claim that this vector can be used as a helper virus for mapping connections into glycinergic network using modified Rabies virus tracing method.

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Somatosensory processing deficits and altered cortico-hippocampal connectivity in Shank3b -/- mice

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Autism spectrum disorders (ASDs) form a heterogeneous group of neurodevelopmental syndromes characterized by repetitive behaviors and social/communication impairments. Recently, hyper/hypo--reactivity to sensory stimuli has been included as a diagnostic criterion for ASD, with 90% of individuals facing atypical sensory experiences. Abnormal tactile response is considered an integral feature of ASDs, and hypo-responsiveness to tactile stimuli is often associated with the severity of ASDs core symptoms. Patients with Phelan-McDermid syndrome (PMS), caused by mutations in the SHANK3 gene, show ASD-like symptoms associated with aberrant tactile responses. However, the neural underpinnings of these somatosensory abnormalities are still poorly understood. Here we investigated, in Shank3b -/- adult mice, the neural substrates of whisker-guided behaviors, a key component of rodents' interaction with the surrounding environment. To this aim, we assessed whisker-dependent behaviors in Shank3b -/- adult mice and age-matched controls, using the textured novel object recognition (tNORT) and whisker nuisance (WN) test. Shank3b -/- mice showed deficits in whisker-dependent texture discrimination in tNORT and behavioral hypo- responsiveness to repetitive whisker stimulation in WN. Notably, sensory hypo responsiveness was accompanied by a significantly reduced activation of the primary somatosensory cortex (S1) and hippocampus, as measured by c-fos mRNA in situ hybridization, a proxy of neuronal activity following whisker stimulation. Moreover, resting-state fMRI showed a significantly reduced S1-hippocampal connectivity in Shank3b mutant mice. Together, these findings suggest that impaired crosstalk between hippocampus and S1 might underlie Shank3b -/- hypo-reactivity to whisker-dependent cues, highlighting a potentially generalizable form of dysfunctional somatosensory processing in ASD.

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Somatostatin neurons in the human prefrontal cortex

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Somatostatin positive cells represent a major population of GABAergic neurons in the human cerebral cortex. Using double labelling immunofluorescence and RNAscope *in situ* hybridization we characterized the somatostatin cell population in the human prefrontal cortex (PFC). We analyzed histological slides from five adult specimens and evaluated the results in two regions of the PFC – Brodmann areas 9 and 14. There were no observable qualitative differences between the two areas and, therefore, the results presented are applicable to both regions of the PFC. After thorough qualitative analysis, we found that somatostatin cells in the supragranular layers were small (soma diameter of approximately 10 μm), had a high expression of somatostatin mRNA and a relatively low expression of somatostatin peptide. Furthermore, almost all of the supragranular somatostatin cells colocalized with calbindin, indicating that somatostatin and calbindin positive cells represent a single cell population in the supragranular layers. In the infragranular layers, somatostatin cells were large (soma diameter of approximately 20 μm) with complex dendritic morphology, typically had a lower expression of somatostatin mRNA and a high expression of somatostatin peptide, and did not express calbindin protein. All somatostatin cells were also NeuN positive, confirming that this is indeed a neuronal cell population. In conclusion, in the human PFC, supragranular and infragranular somatostatin cells represent two distinct subpopulations of somatostatin neurons.

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Psychopathology and resting state functional connectivity in adult ADHD: a resting state fMRI study

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Attention-deficit/hyperactivity disorder (ADHD) is a common childhood psychiatric disorder that often persists into adulthood. Cortese et al. reported altered activity in resting state brain networks in ADHD in individual studies however failed to find significant spatial convergence of results across studies (1). In addition, existing studies focusing on adults barely studied the effect of psychopathological characteristics on resting state functional connectivity.

Adults with a clinical ADHD diagnosis (N = 39) and healthy, adult subjects (N = 17) underwent a 9-minute resting-state fMRI session in a 3T MRI scanner. After preprocessing, we used Seed-to-Voxel based correlation analysis with 28 seed regions (based on subparts of 7 well-known resting state networks). One-way ANCOVA was used with age covariate for group analysis, while regression was used for CAARS scores. Correction for multiple comparison was carried out with a cluster-forming voxel-level height threshold of $p<.005$ and a spatial extent threshold that ensures a cluster-wise FWE at $p<.05$ (2). We used CONN toolbox for preprocessing, denoising and statistical analysis (3).

Left lateral parietal region of DMN, right FEF region of DAN and PPC region of FPN showed altered functional connectivity in ADHD. Furthermore, psychopathological measures, such as CAARS, showed significant effects in the connections of these seed regions.

Despite the fact that altered resting state connectivity in ADHD is heterogenous, our results are in line with all other studies, suggesting some sort of disturbed activity in ADHD which persists to adulthood. Psychopathological association with certain seeds of connections may highlight the importance of those regions.

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Learning of non-visual spatial task in two strains of house cricket *Acheta domesticus* with altered visual pigments synthesis

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The insects' eyes are composed of numerous isolated facets with separate lenses and photosensitive elements present in each. Those facets are separated by pigment cells, containing so-called screening pigments, including two groups of compounds; pteridines and ommochromes. The role of those pigments is speculated to provide optical isolation of the compound's eye facets, thus contributing to visual acuity. As both groups of the pigments are derivatives of tryptophan (TRP), mutations involving their synthesis pathways could cause alteration not only at the level of pigments but other derivatives of TRP, including neurotransmitters [1]. Thus, the mutant strains of insects with defects in this pathway provide valuable models both for studying the role of TRP in insect behaviour as well as the broader scope of pathologies related to this metabolic pathway (as demonstrated by extensive usage of the *white* strain of *Drosophila melanogaster*).

The poster presents data obtained from assays on three strains of *Acheta domesticus* – wild type, *yellow* and *white*. As the nature of mutation involves vision – a non-visual learning task [2] was chosen for the study in order to exclude the possible impairment in vision. The task consisted of finding an inconspicuous cool spot on the arena heated to aversive temperature. It was found that while the learning performance of the *yellow* strain does not differ significantly from the wild type, the *white* strain learned to successfully complete the task significantly faster. Implications of this result as well as further prospects of research are discussed on the poster.

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Normalization of visual responses explains size tuning in the rat collicular neurons

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BACKGROUND: The receptive field of many visual neurons is composed of a central responsive area, the classical receptive field (CRF), and a non-classical receptive field, also called the 'inhibitory surround' (IS). In IS visual stimuli do not induce any response but modulate responses to stimuli within CRF, usually by suppressing them. Therefore, usually, visual response amplitude grows for relatively small increases in the visual stimulus size, smaller than the CRF width, while responses to large stimuli decrease due to suppression by the surrounding IS. The stimulus size corresponding to the maximal response is called an optimal stimulus size. In cortex there is fairly good correspondence between the CRF and the optimal stimulus sizes. In contrast, there is no apparent correspondence between the sizes of CRF and the optimal stimulus in the rat superior colliculus (SC).

METHODS: Extracellular recordings employing tetrodes in the urethane-anaesthetized adult rats (>2 months of age) were used for this study.

RESULTS: We find that in the rat SC the optimal stimulus size changes as the inverse of the stimulus contrast. However, the CRF width is insensitive to contrast changes. These results are explained by normalization of the responses through a divisive modulation of the CRF gain by IS. To explain data on responses to stimuli displaced from the CRF center, an additional step of normalization is necessary, most likely due to saturation to light flux.

CONCLUSIONS: Multiple steps of visual response normalization play crucial role in the visual information processing in rodent SC.

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Heart rate regulation model to capture fluctuating higher-order influence by brain during emotional tasks

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Although there are theoretical models and studies investigating the association of brain activity with changes in heart rate, the existing mathematical and algorithmic models of the heart rate dynamics are mainly limited to simulation of lower-order mechanisms, i.e. in response to autonomic nervous

system activity at sinus node, respiration and baroreflex. The aim of this research was to develop an algorithmic model of heart rate regulation that incorporates higher-order levels than those associated with respiratory arrhythmia and baroreflex. A working primary model with interconnected components of heart rate regulation mechanisms was realized in MATLAB SIMULINK environment. It uses the real human psychophysiological data – R-R intervals and respiration – as the input data. The heart rate dynamics is modelled, fitted to traditional lower-order mechanisms, dynamics unexplained by lower-order mechanisms attributed to the fluctuating influence by higher-order brain regulation. The time moments are found when the presumably relevant activity in higher brain areas changed, the corresponding heart regulation weights are modelled and the higher-order regulation dynamics are returned as the output data. Psychophysiological data from study with emotional tasks were re-used to illustrate capabilities of the proposed model.

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TrkC downregulation increases the number of primary dendrites in opossums' Purkinje cells

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Opossums, *Monodelphis domestica* are born at very immature stage of development. The nervous system of newborn opossum is at a developmental stage comparable to embryonic day 11 in the mouse. We used this animal model, because opossums' development is slower and more expanded in time than in laboratory rodents, thus some molecular mechanisms are easier to capture. Cerebellar Purkinje cells are large GABA-ergic cells known from their unique and impressive dendritic tree. However, the molecular basis of its formation is not yet fully known. It is well established that one of the most important proteins involved in the processes of development and plasticity of neurons are neurotrophins and their receptors. In this study we focused on TrkC receptor which binds to neurotrophin-3.

The aim of this study was to investigate the function of TrkC receptor in the development of neurites in opossums' Purkinje cells.

We found that the peak proliferation of Purkinje cells progenitors occurs at postnatal day 3, therefore we used opossums at this age and prepared primary cerebellar cell cultures. We transfected the cells with TrkC shRNA or control shRNA control plasmids expressing green fluorescence protein (GFP) and cultured the cells for 7 days. Cell cultures were fixed and immunostained with calbindin, a marker for

Purkinje cells and GFP and the colocalization was determined using a confocal laser microscope. Sholl analysis was performed on healthy looking, double-labeled cells.
The statistical analysis revealed a significant increase in the number of primary neurites in Purkinje cells with lowered expression of TrkC receptor in comparison to control cells. Further research is required to understand the mechanism and function of this structural changes.

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Utilization of conditional single lentivirus CRISPR/Cas9 vector for efficient gene knockout generation in adult neurons *in vivo* and *in vitro*

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The study of neurodegenerative diseases, such as Parkinson's disease (PD), relies on animal models, which have certain caveats reflecting the complex aspects of the disease. They are mostly based on genetic basis of the disease or neurotoxines which do not reflect idiopathic forms. Using the CRISPR/Cas9 system, which has recently revolutionized the field of biotechnology, we have created a universal construct that will be used to generate transgenic animals in a more efficient. Single lentiviral, CRE-dependent vector with gRNA targeting the selected protein specific to the disease will be delivered by stereotaxic injection into to the brain of CRE-bearing mice in a specific cell populations.
We provide a standardized workflow for assessing mutagenesis in population of targeted neurons. The efficacy of this approach is demonstrated *in vitro*, in primary dopaminergic mice neurons. We used gRNA targeted EGFP to visualize the effect of the mutation. Lentivirus transduction lowered EGFP expression by approx. 50% (shown by qPCR and fluorescent staining). We also confirmed it *in vivo*, making an attempt to create a pre-symptomatic PD model based on applying Cre-dependent lentiviral vector carrying the *Rrn3* deletion (transcriptional factor, TIF1-A) directly to locus coeruleus in DBHCre mice. We obtained progressive degeneration of noradrenergic neurons restricted to locus coeruleus (up to 30-50% cell loss after 3 months from injection).
We found this approach to be more efficient than conventional gene knockout allowing targeting cells that would be difficult to differentiate and reducing time-consuming animal breeding in classic Cre/loxP mediated recombination.

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Modulatory effects of grafted neuroectodermal stem cells after chronic spinal cord contusion injury

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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. Many of these lesions remain untreated and a chronic injury develops. In this study we investigated the integration and differentiation capacity of neuroectodermal stem cells transplanted into the chronic lesion cavity of the spinal cord and the host cellular reactions to the grafted cells.

NE-4C stem cells (ATCC: CRL-2926) were grafted intraspinally five weeks after a thoracic (T11) spinal cord contusion injury performed in Sprague-Dawley rats. Control animals underwent contusion injury without stem cell transplantation. Five and ten days after transplantation detailed immunohistochemical analysis was performed to evaluate the fate and the modulatory effects of grafted cells.

Five days after transplantation, the grafted cells survived remarkably, formed clusters and a small proportion of the cells differentiated into neurons and astrocytes. At this time point, the NE-4C cells did not migrate away from the grafted area. Ten days after grafting the majority of the grafted cells appeared as nonviable fragments in microglia/macrophage cells. This observation suggests a fast elimination process of the transplanted stem cells. On the other hand, the grafted cells induced significant reduction of microglia/macrophage and astrocytic reactions in the treated groups compared with the control animals. These data provide evidence for the effective modification of the lesion microenvironment despite limited survival of grafted cells after chronic spinal cord injury.

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Keywords: spinal cord injury, chronic contusion, stem cells, secretome

BDNF-induced BDNF release mediates experience-dependent long-term potentiation at mossy cell-granule cell synapse

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Our brains have evolved to adjust behavior in response to new experiences. This crucial process mainly relies on activity-induced modifications of synaptic contacts, such as long-term potentiation (LTP) and depression (LTD), whose underlying mechanisms are remarkably diverse and not fully understood. The brain-derived neurotrophic factor (BDNF) is a key mediator of synaptic efficacy, neural connectivity and use-dependent plasticity. However, despite many years of work, the mechanism by which BDNF/TrkB signaling mediates synaptic plasticity remains unclear.
Within the dentate gyrus (DG), the main entry area of the hippocampus, hilar mossy cell (MC) to dentate granule cell (GC) synapse expresses a robust form of presynaptic LTP. Using restricted genetic deletion to selectively disrupt BDNF expression in either the presynaptic (MC) or the postsynaptic (GC) neuron, we showed both pre- and postsynaptic BDNF are required for LTP in acute mouse hippocampal slices. Using two-photon live imaging of BDNF-pHluorin, a pH sensitive fluorescent BDNF sensor, we found that BDNF released from the presynaptic compartment can elicit postsynaptic BDNF release in a TrkB- and calcium-dependent manner. In addition, activation of type-1 cannabinoid (CB_1) receptors, which are highly expressed in MC axon terminals, negatively regulates presynaptic BDNF release and LTP, likely through inhibition of voltage-gated calcium channels. Finally, we found that BDNF-dependent MC-GC LTP can be elicited *in vivo* following enriched environment exposure.
Altogether, these results suggest that BDNF-induced BDNF release is a key mechanism underlying LTP which might contribute to DG-dependent cognitive functions, as well as epilepsy.

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Effects of caffeine and age on sleep and behaviour in *Drosophila melanogaster*

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Caffeine is known to reduce nighttime sleep in the fruit fly *Drosophila melanogaster* [1]. In case of mammals, caffeine enhances dopaminergic activity by a competitive antagonism to adenosine receptors [2]. In flies, however, the role of adenosine receptors and caffeine on sleep and behaviour is not fully described. We examined the role of caffeine on daytime (siesta) and nighttime sleep in wild-type flies (CantonS) of different age and in transgenic flies with the *dAdoR* gene overexpression or silencing in glia, *tim*-expressing clock cells, and dopaminergic neurons. Our results showed that caffeine reduces sleep during the night and increases siesta in wild-type male flies at different age. In females, however, caffeine increases siesta in old flies (50 days old) but decreases in younger 30 days old ones. We also found that caffeine treatment reduces night sleep, but not siesta in transgenic flies with the silenced

dAdoR gene in dopaminergic neurons and glial cells. In turn the overexpression of *dAdoR* gene in the same cells after feeding with caffeine had no effect on sleep. It means that the regulation of siesta involves adenosine and its receptors but sleep during the night is also regulated by other mechanisms. Apart from that, the effect of caffeine on sleep in the fruit fly varies with age and sex of flies.

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The effect of chronic treatment with 1MeTIQ on frontal and hippocampal BDNF and PSD-95 level in rat model of schizophrenia

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Schizophrenia is a severe mental disorder affecting ~1% of population [1] and may be characterised by positive, negative and cognitive symptoms. However, pathophysiology of the illness still remains unclear. Some studies suggest role of brain-derived neurotrophic factor (BDNF) and post-synaptic density (PSD)-95 in schizophrenia [2]. 1MeTIQ is an endogenous compound, found in mammalian brain, exerting neuroprotective and pro-cognitive action [3], therefore we decided to evaluate BDNF and PSD-95 levels in rats' frontal cortex and hippocampus in animal schizophrenia model.

Male Sprague-Dawley rats were injected with saline, MK-801 (0.1mg/kg), 1MeTIQ (25 or 50 mg/kg) or 1MeTIQ combined with MK-801 (6 groups) for 7 consecutive days. After that time, animals were decapitated and frontal cortex and hippocampus were dissected for assaying BDNF and PSD-95 levels using Western Blot method.

We found lowered frontal BDNF level in all experimental groups. MK-801 decreased BDNF level, however 1MeTIQ (in both doses) did not reverse this effect. PSD-95 level in rat's frontal cortex did not change significantly after drugs treatments. In hippocampus, BDNF level was increased in MK-801 group, comparing to saline. Combined treatments (1MeTIQ and MK-801) decreased BDNF level. Hippocampal PSD-95 level was found to be lowered after combined treatment.

In our study, 1MeTIQ in both doses did not reverse effect of MK-801 and did not restore proteins level. However, more studies are needed to evaluate the mechanism of interaction between BDNF and PSD-95 and their role in schizophrenia, leading to developing new, promising therapies for the illness.

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Effect of air pollution with particulate matter on the expression of genes connected with immunological response in EAE model of multiple sclerosis (MS) in mice

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A high concentration of air pollution with particulate matter (PM) is known to be a risk factor for human health. Exposure to PM causes a pro-inflammatory response, disturbances in antigen presentation, dysregulation of lymphocytes' function and activity. These phenomena can lead to autoimmunological diseases. Multiple sclerosis (MS) is a neurological disease with processes of inflammation and demyelination of axons in which autoimmunological processes play an important role. EAE is the animal model of MS induced by administration of immunogenic peptides from myelin. We aimed to investigate the effect of PM devoid a majority of organic compounds by treatment with cold plasma (LAp120) on expression of 93 selected immune-related genes in the spinal cord in mice in EAE model. The TLDA arrays with TaqMan probes and real-time PCR were used to identify particular genes. We found massive changes induced by EAE whereas changes caused by LAp120 were much smaller, and LAp120 x EAE interaction as well. Statistical analysis with 2-way ANOVA showed that EAE changed the most (85) of examined genes. Whereas LAp120 and LAp120 x EAE changed only 10 genes. Detailed analysis revealed the increase in expression of Hmox1, Stat 1, Stat 6 and Mmp2 mRNAs. Interestingly Igf2 mRNA expression was increased by EAE and next intensified by LAp120. This change of Igf2 mRNA expression may reflect the adaptive, recovery processes in the spinal cord during EAE. Surprisingly, LAp120 seemed to augment these processes. Our study found that LAp120 had only a modest effect on the progression and intensity of EAE.

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Studying the addictive *vs*. natural reward exposure in mice – whole-brain imaging approach

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Whole-brain imaging of optically cleared tissue is a rapidly developing research area. Whereas many successful attempts to clear and image mouse brain have been reported, there is still a limited number of studies where such techniques were used to answer neurobiologically relevant questions[1]. Herein, we report on using this method to study how addictive and natural rewards are processed by the brain. Such rewards are known to activate mesolimbic pathway but their influence on other brain structures is less known. Thus, we were studying effects of the initial exposure to addictive and natural substances on the whole network of the mouse brain.

As a model of addictive reward we chose cocaine intraperitoneal injections, while sucrose self-administration mimicked the natural one. After the behavioral training, mice brains were extracted and underwent iDisco protocol to optically clear the tissue. During this procedure, we immunohistochemically labelled c-Fos protein to mark cells in the brain, which were activated after reward treatment. Then, samples were imaged with a light-sheet microscope. Finally, with use of a ClearMap software we fitted our images into virtual map of the mouse brain to automatically calculate number of c-Fos-positive neurons in the whole brain.

Together, we analyzed over 400 structures and we found that even single exposure to cocaine or sweet water cause excitation of distant parts of the brain. Principally strong activation we found in structures belonging to the reward system. Moreover, we found regions selectively activated by addictive rewards (e.g. visual cortex) or natural ones (e.g. olfactory system).

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The effect of growth factors on the regeneration after spinal cord compression

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The aim of the presented study was to analyze the effect of increased levels of BDNF&GDNF and their receptors TrkB&Gfrα on the spinal cord tissue after Th9 compression (SCI). The increase of growth factors levels was induced by 6-weeks endurance training before trauma. After 6-weeks survival, we monitored the regenerative capacity of spinal cord in SCI and pre-training + SCI groups (T+SCI). Our results showed increased mRNA expression and protein levels for Gap-43 in T+SCI group compared to the SCI

group. In addition, immunohistochemical analysis showed longer Gap43+ fibers and higher density of neurofilaments in the pre-trained animals. We also monitored factors specific for glial-cells (Iba1, GFAP) and oligodendrocytes (APC) in injured tissue. The aggregation of Iba1+ and GFAP+ cells around the lesion site was noticeable in both groups. However, in the T+SCI group we observed visibly more APC+ cells around the epicenter of injury. During survival, we also monitored the BBB-neurological score. Significant differences between groups were recorded on the 8th and 42nd day of survival. These findings suggest that pre-training increases the levels of growth factors and could induce better microenviroment for the regeneration of the damaged spinal cord and thus promote motor activity of paralyzed animals.

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Chirp-evoked haptic and visual responses – a study on basic response properties in healthy humans

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Chirp-modulated signals are the type of short oscillatory signals with monotonically increasing frequency. Such stimulus temporal structure offers an efficient way to stimulate sensory systems across wide frequency bands. EEG chirp-evoked responses can be observed on time-frequency plots, either as momentary changes in power or in phase coherence. In our study we used this type of stimulation in somatosensory and visual domain to elucidate EEG responses in healthy humans. In the somatosensory domain we presented series of haptic, vibro-tactile chirps within 1-35 Hz range delivered by a vibrating haptuator placed on the finger. In the visual domain the stimuli (also within 1-35 Hz range) were delivered by a centrally positioned white LED. We have utilized two types of monotonic frequency progression: linear and logarithmic. EEG inter-trial phase clustering parameter was analyzed. In haptic stimulation condition the strongest responses were observed for both types of progression in two regions: fronto-central, and centro-parietal, contralaterally to the stimulated hand. The response was observed within 15-30 Hz range in both regions. In the visual condition the responses for both types of progression were observed in the occipital region. In this condition, the frequency following responses were observed only for stimulation above 5 Hz. For the lower frequencies we observed event-related responses. This exploratory study confirms that chirp-evoked haptic and visual responses may serve as a method for testing the integrity of the cortical sensory areas.

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Descending control of lumbar spinal cord neurons by rostroventromedial medulla

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The rostroventromedial medulla (RVM) contains several classes of neurons projecting to spinal cord (SC) and is considered critical for the descending regulation of nociceptive pathways [1], yet its mechanisms remain poorly understood. Here we studied mechanisms of this modulation using *ex-vivo* spinal cord elctrophysiology and optogenetic stimulation of descending fibers. Adult mice were injected into the RVM by AAV9-YFP-ChR2 vector, resulting in a strong ChR2 expression in axons within the dorsolateral funiculus and grey matter of the lumbar spinal cord with the highest expression in the laminae I and X. We did patch-clamp recordings of lamina I neurons in the intact spinal cord preparation while stimulating the dorsal roots [2]. ChR2-expressing RVM axons were optically stimulated (10 ms @480 nm, 5Hz) via a microscope objective. Such an approach allowed investigating the inputs from descending fibers and their impact on the primary afferent-driven responses. We found that a proportion of lamina I neurons receive mono- and polysynaptic inhibitory inputs from RVM axons. Importantly, polysynaptic excitatory post-synaptic currents (EPSCs) evoked by dorsal root stimulation were significantly decreased by photostimulation of RVM axons. Moreover, a decrease of monosynaptic EPSCs amplitudes induced by primary afferent stimulation was also observed, implying the direct presynaptic inhibition as a possible mechanism of descending control. Thus, our study suggests complex and diverse ways of RVM descending modulation of lamina I neurons mediated by pre- and postsynaptic mechanisms.

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A single exposure to predator odor induces long-term anxiety but not fear in outbred female rats

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Despite higher prevalence of post-traumatic stress disorder (PTSD) in women, the majority of preclinical neuroscience research has been conducted utilizing male subjects [1]. We have reported that a single exposure to the predator odor 2,4,5-trimethyl-3-thiazoline (TMT) causes anxiety-like behavior via Elevated Plus Maze and Acoustic Startle Response testing 7 days post-TMT [2]. Male rats segregated into stress-Susceptible, -Resilient, and Intermediate phenotypes based on a median split conducted on EPM and ASR behavior also displayed differences in conditioned fear weeks later; Resilient males exhibited increased mGlu5 mRNA expression in the basolateral amygdala (BLA) and prefrontal cortex. Here we sought to determine whether the same would be observed in Resilient females. Sprague--Dawley rats (n=40) were exposed to TMT for five minutes, while Controls were similarly exposed but with an unscented environment (n=14). Behavioral tests (EPM, ASR, light/dark box, and sucrose preference) were conducted 7-14 days later to assess anxiety and anhedonia. TMT-exposed females exhibited a reduction in time spent in EPM open arms compared to Controls. Interestingly in ASR testing, TMT-exposed females habituated to acoustic stimuli, while males do not. TMT-exposed rats showed a reduction in sucrose consumed compared to control rats. Unlike male Susceptible rats who show enhanced contextual fear when re-introduced to the TMT-environment, female Susceptible rats showed no such freezing, nor did Resilient female rats present increased BLA mGlu5. This work indicates that, as in humans, rats exhibit sex-dependent responses to stress. This translational animal model may provide insight into how females are uniquely affected by PTSD.

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Study of Neuronal Network Excitability in Rat Autism Model

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Autism Spectrum Disorder is one of the most frequent neurodevelopmental disorders with the prevalence of 1.7 %, which is characterized by impairments in social interactions and repetitive behavior. Caused by the imbalance of excitatory and inhibitory circuits, morphological and functional changes can be observed in several brain regions e.g. the prefrontal cortex and the hippocampus [1][2]. It is well-established in rats that prenatal valproic-acid (VPA) exposure on embryonic day 12.5 leads to neurodevelopmental aberration with autism-like clinical and behavioral alterations. The aim of this study is to reveal the details of the higher excitability of neuronal networks and single neurons elicited by prenatal VPA treatment.

Wistar rat dams in the autistic group received 500 mg/kg VPA i. p. on gestation day 12.5. Brain slices of 6-week-old and 3-month-old offspring were investigated. Field potential and whole-cell patch clamp recordings were carried out to measure network excitability and single cell activity in the hippocampus and the prefrontal cortex.

Basic excitability of hippocampal networks was altered in the 6-week-old males, however this change could not be observed in 3-month-old males. Single neuron activity was increased mainly in females, both in the 6-week-old and 3-month-old groups but in different fashions.

In conclusion, VPA treatment had different effects regarding to the sex and the age of the animals. It seems that some alterations manifested in 6-week-old animals may be compensated later and also other changes may develop until the age of 3 months.

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The authors declare that they have no conflict of interest

Decreased GCase protein level in cultured primary macrophages in derived from patients with sporadic Parkinson's disease

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Background

The GBA gene encodes the lysosomal enzyme glucocerebrosidase (GCase), which hydrolyzes glucosylceramide and glucosylsphingosine. Variants in GBA are among the most common genetic risk factors for Parkinson's disease (PD). In some cases, reduction in GCase activity has been linked to PD.

Objective

To compare GCase protein level in cultured macrophages derived from patient blood monocytic cells (PBMC) of patients with sporadic PD (sPD), PD patients with GBA mutations (GBA-PD), asymptomatic carriers of GBA mutations (asympGBA) and controls.

Methods

Mononuclear fraction was isolated from the whole blood of GBA-PD (N=10), asympGBA (N=7), sPD (N=11) and healthy controls (N=10) with subsequent differentiated into macrophages using RPMI supplemented with 10% FBS, 1% streptomycin-penicillin and 10 ng/ml M-CSF for 4 days, with daily media changes. Total protein (10µg) was separated by SDS-PAGE and then transferred to PVDF membranes by electroblotting. Primary anti-GBA and anti-GAPDH antibodies were used. Digital images were obtained by the chemiluminescence system ChemiDoc.

Results

In our study, we showed differences in the amount of GCase protein level in PBMC-derived macrophages from sPD and asympGBA compared to controls. A decreased protein level has been observed in asympGBA 0,49 (-0,16 – 1,18) and sPD 0,78 (-0,09 – 1,85) patients compared to controls 1,82 (0,46 – 3,74) (p=0.012, p=0.025).

Conclusions

sPD patients and asymptomatic carriers of GBA mutations are characterized by decreased GCase protein level in cultured primary macrophages.

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The authors have no actual or potential conflict of interest.

Pulse respiration quotient – posture and breathing regime patterns with possible impact for artificially ventilated patients

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Cardiorespiratory coupling (CRC), expressed as pulse-respiration quotient (PRQ, number of heart beats per respiratory cycle) represents a bidimensional autonomic variable (1) most probably reflecting cardiorespiratory ventilation-perfusion efficiency (2). Different physiologic states – supine and standing with spontaneous breathing (Sup and St, respectively), and the same states with slow 0.1Hz paced breathing (Sup01 and St01) are characterized by different patterns of CRC(3). The aim of our work was to investigate 20-minute-by-minute PRQ values dataset (PRQ mean and SD) within the respective state, reflecting the state specific dynamics of PRQ regulatory networks. For 20 healthy adults we computed RRI and respiratory frequency variability from a 20-minute ECG recording (Biopac® MP160), following the protocol of (3). For each physiological state we plotted one minute horizontally averaged values of PRQ mean and SD. Visualy, we observed that PRQ mean notably changes between the states, while SD for all states grouped roughly around the value of 2. We considered PRQ mean and SD as two separate independent variables, potentially differently regulated. We report that PRQ mean significantly increases following the sequence sup-st-sup01-st01, while SD increments following the sequence sup--sup01-st01-st. This might indicate that the measures of variability of autonomic parameters are not always directly corresponding to their tone. If we, hypothetically, asume that PRQ mirrors ventilation/perfusion ratio (2),our postural maneuvers with slow 0.1Hz breathing could enrich cardiorespiratory retraining practice of outmost importance for the patients with decreased capacity for blood oxigenation and integrative cardiorespiratory action, as severely damaged in COVID 19 patients (4).

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The stereological analysis of the human subthalamic nucleus

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The subthalamic nucleus (STN) is a small, lens-shaped structure providing the only glutamatergic input to the basal ganglia circuits. Like other basal ganglia structures, the nucleus is conventionally divided in three functional parts: motor, associative, and limbic territory. This subdivision is particularly useful for planning neurosurgical procedures like deep brain stimulation in which electrodes are implanted in the motor portion of the nucleus. However, the number of STN subdivisions reported in literature varies from 0 to 4 and the intricacies of its cellular composition are not well understood [1, 2] . In order to further explore STN neuronal populations, we performed immunohistochemical analysis of several well-known neuronal markers (NeuN, parvalbumin, calretinin, calbindin, nNOS) and transcription factors implicated in specifying neuronal phenotype (FOXP2, PAX6, NKX2.1) on post mortem adult human STN [3]. Using Stereo Investigator software, we estimated neuronal density, counted the number of immunoreactive neurons for each marker, and described their spatial distribution. The coordinates of neurons and contours of each STN were extracted to enable the final visualization of data using custom Python scripts. Generally, the analysed markers can be divided into two categories: one with density greater than 3000 neurons/mm^3 (nNOS, PAX6, Nissl, FOXP2) and second with density around 2.200 neurons/mm^3 (NeuN, NKX2.1, parvalbumin, calretinin). We didn't observe clearly delineated zones of expression for each marker, but we detected a gradual increase of neuronal density from dorsolateral to ventromedial tip of the nucleus.

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Changes in DNA methylation between chronic and acute anxiety and depressive states

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Stress as an environmental factor plays an important role in the pathogenesis of depressive and anxiety disorders. It is already widely accepted that stress mediates the epigenetic landscape and thus influences gene expression. Among epigenetic mechanisms, DNA methylation has been associated with the onset and development of depressive and anxiety disorders.

The aim of the pilot study was to investigate DNA methylation patterns of candidate genes involved in neurotransmission, stress and neuroplasticity (BDNF, COMT, SLC6A4) in relation to the duration and severity of heterogeneous anxiety-depressive states.

Patients' symptoms were assessed using clinical scales measuring separate symptom dimensions (IDS-C, HAM-A, and Beck-BDI), and DNA methylation was determined in the blood of 25 patients. Linear regression models showed a statistically significant negative correlation ($p < 0.05$) between only one specific amplicon of the COMT gene and clinical symptom severity for all clinical scales (the largest principal component explained 26% of the variance). The correlation between methylation and disease duration (current episode or total lifetime) was not statistically significant.

Based on the correlations between COMT amplicon and symptom expression, we concluded that DNA methylation likely represents a flexible process that depends more on the current stress level than on the duration of the disorder.

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The moderating effect of caregiving experiences on adult romantic relationships: a gene-by-culture investigation

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Parents play a crucial role in child development and foster a less or more adaptive social environment [1]. At a biological level, such affiliative behaviours are regulated by the oxytocin system [2]. This investigation aimed to probe the effect of Oxytocin Receptor Gene (OXTR) and caregiving patterns on the anxiety and avoidance felt in romantic relationships. 313 adults from Western (Italian) and Eastern (Singaporean) cultures filled the questionnaires Parental Bonding Instrument (PBI) and Experiences in Close Relationships-Revised (ECR-R), respectively self-reported measures of perceived quality of relationship with parents in childhood and expectations on close relationships in adulthood. Buccal mucosa samples were assessed, and OXTR rs53576 (Study 1: Western G/G *vs* A-carriers; Eastern A/A *vs* G-carriers) and rs2254298 (Study 2: Western and Eastern G/G *vs* A-carriers) polymorphisms were analyzed. For each study, we considered one ECR-R dimension as a dependent variable, the genetic region as a unique between-subject factor, and the PBI dimensions as covariates within a set of hierarchical multiple regressions (HMR) performed on the Italian and Singaporean samples separately and merged. Study 1 highlighted distinct gene-by-environment and gene-by-culture interactions on the adult attachment [3]. Regarding OXTR rs53576, a differential susceptibility of genotype to the recalled parental bonding is observed between Western and Eastern groups on the levels of felt anxiety and avoidance. In contrast, no significant interactions on the outcomes emerged from Study 2 when OXTR rs2254298 was analyzed. These studies provide further evidence of the asymmetric effects of genetic, environmental and cultural factors on social relationships.

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Analyzing the role of inhibitory neurons in the induction of synchronized patterns in the primary visual cortex using computational modeling approach

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One of the essential questions in neuroscience is how the brain dynamically collects, processes, stores, and retrieves information in a rapidly changing environment. It has been suggested that synchronization among the neurons enables the brain to perform required computations. Therefore, synchronization in the neuronal network could have a vital role in forming perception, learning, and attention. Visual stimulation can lead to rhythmic and synchronous firing patterns in the visual cortex. The various factors that cause and are involved in this process have not yet been clearly identified. It has been hypothesized that the emergence of synchronization in gamma-frequency can be the output of an information-rich and sparse process [1]. Despite the significance of this subject, our knowledge through neuronal synchronization is limited, fundamentally due to the overwhelming complexity of brain networks [2].
We developed a biophysical data-driven model of the L4 in the primary visual cortex using the BMTK toolbox [3]. The model consists of different types of excitatory and inhibitory neurons. Simulation results show that inhibitory neurons contribute to the induction of the synchronized activity in L4 of the primary visual cortex. Here, beside the role of different inhibitory neurons, we investigate various factors such as number of synapses, inhibition delay, and disinhibitory mechanism in the generation of oscillations.
To the best of our knowledge, this study is the first computational biophysical modeling of the L4 layer that simultaneously explores the role of various factors influencing synchronization and their frequency between neurons.

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Evaluation of the effects of sweroside on cognitive performance and oxidative status in the zebrafish (*Danio rerio*) model of Alzheimer's disease

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Alzheimer's disease (AD) is the most common neurodegenerative disorder, being associated with memory disorders and cognitive decline that ultimately affect thought, reason, video-spatial orientation and behavior. AD is associated with mitochondrial dysfunction and a significant difficulty in cholinergic transmission, therefore, there is a special interest in the selection of different compounds capable of regulating acetylcholinesterase (AChE) activity. Recently, it has been discovered that several plant iridoids have significant neuroprotective effects, being able to slow down the process of neurodegeneration. The mechanisms of action of iridoids have been shown to be mediated by the inhibition of cholinesterases including AchE. We focused on one natural iridoid, Sweroside which is described in studies as an iridoid with beneficial effects on the SNC, without adverse effects. The Sweroside was administered to zebrafish, chronically by immersion, daily for 16 days. To induce memory loss and anxiety we administered scopolamine. As a positive control we used Galantamine. Anxiety was measured using the Novel Tank Test (NTT), spatial memory was assessed using by Y-maze test, and recognition memory was assessed using by the Novel Object Recognition Test (NOR). We also evaluated the impact of the Sweroside on the cholinergic and oxidative state of this animal model. Our results show that Sweroside can effectively restore the antioxidant defense mechanism by increasing the level of antioxidant activity in the brain and can improve cognitive dysfunction of amnesic fish by inhibiting AChE, which is also correlated with improved memory parameters, as shown in behavioral approaches (NTT, Y maze and NOR).

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Perceiving an emotionally-charged body

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The mental representation of the body can be explored by motor imagery (MI) tasks, such as the hand laterality task (HLT) [1]. With hands, we interact with our body, with other people, and with the environment. These contacts might cause disgust, for which drivers include body products (e.g., faeces) and body envelope violations (e.g., amputation) [2]. However, not much is known about how disgust changes our MI processes.

In this study, we examined whether there is any difference in the processing of disgust-charged hands versus non-emotionally charged hands when considering MI.
Thirty-six participants completed an online version of a (neutral) HLT [1] and two emotionally charged (disgust) HLT versions, in which hands were partially covered in faeces or with the index finger amputated. Stimuli were presented 0°, 90°, 180° and 270° rotated, to calculate two effects: the effect of stimulus orientation (SO), indicative of visual imagery (VI), and the effect of biomechanical constraints (BC), a MI index.
In both VI (SO – $p < .001$, $\eta^2 = .27$) and MI (BC – $p < .001$, $\eta^2 = .20$) the presence of faeces over hands led to faster performance, in comparison to the other two types of stimuli.
Our results show that body products such as faeces that remind of contamination are more salient when the mental representation of the body is involved, but this salience is not restricted to MI and affects also VI.

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Diverse roles of the various inhibitory cell types in the prelimbic cortex in social bonding

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Although much is known about the neuroanatomy of social bonding, still its functional circuitry is poorly understood. Although many studies indicate a crucial role of inhibition in the prefrontal cortex (PFC) in sociability, still the involvement of the vasoactive intestinal peptide (VIP)-expressing interneurons in that process remains unexplored. Hence, we investigated the effects of the artificial activation of the VIP-expressing cells in the prelimbic part (PL) of the PFC and evaluated its influence on the interest in familiar social stimuli and interactions with conspecifics.
Animals were tested in Eco-HAB, an ecologically relevant, RFID-based system for assessment of sociability in group-housed mice, which enables continuous, individualized measurement of voluntary

behavior. Using genetically modified mice selectively expressing Cre protein under the VIP promoter combined with the PSAM/PSEM-based chemogenetic approach, we performed a time-constrained, cell-specific stimulation of the VIP neurons in the PL and tested subjects social behavior during the 90-minute period following the systemic administration of the drug (PSEM) activating virally introduced artificial ligand-gated ion channels (PSAM).

We show that PL-constrained inhibition evoked by the chemogenetic excitation of the VIP neurons does not alter approach to familiar social stimuli. Moreover, such manipulation of neuronal activity does not change the amount of time animals voluntarily spend together or the way they follow one another throughout the Eco-HAB territory. Notably, selective activation of the parvalbumin-expressing cells in the PL impairs all those aspects of sociability. Taken together, our data point to the diverse roles various inhibitory cell types play in regulation of social bonding.

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Oxytocin modulates the expression of neuronal markers in early brain development

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Aims: The aim of the study was to determine the effect of oxytocin on morphological changes in hippocampal GABAergic and glutamatergic neurons and changes in gene expression of different cell types in the hippocampus during postnatal brain development.

Methods: Incubation of rat primary hippocampal cells with 1 μM oxytocin (48 hours / 8 days) followed by confocal microscopy was used to detect the effect of oxytocin on the length and branching of the GABAergic and glutamatergic neurons. After neonatal oxytocin administration (second and third postnatal day, 0.1 μg/μl oxytocin), changes in gene expression of specific neuronal markers for GABAergic, glutamatergic, serotonergic, dopaminergic and cholinergic neurons, were detected by qPCR at 5, 7 and 9 postnatal day (P5, P7, P9).

Results: Measuring the number of neurite branches by Sholl analysis did not show differences between the control and oxytocin group. Oxytocin treatment significantly decreased the gene expression of GABA marker and marker of cholinergic cells on day P5 and P7 and significantly reduced expression of glutamatergic dopaminergic, serotonergic cell markers on day P7.

Conclusion: These results suggest that oxytocin has an impact on hippocampal neuronal cell fate decision with potential neurodevelopmental consequences.

The authors declare that there are no conflicts of interest.

Brief maternal separation prevents the potentiation of cocaine CPP induced by social defeat stress, but not the deficit of social avoidance observed in defeated mice

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Stress induced by repeated social defeat (RSD) reduces social interaction in mice and enhances the conditioned place preference (CPP) induced by cocaine [1]. The stress inoculation hypothesis presupposes that brief exposure to intermittent stress early in life, for example maternal separation (MS), induces the subsequent development of stress resistance [2, 3]. Our aim was to expose mice to a brief MS in order to promote resilience to the effects of RSD on social interaction and cocaine CPP in adulthood. To this end, four groups of male C57BL/6 mice were used; two groups were exposed to MS for 6h on postnatal day (PND) 9, and another two groups were not (controls). On PND 47, 50, 53 and 56 mice who had experienced MS were exposed to an episode of social defeat by a resident aggressive mouse (MS+RSD group) or were allowed to explore an empty cage (MS+EXPL group). The same procedure was performed with control mice that had not undergone MS (CONTROL+RSD and CONTROL+EXPL groups). Next, all the mice performed the social interaction test (PND 57) and CPP with 1 mg/kg of cocaine (3 weeks after the last episode of RSD). In control mice, exposure to RSD reduced the frequency of social interaction and increased the rewarding effects of cocaine. MS only prevented the effect of RSD on CPP. Our results suggest that exposure to a brief episode of stress early on in life increases the subsequent resilience of animals to the effects of social stress on cocaine reward.

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Empathic reaction to pain in people with a social phobia - a study using facial electromyography

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Social phobia is characterized by an intense fear of social situations and anxiety of reading negative signals and emotions from the facial expressions of other people. Previous research shows that people with social phobia have a distorted perception of emotional expressions and experience increased empathy for negative emotions. However, there is a lack of data on how people with social phobia react to other people's pain. Pain expression is an important social signal often related to anxiety, therefore people with social phobia might be expected to show a strong response to such stimuli.
This study aimed to determine differences in the empathy level measured as a trait and empathy reaction for pain. Participants of high and low levels of social phobia (N=27 and N=32, respectively) watched movies showing various pain situations and during exposure to these stimuli, the electrical activity of the *corrugator supercili* (CS) and *orbicularis oculi* (OO) muscles were measured using facial electromyography (EMG).
The results of the research confirmed the existence of differences in empathy measured as a trait (based on questionnaires) and showed significant differences in empathy measured in the situation of watching people in pain. People with a high level of social phobia assessed the pain more intensely, experienced higher personal distress and empathetic concern than people with a low level of this phobia. The study also showed that there is a significant difference only in the activity of OO muscle in people with a high level of phobia.

The authors declare that they have no conflict of interest.

Short alcohol sensitization results in long-lasting changes in motivation to drink

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Regular alcohol abuse lead to physical and mental dependency described like an alcohol addiction. The neuronal basis of this process are still poorly understood, thus, we investigated the behavioral and molecular consequences of the alcohol treatment.
Mice injected (i.p) with ethanol (2 g/kg) for 7 days, developed context-independent sensitization of locomotor response observed after 7-day withdrawal; and it was further enhanced after 30-day incubation.

Interestingly, this short protocol resulted in higher motivation for alcohol and increased alcohol seeking during withdrawal measured close-to-ethologic conditions in the IntelliCages. Alcohol sensitization was accompanied by structural (analyzed with Serial Block-face scanning Electron Microscopy (SBEM)) and functional (whole-cell patch-clamp) changes of the central nucleus of the amygdala (CeA) glutamatergic synapses and decreased levels of the autophosphorylated form of αCaMKII. In agreement with this observation αCaMKII autophosphorylation-deficient heterozygote mutant mice (αCaMKII-T286A+/-) showed enhanced sensitization as well as increased alcohol consumption in the IntelliCages, and surprisingly lower alcohol seeking during withdrawal and cue relapse.

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Investigation of the cyto- and chemoarchitecture of the central cervical nucleus

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Central cervical nucleus (CeCv) is a spinal cord gray matter nucleus located in upper spinal cord segments (C1-C4) in humans and other vertebrates, within the limits of lamina 7, adjacent to lamina 10 as a continuous column. CeCv neurons receive afferents from neck muscles, joints, semicircular canals, and project to the contralateral cerebellum, and ipsi- and contralateral vestibular nuclei, as shown in animal studies. In this study, we aimed to reveal the cyto- and chemoarchitecture of CeCv for the first time in the human spinal cord. 10% formalin-fixed cord was divided into C1-C4 segments and cut on a cryostat at a thickness of 35μm. Nissl histochemical and CGRP, ChAT, GAD65/67, calbindin, calretinin immunohistochemical stainings were performed; sections were imaged under light microscopy, and analyzed using ImageJ. CeCv neurons were multipolar, ovoid, triangular and fusiform in shape. Average section area of the CeCv was measured as 286.11μm^2. The largest CeCv neurons were 42,6x16,65μm in size, and the smallest 11,84x10,43μm. In all segments, CGRP, ChAT, GAD65/67, calbindin and calretinin immunoreactivities were observed in CeCv neurons. ChAT immunoreactivity was observed in very high, CGRP, GAD65/67, Cb, Cr in high density. When compared with the sections in our earlier spinal cord atlas, we noted that the cytoarchitecture of the CeCv was similar to that of the rat, mouse, marmoset and rhesus monkey [1]. For CGRP, ChAT, Cb and Cr immunoreactivities, interspecies variability was observed. We believe this findings will contribute to literature with detailed novel data for this important precerebellar nucleus.

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Data-driven model of striatal external input

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The basal ganglia play an important role in a variety of functions as decision making and action selection mainly based on input from cortex, thalamus and the dopamine system. Striatum is the input stage of the basal ganglia. It consists of 95% striatal projection neurons of two types (dSPNs and iSPNs) and 5% interneurons including the cholinergic (ChIN), fast-spiking (FS), and low-threshold spiking (LTS). They are all included in our study.

Here we model the extrinsic inputs from cortex and thalamus to the different population of striatal neurons, each of which have different dynamics and synaptic strengths. We simulate detailed multicompartmental cell models with subsets of synapses to mimic neuronal behaviour and reproduce experimental data.

First, we present a data-driven model of the postsynaptic currents mediated by NMDA or AMPA receptors based on whole-cell patch-clamp recordings from striatal neurons obtained during optogenetic stimulation of primary motor cortex, primary somatosensory cortex and the parafascicular nucleus. We describe the dynamics using three exponentials, one for the rising and two for the decay phases. This enabled us to get a very reproducible simulated response during both single traces and with spike trains.

Second, we introduce a classification of the synaptic traces (recorded using the same method) for each input region and each cell type.

Finally, we optimize the parameters of a Tsodyks-Markram model to match the dynamic of the synapses.

These models are being integrated into large scale networks, as part of our ongoing effort to create detailed simulations of the entire striatum.

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The authors declare no competing interest.

Correlation structure between brain regions in working-memory tasks: fMRI fractal and spectral analysis

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False memories are a topic that has enjoyed decades of fascinating research [1]. In this paper, we study with fMRI diurnal variation of short-term memory distortions in four types of experimental tasks [2]: two visual-verbal (V; based on lists of semantically or phonetically associated words) and two non-verbal (NV; based on pictures of similar objects), in memorisation and retrieval phases. Since functional activations have a non-trivial auto-correlation and cross-correlation structure, we quantified the activity of regions of interest (parcellated with 116-region AAL atlas) with the Hurst exponent (H), detrended fluctuation analysis and detrended cross-correlation coefficients [3]. The signals in specific occipital lobe areas depend not only on the type of experimental tasks but also on information memorisation or retrieval. A particularly apparent difference is visible between memorisation in V and NV tasks. In the former, for some brain regions in the Visual II resting-state (RS) network, the H exponents are very close to 0.5, indicating a lack of linear temporal correlations. In the latter, we observe persistent behaviour. The reduction of H in tasks relative to the spontaneous brain activity in RS is significant in many brain areas. We additionally uncovered regionally coordinated changes by comparing distributions of eigenvalues of cross-correlation matrices. These results were strengthened by grouping eigenvalues according to their eigenvector similarity rather than their natural order. The detrended correlations were more sensitive than linear ones, showing the greatest differences between: RS and other tasks, memorisation and retrieval, V and NV tasks.

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Effect of acute alcohol intoxication on1/f neural noise in lateral septum

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Brain activity shows both oscillatory dynamics that typically is analyzed in the time-frequency domain to describe oscillatory phenomena and the broadband scale-free background activity characterized as 1/f neural noise or pink noise of the brain. Neural noise changes were detected in neurodegenerative and psychiatric disorders. Dynamics of neural noise is sensitive to age, specific neurotransmitter systems and drug-induced changes including alcohol [1, 2]. Lateral septum is a part of limbic system which is involved in formation of alcohol use disorders (AUDs). We tested the changes in neural noise induced by acute alcohol intoxication in LS for whole spectrum (1-200 Hz) and frequency range refers to beta band (12-30 Hz). Five male Wistar rats were implanted with intracranial electrodes and local field potential (LFP) signal was collected or baseline activity and activity induced by acute ethanol intoxication (2 g/kg). Change in neural noise dynamics was assessed as a change in slope of linear regression fit of power spectral density curves in double logarithmic scale.Our findings showed that alcohol resulted in flatten of scale free 1/f signal in LS for whole spectrum range, which is interpreted as increase in neural noise. At the same time we observed decrease of neural noise for beta frequency range. As was reported previously, alcohol alters the nonlinear structure of brain activity and increase noise, which is consistent with the change for whole spectrum, except beta frequency range which may by reflect activation of GABA transmission in response to alcohol [3].

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Discovering the brain regions in Osteoarthritis patients responsible for chronic pain sensation using convolutional neural network

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Chronic pain is a complex phenomenon, which is still not sufficiently explored. Chronic pain occurs in various illnesses, such as osteoarthritis. Being the most prevalent form of arthritis, osteoarthritis occurs due to the weakening of the protective cartilage that cushions the bones' ends over time. This paper investigates the effect of chronic pain in the brain, employing deep learning algorithms using resting-state fMRI data of osteoarthritis pain patients and healthy controls. The dataset used in this study consists of fMRI data of 51 pain patients and 20 healthy subjects. This study proposes a deep learning-based computer-aided diagnosis framework comprising Multi-Layer Perceptron and Convolutional Neural Network to classify chronic pain-affected osteoarthritis patients from healthy controls. Among the investigated algorithms, the study finds that the CNN outperformed and yielded the highest accuracy of around 85%. The algorithms' high performance not only shows distinguishable features within the data, but may also increase on a higher number of data. Furthermore, the affected brain areas have been investigated. This study successfully identified a few regions such as the occipital lobe, the superior frontal gyrus, cuneus, middle occipital gyrus, and culmen, which were never mentioned in literature. This study is the first of its kind to explore the applicability of deep learning algorithms for investigating the brain regions distinguishable in osteoarthritis patients having chronic pain. This work's outcome may contribute to the research of osteoarthritis pain patients and support fMRI-based pain recognition, leading to improved clinical intervention for chronic pain patients.

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EEG-behavioral phenotyping of three mouse models of autism

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Synapses are essential structures shaping the activity of the nervous system. Dysfunctions in the synaptic protein's network are related to numerous disorders. We studied the brain electrical activity and behavioral reactions in three mouse strains with the mutations in genes related to autism spectrum disorder (ASD) in humans. The genes (and corresponding proteins) were: STXBP1 (Munc18-1), NLGN4 (Neuroligin-4), and NRXN1 (Neurexin-1).
The brain electrical activity (EEG) was recorded from the cortical surface using NeuroLogger (NewBehavior AG) during staying at home cages and several behavioral tests. We expected to find the disturbances related to ASD symptoms in humans.
First, we analyzed the baseline EEG (at the home cage) and circadian activity patterns. No changes in sleep duration and structure were revealed. STXBP1-mutants are characterized by an increased probability of spontaneous paroxysmal activity (hypersynchronous theta activity, spikes, and seizure-like paroxysms), which indicates an imbalance between excitation and inhibition in cortical networks. NRXN1-mutants had greater levels of high-frequent EEG activity (beta band) showing greater cortical arousal.
Surprisingly, no prominent autism-related differences in social behavior were revealed between controls and mutants in all strains. The most essential behavioral effect was found in reaction to novelty in NRXN1-mutants: a strong but transient behavioral interest in the exploration of the object. Such exploration was related to an increase in the theta band peak frequency in controls but not in mutants. This may indicate the lack of hippocampal activation related to the exploration of new objects in NRXN1-mutants, which underlies the novelty processing abnormalities in autism.

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Impact of mitofusin 2 in the nucleus accumbens on motivated behaviour and underlying neurobiological mechanisms

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Mitochondrial dysfunction is increasingly being implicated in the aetiology of psychiatric disorders, but the underlying cellular and molecular mechanisms have not been fully identified [1]. Recent data from animal research indicates that high anxiety is associated with alterations in the mitochondrial fusion

protein mitofusin 2 (Mfn2) in the nucleus accumbens (NAc) [2], a part of the ventral striatum essential for motivation and goal-directed behaviour. Here, the consequences of downregulated Mfn2 levels in the NAc are investigated for motivated behaviour, dendritic structure and synaptic physiology of NAc medium spiny neurons (MSNs).

Upon Mfn2 downregulation in D1-MSNs via tamoxifen treatment or viral-mediated vectors, animals' performance was evaluated in the forced swim test, an effort-based motivation task. The results showed that Mfn2 deletion in NAc D1-MSNs promotes the adoption of passive coping behaviours, as indicated by increased immobility. This was associated with a decreased activation of NAc shell neurons during the task, as revealed by cFos mapping. In *ex vivo* electrophysiological recordings, we found that -as compared to wild-type D1-MSNs- D1-MSNs with *mfn2* deletion show higher intrinsic excitability, reduced excitatory synaptic inputs and reduced dendritic complexity.

These results indicate that Mfn2 downregulation impacts the morphology and electrophysiological properties of accumbal D1-MSNs, leading to impaired engagement of the NAc during effort-related behaviour. Our findings can illuminate the understanding of the mechanisms underlying motivational disorders and pave the way for the development of novel treatment strategies to depression.

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Visual P300 as an index of cognitive functioning in individuals with aphasia

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Aphasia is an acquired impairment of language functions resulting from a brain lesion. It is often accompanied by deficits in non-language cognitive functions, such as attention, working memory and executive functions which may intensify language difficulties and hinder the rehabilitation process.

P300 is a large positive ERP wave appearing after the occurrence of infrequent stimulus. Its latency, reflecting stimulus classification speed, is considered an index of cognitive decline in many clinical dysfunctions, including stroke.

The study included 26 participants with post-stroke aphasia. Several language and non-language cognitive functions were assessed behaviourally. ERPs recording was performed using visual Go/No-Go task. Signal from electrodes on the midline of the scalp was analysed. P300 was identified in 300-600ms time window.

Neither P300 latency nor amplitude was associated with age, post-stroke time and lesion volume. In contrast, shorter P300 latency was associated with better planning ability, divided attention, spatial working memory, psychomotor speed and the auditory speech comprehension (evidenced in Token Test). These results showed that P300 latency is a sensitive index of general cognitive functioning in subjects with aphasia. The associations were observed with both non-language cognitive functions and higher level language function, such as comprehension of complex sentences.

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Partial kappa-opioid receptor agonist nalmefene regulates social behaviours

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The endogenous opioid system plays an important role in the modulation of social interaction and bonds formation. Here we investigated the effects of nalmefene, a partial kappa-opioid agonist, on social reward and motivation to interact with another animal. We tested C57BL/6 mice in the social conditioned preference place test (sCPP) and social interaction in the open field test (SI). In the sCPP, 31-35 days old male mice were conditioned to associate one context with group housing and another with isolation. The rewarding effects of social contact were assessed by comparing preference for a context associated with group housing before (pre-test) and after (test) the conditioning. In the SI, we tested adult males and females. Interaction with an unfamiliar, same-sex mouse was measured in 10 minutes-long sessions. In both procedures, nalmefene (1 mg/kg) was injected one hour before testing. In the sCPP, mice showed preference to the socially-associated context typical for the early-adolescence period. No preference was observed after nalmefene treatment and the effect was reversed by kappa-opioid antagonist – norbinaltorphimine (10 mg/kg). In the SI, nalmefene resulted in shortened distance between interacting animals and extended cumulative duration of interaction. The effect was restricted only to females. Our results show the ability of nalmefene to differentially modulate social behaviours via its action through the kappa-opioid receptor. We hypothesise that the effects can depend on the motivational state before the test or the familiarity with a stimulus animal.

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Differences between the hippocampus and neocortex in the autophagy response to hypobaric hypoxia

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Autophagy is a highly regulated mechanism of degradation and recycling of misfolded proteins and organelles in the cell. Neurons are highly differentiated cells with extended processes, and therefore their functioning largely depends on the mechanisms of autophagy. Autophagy becomes extremely important under different harmful conditions, including hypoxia. In the present study we investigated the autophagy response of different brain regions (hippocampus and neocortex) of rats to hypobaric hypoxia (180 mm Hg, 3 h). We used immunohistochemistry to reveal LC3 protein levels and RT-PCR analysis to assess the expression of the related gene *map-lc3*. First, it was found that LC3 turnover was higher in the CA1 field of the hippocampus rather than neocortex of control rats. Second, in the hippocampus a decrease in LC3 levels was found on the first day after hypoxia which was restored to control values by the third day. Together with this the expression of *map-lc3* increased by the 3 day after the exposure. No changes in protein or gene expression levels were found in the neocortex after hypoxia. The data obtained indicate that autophagy mechanisms differ in the cells of hippocampus and neocortex both in normal conditions and under hypoxia challenge. We suppose that autophagy is activated by hypoxia leading to quick degradation of LC3 in the cells of the hippocampus. As a result *map-lc3* expression increases to compensate the lack of protein. In the neocortex the large LC3 intracellular store may provide the response to hypoxia without intensification of gene expression.

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Different faces of neurons expressing dopamine receptors in motor cortex – their laminar distribution, electrophysiological properties and role in skilled forelimb reaching

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Motor cortex comprise the primary descending circuits for flexible control of voluntary movements and is critically involved in motor skill learning. However, due to the complexity of motor cortex circuits, precise mechanisms of motor control and skill learning are still not well understood.

Here we have used transgenic mice, electrophysiology and neural tract-tracing methods to target genetically defined cell types expressing D1 and D2 dopamine receptors. We observed that D1+ and D2+ neurons are organized in a separate, largely non-overlapping populations, as evidenced by the laminar distribution of their cell bodies, colocalization and projection patterns. Moreover, based on *ex vivo* patch-clamp recordings we shown that D1+ and D2+ cells have distinct electrophysiological properties. Finally, we observed that chemogenetic inhibition of D2+, but not D1+ neurons disrupts skilled forelimb reaching in adult mice.

These results suggest that dopamine receptor-expressing cells in motor cortex are organized into separate, non-overlapping circuits and that they play specialized roles in fine motor control. We believe that a better understanding of the function of these dopamine-sensitive circuits can be a key to the development of new and more effective therapies for people suffering from neurological disorders and stroke.

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Alterations of synaptic mitochondria in the brain of the autistic-like rats induced by maternal immune activation during pregnancy

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Maternal immune activation (MIA) has been implicated as a risk factor for the development of autism spectrum disorders (ASD), however, the molecular links between maternal infection and autistic phenotype remain unclear. Mutations in synaptic proteins and alterations of mitochondrial functions are considered important pathogenic factors in ASD. Due to the critical function of synaptic mitochondria in the mechanisms of neurotransmitter release and synaptic plasticity, our study aimed to analyze the structure and function of these organelles in the brain of adolescent rats prenatally exposed to MIA.

MIA model was induced by a single intraperitoneal injection of lipopolysaccharide (LPS, 100 µg/kg b.w.) to pregnant rats at embryonic day 9.5. On the 52-53 post-natal day male offspring were decapitated and the synaptosomes isolated. Transmission electron microscopy, spectrophotometric and spectrofluorimetric analysis were used to determine mitochondrial ultrastructure and function, respectively.

Electron-microscopic study demonstrated ultrastructural changes in synapses of rats prenatally exposed to MIA, such as diminished packing density of synaptic vesicles in presynaptic area, as well as blurred and thickened structures of synaptic clefts. Moreover, alterations of synaptic mitochondrial morphology, including fragmented cristae, expanded matrix compartment, and membrane disruption were observed in both cerebral cortex and hippocampus of MIA offspring. Our results also demonstrated se-

vere impairment of mitochondrial function in MIA-affected offspring - reduced mitochondrial membrane potential and ATP level. Concomitantly, the activity of Complex I was decreased.
In conclusion, MIA-evoked alterations in synaptic mitochondria reveal potentially important aspects of the mechanism linking neuroinflammation and ASD pathology.

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Maternal immune activation leads to alterations of mitochondrial biogenesis and dynamics in offspring brain development

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Maternal immune activation (MIA) has been linked to an increased risk of autism and other neurodevelopmental psychiatric disorders. Infections during pregnancy activate the mother's immune system and alter the fetal environment, but links between infection-induced altered fetal development and CNS dysfunction in the offspring are unknown. Recent evidence suggests that mitochondrial dysfunction could participate in the development and clinical features of autism.
Here, we investigated the developmental changes of mitochondrial dynamics and biogenesis in the rodent MIA model. Pregnant rats (9,5 days of gestation) were subjected to lipopolysaccharide (LPS; 0.1mg/kg, intraperitoneally) and the offspring were sacrificed on 7th or 52-53rd, post-natal day (PND), and the brains were isolated. The biochemical and molecular biology methods were applied.
Our results indicate that MIA induces age-dependent alterations of the fusion-fission regulatory proteins in the offspring brain. At 7 PND we observed up-regulation of mitofusin 1 (Mfn1) and mitofusin 2 (Mfn2), with a concomitant increase in dynamin-related protein 1 (Drp1) and fission1 (Fis1). When offspring reach adulthood (52-53 PND), the level of fusion proteins (Mfn1 and Mfn2) significantly decreased in the brain of MIA offspring, whereas the expression of fission regulator - Drp1 remained increased. Moreover, we observed the decrease of the activity of citrate synthase, a common marker of mitochondria level, which is accompanied by the down-regulation of the mitochondria biogenesis regulators, Pgc1α and Tfam.
These findings provide evidence that mitochondrial dynamics and biogenesis are compromised in offspring prenatally exposed to MIA and that mitochondrial dysfunction occurs during adolescence.

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The Possible Effects of the Wobbler Mutation on miRNA Deregulation

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Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease, which is characterized by rapid progressive degeneration of motor neurons in the central nervous system (CNS). Wobbler mouse, which has a spontaneous (*wr*) mutation in Vps54 gene, can be used to study ALS *in vivo*. VPS54 is one of the components of Golgi associated retrograde protein (GARP) complex and it takes role in retrograde vesicular transport of molecules from early/late endosomes to recycling endosomes and trans Golgi network (TGN). The *wr* mutation causes to the destabilization of VPS54 protein, which in turn destabilizes the GARP complex. As a result, wobbler mice show similar symptoms and cellular defects to ALS patients, in terms of vesicle trafficking defects, impaired axonal transport, protein aggregations and mitochondrial dysfunction. Additionally, miRNAs take role in the gene regulation responsible for several cellular pathways including neuronal survival, differentiation and apoptosis. Deregulation of miRNAs can make neurons more vulnerable to oxidative stress and contribute to the development of neurodegenerative diseases. So far, many different miRNAs have been associated to ALS. In this study, we dissected the ventral horn of the gray matter from the spinal cord by using laser micro dissection microscopy and performed qPCR for miRNAs which have been associated with motor neuron degeneration and neuroinflammation in the literature. The downstream pathways of these miRNAs will be validated by further experiments. Overall, we aim to unravel the effects of the *wr* mutation on miRNA deregulation by using wobbler mice.

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Modelling *ARID1B*-related Coffin-Siris syndrome using iPSC-derived neurons

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Abstract

Mutations in genes encoding the subunits of the BAF chromatin remodelling complex underlie a spectrum of neurodevelopmental disorders (NDDs). *ARID1B* is the most commonly mutated gene in patients with intellectual disabilities (ID), ranging from a syndromic form of ID, Coffin-Siris syndrome (CSS), to autism spectrum disorders (ASD). The mechanism by which disruption of *ARID1B* and the BAF complex leads to various disorders remains unclear. We generated excitatory glutamatergic neurons from

induced pluripotent stem cells (iPSCs) derived from CSS patients with mutations in *ARID1B*, as well as an *ARID1B*$^{+/-}$ CRISPR line generated from control iPSCs. Using Micro-electrode arrays (MEAs) to investigate the spontaneous neuronal network activity of the neurons, we found that *ARID1B*$^{+/-}$ neurons showed delayed developmental trajectory in comparison to control neurons. In support of this phenotype, network activity of *ARID1B*-deficient neurons showed a differential response to blockage of glutamate receptor. Single cell electrophysiological recording and immunofluorescence data also suggests altered synapse development in *ARID1B*-deficient neurons. These results give insight into the molecular mechanism by which loss-of-function of *ARID1B* leads to different subtypes of NDDs.

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The effect of food restriction on the number of parvalbumin-expressing neurons in the cortex of 5xFAD mice

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Numerous beneficial effects of food restriction on brain aging and age-related neurodegenerative diseases such as Alzheimer's disease (AD) are well documented. Since AD is characterized by decades--long, clinically silent prodromal phase of the disease, the aim of the present study was to examine the effects of preventive intermittent, every-other-day (EOD) feeding regimen in 5XFAD mice, well characterized animal model of AD. Comprehensive analysis of parvalbumin-expressing (PV) neurons in different parts of the cortex was performed by immunohistochemistry, in 6-month-old female 5xFAD transgenic (Tg) mice and their non-transgenic littermates exposed to *ad libitum* (AL) or EOD feeding regimen for 4 months. The number of PV neurons was determined independently in the *retrosplenial dysgranular cortex*, *retrosplenial granular cortex*, *parietal cortex*, and *somatosensory cortex*. Immunohistochemical analysis revealed loss of PV neurons in the *retrosplenial dysgranular* and *parietal cortex* of Tg-AL mice in comparison to non Tg-AL animals. On the other hand, EOD feeding increased the number of PV neurons in all cortical subregions except *retrosplenial granular cortex* (in which the number remained unchanged) in comparison to age-matched Tg-AL animals. In addition, there was no change in the number of PV neurons among non-Tg mice regardless of the feeding regimen applied. The present study indicates that every-other-day feeding regimen can ameliorate PV neuronal loss, and have important role in further understanding of neural basis of AD-like-associated cognitive impairments in 5xFAD mouse model of AD.

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Effects of biguanides on mitochondrial permeability transition pore opening in intact brain cells

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Mitochondrial permeability transition pore (MPTP) is thought to be involved in ischemia/reperfusion-induced cell death in various organs, including the brain. Thus, inhibition of MPTP is considered as a target for neuroprotection. A biguanide compound metformin has been found to exert neuroprotective effects possibly involving modulation of mitochondrial functions. The aim of this study was to investigate the effects of various biguanide compounds - metformin, phenformin, imeglimin on ionomycin-induced MPTP opening in intact brain cells.

Cultures of isolated rat neuronal and astrocytic cells were pre-treated with biguanides and were loaded with calcein-AM and $CoCl_2$. Then ionomycin was added allowing entry of excess Ca^{2+} into cells and triggering MPTP opening which was observed as subsequent loss of mitochondrial calcein fluorescence measured with fluorescence microscope.

We found that in neurons 2-3 mM metformin protected against ionomicin-induced MPTP opening. Phenformin exerted partial protective effect at 50 μM and at 100 μM concentration fully prevented MPTP opening. Imeglimin exerted partial protection at 1 μM and completely blocked MPTP opening at 10 μM concentration. In astrocytes, 2-3 mM metformin, 1-10 μM imeglimin and 50-100 μM phenformin partially suppressed ionomycin-induced MPTP opening.

Our data suggest that metformin, phenformin and imeglimin prevent MPTP opening in intact neurons and astrocytes.

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The role of parathyroid hormone 2 receptor in depression-like behaviour caused by psychosocial stress

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Chronic psychosocial stressors are major aetiological risk factors for depression. In mice, one form of environmental manipulation proposed to model aspects of human psychosocial stress is chronic social defeat (CSD). The objective of the study was to identify a key component of the evolving depression-like behaviour following CSD, parathyroid hormone 2 receptor (PTH2R). PTH2R is a G-protein coupled receptor whose endogenous ligand is tuberoinfundibular peptide 39 (TIP39). Both the receptor and its

ligand are abundant in limbic brain regions, such as the infralimbic cortex, lateral septum, amygdala, paraventricular hypothalamic nucleus. We previously demonstrated that PTH2R KO mother mice showed increased depression-like behaviour (Gellen et al., 2017). Based on the previous findings, we hypothesized a role of the PTH2R in depression-like behaviour provoked by psychosocial stress. We applied a 10 days exposure of continuous presence of a dominant intruder mice separated by a divider in the home cage, with brief daily experience of actual physical attack and defeat. This protocol leads to the manifestation of depression-like phenotype in stress-treated mice (Golden et al.,2011). PTH2R KO male mice and their WT male littermates were compared to assess symptoms of depression-like behaviour in various behavioural tests, such as anhedonia, despair, social aversion, anxiety and abnormalities in eating behaviour. We found differences in some aspects of behaviour of stressed PTH2R-KO mice compared to stressed WT animals. The data suggest that the PTH2R may contribute to the development of mood disorders.

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The modulation of thalamic reticular nucleus neurons by corticotropin-releasing hormone

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The thalamic reticular nucleus (TRN) is a main modulator of thalamic function. It is known as a sleep spindle pacemaker, generating brief oscillations that appear during NREMS. Stress can negatively impact sleep, which can contribute to psychiatric disorders. Previous literature indicates that the corticotropin-releasing hormone receptor 1 (CRHR1) is highly expressed in TRN neurons[1]. How the stress neuropeptide, corticotropin-releasing hormone (CRH), affects TRN function has not been explored.

Using *in situ* mRNA hybridization, we found that CRHR1 has a significantly higher expression in TRN parvalbumin-positive neurons compared to somatostatin-positive neurons. Parvalbumin-positive TRN neurons are known to play a major role in sleep spindle generation by firing low-threshold calcium bursts that entrain the thalamocortical loop.

We investigated the effects of CRH on TRN neuron firing pattern (bursts *vs*. tonic firing) using *ex-vivo* electrophysiology. CRH (200nM-1μM) induced a firing modality shift in TRN neurons mediated by CRHR1, by decreasing the number of bursts.

Employing transgenic mice (CRH_IRES_Cre) and viral methods (rAAV2), we mapped the source of endogenous CRH projections to the TRN. Our data indicate diverse, central sources of CRH, and none from hypothalamic regions.

We found that CRH decreases the TRN neuron's propensity for bursting, suggesting a potential impact on sleep spindle generation that we will assess in future *in-vivo* recordings. Revealing the interaction between the CRH system and sleep regulation at the TRN may help advance our understanding of how stress affects sleep spindles, and their related functions in sleep stability and cognitive abilities, processes often impaired in psychiatric disorders.

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Individuals with blunted cortisol responsiveness to a laboratory stressor display low fear conditioning responses

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There are individual differences in the physiological stress responsiveness to stressors. Studies based on the delivery of laboratory stressors typically find a percentage (around 15%) of subjects that show blunted cortisol levels as opposed to a larger proportion mounting a more 'normative' cortisol response. Here, we aimed at characterizing potential differences between these types of participants in a fear conditioning and extinction paradigm. To this end, we probed a database in which participants engaged in two separate visits (fear conditioning on day 1, stress exposure in virtual reality on day 2). Our cohort contained 85 healthy male participants split into cortisol responders (N=40) and non-responders (N=45) according to their cortisol changes between successive saliva samples obtained during experimental day 2: if their cortisol changes did not rise above the 1.5nmol/L threshold, participants were defined as non-responders. The fear conditioning paradigm consisted of 4 blocks: habituation, 2 acquisitions and extinction. Significantly lower skin conductance responses (SCRs) were observed across all trials and to both conditioned stimuli (CS+/CS-) in cortisol non-responders. In particular, compared to responders, non-responders showed a more accentuated SCRs reduction in response to CS+ from the second acquisition block onwards, displaying signs of early recovery. Furthermore, non-responders felt significantly less anxious about the CS+ when asked to rate their feelings following extinction. Our results support recent interpretations of the psychobiological phenotype of healthy subjects showing blunted cortisol responses to laboratory stressors pointing at a more efficient coping and early recovery. However, further studies are warranted to investigate this hypothesis.

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The amyloid precursor protein intracellular domain affects hippocampal synaptic plasticity in Alzheimer's disease: a computational modeling study

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Alzheimer's disease (AD) is an irreversible and incurable brain disorder, characterized by progressive memory loss and cognitive dysfunction. In early AD, the alterations in amyloid precursor protein (APP) processing and clearance of APP peptides are observed. Increased APP levels lead to the production of AD related peptides such as amyloid beta, and the amyloid APP intracellular domain (AICD) at higher concentrations [1]. It was recently shown that AICD modifies intrinsic excitability of hippocampal CA1 pyramidal neuron and impairs synaptic plasticity in AD [1].

The aim of this study is to investigate the effect of the pathological AICD levels on long-term potentiation (LTP) and long-term depression (LTD) in a detailed computational model of a CA1 pyramidal neuron. We used a detailed compartmental model [2] and included the influence of the elevated AICD levels by increasing the conductances of SK channels and L-type calcium channels. At a synaptic level, the contribution of the GluN2B-containing NMDA receptor (NMDAr) was also increased. A modified NMDAr dependent voltage-based model of synaptic plasticity [3] was used to analyse synaptic strengths at clustered Shaffer collateral synapses.

The results support the experimental indication that pathological concentration of AICD leads to LTP disruption and leaves LTD intact in AD. The model provides insight into the complex interactions in AD pathophysiology and suggest the conditions under which the synchronous activation of a cluster of synaptic inputs targeting the dendritic tree can concur in generating the observed signal at the soma after a LTP conditioning period.

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Complicated grief in a 68 year-old patient with a resistant depressive episode: a case report

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Depression in elder patients often presents with clinical features that significantly differ from those found in depressed younger adults [1] and, in elder patients presenting with a first episode of depression, neurological conditions should be systematically excluded before establishing a psychiatric diagnosis [2].
We present a case report of a 68-year-old woman who consulted the psychiatric emergencies for a sub-acute anxiety syndrome that rapidly evolved during the first days of hospitalisation in a psychiatric hospital. The patient developed psychotic symptoms including persecution delusions and Cotard's syndrome. Later on, she developed gaze difficulties and breathlessness. We conducted several neurological investigations including a thorough neurological examination, an MRI scan, an EEG, an extensive analysis of the cerebrospinal fluid, and a PET scan, all of which were normal. The patient was treated with three lines of antidepressants —sertraline, trazodone, and amitriptyline—, as well as with aripiprazole, but showed no signs of response or remission, and electro-convulsive therapy (ECT) was initiated. The patient showed a rapid improvement with this therapy, but some weeks later she developed manic symptoms, after which we stopped the treatment with amitriptyline. One month later, the patient still presented hypomanic symptoms but could go back home. One year later, she maintains her treatment with aripiprazole and ECT and has not shown any signs of relapse.
This case report describes an atypical presentation of a first late-onset depressive episode that responded to ECT.

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Glial clocks contribute to the sleep regulation in *Drosophila melanogaster*

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In *Drosophila melanogaster* the main role in maintaining circadian rhythms plays the pacemaker located in the brain, however, peripheral oscillators, including glial cells, are also important components of the circadian system. In the present study we investigated an impact of glial oscillators located in astrocyte-like glia, the chiasm giant glia of the optic lobe, the epithelial and marginal glia on sleep. Our

data suggest that disruption of the clock in specific glia-types causes changes in the duration of sleep. Moreover, the oscillators located in astrocytes and in the optic chiasm glia affect the complexity of the clock neuron (sLNvs) terminals in the dorsal brain.
Our data suggest that sleep regulation depends on a proper glial-neuronal communication. Silencing of the PDF (a clock neurotransmitter) receptor gene *pdfr* in specific glia types decreased sleep level during the day. Moreover, decreasing the strength of septate junctions in the epithelial glia by silencing of Discs-large (DLG1) encoding gene *dlg1* increased the amount of sleep during the day.
These results show that regulation of sleep by glia is very complex and requires bidirectional communication between the pacemaker neurons and specific glia types.

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Effect of rTMS on EEG and brain-derived neurotrophic factor (BDNF) concentration changes in treatment resistant depression patients

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Treatment resistant depression (TRD) cases are usually treated with repetitive transcranial magnetic stimulation (rTMS). However exact therapeutic mechanisms of rTMS remain elusive. Therefore a an optimization of rTMS procedure and a search for possible therapeutic markers is needed [1].
Aim of this study was to explore the physiology of drug resistant depression by studying biochemical markers, related to pathology manifestation, and rTMS therapy induced reorganization of the brain.
25 TRD patients, subjected to rTMS and 20 healthy control subjects participated in the study. Left dorsolateral prefrontal cortex was targeted applying 10 Hz or intermittent theta burst (iTBS) stimulation. Levels of BDNF concentration for patients were measured 3 times and for healthy participants once using ELISA sets. EEG was recorded for 10 min. using 20 electrodes, placed according to international 10-20 system. Power spectrum of 30 s baseline EEG intervals was evaluated in 5 frequency bands MADRS and HAM-D tests were used to evaluate the changes of clinical symptoms..
Initial patient BDNF concentration levels before rTMS treatment were smaller than those of healthy control group without the statistical significance. rTMS produced a steady increase of BDNF concentration during the treatment course, however it held no correlation with the clinical improvement. Instead we have observed positive correlation between BDNF concentration and low frequency EEG band spectral power, particularly in the delta band. Significant positive correlation was detected between measured EEG global delta band power and all BDNF measurements, third BDNF measurement also correlated significantly with the theta band power.

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Functional consequences of homozygous p.Gln121X mutation in human *Peroxiredoxin-5* gene

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Peroxiredoxins (PRDXs) are thiol-dependent peroxidases that are major antioxidant enzymes and redox modulators of cell signaling in mammalian cells. Peroxiredoxin-5 (PRDX5) is the only atypical 2-Cys PRDX in mammals. PRDX5 exhibits a wide subcellular localization and high expression levels in neuronal and glial cells in the central nervous system. Recently, a homozygous nonsense mutation (p.Gln121X) in *PRDX5* gene has been identified in two young sisters suffering from severe motor and mental retardation, cerebral atrophy and epilepsy, suggesting an important role of PRDX5 in human central nervous system. To examine the putative functional consequences of PRDX5 p.Gln121X mutation, fibroblasts from both patients carrying the homozygous mutation were cultured *in vitro*. Western blotting analyses of PRDX5 protein showed that the full-length or the truncated protein are not expressed in these cells. RT-qPCR analyses of *PRDX5* mRNA revealed that *PRDX5 p.Gln121X* transcripts are degraded. RT-qPCR analyses also showed that transcripts are degraded by nonsense-mediated mRNA decay (NMD) as revealed by NMD inhibition using UPF1 siRNAs. Moreover, our results suggest also that if a truncated protein can be expressed in other cell types, this protein is degraded by the proteasome as shown with the use of MG132 proteasome inhibitor. Altogether, our results suggest that homozygous p.Gln121X mutation in human *PRDX5* gene triggers nonsense-mediated mRNA decay or proteasome-dependent degradation of the truncated PRDX5 protein.

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Chronic treatment with L-thyroxine decreases the level of cleaved caspase-1 (p20) in the frontal cortex and hippocampus in an animal model of coexisting depression and hypothyroidism

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Supportive treatment with thyroid hormones is known increase the clinical efficacy of antidepressants in patients, suffering from treatment-resistant depression, however the exact mechanisms, by which these hormones act were not extensively studied. Since increased activity of caspase-1 in the brain is known

to intensify neuroinflammation and contribute to cognitive decline and since both of these processes are evident in depression, therefore in present study we determined the effects of venlafaxine and/or L-thyroxine on the levels of caspase-1 in the brain frontal cortex and hippocampus in an animal model of co-occurrence of depression and hypothyroidism.
Study was performed Wistar-Kyoto rats (WKY, model of endogenous depression) which received 0.05% 6-n-propyl-2-thiouracil (PTU) in drinking water for 6 weeks. During last 3 weeks, some animals were treated with either venlafaxine (VEN, 20 mg/kg, *i.p.*), L-thyroxine (L-T4, 1.5 mg/kg, *i.p.*) or combination of L-T4 and VEN. The levels of caspase-1 were determined using Western blot analysis.
Six weeks of PTU administration resulted in an increase in the level of cleaved caspase-1 (p20) in both the frontal cortex and hippocampus of WKY rats. Treatment with L-T4 alone and combined with VEN was shown to reverse this effect in both of examined brain structures. In summary, our study demonstrated that PTU-induced thyroid hormone deficiency can increase the activity of caspase-1 in the applied rat depression model and also inhibiton of its activation by L-T4 may be one of the possible mechanisms of antidepressant action of thyroid hormones.

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Early-life changes in prefrontal cortical spontaneous activity and recency memory in the MAM model of schizophrenia

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Schizophrenia is a common, severe and multifactorial neuropsychiatric disorder, for which current medication mainly focuses on treating the positive symptoms of the disease. In our study we aim to identify early-life neurophysiological changes conceivably evident in the methylazoxymethanol acetate (MAM) mouse model of schizophrenia compared to control mice (saline-treated) [1]. Our experiments included neonatal (P8-P11), juvenile (P15-P21) and adolescent (P40-P45), female and male C57BL/6J mice. MAM or control mice were decapitated and prefrontal cortical (PFC) brain slices were acquired for extracellular local field recordings, followed by analysis for neuronal oscillations present in the recordings. Adolescent MAM and control mice performed the temporal object recognition (TOR) task, and afterwards the mice were used for electrophysiological recordings. Our results indicate a significant reduction regarding the baseline neuronal oscillations of delta, theta, alpha and beta rhythms in neonatal MAM mice, but not in juvenile or adolescent MAM mice, compared to controls. In control adolescent mice, ketamine application in PFC brain slices increased the beta and gamma frequencies; however, in MAM adolescent mice ketamine reduced the contribution of these frequencies. Finally, adolescent

MAM mice exhibit a significantly reduced discrimination index compared to control mice in TOR task. In conclusion, early-life alterations of neuronal oscillations could affect prefrontal cortical development and lead to cognitive deficits (TOR deficits) observed in adolescence.

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Characterization of the Y2 receptor antagonist SF-11: possible mechanisms underlying its antidepressant-like effect in the astroglial degeneration model of depression in rats

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Our recent study has shown for the first time that a new brain penetrant Y2 receptor (Y2R) antagonist SF-11 decreased the immobility time in the forced swim test (FST) after acute peripheral administration (10 and 20 mg/kg, *i.p.*) in rats, indicating its antidepressant potential [1]. Thus, Y2Rs may represent very promising targets in the treatment of depression [2]. In the present study, we evaluate antidepressant-like effect of SF-11 in the astroglial degeneration model of depression to fully elucidate the possible mechanisms of the Y2R-mediated antidepressant-like effect.

The model of depression was developed by ablation of medial prefrontal cortex (mPFC) astrocytes through administering the astrocytic toxin L-alpha-aminoadipic acid (L-AAA, 100 μg/2 μl) to Sprague-Dawley rats, twice, on day 1 and 2. SF-11 (10 mg/kg, *i.p.*) was administered once 1h before the FST.

We observed that L-AAA induced an increase in the immobility time in the FST, which indicates depressive-like effect of this compound. Compared with the L-AAA-treated group, SF-11 reversed depressive-like behavioral changes in the FST, indicating its antidepressant-like effect. We also found that L-AAA injected into the mPFC strongly reduced the GFAP protein level in this structure and this decrease was reversed by acute administration of SF-11. Furthermore, we observed that the mechanism of antidepressant-like effect of SF-11 was most likely associated with the influence on brain-derived neurotrophic factor (BDNF) protein expression as well as on the extracellular glutamate level.

These mechanistic findings provide further evidence for the potential role of Y2R antagonist SF-11 in therapy of depression.

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Functional ultrasound imaging to study non-clinical pathological models: Application to attention-deficit/hyperactivity-disorder

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Functional ultrasound (fUS) is a novel instrument allowing the imaging of brain activity through monitoring of cerebral blood volume (CBV) dynamics [1; 2]. This innovative technique has not yet demonstrated its full potential in non-clinical model evaluation. Here, we assess and compare the brain activity of a rat model of attention-deficit/hyperactivity-disorder i.e the spontaneously hypertensive rat (SHR) and its appropriate control strain with the similar genetic background, the Wistar-Kyoto (WKY) rat using this original imaging approach.

Rats were anesthetized with intraperitoneal injection of ketamine/medetomidine and placed in a stereotaxic frame. Body temperature was maintained using a heated blanket. Doppler images, proportional to the CBV, were acquired every second using plane waves compounding with a small animal fUS system (Iconeus, France). After a baseline session, rats were subjected to episodic visual stimulation (30 s baseline followed with 30 s stimulation). Significant CBV changes from the different regions and functional connectivity were measured.

Cerebral Blood Volume responses during visual stimuli were found to be specifically correlated with the visual stimulus time profile in visual cortical regions in both groups. More, CBV variations within the primary or mediolateral secondary visual cortex were significantly higher in SHR rats in comparison to WKY rats during stimulation. In addition, this study also pointed out to differential functional connectivity patterns between these two groups.

These results highlight the interest of fUS imaging to evaluate brain activity modifications and alterations in rodent pathological models.

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MD, BV, FM and MC are full-time employees of Theranexus company. The other authors (LZ and MV) declare no financial conflict of interest.

Control of the activity of midbrain dopaminergic neurons activity by the nucleus incertus of the brain stem – electrophysiological and anatomical studies in rats

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Dopaminergic (DA) neurons involved in the control of animals' motivation and motor functions are located in the ventral tegmental area (VTA) and substantia nigra pars compacta (SNc). Based on recent studies, we have hypothesized that one of the sources of signals controlling dopaminergic system may be brainstem nucleus incertus (NI) involved in aversive stimuli processing and stress response generation. Consequently, our experiments were aimed to scrutinize so far unknown neuronal pathway linking NI and the midbrain dopaminergic system in the Sprague Dawley rats. To achieve that, single-unit extracellular and juxtacellular recordings of DA neurons' firing were combined with optogenetic activation of NI neurons innervating VTA/SNc. Prior to electrophysiological experiments, two viral vectors, one carrying Cre recombinase gene (CAV-Cre; retrograde) and the other carrying genes for the red light-sensitive cationic channel and fluorescent protein (AAV-DIO-Chrimson-tdTomato), were stereotaxically injected into animal's VTA/SNc and NI respectively. To visualise the anatomy of the studied pathway, antero and retrograde tracing studies were conducted. For anterograde tracing, NI was injected with AAV1 viral vector transsynaptically carrying Cre gene and VTA/SNc was injected with vector containing Cre-dependent gene for red fluorescent protein (mCherry). Our electrophysiological observations revealed that there is a subpopulation of VTA/SNc DA neurons that respond to NI activation with fast, short duration inhibition. In turn, anatomical data confirmed the existence of a monosynaptic neural input from NI to VTA/SNc DA neurons. Taken together, our study demonstrated the existence of, so far unknown, neuronal pathway that directly inhibits the activity of the midbrain DA neurons.

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The electrophysiological properties of the motoneuron do not differ between male and female rats

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Studies on rat muscles show striking differences between male and female animals with respect to basic morphometric properties, number and contractile properties of motor units (Drzymała-Celichowska et al. 2012). Moreover, the size of neurons influence their electrophysiological properties and smaller neurons have higher input resistance and reveal higher excitability. The differences in soma diameters of α-motoneurons (Mierzejewska-Krzyżowska et al. 2019) which are smaller in females suggest that there are possible sex-related differences in their basic properties as the: basic membrane potentials, threshold properties, rhythmic firings at the intracellular current injection. Furthermore, electrophysiological analysis of males and females motoneuron membrane properties which to some extent correlate with the contractile properties of innervated motor units has never been attempted. Importantly, the electrophysiological properties of spinal motoneurons such as their excitability and ability for rhythmic discharges is crucial for recruitment and force development during the motor unit contractions. Therefore, the present study aimed to verify possible sex differences in the basic electrophysiological properties of the motoneuron for better understanding of differences in motor control processes between males and females. Intracellular recordings from multiple antidromically identified rat motoneurons were performed on 65 and 70 male and female motoneurons, respectively. The study has indicated that morphometric properties of motoneurons do not impact all of the studied basic electrophysiological properties of motoneurons: resting membrane potential (RMP) and spike amplitude, action potential (AP) duration, input resistance (IR), rheobase and rhythmic firing properties of motoneurons.

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Changes in NMDA receptor subunit composition accompanies the hypoxia-induced plasticity of the excitatory retinocollicular neurotransmission

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Hypoxia is the main accompanying factor of numerous diseases. Identification of prepathological processes and mechanisms underlying *the* early stage of hypoxic injury of the retinocollicular pathway will benefit the future treatment of navigation, orientation, and visual attention impairments [1].

We developed the *in vitro* model of the reinocollicular pathway - a coculture of dissociated retinal cells and superficial superior colliculus (SSC) neurons. Using paired patch-clamp technique, we recorded retinocollicular signals transmission under normal and hypoxic conditions *in vitro.*

We showed that hypoxia induces long-term potentiation (LTP) of NMDA neurotransmission and leads to a reduction of voltage-dependent magnesium blockade of evoked NMDA response. All currents were analyzed in terms of their kinetic characteristics, such as decay time. This analysis revealed that hypoxia-induced LTP is accompanied by a rapid and irreversible decrease of the decay time of NMDA currents (from 37.2 ± 5.4 ms to 18.5 ± 7.2 ms, respectively).

The hypoxia-induced pathological LTP of NMDA neurotransmission mediates an increase of calcium ions influx and leads to apoptotic cell death. The reduction of the magnesium blockade enhances the pathological effect of LTP and causes an additional injury.

Withal, the hypoxia-induced decrease of the decay time of NMDA current leads to the reduction of the calcium ions influx. Since different subunit composition determines the kinetic characteristics of the postsynaptic currents [2], this indicates that hypoxia causes a rapid increase in NR2A-to-NR2B subunit ratio at the retinocollicular synapses. The results obtained might be targeted to prevent hypoxia-involved lesions of the retinocollicular pathway.

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Sestrines as a target of therapeutic strategies in ischemic strokes: a systematic review

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Background: Damage to DNA or proteins causes the activation of transcription factors, which in turn cause an increased expression of a described group of proteins – sestrins. Sestrins appear to be an important element of antioxidant protection and an important target of therapeutic strategies, including in stroke. *Objectives*: This study aimed to assess therapeutic strategies for ischemic stroke with the potential use of sestrins. *Design*: Systematic review. *Data sources*: Searched MEDLINE (via PubMed), Embase, Cochrane Central Register of Controlled Clinical Trials, and the Cochrane Database of Systematic Reviews for relevant studies published since database inception to May, 2021. *Study eligibility criteria*: Literature published in English in the last ten years. *Analysis*: Evidence from *in vitro*, *in vivo* and *in silico* research. Randomized controlled trials involving sestrins was also considered. *Findings*: 324 publications met the inclusion criteria and were pooled. *Conclusions*: The results of experimental model studies of acute stroke showed a strong induction of sesterin2 in cortical areas, which may indicate its potential importance in cerebral ischemia. According to *in vivo* studies, SESN2 can protect hippocampal CA1 neurons from apoptosis induced by transient global ischemia (TGI) by regulating RpS6 phosphorylation. It has been shown that SESN2 can act as an endogenous protective mechanism in cerebral ischemia by influencing the expression of RpS6, modulating the oxidative state and influencing the state of neuronal damage. Accordingly, it is postulated that sesterin2 and RpS6 may exert a protective effect to counteract the deleterious effects of ischemia and reduce neuronal damage.

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Impact of low-level learning of the stimuli on conflict effects in MSIT

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Impact of the intrinsic regularities (ratios or sequences) in stimuli trains within cognitive tasks can compromise the study of designated cognitive function [1]. Here, we focus on conflict resolution and check if the extended Multi-Source Interference Task, used for probing conflict, is robust for such low-level learning confounds. The task comprises stimulus-stimulus (presence of flankers in addition to target symbols) and stimulus-response (spatial incongruence of the target and the corresponding response button) conflicts both separately (on different trials) and simultaneously (both types of conflict in a single trial) [2].

To determine the impact of the contingency bias (unequal stimulus-response associations stemming from the ratio of stimuli) and feature-binding (sequential effects – interference from alternating or repeating features) we conducted two experiments with different task designs. A standard design, used all combinations of stimuli, comprising (unavoidably) less stimuli variants in congruent than in incongruent trials. A contingency-equalized design used several parallel subsets of experimental stimuli, to present each unique stimulus equally often.

Reaction times showed large conflict effects elicited by incongruent trials. These effects were not compromised by removing the contingency bias (no significant differences between task designs). We observed an impact of feature-binding upon the behaviour, e.g. slower RTs for trials with flanker repetitions, regardless of the changes of any other features (repetitions of target or its position). However, these effects were substantially smaller than RT differences between incongruent and congruent trials. In sum, despite the biases within design, conflict effects in MSIT cannot be explained by lower-level learning confounds.

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Ventral caudal nerve motoneurons: quantification study

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The peripheral nerve injury represents a challenging condition in the field of regenerative medicine and effective therapy which would result in a complete functional recovery is still not found. In experimental research, several models of nerve injury are used and various parameters are considered when evaluating therapy efficacy. In our group, we established the model of rat ventral caudal nerve for studying nerve injury. Besides axon number, g-ratio and basic electrophysiology, the number of motoneurons in the spinal cord is an important parameter for model characterization. The aim of this study was to map out and quantify ventral caudal nerve motoneurons using fluorogold retrograde labeling. Six adult male Wistar rats underwent transection of the caudal nerve and 5 mm polyethersulfone tube was applied on the proximal stump of a nerve and injected with fluorogold (4%). After 7 day of survival, rats were sacrificed and spinal cords processed by RetroDisco whole-mount clearing technique [1]. Quantification of neurons has revealed an average number of 178,4 cells in the ventral horn of the spinal cord. The cells were dispersed through segments S1 – Co3. This data will serve as baseline for our future experiments.

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Modulation of the visual information processing in the frequency domain with periorbital pulse current stimulation in healthy humans

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Aim: To explore how a periorbital pulse current stimulation (pPCS) affects visual information processing in healthy humans.

Methods: Participants (n=32, 16 females, 25.7±3.7 yrs) were divided randomly into gender- and age--balanced groups. Visual evoked potentials (VEP) were recorded in response to checkerboard pattern reversals (CPRs) displayed alone (sham group) or preceded directly with pPCS (experimental group) of different duration (12.5, 25, 50 and 100 ms) and amplitude (100 and 200 μA). We used Python to analyse frequency characteristic of the visual responses to CPR (1 s post-stimulus window) in the occipital electrodes ('O1', 'O9', 'OI1h', 'Oz', 'OI2h', 'O10', 'O2') and its modulation with pPCS.

Results: Passive viewing of CPR had a generally non activating effect as evidenced by significant increase in alpha power in the sham group. In the experimental group, pPCS caused power decreases from pre-stimulation level, similar for all current pulse parameters. The changes were significant in comparison to the sham group: delta (1-4 Hz, p=0.0002), theta (4-8 Hz, p=0.0006) and alpha (8-14 Hz, p=0.0214) bands. The effect of pPCS was visible also for no-stimulation trials introduced within the series of pPCS presentations, and faded away after 3 minutes from the last current pulse application. Time-frequency representations revealed no particular time windows responsible for these global power changes.

Conclusions: Short-term plastic (STP) changes induced by pPCS of different parameters were not distinguishable probably due to the overlap of STP response curves. Observable cumulative effects didn't exceed a single stimulation session.

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The authors declare no competing financial or other interests.

Ghost sensations in hands and feet

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When you are trying to relax, your attention might shift to various "ghost" sensations across your limbs, such as shaking or numbness [1,2]. Those spontaneous sensations (SPS) might not be the same across our limbs as there are sensory, anatomical and functional differences between them [3]. The present study aimed to answer the question of whether SPS present differently in hands and feet. We designed an online SPS questionnaire to measure the general tendency to experience SPS in our limbs,

named SPS_{Trait}, and the experience of SPS while taking the questionnaire, named SPS_{State}. Eighty one participants (age $M = 30.80$, $SD = 10.97$) took part. Data from the SPS_{Trait} and SPS_{State} were analysed separately using a repeated measures ANOVA design with Side (left, right) x Limb (hand, foot) as factors. No significant main effects or interactions were found (all $ps > .05$). We also explored the relationship between SPS_{Trait} and SPS_{State}. The results indicated a significant positive relationship between SPS_{Trait} and SPS_{State}, $r = .57$, $p \leq .0001$, and this relationship was significant when analysing limbs and sides separately (e.g. $SPS_{TraitHand}$ correlated with $SPS_{StateHand}$, $r = .59$, $p \leq .0001$). Our results indicate that SPS are perceived similarly in hands and feet, and on both sides of our body, for both SPS_{Trait} and SPS_{State}. The relationship between SPS_{Trait} and SPS_{State} suggests that our individual ability to attend to ghost bodily sensations is stable and it is closely related to how we experience our body as a whole.

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Biophysical properties of Ca^{2+}-dependent hippocalcin translocation in HEK 293 cells

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Hippocalcin (HPCA) is a neuronal calcium sensor protein, which Ca^{2+} binding leads to its conformational changes and translocation of HPCA from a cytosol to cellular membranes where it interacts with its specific targets [1]. In spite of importance of HPCA signaling in neuronal functioning, biophysical properties of HPCA translocation in a physiological range of Ca^{2+} concentrations in the cytosol ($[Ca^{2+}]_i$) have not been studied yet. In this work we studied a dependency of HPCA translocation to the plasma membrane and trans-Golgi network of HEK cells on $[Ca^{2+}]_i$. High-resolution confocal microscopy permitted direct observation and quantification of spatio-temporal patterns of translocation of fluorescently-labeled HPCA and $[Ca^{2+}]_i$ changes in individual cells. Laser-induced Ca^{2+} uncaging from NP-EGTA was used to control $[Ca^{2+}]_i$. Fast homogeneous increases in $[Ca^{2+}]_i$. resulted in heterogeneous HPCA translocation to a certain sites in the plasma membrane and trans-Golgi network indicating to diverse affinity of HPCA binding in different membranous loci. Slow decay of $[Ca^{2+}]_i$. after uncaging (about 200 s) allowed to obtain a Ca^{2+}-dependency of HPCA translocation, which was in a range of physiological values of $[Ca^{2+}]_i$. At the

saturating level of $[Ca^{2+}]_i$. an estimated maximal membrane fraction of HPCA was 27 ± 4%, which was at least four-fold higher than one at a basal level of $[Ca^{2+}]_i$ [2]. We have concluded that in a physiological range of Ca^{2+} concentrations in the cytosol HPCA can function as a site-specific Ca^{2+} sensor having a wide dynamic range for a precise decoding of complex spatio-temporal patterns of $[Ca^{2+}]_i$ changes.

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Psychophysiological components of stress resistance of qualified athletes

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The solution to problem of achieving high levels of special physical performance and maintaining the health of athletes, demands new ways. The constant monitoring of the current state of psychophysiological functions in athletes (in order to predict the risk of injury) and their mental training (in order to health maintenance) allow maintaining an active sports life. The aim of the current study was a comparative analysis of the current status of psychophysiological functions of highly qualified athletes specialising in sports with varying degrees of extremeness (high risk).

The study involved 18 high-class athletes (snowboarding, alpine skiing, cross-country skiing) aged 15-42. To determine the state of the sportsmen's psychophysiological functions, the diagnostic complex "Diagnost-1" (by M. Makarenko, V. S. Lizogub) was used. We have analyzed latent periods of a simple visual-motor reaction, a simple and complex reactions of choice, the effectiveness of sensorimotor activity, the accuracy of the reaction to a moving object and the ratio of advance and delay reactions, as well as the basic properties of the nervous system (in particular, the functional mobility of nervous processes and the strength of nervous processes).

It has been shown that athletes with a higher degree of extreme sports activity and the risk of injury (snowboarders and skiers) are characterized by higher indicators of sensorimotor endurance, the strength of nervous processes. The level of the functional state of the central nervous system was also higher among snowboarders and skiers.

Keywords: highly qualified athletes, high risk, injury, endurance, of nervous processes

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A disclosure of conflicts of interest No.

Neuropathology of graded severe spinal cord compression in rat with low spontaneous motor recovery

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The proper selection of experimental model is the key factor of successful therapeutic development for patients with spinal cord injury. Hence, the main aim of our study was to specify a standard model of spinal cord compression that satisfactory mimics a progress of human traumatic injury and also overcome the problem of rodent rapid spontaneous motor recovery. Adult Wistar female rats underwent graded compression at Th9 level lasting 15min induced by 30, 40 or 50g weighted impactor. During the 28-day posttraumatic period, the progressive recovery of voiding as well as the regeneration of hind-limb motor function that involves a rapid and slow recovery phase were detected in all experimental groups. Primary mechanical impact causes direct and indirect blood-spinal cord barrier disruption measured spectrofluorometrically that was evident mainly in the lesion site with gradual decline in cranio-caudal direction. After 4 weeks, the grey and white matter degradation and cystic cavitation were evaluated by histological Luxol fast blue/Cresyl violet staining. The force-dependent massive tissue loss measured mostly in the injury epicentre with progressive decrease in cranial and caudal segments was accompanied with the extensive cystic cavitation, demyelination and astrogliosis occurred predominantly in white matter below the lesion site. The severe compression sufficiently mimics traumatic neuropathological conditions in human with highly reproducible damage leading to low spontaneous recovery so it represents the optimal experimental model for a basic pathophysiologic research as well as the development of potential therapy.

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Biological sex evokes distinct behaviour-communication deficits in a mouse model of autism spectrum disorder

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Autism spectrum disorder (ASD) is a neurodevelopmental condition characterized by deficits in social interaction and by stereotyped behaviours. Particularly, impaired communication is a hallmark of ASD and is mainly studied in animal research through the analysis of ultrasonic vocalizations (USVs). Male bias in ASD is well-recognized, with a 4:1 ratio of diagnosed boys and girls, making it relevant to explore the effect of biological sex on ASD-like behaviour. Here, male and female *Tsc2*$^{+/-}$ mice, an established genetic animal model for the study of ASD, were used. We assessed simultaneously USVs and behaviour produced in social environment, as well as during repetitive/stereotyped tasks. Preliminary results indicate a less complex vocal repertoire in female *Tsc2*$^{+/-}$ mice in social environment. They showed a significantly greater proportion of USVs emitted while socializing rather than non-socializing, in comparison to their wild-type (WT) littermates. Additionally, *Tsc2*$^{+/-}$ females spent a greater average time socializing. Regarding repetitive tasks, *Tsc2*$^{+/-}$ mice presented a more stereotyped behaviour, and mutant males produced less complex USVs compared to male WT. Our study demonstrated that differences in communication during social tasks occur between females, while these differences between males arise in repetitive tasks.

These results suggest a sex-dependent behaviour-communication link and a potential use of USVs as biomarker for behaviour deficits.

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Propagating slow waves in the thalamus of anesthetized rodents

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Propagating waves of activity were found in a variety of brain regions, including the neocortex, the hippocampus and the thalamus; and were described in many species such as turtles, rats, cats, monkeys and also in humans. Traveling waves have been reported in *in vitro* and in *in vivo* experiments, in both

anesthetized and awake states. Several brain rhythms behave as traveling waves, including the alpha and theta rhythm, sleep spindles, or the slow oscillation. The latter brain oscillation has a thalamocortical origin; however, since the access to the thalamus with methods having a high spatial resolution and a large tissue coverage is limited, propagating slow waves were investigated mainly in the neocortex. Although propagation of sleep spindles was described in the thalamus of cats *in vivo*, thalamic traveling slow waves were found only *in vitro*, in brain slice preparations. Thus, there is a lack of *in vivo* studies investigating thalamic propagating waves. In this study, we used multi-shank and high-density silicon probes to examine whether propagating slow waves can be detected in the thalamus of anesthetized rats and mice. We recorded spiking activity from multiple thalamic nuclei simultaneously, then wave propagation was assessed based on the analysis of the multi-unit activity (e.g., propagation of population activity) and single-unit activity (e.g. spatiotemporal firing sequence of thalamic single units). Our preliminary results suggest that slow wave propagation can be detected only in certain thalamic nuclei (e.g., VPL/VPM) and traveling waves in these nuclei usually have a preferred propagation direction.

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Reward processing related theta oscillations in schizophrenia

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It is known that motivational deficits are one of the main characteristics of schizophrenia (SCH). Theta oscillations have been linked to outcomes in positive reinforcement in the Monetary Incentive Delay (MID) task (1). Considering the GABAergic abnormalities in SCH, which is thought to be related to altered theta activity (2), in this study it was hypothesized that patient and healthy control (HC) groups would respond differently in reward processing during the MID task. Event-related oscillations (ERO) were recorded during the task in 21 patients (15 males) with SCH and sex-, education-, age-matched 22 HC. In the MID task, a total of 450 trials (control, punishment, and reward conditions) were completed. The maximum peak-to-peak theta amplitudes (4-8 Hz) were measured at the midline electrode positions

(F_z, FC_z, C_z, CP_z, P_z, O_z). Repeated measures (RM) ANOVA was carried out with IBM SPSS. The mean age was 37.71 (±9.01) and the duration of education was 11.14 (±3.26) years for the patients. PANSS sum score was 68.24 (±17.03) and BNSS sum score was 41.33 (±13.12). RM ANOVA of theta ERO revealed group and condition interaction [$F_{2,82}$= 4.193; p=0.018]; indicating decreased theta oscillation in reward condition for individuals with SCH than HC (p=0.043). In our study, we found that patients had significantly lower theta activity in reward condition. Earlier studies linked increased theta activity with the reward anticipation HC (1). Therefore, this result can be thought as an indicator of impairment in GABA function and top-down processes regarding sensory-motor integration in SCH (2, 3).

Keywords: motivation, reward expectancy, schizophrenia, theta oscillations

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Quantitative analysis of the stroke specific marker gene expression in the peripheral blood after induction of the ischemic tolerance

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Ischemic damage can be reduced by a conditioning process that induces a neuroprotective effect of ischemic tolerance (IT). TM4SF1 regulates cell development, cell growth and motility, cell cycle and apoptosis. Currently it was observed that gene expression of TM4SF1 in brain and peripheral blood correlates very specifically. Moreover, TM4SF1 was identified as a brain ischemia specific marker. The aim of this study was to quantify the changes in TM4SF1 gene expression in peripheral blood after global cerebral ischemia and its correlation after induction of remote postconditioning using rat model. The following experimental cohorts were studied: sham, global cerebral ischemia, global cerebral ischemia with early remote postconditioning (1 hour after ischemic attack) and global cerebral ischemia with delayed remote postconditioning (1 day after ischemic attack). We analyzed the expression level

of TM4SF1 gene in peripheral blood 3 days after ischemic attack. In cerebral ischemia, we observed a significantly increased rate of TM4SF1 expression. In early remote postconditioning the increase in expression was more modest, and in delayed remote postconditioning, the increase in expression was the lowest. In the present work we found that postconditioning reduces the expression level of TM4SF1, which is also associated with reduced rate of apoptosis. This neuroprotective effect was observed in higher level in the delayed form of postconditioning. Our results may help to clarify more details about IT mechanisms at the molecular level and play a key role in identifying specific mechanisms of postconditioning at the molecular level.

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Evaluation of visual working memory hemispheric laterality: psychophysical study

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It is known that visual stimulus projected directly to the specialized hemisphere can be processed more efficiently. Using a visual half field paradigm, it has been demonstrated the functional hemispheric laterality of language and some other cognitive functions. However, the knowledge about a lateralization of visual working memory (VWM) is limited. In this study, we investigated VWM asymmetry assessing participants' behavioural metrics, evaluating links between VWM, handedness and sex.

99 volunteers (40 men, 21.75 ± 2.44 years) performed change detection VWM task based on visual half-field paradigm and completed questionnaires.

The results of the study revealed that VWM capacity was higher when stimuli were presented to the RVF/LH (3.05 ± 0.5) as compared to LVF/RH (2.93 ± 0.53, $p < 0.011$), however this effect was stronger for women. Accuracy was significantly higher and responses faster when the stimuli were presented in the RVF/LH (76.34 ± 10.3; 886 ± 154 ms) than to the LVF/RH (67.92 ± 11.91; 915 ± 161 ms, $p < 0.0001$) regardless of sex.

Our study showed the advantage of the left hemisphere for VWM processing. However, study design does not answer to the question if encoding or retrieval processes are lateralized to the left hemisphere.

Disclosure of potential conflicts of interests:

Rimantė Gaižauskaitė, Indrė Pretkelytė, Ramunė Grikšienė no competing interests to disclose.

Effects of chronic nerve cuff electrodes and low threshold electrical stimulation on rat tibial nerves

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Numerous experimental data point to therapeutic effects of electrical stimulation of damaged peripheral nerves. The cuff electrodes implanted around the nerve are often used for chronic stimulation but their long-lasting contact with the nerves may cause unwanted effects.
To assess these effects the cuff electrodes were implanted around tibial nerves bilaterally (cuff) and a 7-day low-threshold stimulation was added unilaterally in the 4th week (cuff+stim). Non-implanted group was used as control.
The effects were evaluated on photomicrographs captured with use of light microscope (obj. 40x) from semi-thin epon sections (500 nm) stained with toluidine blue. The total number of myelinated axons in the nerves, their cross-sectional area (>1μm^2), mean area and circularity were measured.
The average number of axons in control nerves was 5442±579; it tended to be lower by 10% in cuff and cuff+stim groups. The mean axons area in the control nerves was 31.6±8.9 μm^2 and it tended to decrease by 10% in both cuff groups. There was more small axons (1-10 μm^2) in expense of large ones (>50 μm^2) in cuff groups ($p \leq 0.05$, *Student's t-test*). The nerve cross-sectional area positively correlated with axons mean area (r=0.85, p=0.030, *Student's t-test*). The distribution of fibers with different cross-sectional areas showed a random pattern in all groups. Further studies with the use of EM should clarify whether increased number of small axons stems from regenerative processes taking place after long-lasting presence of cuff around the nerve.

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Title Two-Photon Optogenetics Protocols for Neuronal Cell Input Investigations

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Understanding how a neuron processes incoming inputs requires measurement of two components: first, its complete synaptic connectivity map, defined by the number of inputs, the cell types of presynaptic neurons, their physiological properties, and the distribution of their strengths in space; and second, a complete description for how input summation across these presynaptic sources drives neuronal input/output transformations in the intact brain. This can be achieved by combining fast two-photon optogenetic stimulation with near cellular resolution with whole-cell electrophysiological recording. We integrated

3D scan-less holographic optogenetics with temporal focusing (3D-SHOT) [1] and cell type-specific expression of the fast and potent ChroME and ChroME2.0 cation opsins [2] with whole-cell electrophysiology, to rapidly and repeatedly prove monosynaptic connections in a 3D volume. First, we identify unitary synaptic connections from subpopulations of neurons to map local synaptic input to single neurons. Then, we examine the dynamics of input summation by stimulating variable numbers of identified presynaptic neurons. These experiments will define how the source, number, and dynamics of presynaptic activity drive postsynaptic spiking. By executing this approach *in vivo* we will be able to directly address how single neurons integrate their inputs to enable fundamental aspects of neural computation.

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Number, density and morphometry of muscle spindles and intrafusal muscle fibers in medial gastrocnemius of male and female rats

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The experiments aimed to determine the sex differences in number and density of muscle spindles as well as their morphometric properties for rat medial gastrocnemius muscle. The muscles were cut into 5-10 and 20 μm thick sections and muscle spindles were identified and counted. The number of muscle spindles was similar for males and females and amounted to 14.45±2.77 and 15±3.13, respectively ($p > 0.05$). The mass of studied muscle was 38.89% higher in males (1.08 g vs 0.66 g in females) and therefore the density of these receptors was higher in females as one spindle on average occurred per 79.91 mg of the muscle mass in males and 51.14 mg in females ($p < 0.01$). The diameter of intrafusal muscle fibers in males amounted to 5.16±2.43 μm and in females 5.37±2.27 μm, the number of intrafusal muscle fibers in males amounted to 5.57±2.20 μm and in females 5.60±2.16 μm, the shorter muscle spindle diameters for males amounted to 25.85±10.04 μm and for females 25.30±9.96 μm whereas longer diameters for males amounted to 48.99±20.73 μm and for females 43.97±16.96 μm ($p > 0.05$ for all these morphometric properties). The sex differences in number, morphometric properties and density of muscle spindles in rat medial gastrocnemius predominantly concern only density of muscle spindles.

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Mitochondria, adult hippocampal neurogenesis, and anxiety

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In the adult mammalian hippocampus, new neurons arise from stem and progenitor cell division, in a process known as adult neurogenesis. These new granule neurons have been linked to behaviors depending on hippocampal function, such as spatial navigation, learning, anxiety, stress regulation, and social cognition.

Adult hippocampal neurogenesis is increased by antidepressants, and is required for some of their behavioral effects. However, it remains unclear whether expanding the population of adult-born neurons is sufficient to affect anxiety and depression-related behavior.

Moreover, emerging evidence indicates that mitochondria can regulate stem cell fate decisions and are crucial for adult neurogenesis However, the roles of mitochondrial proteins or functions in adult neurogenesis remain barely investigated.

In this study, we aimed at understanding the molecular mechanisms that link basal anxiety with differences in mitochondrial function in stem cells and newly generated neurons in the dentate gyrus of the hippocampus. We found that expression levels of mitofusin 2 (MFN2), a mitochondrial membrane protein that participates in mitochondrial fusion and contributes to the maintenance and operation of the mitochondrial network, are altered in high anxious animals. These findings are observed along with changes in mitochondrial function and morphology, along with a dysregulation of adult neurogenesis.

The identification of the molecular mechanisms linking high anxiety with impaired neurogenesis will advance our understanding of the neuropathology underlying stress-related disorders.

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Object categorization depends on category level and forward mask congruency

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Visual categorization is the ability to assign a sensory stimulus to a discrete category. Each stimulus can be attributed to different levels of abstraction: the basic (e.g. chair), the superordinate (e.g. furniture), and the subordinate (e.g. office chair). The categorization process differs according to the category level, but the cognitive mechanisms underlying it remain unclear.

In this research, we investigated how the category level (basic or superordinate) and congruent (another man-made object) or incongruent (animal) forward mask altered the object categorization process. The basic-level categorization performed faster than the superordinate one. The early occipital and temporal N50 components depended on both the categorization level and the congruence between the mask and the stimulus. We assume that these differences indicate anticipatory attention, depending on the categorization level. The occipital and temporal P130 and the frontal N150 amplitudes were higher when masking by incongruent images, which, possibly, reflects a more efficient separation of significant and insignificant information at the perceptual stage. The central late positivity and frontal N400 amplitudes were higher at the superordinate-level categorization, and the frontal P300 amplitude was higher at the basic-level one. The data obtained indicate the important role of the central and frontal areas in the categorization task and the change in their activity at the later processing stages, depending on the categorization level. In occipital and temporal areas, the categorization level effect appears very early, altering the perceptual stimulus processing.

The authors declare no conflict of interest.

Regulation of the glycolysis process in the brain in an animal model of coexisting depression and hypothyroidism: combined efficacy of venlafaxine and L-thyroxine

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Among the hormones whose altered level or action can lead to metabolic changes in the brain, thyroid hormones are gaining renewed interest. Additionally, current clinical evidence indicates that supportive therapy with these hormones in the treatment of depression, in which brain dysregulated metabolism was shown [1, 2], may have beneficial effects [3]. We determined the impact of venlafaxine and/or L-thyroxine (L-T4) on the level of lactate, pyruvate, and pyruvate dehydrogenase in an animal model of coexisting depression and hypothyroidism, namely, Wistar-Kyoto (WKY) rats treated with propylthiouracil, in the frontal cortex. In the used model, the glycolysis process was inhibited when compared to the depression model (WKY rats). In the examined brain structure, it was proven by the reduced levels of pyruvate and lactate in the cytosolic fraction. The decreased lactate level was normalized by venlafaxine, L-T4, and by the co-treatment, while the downregulated level of pyruvate was increased more strongly in the frontal cortex of animals receiving venlafaxine+L-T4 than those receiving venlafaxine, which indicated that L-T4 can intensify the effect of venlafaxine. Similarly, the level of pyruvate dehydrogenase in the mitochondria-enriched fraction was diminished in the model of coexisting depression and hypothyroidism but only co-treatment with L-T4 and venlafaxine reversed this unfavorable change. Our results demonstrated that the use of L-T4 as adjunctive therapy for depression may normalize neuro-

transmission not only by acting on neurons but also by regulating the function of astrocytes preventing disturbances in the glycolysis process.

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A method for multiplex fluorescent RNAscope hybridization combined with immunofluorescence on PFA-fixed, free-floating muscle fibers, and spinal cord slices

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Here we provide a protocol for a combination of fluorescence *in situ* hybridization (FISH) and immunofluorescence (IF) on free-floating spinal cord sections and muscle fibers of adult rat. We applied TSA-based Fluorescent RNAscope®, which is a novel FISH assay able to detect single transcripts [1]. This protocol allowed us to determine the origin of the transcript in the spatial context of marker proteins with subcellular resolution, in two components of the motor unit – motoneuron (MN) and neuromuscular junction (NMJ). To settle the method we used tissues from 12 young adult male Wistar rats. We modified the protocol of the producer (Advanced Cell Diagnostics, US), adjusting protease digestion parameters, probes concentration, and amplification time. On the tissues designated and fixed for IF we were able to visualize mRNA of BDNF and its two receptors: TrkB and p75, imaging them simultaneously with 3 proteins in the traced MNs and NMJs. The proteins were chosen to reveal origin of the transcripts: oligodendroglial precursors (NG2), oligodendrocytes (Olig2), and microglia (Iba-1) markers. Sections were imaged with Leica SP8 TCS confocal microscope in Lambda mode and processed using spectral unmixing method. Deconvolved images were subjected to qualitative and quantitative analysis by the means of Imaris software.
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Comparison of the effects of open- and closed-skill exercise on cognition and peripheral proteins

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Exercise modes can be divided into open- and closed-skill exercises. Previous research indicates that open and closed-skill exercise modes might create different effects on cognition and peripheral protein signals [1,2]. Our study aimed to compare the effects of long-term participation in an open and closed--skill exercise on cognitive functions and Brain-derived neurotrophic factor and Cathepsin B levels. Fencers (open-skill), swimmers (closed-skill), and sedentary controls (n = 18/18/18) between 18-25 years old participated in the study. Visuospatial working memory, verbal fluency, and selective attention task were performed. Brain-derived neurotrophic factor and Cathepsin B levels were determined both resting and after an acute bout of aerobic exercise by ELISA in serum. The results showed that fencers performed superiorly on some part of visuospatial working memory, verbal fluency, and selective attention tasks compared to swimmers and sedentary controls. Moreover, athlete groups showed higher scores on visuospatial working memory and selective attention tasks than sedentary controls. Resting serum Brain-derived neurotrophic factor level was not significant between the groups, but Cathepsin B was higher in fencers than swimmers and sedentary controls. After an acute bout of aerobic exercise, the peripheric protein signal response was significantly higher in athletes, particularly in the open-skill group for Cathepsin B. This study provided noteworthy results that more cognitively challenging exercise may provide more gains for some aspects of cognitive functions and peripheral protein signals related to cognition.

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The authors declare that there is no conflict of interest.

Social behaviour and ultrasonic vocalisation in male TPH2- knockdown rats

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Serotonin is a monoamine, which appears early during neurodevelopment. Brain serotonin level manipulations are associated with central nervous system processes, including social behaviour. Tryptophan hydroxylase 2 (TPH2) is a rate-limiting enzyme of serotonin synthesis. Therefore, downregulation of this enzyme results in central serotonin depletion.

The goal of the study was to examine social behavior and communication of genetically modified rats, TPH2-inducible shRNA knockdown rats (TPH2-KD). TPH2-KD model allows downregulation of serotonin synthesis in the rat brain during adulthood, in contrast to animals with constitutive life-long serotonin depletion.

To anayze social interaction two unfamiliar rats were placed in the open field arena, and their social behaviour was recorded. Additionally, 50-kHz ultrasonic vocalisations were measured and their acoustic parameters were characterized.

We report that TPH2-KD rats demonstrated disturbed patterns of social behaviour and communication. TPH2-KD and TPH2-WT rats showed a comparable number of episodes, but there were significant differences in the distribution of separable behaviours. Moreover, TPH2-KD rats emitted significantly less 50-kHz USVs, characterized by narrower bandwidth and shortened peak frequency compared to controls.

The present study confirms the role of central serotonin in the regulation of social behaviour and communication.

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Functional heterogeneity of the social brain: focus on the Theory of Mind network. FMRI study. *Theory of mind, social cognition, social brain, theory of mind network*

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Theory of Mind (ToM) is referred to as a cognitive capacity to infer mental states of others which enables effective social interaction. A dedicated network of brain structures hypothesized to underlie ToM has been indicated. However, there is still an ongoing debate whether ToM comprises a single entity or refers to a family of abilities in terms of the specificity of functions involved. As a result, tasks regarded as the ToM network localizers vary substantially and deliver inconsistent results regarding the activation

patterns. This might hamper the inference on neural mechanisms of ToM which are widely studied in clinical populations, especially autism spectrum conditions. Examining the patterns of neural response to tasks covering different aspects of ToM within one group of subjects might contribute to forming a coherent picture of what ToM is and precisely defining it's neural correlates.

In a current study group of healthy adults (N=61, age: M=27,22 SD=7,92) engaged in a battery of 5 ToM tasks of varying complexity during a session of functional magnetic resonance imaging (fMRI) in 3T Siemens PRISMA scanner. The data analysis was conducted in SPM toolbox and Matlab with the use of whole-brain and regions-of-interest (ROI) approach.

Task-related differences in the responses of ToM network were found, revealing the hierarchical pattern of activation depending on the level of complexity of the social-cognitive processes evoked by the task, with an overlap in temporo-parietal junction ROI. Our results support the notion of ToM as a single capacity, however, relying on various complementary abilities.

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The role of bursts in sensory differentiation in the neural networks of dissociated cortical culture

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The capacity of neural tissue to differentiate the sensory signals determines how we recognise the world diversity. Dissociated cortical culture (DCC) homed in a multielectrode array allows to mimic neural networks of the brain and use it for investigation of neural computation processes [1, 2]. We were interested in whether the neural circuitry of DCC was capable of sensory differentiation and to determine the role of bursts in the responses to preferred stimuli.

DCC from the 7th to 60th day of *in vitro* cultivation were used for the study. In order to simulate a variety of sensory inputs, 300mV of single, paired-pulse (20ms interstimulus interval), 1, 5, 10, 20 and 50Hz stimuli for 1sec were repeated after every 30secs from effective pairs of electrodes; Activity was registered from all active channels.

Results showed that on average from the 10th day of cultivation neurons trended to one of the given stimulus paradigms, generating or increasing level of activity, while eliminating responses to other kinds of

stimuli. Single-unit responses included both early (<300ms) and late (>300ms) tonic and burst elements, however, repetition of the preferred stimuli elicited the early responses became more frequent. In many cases, relocating the stimulator electrodes elicited change of active channels and/or altered activity level. Data shows that neural circuits of DCC have high selectivity to physical properties and spatial position of the sensory inputs and produces early and late responses that include burst elements that may serve as the robust mechanism for reinforcement of the coding information.

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The authors declare that there is no conflict of interest.

Epileptiform GluN2B-driven excitation in hippocampus as a therapeutic target against temporal lobe epilepsy

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NMDAR is an ionotropic glutamate receptor that forms the foundation of excitatory synaptic transmission. The receptor properties are strongly determined by its subunit composition[1]. One of the NMDARs' subunits is GluN2B which displays restricted and spatially different expression in the mature brain. GluN2B-containing NMDARs are present in the hippocampus – the structure playing a major role in temporal lobe epilepsy (TLE)[2]. However, the contribution of GluN2B to pathophysiology of TLE has been only briefly investigated. Here, we report the functional alterations of GluN2B-containing NMDAR receptor in hippocampus in distinct mouse models of temporal lobe epilepsy. In particular, we show GluN2B impact on excitatory feedback at granule cells. Based on these results we propose the mechanism-oriented effective antiepileptic strategy that selectively antagonizes GluN2B-containing NMDARs with ifenprodil. Collectively, our research identifies GluN2B as one of the instrumental factors in pathogenesis of temporal lobe epilepsy and associated recurrent seizures. Furthermore, our study indicates the prospective antiepileptic properties of ifenprodil.

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Effect of simultaneous exposure to western diet and wheel running on the cortical and hippocampal levels of glucose transporters in female rats

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Introduction: Recently, more attention has been paid to the contribution of an unhealthy diet to the development of the central nervous system. Western diet (WD)-induced adverse effects in the brain seem to be related to disturbances of brain energy metabolism. Growing evidence supports the role of physical activity as a brain and nervous system disease-preventing factor.

Aim: The goal of the study was to investigate the effects of simultaneous exposure to forced wheel running and WD on the cortical and hippocampal levels of glucose transporters (GLUT1, GLUT3, GLUT8) in the female rats.

Methods & Materials: 9-weeks old female Long Evans rats (n=6) for 6 weeks received snacks typical for human WD alongside with standard rodent chow (WD group). During this time 5 animals were also subjected to forced wheel running (WD/EX group). Animals in the control (CTR) group received standard rodent chow (n=6). Western blot analysis was performed in samples collected from frontal cortices and hippocampi of the animals.

Results: We did not observe statistical changes in the GLUTs in both brain regions of animals fed with a western diet. However, we report an almost 2-fold increase of a cortical level of GLUT3 ($p=0.04$), a major neuronal glucose transporter, in animals fed with the WD and exercised in wheels (WD/EX group), as compared to the WD group (ANOVA $p=0.028$, $F(2,12)=4.872$). The level of GLUT8, which is expressed mostly in neurons, was increased in hippocampi of exercised animals fed with the western diet (ANOVA $p<0.0001$, $F(2,12)=44.16$). Namely, a 1.84 fold increase was significant as compared to the control ($p<0.0001$), and to the WD group ($p<0.0001$).

Conclusion: We conclude that our preliminary data provide a valuable basis for deeper investigation of changes in brain structure and function induced by western diet and physical activity.

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Impact of aging on the structure and NMDA receptor expression of somatostatin expressing hippocampal interneurons

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Aging is a natural process related to the gradual loss of physiological and social functions. Understanding the neurobiology underlying age-related impairment is essential given the growing elderly population. One of the most studied brain structures affected by aging is the hippocampus, known to be involved in different cognitive processes. While the aging-associated quantitative changes in dendritic spines of hippocampal pyramidal cells have already been studied, the relationship between aging and the structural dynamics of hippocampal interneurons remains relatively unknown. Spines are not a frequent feature in cortical inhibitory neurons, but these postsynaptic structures are abundant in a subpopulation of somatostatin expressing interneurons, particularly in *oriens-lacunosum moleculare* (O-LM) cells in the hippocampal CA1. Previously we have shown that the spines of these interneurons are highly plastic and influenced by NMDA receptor manipulation. Thus, in the present study, we have investigated the impact of aging on these interneurons. The analyses were performed in 3-, 9-, and 16-month-old GIN mice, a strain in which somatostatin positive interneurons express GFP. We studied the changes of dendritic spines, *en passant boutons*, and NMDA receptors (GluN1 and GluN2B) using confocal microscopy and image analysis. We observed a significant decrease of the dendritic spine density in 9-month-old when compared with the 3-month-old animals. We also observed a decrease in the expression of the GluN2B subunit, but not of that of GluN1, during aging. These results will constitute the basis for advanced studies of the structure and connectivity of interneurons during aging and their contribution to cognitive decline.

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Peripheral blood monocytes and neutrophils number changes after subthalamic nucleus deep brain stimulation applied in a rat model of early Parkinson's disease

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Introduction:

The immune activation in Parkinson's disease (PD) is not only dependent on neuroinflammation but also involves peripheral immune cells, found in the blood as well as infiltrated into the brain [1]. Understanding the involvement of peripheral cells in PD is essential for the development of immunomodulatory therapies, which might modify disease progression. Subthalamic nucleus deep brain stimulation (DBS-STN) is most effective treatment for late PD motor symptoms [2]. The animal data showed that DBS-STN have substantial neuroprotective properties [3].

Aim:

Considering the close association between neuroinflammation and peripheral immune cells activation during PD progression, we hypothesized that neuroprotective properties of DBS-STN may be related with blood peripheral monocytes and neutrophiles number changes in a rat model of early PD.

Methods:

Male Wistar rats were implanted unilaterally for DBS-STN and received intranigral (substantia nigra pars compacta, SNpc) infusion of 6-OHDA. After recovery, rats were subjected to DBS- STN for 7 days (1h daily, n=6) or SHAM stimulation (control, n=6). The peripheral blood samples were analyzed using automated hematology analyzer (Cell Dyn 3700). PD model have been verified by the detection of tyrosine hydroxylase positive neurons in SNpc.

Results:

We found that the percentage and number of peripheral blood monocytes and neutrophils after DBS--STN applied in PD rats were elevated. However, differences between control and DBS-STN groups did not reach statistical significance.

Conclusion:

The obtained results suggest a potential peripheral mechanisms of DBS-STN immunomodulation, which may have neuroprotective abilities.

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Characterization of macrophages/microglial activation in the Spared Nerve Injury versus Spinal Nerve Ligation pain models

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Introduction: Peripheral neuropathic pain involves macrophages/microglial activation it the spinal cord (SC) and dorsal root ganglia (DRG), as well as interactions between these cells and neurons, which can be mediated by cytokines such as CCL2. The aim of this study was to investigate macrophages/microglial activation and CCL2 expression after two neuropathic pain models: spared nerve injury (SNI) and spinal nerve ligation (SNL).

Materials and methods: SNI and SNL pain models were performed on CX3CR1-GFP transgenic mice, while sham conditions involved exposing the nerves without damaging them. Seven days after SNI and five days after SNL, L3, L4, L5 DRG and their corresponding SC segments were collected and sectioned. DRG sections were immunostained using antibodies against NF200 or CGRP and CCL2, while SC sections were quantified for CX3CR1-GFP microglia.

Results: Our results show that SNI and SNL induce microglia activation at the SC level, in the dorsal and ventral horns and that the distribution of activated microglia changes from L3 to L5, as well as between SNI and SNL. At the DRG level, CCL2 is mostly secreted by large NF200(+) neurons and to a lesser extent by small CGRP(+) neurons, and nerve injury does not cause major changes in its expression. After SNI, macrophage activation is mostly observed in L3/L4 DRGs, while after SNL this activation is mostly restricted to L5 injured site, as well as neighboring L4.

Conclusions: Both SNI and SNL pain models induce macrophages/microglial activation but with different distributions of activated cells at the SC and DRG level.

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The authors declare that there is no conflict of interest.

Inhibition of histone deacetylase class IIa with TMP-195 suppresses rotenone and 6-OHDA induced inflammation in PC12 and SH-SY5Y cells

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Histone modifications are a hot topic for understanding possible epigenetic mechanisms in neurodegenerative diseases, especially Parkinson's disease (PD). In these modifications, histone acetylation status comes forward with the rise of clinical usage of histone deacetylase inhibitors. However, due to the overall effects of histone deacetylase enzymes in different classes, knowledge about their effects on PD is still limited. Therefore, the current study investigates the effects of TMP-195, which is a class IIa selective histone deacetylase inhibitor, on rotenone and 6-OHDA (hydroxydopamine)-induced neuroinflammation in PC-12 and SH-SY5Y cells. Cells were treated with rotenone, 6-OHDA, and TMP-195 for 24 hours, then cell viability was assessed. After determining treatment doses, cells were incubated with rotenone, 6-OHDA, rotenone+TMP-195, and 6-OHDA+TMP-195 for 24 hours. Next, mediums were isolated, and tumor necrosis factor- α (TNF-α), interleukin 1-β (IL-1β), and interleukin-6 (IL-6) levels were determined by enzyme-like immunosorbent assay (ELISA). TMP-195 did not significantly affect the cell viability at the 1, 3, 10, and 30 nM concentrations. Additionally, TMP-195 at the dose of 30 nM significantly inhibited rotenone and 6-OHDA induced increase in tumor necrosis factor- α (TNF-α), interleukin 1-β (IL-1β), and interleukin-6 (IL-6) levels. Our results suggest that TMP-195 markedly inhibited rotenone and 6-OHDA-induced neuroinflammation in both PC12 and SH-SY5Y cells.

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Neural basis of anticipation and premature action in the frontal cortex

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Planning and anticipating motor actions can increase behavioral performance[1], however, it can also lead to premature actions[2]. Although the anterior lateral motor cortex (ALM) is known to contribute to correct motor planning[3], it is currently unknown whether it is also involved in premature motor output. Here, this was addressed by using whole-cell patch clamp recordings from layer 2/3 (L2/3) pyramidal neurons within the ALM while mice were performing a cued-sensory association task. During both cor-

rect and premature performance in the task, a robust voltage response was evoked in L2/3 pyramidal neurons during the auditory cue. This cue-evoked voltage response was context dependent and greater during premature behavior. Optogenetically suppressing ALM during the cued-sensory association task lead to enhanced behavior, with fewer, and more delayed, premature responses and faster correct responses. Taken together, our findings suggest that ALM plays a critical role in anticipation by not only contributing to motor planning but by also driving premature actions.

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Autistic features in adult rats with modeled autism

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Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by deficits in communication and the presence of repetitive behaviors. A growing body of evidence has also shown that ASD is linked to GABAergic system dysfunction. Despite ASD is a long-life disorder, most research focuses on symptoms at a young age. Therefore, we investigated whether autistic symptoms persist into adulthood. In addition, levels of parvalbumin (PV) and glutamate decarboxylase (GAD) in the brain were examined.

Methods:

Pregnant Sprague-Dawley rat dams received a single *i.p.* injection of valproic acid (VPA, 500 mg/kg) or vehicle at gestational day 13. The 45-47 days old rats were then tested using the odor preference test. Furthermore, locomotor activity and repetitive behaviors were automatically scored using actometers. Additionally, levels of PV and GAD in the brain were measured using ELISA.

Results:

There were no differences in odor preferences between control and autistic-like rats. Notwithstanding, VPA rats tended to be less interested in the exploration of the bedding, which was reflected in the diminished number of vocalizations emitted during the exploration. Additionally, autistic-like rats have increased locomotor activity and the number of repetitive behaviors. We have also observed changes in GAD and PV levels in the cortex and hippocampi of female ASD rats.

Conclusions:
ASD symptoms in animals exposed to VPA persist into adulthood. It concerns not only behavior but also changes at the cellular level. Additional research is needed to accurately analyze the changes caused by ASD, especially when it comes to alterations at the molecular level.

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The authors declare that there is no conflict of interest.

Gender and the phase of estrous cycle dependent regulation of brown adipose tissue activity by brain uroguanylin

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Postprandial activation of brown adipose tissue (BAT) is gender and age dependent (1, 2). Since the uroguanylin (UGN), as an agonist of ganylate cyclase C (GC-C), leads to browning after prolonged i.c.v. application and it is released from the gut after a meal, in this study we determined the acute activation of BAT by UGN. In this study, male and female C57Bl/6NCrl were used. The activity of BAT was determined by infrared thermography (FLIR T-1020). The expression of UGN in hypothalamus upon insulin or GLP-1 stimulation was determined by GUCA2B ELISA Kit. GC-C was localized in POMC and dopaminergic neurons in Arcuate nucleus of hypothalamus by immunohistochemistry. In older animals five time smaller amount of UGN i.n. significantly increase BAT activity when compared to *i.p.* application. This activation was smaller in female animals in diestrus and not present in estrus. Insulin and GLP-1, 2h after i.n. application decreased pro-UGN expression in hypothalamus. Differences in BAT activation due to estrous cycle could be explained by increased and different pattern of expression of GC-C in hypothalamus in female mice in diestrus (3). Application of insulin or GLP-1 decreases UGN expression in hypothalamus. When insulin or analogues of GLP-1 are used in treatment of diabetic patients, the decrease of BAT activity and glucose expenditure by BAT, via decrease of UGN expression, could be expected. This study could lead to development of medicaments for activation of BAT for treatment of hyperglycaemia in diabetic patients, which will improve glucose metabolism and postpone insulin application.

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Utilizing *c-fos* to identify brain regions and cell populations recruited during incubated cocaine craving in rats

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A time-dependent increase in cocaine seeking has been observed in animal models of cocaine abuse, as well as in abstinent cocaine users. This phenomenon, also termed 'incubation of cocaine craving,' is thought to contribute to high rates of relapse in cocaine use disorder. And while several studies have explored neural substrates of incubated cocaine craving, a systematic investigation of circuitry recruited with early vs. late cocaine-seeking has not been conducted. In the current study, we used *c-fos* to map neural activity related to cue-elicited cocaine seeking after prolonged (45 days) vs. brief (1 day) abstinence. Adult male Sprague-Dawley rats were trained to self-administer cocaine (1-to-6 h/day) for 18 days. After 1 or 45 days of abstinence, rats were re-exposed to cocaine-paired context and cues, or kept in their home cage. Subsequently, *c-fos* mRNA was measured by fluorescent in situ hybridization (FISH, RNAscope) at the three coronal plains that contain key regions implicated in cocaine seeking. As activation of metabotropic glutamate receptor 5 (mGlu5) is known to play an important role in cocaine-induced behaviors, multiplex FISH was utilized to further categorize *c-fos* expression limited to the mGlu5-expressing subpopulation of cells. Multiple brain areas, including the dorsal hippocampus, basolateral amygdala, and ventral orbitofrontal cortex, were selectively activated during late/45d (but not early/1d) cocaine seeking. Follow-up correlational analysis was used to uncover multi-regional networks that are co-activated during late cocaine seeking. Future experiments will evaluate the causal role of identified neural circuits and mGlu5-expressing cells within these circuits in regulating incubated cocaine craving.

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Anatomical dissection of the connectivity between the ventral tegmental area and basal forebain nuclei in mice

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Neuromodulatory systems of the brain can synchronously modulate brain regions at multiple time scales. Early anatomical studies of the midbrain dopaminergic system suggested a direct connection between the dopaminergic neurons of the ventral tegmental area (VTA) and cholinergic basal forebrain

nuclei (BF) [1,2] which could serve as an anatomical basis for synchronized activity of these two neuromodulatory systems. However, these studies did not completely reveal the projection topography and target selectivity of the major neurotransmitter systems arising from the VTA and innervating the BF nuclei.

To address this question, we performed cell-type specific anterograde tracing in transgenic mouse lines to examine the projection patterns of VTA dopaminergic, glutamatergic [3] and GABAergic neurons. We found that these cell types differentially innervate BF nuclei; the densest dopaminergic and glutamatergic innervation could be observed in the lateral part of the ventral pallidum (VP) and VTA-GABAergic fibers innervated the VP and the Diagonal Band of Broca (DBB). We found prominent VTA-glutamatergic innervation of the medial septum as well in contrast to the low density of VTA-dopaminergic fibers in that area. Moreover, we found coexpression of dopaminergic (TH) and glutamatergic (vGluT2) markers specifically in the ventral pallidum, raising the possibility of glutamatergic-dopaminergic cotransmission. In the future, we would like to identify the BF target cell types of the VTA projections, which might reveal a monosynaptic connection between the dopaminergic and cholinergic neurons as well.

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Functional segregation of hippocampal subfields across the proximodistal axis in novel spatial and novel spatial cue learning

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Hippocampal long-term plasticity is tightly linked to learning about generalized changes in space, or novel content of an environment [1,2]. These processes are accompanied by the expression of immediate-early genes that is rapidly and dynamically triggered as response to neural activity [3]. Here, we investigated the effect of different forms of spatial learning on the expression of Arc and Homer1a mRNA and compared it within the proximodistal axis of the rat hippocampus. We employed fluorescent in situ hybridization and subsequent high-resolution imaging technique to map the activity of hippocampal

subfields during exploration of novel spatial environment or novel object configuration. These techniques are based on the detection of experience-dependent nuclear encoding of Arc and Homer1a mRNA. We showed that learning about general aspect of space resulted in potent and significant induction of immediate-early genes in all hippocampal subfields. By contrast, novel configuration of objects triggered subregion-specific expression of Arc and Homer1a mRNA. Our results demonstrate the functional segregation of the hippocampal subfields in the encoding of different aspects of spatial information.

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Exclusive targeting of extrasynaptic NMDA receptors ameliorate tactile allodynia after nerve injury in mice

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The mechanisms of neuropathic pain are associated with glutamatergic excitotoxicity [1]. This process of neuronal damage is mediated by tonic overactivation of N-methyl-D-aspartate receptors at extrasynaptic sites (eNMDAR) [2]. Selective blockade of eNMDAR is a potential way of preventing the excessive pathological activation that associated with pain development while normal synaptic transmission is preserved. Considering these facts, we used an exclusive nanostructured antagonist of eNMDARs – AuM - that due to its extended dimensions cannot enter the synaptic cleft and reach synaptic NMDARs, but can effectively block eNMDARs [3]. In this work we studied the effect of eNMDA receptors blockade on mechanical allodynia in 2-month old mice after right-side spared nerve injury (SNI). We stereotaxically injected 0.5 µl of 25 nM AuM or the same amount of saline into the right spinal dorsal horn. After that, mice were subjected to mechanical sensitivity testing by von Fray filaments starting from 3 to 25 day after SNI. Different experimental schemes of AuM-treatment (5 days after SNI or simultaneous with SNI modeling) were probed to figure out the therapeutic effects of spinal eNMDA inhibition. Our data

revealed that the SNI animals treated with AuM right after the injury show significant slowing down of allodynia progressing compared to saline-injected SNI animals. Post-SNI (5 days) treatment display significant reduction of hypersensitivity. Our findings indicate the involvement of eNMDAR at different stages of SNI – induced pain development and offer the opportunity to develop novel therapeutics to ameliorate the impact of nerve injury.

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Conflict of interest: Anna Honcharova, Alex Savchenko, Elena Molokanova, and Nana Voitenko declare that they have no conflict of interest.

Morphofunctional comparison of neocortical layer III pyramidal neurons from human mutant tau transgenic rTg4510 and control mice

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Transgenic rTg4510 mice express the P301L mutant tau protein, a hallmark of frontotemporal dementia (FTD). By 9 months of age, rTg4510 mice recapitulate many pathological features of FTD at the molecular, microscopic, and behavioral level. Layer III frontal pyramidal neurons of these transgenic (TG) and age-matched wild-type (WT) mice were morphofunctionally compared by using the new method of morphofunctional matrices (MFM). Based on 3D morphological reconstructions and electrophysiological data, segmental cable models of neurons were set up in the NEURON software to simulate subthreshold dendritic signaling. Postsynaptic potentials (PSP) were simulated by current injections at different dendritic sites and transfers and delays of PSPs between dendritic sites and the soma were computed. MFMs, representing association of path distances of dendritic sites and transfers or delays of PSPs between dendritic sites and the soma, were calculated for each TG and WT cell. Neurons were grouped by cluster analysis based on the similarity of the neurons' MFMs. Degree of separation of neurons into two groups was quantified by homogeneity indices. We found that separation of the TG and WT neurons is not statistically significant (Wilcoxon test, p=0.314, n=100) due to their high morphofunctional similarity.

This result suggests that alterations in neural network functions in FTD are primarily due to alterations in synaptic connections rather than in morphofunctional properties of neurons in the affected neural networks.

MFMs use normalized distances, transfers and delays. For comparison of TG and WT neurons by using absolute scales, see our accompanying poster that reached identical conclusion.

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Revealing the impact of chemical fixation on brain tissue using Super-Resolution Shadow Imaging (SUSHI)

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Chemical fixation using paraformaldehyde (PFA) is a commonly used technique to preserve biological samples and is the first step in standard protocols of immunohistochemistry and electron microscopy. However, chemical fixation does not only faithfully preserve the sample, but can introduce serious fixation artifacts. In the case of transcardial perfusion of the brain with PFA, a nearly complete depletion of the extracellular space (ECS) accompanied by a heterogeneous mix of tissue swelling and shrinkage have been observed. While these effects can dramatically affect the microanatomical relationships inside the brain tissue, they have never been observed directly and quantified via a 'before-and-after' type of experiment, leaving us with little knowledge about which (extra)cellular structures are particularly sensitive. This is owing to technical difficulties in resolving the fine structures of ECS and small cellular structures such as dendritic spines and astrocytic processes.

Using the recent SUSHI (Super-Resolution Shadow Imaging; [1]) technique, which enables us to image ECS in living brain slices at the nanoscale, we established a protocol to track the changes in brain tissue morphology upon PFA chemical fixation over time. By combining SUSHI with positive labeling of neurons and/or astrocytes, this technique also allows us to observe how neurons and astrocytes change in parallel with the changes in ECS. This study provides the first detailed characterization of the response of brain tissue to chemical fixation, which helps us better understand the limitations of current PFA-based protocols and a useful read-out for improving them.

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Brain state dependent responses of midbrain dopaminergic neurons' to the aversive stimulus

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Dopaminergic neurons of the ventral tegmental area (VTA) and substantia nigra pars compacta (SNc) constitute the stem of reward and motivation system in the mammalian brain. Midbrain dopaminergic neurons have long been thought to respond uniformly to aversive or noxious stimuli, displaying short latency, transient pause in firing. However, later studies revealed a subpopulation of VTA and SNc dopaminergic neurons that exhibit excitatory responses to aversive stimuli. Given that both level and pattern of VTA and SNc dopaminergic neurons' activity depend on altering brain states under urethane anaesthesia, we hypothesized that midbrain dopaminergic neurons' responses to the aversive stimuli are also dynamically modulated. We carried out *in vivo* recordings of dopaminergic neurons' responses to the electrical footshocks in urethane anaesthetized rats. In order to verify dopaminergic character of recorded neurons, we used single-unit juxtacellular recording-labelling technique (wild-type SD rats) or optotagging (TH-Cre rats). Consistently with previous studies, we recorded two subpopulations of VTA and SNc dopaminergic neurons – excited or inhibited by the footshocks. Interestingly, our observations led us to discover the third, previously unknown, population of dopaminergic neurons, which is characterized by dynamic, brain state dependent changes in the type of response to electrical footshocks. These dopaminergic neurons are inhibited by aversive stimulus during REM-like brain state but change their response to excitation during NREM-like brain state. The results of our research verify and supplement the current knowledge on the coding and processing of aversive events by midbrain dopaminergic neurons.

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The authors declare that there is no conflict of interest.

Alterations in distribution and quantity of EAATs transporters of blood cells populations after remote ischemic preconditioning

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Glutamate homeostasis disruption was observed in the pathology of many chronic neurodegenerative diseases, as well as traumatic brain injuries. Our previous results have suggested that neuroprotection mediated by rapid remote ischemic preconditioning (RIC) could lie in the accelerated release of glu-

tamate from nerve tissue to the blood, which preserves neurons from the exposure to the glutamate toxicity. The fate of glutamate outside the brain of RIC treated animals remains unknown.
Our previous results showed involvement of EAATs transporters in increased glutamate scavenging capacity in stimulated blood cells by remote ischemic conditioning. In this paper, we observed alterations in EAATs quantity (western blot analysis), distribution (imunocytofluorescent labeling) and influence of newly synthesized proteins (resting glutamate measurement after inhibition of protein synthesis).
We detected alterations in the fluorescence intensity of EAAT3 on erythrocytes after exposure to glutamate in their surroundings. RIC evoked an increase in EAAT2 and EAAT3 fluorescence intensity on lymphocytes. All the changes were diminished with protein synthesis depletion. In both, the transfer depends on the preserved synthesis of proteins, while the density of transporters remained unchanged. On the contrary, we detected increased density of EAAT1 transporters in platelets.
We can conclude, that the increased scavenging capacity of the erythrocytes and lymphocytes is the most probably caused by transmembrane trafficking of existing EAAT2 and 3 (that is dependent on the synthesis of its proteinaceous modulator). RIC stimulated platelets scavenged glutamate more effectively than untreated counterparts probably due to the overexpression of EAAT1 transporter and other EAATs independent mechanisms.

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scConnect: a method for exploratory analysis of cell-cell communication based on single cell RNA sequencing data

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All multicellular organisms depend on cell-to-cell communication. This is often achieved using evolved ligand and receptor pairs, which provides the specificity needed to conduct paracrine signaling and still only affect the intended cell types expressing the correct receptors. Here, we have developed a method (called scConnect [1]) that utilize gene expression levels of cell types in single cell RNA sequencing (scRNA-seq) experiments to estimate expression of small molecular ligands, peptidergic ligands and receptors. This method creates interactions between cell types expressing known ligand and receptor pairs and stores this information in a multi-directional graph. The graph can be analyzed using a web application allowing the user to filter out unspecific interactions and drill down to investigate connections of interest. We demonstrated this method on a brain dataset [2], identifying both well-known interactions between brain regions, and novel interactions that can be used for hypothesis generation. We could also detect expected interactions in a human melanoma dataset [3] and identified differences in

chemokine signaling between two types of malignant cell types. In conclusion, scConnect can be used in any project utilizing scRNA-seq data and provide valuable information about the ligands and receptors expressed, and how these could contribute to the cell-to-cell communication in the tissue.

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Effects of metformin, cyclosporine and rotenone on activation of developing brain microglia under normoxic and hypoxic conditions

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Hypoxia may affect neural tissue via microglia, however, mechanisms and consequences of microglial activation during hypoxia are not well understood. We aimed to investigate effects of inhibitors of mitochondrial complex I – metformin and rotenone, and classical inhibitor of permeability transition pore (mPTP) on microglial cells. Primary rat microglial cultures at 7–11 DIV were treated with metformin (Met, 3 mM), cyclosporin (CsA, 10 μM) and rotenone (Ro, 5 nM) under normoxic or hypoxic (2% O_2) conditions for 24 h. We show that 24 h hypoxia reduced number of microglia and had a cell viability--reducing effect compared to normoxia. Met had no effect on viability and number of cells, CsA under hypoxic conditions tended to decrease both –viability and cell number, while Ro decreased cell viability but had no effect on microglia cell numbers. Hypoxia increased glutamate in microglia culture media, and pre-treatment with Met but not Ro or CsA tended to reduce glutamate levels after hypoxia. We also found that none of the compounds effectively blocked mPTP opening in intact cells. Calcium dependent fluorescence measurements showed spontaneous calcium spikes; their generation was suppressed by CsA or trolox (0.1 mM), and enhanced by Ro, suggesting that Ca^{2+} spikes were mediated by mPTP opening. Hypoxia increased the frequency of Ca^{2+} spikes, while Met reduced the effect of hypoxia.
In conclusion, our results suggest that hypoxia facilitates opening of mPTP, slightly reduces microglial viability in monotypic cell cultures and causes release of glutamate into culture medium which may be reduced by Met.

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The authors have no conflict of interest to report.

Anodal trans-spinal direct current polarization facilitates spinal MN firing in SOD1 G93A mouse model of Amyotrophic Lateral Sclerosis

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Excitability disruptions leading to spinal motoneuron (MN) degeneration, are the most prominent feature of amyotrophic lateral sclerosis (ALS). It was recently shown that restoration of the decreased levels of synaptic excitation with chemogenetics, reduces the levels of disease markers in SOD1 G39A mouse model of the disease. Interestingly, these positive effects were only seen when the MN firing levels were also increased. In opposition, no positive effects were seen, when the increase of synaptic excitability was countered by a parallel decrease of MN intrinsic excitability limiting its activity. This points to a crucial role of MN firing in maintaining cell physiological profile. Here we present a novel method of altering spinal MN's firing properties with trans-spinal direct current stimulation (tsDCS). 15 minutes of 30μA, anodal tsDCS was applied to pre-symptomatic SOD1 G93A mice while performing intracellular recordings of spinal motoneurons *in vivo* under general anesthesia. Immediately after the polarization onset, MNs membrane potential was depolarized, the threshold for firing was decreased, the slope of the I-V curve was increased and the cell continued to discharge at lower intracellular current stimulation intensities. These signs of increased intrinsic excitability were visible during the entire course of the current application. Most interestingly, after the polarization current was switched off, the cell's membrane potential returned to pre-polarization values, however, the increased excitability signs such as the decreased threshold for firing were still visible in the same cell up to 15 min. after polarization. TsDCS may therefore be an attractive new approach for disease management.

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Conflict of interest: The authors are not aware of any conflict of interest that can be linked to this study

Neuroprotective potential of low-basicity 5-HT7 receptor agonists: A study on human neuroblastoma SH-SY5Y cells

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The serotonin receptor 5-HT7 is widely distributed in the brain playing multiple functions in physiological and pathological conditions. However, its role in neuroprotection still remains poorly recognized. The present study was undertaken to test potential neuroprotective effects of the commercially available 5-HT7 agonist, 5-carboxamidotryptamine (5-CT) and antagonist, SB269970 as well as new low-basicity

5-HT7 agonists (AH-494, AGH-194 and AGH-238) synthesized by the Department of Medicinal Chemistry IP PAS, on human neuroblastoma SH-SY5Y cells. The cells (undifferentiated and retinoic acid (RA)-differentiated) were pretreated with different concentrations (0.001-1 uM) of 5-HT7 ligands for 30 min, followed by 24 h of treatment with the oxidative stress inducer, hydrogen peroxide (H_2O_2) or DNA damage/apoptosis inducer, doxorubicin. The data showed that 5-CT (0.01 and 0.1 uM), AH-494 (0.01 uM) and AGH-238 (0.01 and 0.1 uM) partially attenuated the H_2O_2-induced cell damage in undifferentiated SH-SY5Y cells as confirmed by the cell viability WST-1 assay. However, this effect was not connected with attenuation of H_2O_2-induced caspase-3 activity and protection was not observed in RA-SH-SY5Y cells. We did not find any protective effects of any of the tested 5-HT7 ligands against the doxorubicin-induced neurotoxicity. These findings showed that some 5-HT7 receptor agonists could be neuroprotective against oxidative stress-induced cell damage in neuronal-like cells and some of its mechanisms could be shared with retinoic acid. These results justify continuation of pre-clinical studies on 5-HT7 receptor as a potential drug target for the treatment of neurodegenerative disorders.

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Cell-type-specific localization of kynurenine aminotransferase-2 in the mouse brain

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Kynurenic acid (KYNA) plays an important role in neuroprotection and neuromodulation due to its broad-spectrum receptor modulatory effects. In many neurodegenerative and psychiatric disorders, abnormal levels of KYNA have been observed. KYNA production is catalyzed by enzymes called kynurenine aminotransferases (KAT). So far, four KAT isoforms (KAT-1,-2,-3,-4) have been identified from which KAT-2 is described as the major biosynthetic enzyme of KYNA both in the murine and the human brain. The treatment of diseases affected by abnormal KYNA levels requires the manipulation of the kynurenine system. Since KAT-2 has the ability to regulate KYNA levels, it could be advantageous to study the tissue- and cell-type-specific localization of the enzyme. Previous studies found that in the rat brain KAT-2 is localized not in neurons but in astrocytes [1]. In the mouse brain, however, KAT-2 expression was observed in neurons too [2]. This study aimed to further investigate the cell-type specific localization of KAT-2 in the mouse brain using fluorescent immunohistochemistry. We observed a broad KAT-2 distribution in the whole mouse forebrain and cerebellum. The results show that the most prominent KAT-2$^+$ cells in the mouse brain are GABAergic neurons. Most of these KAT-2$^+$ cells showed complete overlap with many GABAergic neuronal markers while using double immunolabeling. The present study is the first to report

data about the cell-type-specific neuronal localization of KAT-2 in the mouse brain. These anatomical results are supporting future pharmacological and kynurenergic manipulation studies in mice.

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A method to induce low and high flow states while keeping participants on task

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When the challenge of a task meets the skills of the person doing it, a specific mental state, called flow, can emerge. While the psychologic aspects of this mental state have been well-defined, the neural and physiological correlates leading to its emergence have yet to be unraveled. Currently, experimental investigations of these correlates usually contrast conditions of different challenge levels, with the risk of inducing boredom or frustration in some conditions, both known to lead to disengagement, or off-task behavior. Thus, it remains unclear whether previously observed differences ascribed to flow may rather reflect differences in how much participants stayed on-task between the high and low flow conditions being contrasted.

To remedy this, we present a method to induce states of low and high flow while controlling that participants remained on task in both conditions. Using an action video game, we contrasted a condition where the challenge was matched to the skills of the participant to one where the challenge surpassed their skills. Participants report significantly different levels of flow state while reporting similarly trying their best – and thus remaining on task- in both conditions. Using this paradigm, we also investigated potential physiological correlates of flow. While our data confirmed the known difference between an off-task, rest condition versus our low and high flow conditions, they did not distinguish between the latter two. In light of the conflicting findings regarding the physiological correlates of flow, we propose that the present method provides a methodological advance for manipulating flow.

Keywords: Psychological flow, Video Game, Psychophysiology, Intrinsic Motivation, Effort

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Stem cell markers in transient fetal zones of the developing human cerebral cortex

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Transient fetal zones are a characteristic feature of the developing human cerebral cortex. Proliferative ventricular (VZ) and subventricular (SVZ) zones are a major pool of neuroepithelial stem cells which are neural and glial precursors. After being born in proliferative zones, postmitotic neurons migrate through the intermediate (IZ) and subplate (SP) zone to reach their final target - the cortical plate (CP). Interestingly, beside in the proliferative zones, stem cell markers are also found in other transient fetal zones.

The aim of our study was to identify stem cells and cells with proliferative capacity in the human non-proliferative, connectivity-rich synaptic SP [1] during early and mid-fetal period utilizing double and triple immunofluorescence. Therefore, we used SOX2 as a transcription factor responsible for upholding the identity of neural stem cells [2] and Nestin as an intermediate filament protein present in dividing cells during early neurogenesis. Our results showed that both SOX2 and Nestin are also expressed in the non-proliferative SP zone during early and mid-fetal development, which opens a question about their neurodevelopmental role in corticogenesis. In conclusion, presence of SOX2+ cells outside proliferative zones suggests possible different roles of these cells other than proliferative.

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Novel Data Acquisition System for Measuring and Evoking Neural Activity Using Multi-Electrode Arrays and Dedicated Electronics

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We present a novel Data Acquisition (DAQ) system intended to work with custom-designed Application Specific Integrated Circuit (ASIC) called Neurostim-3 [1] and Multi-Electrode Arrays (MEAs). This hardware and software combination allows simultaneous electrical stimulation and brain activity recording from up to 512 independent channels with 40 kHz data sampling rate. It can generate time-intensive sequences of microstimulation pulses with a maximum resolution of 0.64 µs which can be further optimised for artefacts reduction using information about the measured impedance of each electrode [2]. DAQ communicates with the hardware, saves data for later investigation and conveniently visualises recorded waveforms while the experiment is running. The software was carefully tailored to take advantage of underlying ASIC capabilities with special consideration of real-time operation with no time-consuming data precomputation requirement. We characterised Neurostim-3 signal processing quality using this software. The results show signal gain linearity in a wide range of input signal amplitudes irrespective of the signal gain applied. Moreover, a high-pass cut-off frequency can get below 1 Hz, which can be useful for recording Local Field Potential (LFP). The system was tested in a series of pilot *in vivo* experiments during which physiological data was acquired from two MEAs (128 channels) inserted to the thalamus and cortex of an anesthetized rat. Recorded signals—spontaneous and evoked by tactile and electric stimulation—were typical for investigated structures, which proved that the presented system is a powerful tool for investigating information processing in the brain.

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Can we model simultaneous brain and retinal ischemia with the filamentous middle cerebral artery occlusion method?

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Approximately 65% of the ischemic stroke patients suffer from transient or permanent visual impairment. Several studies have shown the evidence of retinal dysfunction following middle cerebral artery occlusion (MCAO) [1, 2]. However, the disruption of retinal blood supply via MCAO remains poorly investigated since there is no clear consensus on the origin of the ophthalmic artery (OA) in rodents [3]. The aim of our study was to clarify if and which of the two modifications of MCAO induce retinal ischemia by longitudinal *in vivo* magnetic resonance (MR) assessment of cerebral and retinal vascular perfusion and the resulting ischemic injury. To compare the brain and retinal ischemic lesion development, two MCAO approaches, the 30-minute modified Koizumi and Longa methods were applied on four months old male C57Bl/6J mice. In sham-operated, the filament was immediately withdrawn. Seven days prior, and on the 2nd, 9th, and 35th day after MCAO the animals were scored for neurological deficit followed by MR imaging using a T2-weighted anatomical scan and a T2-map of the brain and the ipsilateral eye and a 3D-TOF whole-brain angiography. MCAO significantly reduced the blood flow to the MCA and completely reduced the blood flow through the pterygopalatine artery, which gives rise to the OA. The retinal responses to ischemia corresponded to those in the brain when the Koizumi method was performed. The short occlusion with prolonged hypoperfusion resulted in simultaneous brain and retinal ischemia. The Longa method resulted only in brain ischemia, with greater tissue loss compared to the Koizumi method.

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Sensorimotor functions in condition of altered dopaminergic control in DAT-KO rats

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Brain and spinal neuronal sensorimotor networks modulate by different monoamines, especially dopamine [1]. Alteration in the dopaminergic system affect the coordination, motor reactions rate, muscle tonus and locomotor activity [2]. The control of extracellular Dopamine (DA) levels and maintaining the DA stores is realized by dopamine transporter (DAT), which carries released DA back into the neurons. Lack of the dopamine transporter leads to prolonged extracellular life time of released DA that is caused increase in the striatal basal extracellular DA levels [3]. Here DAT knockout rat model was used for investigation of changes in dopaminergic control of CNS function. Sensorimotor functions were evaluated during walking on treadmill. The medial-lateral displacement of pelvis, durations of phases of locomotor cycle and angle of hindlimb joints were analyzed. Motor coordination was assessed on balance beam test. DAT-KO rats demonstrated significantly less duration of stance in locomotor cycle during walking on treadmill compared to WT (t – test, $p < 0.05$). In addition, we observed higher flexion of the ankle joint during different subphases of locomotor cycle (Mann-Whitney test, $p<0.05$). The described kinematic features of the limbs movement were accompanied by a decrease of the medial-lateral pelvis displacement (Mann-Whitney test, $p<0.05$). DAT-KO rats had better motor coordination in Balance beam test. The percentage of falls in DAT – KO rats was lower (Fisher's exact test, $p<0.05$) than in WT. It can be concluded that increasing the amount of the extracellular dopamine leads to specific alterations to the locomotor control and dynamic balance.

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Reduced density of γ-aminobutyric acid (GABA) interneurons co-expressing calbindin in the amygdala as one of the possible mechanisms of increased anxiety in estrogen receptor β-deficient mice

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The previous studies have shown that the female oestrogen receptor β (ERβ) knock-out mice display increased anxiety-like behaviours associated with a reduced threshold for the induction of synaptic plasticity in the basolateral amygdala. A reduced threshold in this brain region may indicate decreased γ-aminobutyric acid (GABA) activity that could lead to exaggerated responses to normally innocuous stimuli. Thus, alterations in the GABAergic population in the amygdala could be one of the possible mechanisms of increased anxiety in ER-β knock-out mice.

To test this hypothesis the amygdala sections were analyzed in wild-type and ERβ knock-out (adult female mice and processed by immunohistochemistry to visualize calbindin (CB+), parvalbumin (PV+) and calretinin (CR+) neurons, main subsets of the GABAergic population in the amygdala. These neurons were then counted in six nuclei (lateral, basolateral, basomedial, medial, central, and cortical), and the density values were statistically compared between wild-type and knock-out mice.

The results showed that in ERβ knock-out mice, the density of CB+ neurons were significantly reduced in all studied amygdala nuclei while the populations of PV+ and CR+ neurons were not affected. However, in the medial and cortical nuclei slight reduction of CR+ cells were also observed but the difference was not significant.

In conclusion, deficits in the CB+ population which form in the rodent amygdala the largest subset of GABAergic neurons indicate that in the ERβ knock-out female mice the inhibitory tone may be insufficient to maintain proper excitatory/inhibitory balance. This imbalance could lead to increased anxiety-like behaviors observed in these mice.

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Experimental middle cerebral artery occlusion (MCAO) for studying hippocampus–associated mechanisms of post-stroke disorders: a comparative study of two models

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Introduction. There is a growing body of evidence that cortisol disbalance plays a key role in brain diseases. However, corticoid-dependent mechanisms involved in the development of post-stroke disturbances, in particular, depressive and cognitive disturbances, remain obscure. A chronic increase of cortisol level in ischemic stroke patients predicts worse outcome and accelerated cognitive decline [1, 2]. It is hypothesized that patients with focal brain injury, high cortisol may induce distant hippocampal damage associated with neuroinflammation and underlying delayed depression and cognitive decline [3]. Our research aimed to explore rat MCAO models with different corticosterone response for studying mechanisms of distant hippocampal damage.

Materials and methods. Koizumi (K) and Longa (L) MCAO models were used. Eighty-nine male Wistar rats were divided into control, sham-operated, and MCAO groups. Corticosterone and IL-1β were measured in brain regions and blood serum of rat 72 hours and 3 months after MCAO.

Results. Neurological deficit and infarct volumes were similar in K-MCAO and L-MCAO. Corticosterone levels in blood, hippocampus, and frontal cortex (contra- and ipsilateral hemispheres) in K-MCAO were significantly higher compared to controls and shams. In L-MCAO, corticosterone increased only in ipsilateral hippocampus 72h after MCAO. IL-1β level increased significantly only in ipsilateral hippocampus of K-MCAO rats 72h after MCAO. In K-MCAO, corticosterone level in blood remained higher compared with control and L-MCAO even 3 months after MCAO.

Conclusions. The data suggest the validity of comparative studies using K-MCAO and L-MCAO to explore mechanisms of glucocorticoid-dependent hippocampal damage after stroke.

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Cross-whisker adaptation of neurons in layer 2/3 of the rat barrel cortex

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Neurons in the barrel cortex respond preferentially to stimulation of one principal whisker and weakly to several adjacent whiskers. Such integration exists already in layer 4, the pivotal recipient layer of thalamic inputs. Previous studies show that cortical neurons gradually adapt to repeated whisker stimulations and that layer 4 neurons exhibit whisker specific adaptation and no apparent interactions with other whiskers. This study aimed to study the specificity of adaptation of layer 2/3 cortical cells. Towards this aim, we compared the synaptic response of neurons to either repetitive stimulation of one of two responsive whiskers or when repetitive stimulation of the two whiskers was interleaved. We found that in most layer 2/3 cells adaptation is whisker specific. These findings indicate that despite the multi-whisker receptive fields in the cortex, the adaptation process for each whisker-pathway is mostly independent of other whiskers. A mechanism allowing high responsiveness in complex environments.

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Expression of Angiotensin II Receptors in the Hypothalamus Pituitary Adrenal Axis after Spinal Cord Injury

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Angiotensin II (Ang II) is the main effector peptide of the Renin-Angiotensin System and important stress hormone. Both major types of Angiotensin receptors – AT_1 and AT_2 are present within the Hypothalamus-Pituitary-Adrenal (HPA) axis, the major system of endocrine stress. Spinal cord injury (SCI) leads to immediate activation of the HPA axis with systemic effects largely dependent on the localization of the injury. In order to better understand the role of Ang II and its receptors in the HPA axis under pathological conditions, we decided to investigate their expression at selected time intervals (1, 3, 7, 14, 21, 28 day) post-injury within the axis in adult Wistar female rats. The 40g compression at the Th9 spinal level lasting 15 minutes was used as the SCI model, the sham-operated controls underwent laminectomy only. The paraventricular nucleus (PVN), pituitary as well as adrenal glands were analyzed by the method of Angiotensin receptor binding. Separated plasma from collected blood was used for determination of Ang

II concentration in circulation. Plasma concentrations of Ang II were increased only in the first three days in both spinal cord injured and sham operated rats. Our results showed changes in receptor expression at all levels of the axis, with significant increase in AT_1 expression three days after SCI in the PVN and pituitary gland and more dynamic changes in AT_1 and AT_2 in adrenal glands.

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Gyrophoric acid, a secondary metabolite of lichens, may influence the brain structures and related forms of behavior

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Gyrophoric acid (GA) is a secondary metabolite of various lichens. Our study aimed to reveal the effects of GA on brain structures and related behaviors. The ability of GA to cross the blood-brain barrier (BBB) was analyzed using isolated endothelial cells (BMEC) from two-week-old Wistar rats. Then, the potential antidepressant effects of GA were tested on Wistar rats with immobilization stress-induced depression. Furthermore, the potential antitumor effects of GA in Fischer F344 rats with chemically induced brain cancer were analyzed. The behavior of rats from all experiments after daily administration of GA (10 mg/kg body weight) was tested in Elevated Plus Maze (EPM), Open Field Test (OFT), and Morris Water Maze (MWM). Our preliminary results indicate that GA crosses BBB and thus could directly affect brain structures. During the depression, GA potentiated the process of neurogenesis, assessed as a significant increase in Ki67 positive cells in the hilus of the hippocampus ($P<0.01$). Besides, GA influenced the number of mature NeuN positive neurons in the CA1 layer of the hippocampus ($P<0.01$). The behavior showed increased time spent in open arms in EPM. Moreover, our preliminary results show that GA was not able to influence the occurrence or size of brain tumors. In EPM, time spent in open arms increased significantly ($P<0.001$) compared with untreated group; center crossing and grooming activity remained at the level of healthy animals. Our results are the first evidence that GA could affect some brain structures and influence animal behavior.

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Searching for alternative model systems in investigation of excitability

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Monitoring and investigating regulation of single cell electrical excitability require an appropriate model system. We present a low-cost simple and robust plant model system which can be routinely employed in ion channel research as an alternative to animal model systems.

Characeaen macroalga *Nitellopsis obtusa* provides a plethora of high-resolution approaches revealing the mechanism of action potential (AP) generation, modulation and transmission. Here we present how specific electrophysiological parameters are altered by inhibitors of human two-pore channels (TPC): verapamil, tetrandrine, and NED-19, also non-specific action of organic solvent DMSO.

Two-electrode voltage/current clamp technique allowed to investigate modulation of parameters of action potentials and excitation current transients. Patch clamp technique was used to test the effects on single channel activity. It is also possible to investigate parameters of AP propagation and transmission using intracellular or extracellular recording techniques.

Intracellular recordings showed distinct effects by the inhibitors on shapes of APs and transmembrane ion fluxes during excitation. Particularly noteworthy are the depolarization of AP excitation threshold and prolongation of repolarization. Single channel recordings revealed that K^+ currents from the vacuole were not involved. Lethality of higher concentrations of the inhibitors was also confirmed by the used electrophysiological approaches. Additionally, it was assessed that DMSO does affect some of the excitation parameters, thus, an additional control should be considered.

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Rewarding effects of social interactions in adolescence are modulated by mu opioid receptors

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Adolescence is associated with a complex pattern of behavioural changes including decreased interest in bonding with family members. Here we investigated the rewarding effects of interactions among siblings across adolescence in male mice (postnatal days 35-42). The second goal was to test the role of the μ-opioid system in modulating social reward in adolescence. To study changes in rewarding value

of social interactions in C57BL/6J male mice a social conditioned place preference procedure (SCPP) was employed. We found that social interactions with siblings are rewarding for mice in their early and late adolescence, but not mid-adolescence (PD 37-39). Furthermore, the administration of the μ-opioid receptor antagonist – cyprodime – reversed the decline in rewarding effects of social interactions with siblings in mid-adolescent individuals. This observation may indicate the existence of a critical period for social interactions development between postnatal days 37 and 40. We hypothesize that μ-opioid receptors regulate social re-orientation from family to peers during adolescence. Thus, the research is also a good starting point to study the effects of other drugs on social reward and to determine to what extent and through which receptors the opioid system modulates social interactions.

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Parvalbumin interneurons and perineuronal nets in the hippocampus and retrosplenial cortex of a murine double hit model for schizophrenia

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Although the etiology of schizophrenia is not fully understood, early life aversive experiences and alterations in neurodevelopment are considered predisposing factors. Certain brain regions affected by early life stress are also altered in SCZ, including the hippocampus and, interestingly, the retrosplenial cortex (RSC). Studies in patients and animal models have found alterations in parvalbumin (PV) expressing interneurons, making them good candidates to study the neurobiological basis of this psychiatric disorder. Some of these alterations may be mediated by perineuronal nets (PNNs), specialized regions of the extracellular matrix, frequently surrounding PV+ interneurons. Here, we have used a murine double hit model combining a single perinatal injection of NMDAR antagonist (MK801) to disturb early postnatal development and post-weaning social isolation as an early life aversive experience. We have investigated the effect of the model and each of its factors on PV+ cells, and PNNs in the hippocampus and RSC of adult male mice, using unbiased stereology. In the CA1, but not in the CA3 region of the hippocampus, the number of PNNs and PV+PNN+ cells was affected by the treatment with MK-801. In the RSC, we observed a significant impact of isolation, treatment, and the interaction of both interventions on the number of PV expressing interneurons, PNNs, and PV+PNN+ cells. The present model constitutes a useful tool to investigate the effects of postnatal neurodevelopment and early life aversive experiences and the basis ofon schizophrenia. Our results may constitute the basis for further studies on PV expressing interneurons and PNNs in this disorder.

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Extremely low-frequency electromagnetic field (50 Hz) alters oxidant/ antioxidant balance in rat's brain

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Previous reports showed the stress-inducing properties of extremely low-frequency electromagnetic field (ELF-EMF), including disturbance of oxidative status of organism [1, 2]. However, there is evidence for the potential of ELF-EMF to activate the organism's antioxidant defense [3].

The aim of our study was to determine the effect of ELF-EMF (50Hz) on oxidant/antioxidant balance in the rat's brain. We hypothesized that the influence of ELF-EMF may be different depending on the strength of the field. Moreover, the direction of changes can be different. ELF-EMF may trigger adaptive response – the increase of antioxidant defense or may sensitize the organism to subsequent stress event due to the enhancement of oxidative stress.

Wistar rats were exposed for 1, 2, or 3 weeks to ELF-EMF of 1 or 7mT. Control animals were subjected to the same experimental procedure as the exposed groups, except ELF-EMF exposure. After each exposure brains were removed and the levels of protein carbonyl groups (CP), and total antioxidant capacity (TAC) were determined.

The level of CP was significantly elevated in animals exposed to 1mT only after 1st exposure, but in 7mT groups the level of CP was elevated also after 2nd and 3rd exposure. After exposure to 1mT the levels of TAC were comparable to the control values, but ELF-EMF of 7mT caused the decrease in the TAC level.

We concluded that the organism may adapt to ELF-EMF of 1mT, while 7mT exceeds the adaptive capacity of the organism and induces oxidative stress.

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Effect of STIM proteins on the co-localization of NMDA receptors with early endosomes in the process of NMDA receptor endocytosis

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Stromal interaction molecules (STIMs) are transmembrane proteins located mainly in the membrane of the endoplasmic reticulum (ER). In the absence of Ca^{2+} in the ER, STIMs are activated and participate in store-operated Ca^{2+} entry (SOCE). *N*-methyl-D-aspartate receptors (NMDARs) are the main neurotransmitter activated calcium channel, however SOCE is also present in neurons. NMDARs are heterotetramers composed of NR1, NR2 (NR2A-NR2D), and NR3 (NR3A-NR3B) subunits. The activity of NMDARs embedded in the cell membrane can be modulated by their internalization. We recently demonstrated that STIMs associate with NR2 and reduce Ca^{2+} influx through these receptors [1]. We assume that STIMs participate in NMDAR endocytosis. First, in primary neuronal culture, under various experimental conditions the co-localization of NMDAR subunits (NR1, NR2A and NR2B) with an early endosome marker was studied by immunocytofluorescence. We show that acute treatment with 50 μM NMDA + 100 μM glycine increases NMDAR co-localization with endosomes, indicating NMDAR endocytosis. Moreover, it is the NR1 that shows the highest degree of internalization after 15 minutes of stimulation in comparison to 5 and 30 minutes. To investigate the effect of STIM on NMDAR endocytosis, we reduced STIM proteins with lentiviral vectors that deliver shRNA specific for STIM1 or STIM2. One shRNA was selected for each STIM protein based on the efficiency of silencing of endogenous expression. Next, we will examine the degree of co-localization of NMDAR subunits with endosomes in neurons lacking STIM proteins to indicate whether NMDAR internalization is regulated by STIM proteins.

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Study of the dynamic interactions between glucocorticoids and brain-derived neurotrophic factor on brain cell physiology and stress adaptation

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Glucocorticoids (CORT) and neurotrophins, like brain-derived neurotrophic factor (BDNF), share common downstream pathways, through which they regulate glutamate neurotransmission, adult neurogenesis, and brain cell adaptative responses to stress [1-3]. These fundamental neurobiological processes are highly involved in common neuropathological conditions, including Alzheimer's disease. In the present work, we attempt to develop an *in vitro* system to evaluate the differential spatiotemporal interplay between CORT, BDNF and neurotrophin analogues on brain cells, under baseline and toxic conditions. For this purpose, we have set up primary cultures of adult neural stem cells (NSCs), hippocampal neurons and primary glial ones. Subsequently, the different cell types were characterized regarding the expression of BDNF receptor, TrkB, as well as glucocorticoid (GRs) and mineralocorticoid receptors (MRs); NSCs and primary astrocytic cultures favor the expression of GRs (~30 and ~4 times higher than MRs, respectively), contrary to neurons (~4 times higher MR expression). Moreover, the neurogenic and neuroprotective role of endogenous and synthetic neurotrophin molecules was identified under neurotoxic conditions (presence of the oligomeric β-amyloid) in the NSCs and neuronal cultures. In our running experiments, we seek to unravel the transcriptional and translational effects of CORT pulsatility (as opposed to constant CORT exposure or absence of CORT stimulation) on BDNF and TrkB levels in co-culture experiments, which will allow us to simultaneously study these effects and the interplay between the different brain cell types against neurodegenerative and stressful stimuli.

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Conflict of interest

The authors declare no conflicts of interest.

Gender and age peculiarities of electromyographic and anthropometric indices in qualified biathlon athletes

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The problem of locomotor skills teaching is in the focus of researchers attention due to the large amount of facts of neuromuscular system plasticity. At the same time, influence of gender and age factors on adaptation to physical exercise remains insufficiently studied. The objective of the research was to assess gender and age peculiarities of electromyographic and anthropometric indices and to reveal the correlation between above indices in qualified biathlon athletes (22 men and 22 women, 16-30 years of age, M_{age}=20.5, SD=3.8). The method of H (Hoffmann) reflex of soleus muscle was performed using neurodiagnostic complex Nicolet Biomedical Viking Select (Viasys Healthcare, USA). Two-factor analysis of variance considering two between-subjects factors (gender and age) was carried out in the SPSS 17.0. It was found that soleus H- and M-responses thresholds were higher, and maximal H- and M-responses amplitudes were smaller in women than in men. This might be due to thicker subcutaneous fat tissue and less muscle fiber volume in female body in comparison with males. M-responses amplitudes were smaller in juniors than in adult athletes. This might be the evidence of muscle hypertrophy under the influence of long-lasting physical exercise. Significant negative Pearson correlation was revealed between: height and body mass – H- and M-responses thresholds. Significant positive correlation was revealed between: height and body mass – H- and M-responses amplitudes. Hence, gender and age peculiarities of electromyographic indices might be a consequence of corresponding anthropometric differences. Obtained data demonstrate that gender and age should be considered during the electromyographic study.

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Exploring novel mitochondria-targeted compounds for neuropsychiatric disorders

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Mitochondria are key players in bioenergetic and biosynthetic pathways and mitochondrial dysfunction is implicated in several psychopathologies, including stress and anxiety [1, 2]. Therefore, pharmacologically targeting mitochondria is of great translational value. We have previously shown that selective mitochondrial targeting exerts anxiolytic effects [3]. Along these lines, we have now synthesized a modified version of the antioxidant hydroxytyrosol in order to selectively target mitochondria. We then assessed the properties and therapeutic potential of mitochondria-targeted hydroxytyrosol *in vitro* and *in vivo*. We investigated mitochondria-targeted hydroxytyrosol administration effects on cell proliferation, metabolism and oxidative stress readouts. Our results show that mitochondrial-targeted hydroxytyrosol modulates key components of glycolysis, the Krebs cycle and oxidative phosphorylation, along with changes in oxidative stress-related markers without affecting mtDNA copy number. Furthermore, it can also be administered chronically *in vivo* with no physiological side effects. Taken together, we present a mitochondria-targeted antioxidant which effectively modulates key bioenergetic pathways and propose an underlying mechanism of its action with therapeutic potential for translational applications in neuropsychiatric disorders.

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Early distant hippocampal damage after brain trauma in rats: Involvement of immediate seizures and corticosterone elevation

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Hippocampal damage after traumatic brain injury (TBI) is associated with late posttraumatic conditions, such as depression, cognitive decline and epilepsy [1]. Mechanisms of selective hippocampal damage after TBI remain obscure. We hypothesize that selective vulnerability of the hippocampus may be at least partially explained by stress and excitotoxicity as a result of immediate posttraumatic seizures [2]. TBI was modelled using lateral fluid percussion brain injury (LFPI) in 146 adults male Wistar rats. Immediate posttraumatic seizures were recorded and analyzed. The animals were sacrificed on days 1, 3, 7, and 14 after craniotomy. Levels of corticosterone (CS) and IL1- β in blood and the hippocampus were measured using ELISA. Brain sections were stained using Nissl and anti-IBA staining.

IL-1β was elevated only in the ipsilateral hippocampus on day 1 after trauma. Neuronal cell loss in the hippocampus was demonstrated bilaterally; in the ipsilateral hippocampus it was evident on day 3, in the contralateral on day 7. Microglial activation was evident in the hippocampus bilaterally on day 7 after TBI.

The duration of immediate seizures correlated with CS elevation. CS peak was detected on day 3 in blood, the ipsilateral and contralateral hippocampus and correlated negatively with microglial cells density in hippocampus. On day 7 the level of CS correlated positively with level of IL-1β in contralateral hippocampus.

The data suggest potential association of immediate post-traumatic seizures with CS-dependent neuroinflammation-mediated distant hippocampal damage.

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Neurons in the medial xprefrontal cortex encode social status of the familiar conspecifics

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Maintenance of group hierarchy lays a foundation for stability in many social species. Ability to recognize social status of one's conspecifics is critical for that process. Still, little is known about the neural mechanisms underlying responding to stimuli indicating a position in hierarchy of familiar individuals[1]. Although studies have shown that that the medial prefrontal cortex (mPFC) is involved in the dominance-based interactions in mice, its role in encoding social position of the group members remains unclear. We investigated how olfactory information about the social status of cagemates is encoded in the mPFC. Hierarchy of group-housed mice was tested in Eco-HAB[2], a semi-naturalistic environment for automated assessment of social behavior, by restricting access to 10% sucrose in time and space. Next, we presented the scents of the dominant and submissive conspecifics to the individuals from the middle of group hierarchy, while simultaneously recording single unit activity in their mPFC with silicone probes (Neuropixels).

We show that mPFC is strongly activated by the olfactory stimuli indicating social status. Moreover, olfactory stimuli from dominant and subordinate mice elicit diverse patterns of neuronal responses. Specifically, odor of the dominants evoked stronger populational activation than odor of the subordinates. However, we also found a significant subpopulation of cells, which responded preferentially to the latter. In conclusion, mPFC neurons encode information about the social status of familiar conspecifics. Notably, stronger populational activation to the odors of dominants suggests that information about high social status may be distinctly important and further facilitate adaptive social responses.

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Factors associated with vertebral artery tortuosity in non-diabetic patients with sporadic cerebral small vessel disease

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Cerebral small vessel disease (SVD) accounts for approximately 20% of all strokes and up to half of all dementias [1]. Also is known, that vertebrobasilar dolichoectasia is associated with MRI-defined markers of SVD [2]. This study aimed to reveal the factors associated with vertebral artery (VA) tortuosity. 59 consecutive non-diabetic patients (18 (30.5 %) men, mean±SD (Me) of age – 58.0±9.5 (59.0) years) with sporadic SVD were retrospectively analyzed. Carotid and vertebral arteries were imaged bilaterally with a standardized protocol by ultrasonography. Common carotid intima-media thickness (CC-IMT), VA tortuosity presence, middle cerebral artery pulsatility index were assessed. Systolic blood pressure (BP), diastolic BP, lipid profile (total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, triglycerides), fasting plasma glucose, fibrinogen, total white blood cell count, absolute neutrophil count, erythrocyte sedimentation rate (ESR), estimated glomerular filtration rate were determined. Logistic regression was used to evaluate factors associated with VA tortuosity. Statistical significance was set at $p<0.05$. 54 (93.1 %) patients had VA tortuosity. Univariate analysis showed that the presence of VA tortuosity was positively associated with CC-IMT (OR 138.6, 95% CI 1.0-18723.6, p=0.049) and age (OR 1.1, 95% CI 1.0-1.3, p=0.025) among the other factors determined including patient sex. On the other hand, only age was significantly associated with the presence of VA tortuosity by multivariate logistic regression (B=0.219, OR 1.2, 95% CI 1.0-1.5, p=0.035). We report that CC-IMT and age are associated with VA tortuosity presence and, accordingly, with sporadic SVD.

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Using simultaneous tDCS and auditory closed-loop stimulation to modulate slow wave sleep

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In humans, auditory closed-loop stimulation (ACLS) targeting the Up state of ongoing sleep slow oscillation (SO) enhanced endogenous SO, and thalamocortical spindles, but not consistently memory retention [1,2]. Efficacy of ACLS to influence brain rhythms and memory retention may depend upon physiological refractory mechanisms within the thalamo-cortical spindle generating system and/or (individual) cortical excitability levels [3]. This within-subject study employs transcranial direct current stimulation (tDCS) while delivering ACLS to investigate the potential neuromodulatory impact of cortical activity on overnight memory consolidation and post-sleep learning of ACLS. Participants received either ACLS or anodal tDCS+ACLS during nocturnal sleep after learning and before recall of two memory tasks (a non-sense word paired-associate and a figure paired-associate task). To assess post-sleep encoding subjects performed a word paired-associate task. A final session measured cognitive ability. TDCS+ACLS did not affect behavioural performance overall. However, applying cognitive ability as a covariate revealed a significant Condition X Cognitive ability interaction ($p=0.007$, $N=15$, ANOVA). Individuals with higher Cognitive ability revealed poorer retention performance on the non-sense word paired-associate task in tDCS+ACLS compared to ACLS ($p=0.026$, Wilcoxon rank test), whereas individuals with lower cognitive ability showed no significant difference ($p=0.60$). The tDCS+ACLS condition tended to induce larger amplitude SO hyperpolarisation Down states than ACLS alone ($p<0.05$, t-Test). Within tDCS+ACLS, tDCS On- vs. Off-periods increased ACLS-induced peak-to-peak SO amplitude ($p<0.05$, t-Test). Preliminary results support previous findings on the influence of cognitive ability on the efficacy of non-invasive stimulation, and a potential influence of anodal tDCS in a homogenous subset of subjects.

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Effect of different electrical impulses on the neuronal activation in the visual system

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Noninvasive current stimulation (nCS) has a significant potential for inducing neuroplastic changes and is increasingly used to support recovery from brain dysfunctions. In the visual system, a transcorneal alternating current stimulation (tACS) is used, where stimulating electrodes are placed on the eyeball.
To understand the activation pattern evoked by tACS we used multichannel electrodes and recorded visually and electrically evoked potentials (VEPs and EEPs) from the contralateral superior colliculus (SC), lateral geniculate nucleus (LGN) and primary visual cortex (VCx) in a rat under urethane anesthesia. We tested two stimulation electrode placements: eyeball-eyeball and eyeball-neck; and various impulse patterns: rectangular biphasic and monophasic (positive and negative) and sinusoidal biphasic (2 ms and +/-400 μA per phase in all cases, N=300 repetitions). EEPs were compared with VEPs evoked by white LED flashes (N=300) recorded after each series of electrical impulses.
In all structures, all tACS patterns evoked EEPs of significantly shorter latency and different shape then corresponding VEPs. The strongest shape difference was observed for the LGN and SC wherase weakest for the VCx. Responses to various electrical pulse patterns were similar in the VCx and SC but not in the LGN. The properties of EEPs response were in most cases similar for the two configurations of electrode placement. However, for negative monophasic pulses similarity between both electrode montages was less than for other types.
Our results show that the impact exerted by tACS in the visual system differs for particular structures and mostly depends on the parameters of the current impulses.

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Search for a molecular mechanism of clozapine action in ketamine-induced cognitive impairment in mice.

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Prefrontal cortex (PFC) dysfunction is a major contributor to the symptoms of schizophrenia and includes impaired ability to alter the perceptual set of attention. Furthermore, the cognitive impairments associated with schizophrenia are closely linked to dysfunction of the PFC. Administration of the non-competitive NMDA receptor antagonist, ketamine (KET), produces behavioral effects in healthy humans that resemble the symptoms of schizophrenia in patients, what has been adapted to animal model as the attentional set shift task (ASST) [1]. In this test, administration of the atypical neuroleptic, clozapine (CLZ), was shown to reverse the cognitive impairment induced by KET administration [2].
In search of the molecular mechanisms of action of CLZ in ASST, we studied the expression of selected genes and proteins associated with the GPCR signaling pathway in the mouse PFC. RT-PCR method using appropriately designed TaqMan Primers and Western Blot technique were used for the study.
Our results indicate that CLZ increases expression of both mRNA encoding βarrestin1 and protein level, and this effect correlates with ERK 1/2 protein levels. Although there are data indicating the role of 5HT2A receptor in the mechanism of Erk1/2 activation, our results, obtained by autoradiographic analysis of [3H] ketanserin binding, showed no significant changes in the level of this receptor in PFC following CLZ administration. On the other hand, we show that CLZ increased heterodimerization of 5HT1A-D2 receptors (using Proximity Ligation Assay) in PFC [3], and this effect may be responsible for activation of the ERK1/2 pathway.

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The authors declare that there is no conflict of interest.

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Event-Related Potentials under Conditions of Cessation of the Motor Programs in Humans

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Inhibition in the central nervous system is considered a key link in the executive control of cognitive and behavior in humans. That active inhibition is responsible, to a significant extent, for voluntary cessation of the prepared movement. To study such inhibition the Stop-Signal paradigm [1, 2] is frequently used. In this paradigm, while receiving the presented Stop signal, the subject should stop the initiated reaction to the Go stimulus.

The objective of our research was to identify the gender-specific features of the amplitude-time characteristics of the event-related potentials (ERPs) under conditions of cessation of the programs of manual movements (the Stop-Signal task paradigm) in healthy and right-handed volunteers, men (n=32) and women (n=33), aged 18–23 years. The latency and amplitudes of N2 and P3 components of ERPs in the response to launch or stop of the motor program of finger flexes were investigated. ERPs were analyzed in the frontal, central, and parietal lobes of the cortex.

It was established that in the situation of cessation of manual movement the right hemispherical predominance of latency of the N2 component was recorded in men and women in the parietal leads. Lower latency and higher amplitude of the P3 components in men were found in the right frontal and parietal and left central areas of the cortex, in women – in the left frontal lead. The highest amplitude of subcomponent P3 in the left hemisphere in all areas of the cortex was observed in men, in the left parietal area - women.

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There are no conflicts of interest in that research.

Mice and rats can detect localization of food found by a conspecific

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Animals use socially acquired information to guide their behavior in the environment, e.g. search for food [1]. I have developed a paradigm, called Socially Transmitted Place Preference (STPP) to study the neural basis of social information transfer. In my paradigm, animals (rats or mice) interact with a

conspecific (demonstrator) that has found food in one of two different chambers of a standard place preference cage. I show that recipients of social information develop a preference for the chamber where the food was found by a conspecific. This information is probably extracted from the breath odor of a demonstrator. Socially acquired information has an effect on the exploratory behavior of the recipients; using fiber photometry I also show that the exploration of chamber where the food is expected to be found is associated with an increased activity of olfactory tubercle, a brain area associated in processing the rewarding value of an odor [2].

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The use of machine learning in suicidal behaviour research

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Suicidality is a complex behavioural condition affecting the well-being of millions of people worldwide. We previously conducted a pilot DNA sequencing study in which we observed numerous differences in genome-wide DNA methylation status between suicide victims and the control group [1]. In the present study, we aimed to apply the novel machine learning approach to re-analyse the data obtained in the DNA methylation pilot study.

Feature ranking is concerned with identifying the parts of the input space (e.g., variants or physiological features) that are critical for discriminating between observed classes. In this work, we performed feature ranking based on mutual information [2]. The output of this procedure is individual features (CpG positions in the genome), each of which is scored with a single real number. We used feature ranking to rank the data from most relevant to least relevant features for classification.

We generated a model that can discriminate data from suicide victims and the control group based on the top 250 features (CpG positions in the genome). The model can separate both group subjects with 76% accuracy. Additionally, gene ontology analysis of the 250 CpG positions in the genome revealed enrichment for pathways involved in inflammation and polyamine system.

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Association between cortical thickness and complex real-time strategy video game skill acquisition

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Studies about the influence of individual differences on complex video game (VG) skill acquisition have attracted attention in recent years, however, there is still no unanimity on the effectiveness of such training for particular subjects. Individual differences in neuroanatomy, especially in gray matter may be related to variability in successful VG learning. The goal of our research was to analyse the relationship between complex Real-Time Strategy (RTS) VG skill acquisition and Surface Based Morphometry (SBM) measures such as cortical thickness (CT) in the number of brain areas previously found to be significant predictors of VG learning. CT of 17 participants who completed 30 h of training was calculated using the CAT12 toolbox for SPM (cyt). Attentional processes, perceptual processes and cognitive-motor speed were measured using cognitive-motor indicators extracted from VG such as Perception-Action-Cycles per minute (PAC) and Actions per minute (APC) [1]. We confirm the importance of the cingulate gyrus, which controls executive functioning and attention maintenance [2], frontal areas responsible for cognitive flexibility as well as parietal areas which were linked to control of movement and spatial attention [3] for the successful complex RTS VG skill acquisition. Such knowledge can help researchers to better adjust training regimes and selection procedures for esport professionals, create personalized training for healthy people who want to increase their efficiency and tailored rehabilitation programs for patients requiring compensation for loss or reduction of cognitive functions.

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The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Xanthotoxin affects depressive-like behaviors in female and male swiss mice – mechanisms underlying this effect

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Aims: Natural compounds may be an interesting approach to the effective treatment of central nervous system disorders. Furthermore, depression is characterized by sex differences in their prevalence, symptomatology and treatment response. Thus, this study aimed to evaluate the antidepressant properties of naturally derived furanocoumarin – xanthotoxin for both male and female Swiss mice.

Methods: The forced swimming test (FST) was performed on male and female Swiss mice using Kinoscope software. High-Performance Liquid Chromatography (HPLC) was used to evaluate the level of noradrenaline, serotonin and its metabolite in the prefrontal cortex and the hippocampus of the mice treated with xanthotoxin (2.5, 5, 12.5 mg•kg^{-1}).

Results: The results of the study showed that xanthotoxin administered at a dose of 12.5 mg•kg^{-1} diminished the level of depressive-like behavior only in the male group. We observed sex differences in the level of serotonin, metabolism of serotonin, and noradrenaline in the hippocampus after coumarin treatment (12.5 mg•kg^{-1}). Additionally, a significant increase in the level of serotonin and noradrenaline in the prefrontal cortex in both male and female groups was observed. The level of metabolism of serotonin was decreased by xanthotoxin (2.5, 5, 12.5 mg•kg^{-1}) in the hippocampus and the prefrontal cortex of both sexes.

Conclusions: Our study indicated for the first time the sex-dependent antidepressant effect of xanthotoxin. The coumarin decreased depressive-like behavior only in male mice. We revealed that changes in the level of serotonin and noradrenaline may underlay observed effect.

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All authors claim that there are no conflicts of interest.

The influence of co-administration of ligands of cholinergic and cannabinoid receptors on the anxiety-related responses in mice

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Dysfunction of the cholinergic system is associated with the development of many mental diseases, including Alzheimer disease (AD) – with memory loss and emotional disturbances (e.g., anxiety). Current therapy of AD mainly alleviates cognitive symptoms. One of the possible strategies for the modulation of emotional problems is connected with endocannabinoid system (ECS). Several findings suggest that ECS, through the cannabinoid (CB) receptors, is involved in the modulation of anxiety-related behavior [1]. However, the interaction between cholinergic system and the ECS in the context of anxiety-related responses remains poorly understood.

The aim of the study was to determine the influence of CB receptor ligands on the anxiety-related behavior in mice in the context of the interactions with cholinergic system. We examined an impact of CB1 receptor agonist (oleamide), CB1 receptor antagonist (AM 251), CB1/CB2 receptor agonist (WIN 55,212-2), as well as CB2 receptor agonist (JWH 133) and CB2 receptor antagonist (AM 630) on the anxiety-related behavior modified by an acute administration of scopolamine, a cholinergic receptor antagonist. To assess and understand the anxiety-related effects in mice we used the elevated plus maze (EPM) test. We revealed that acute administration of oleamide (5 mg), WIN 55,212-2 (0.25 mg/kg) or AM 630 (0.25 mg/kg) attenuated scopolamine-induced anxiogenic effect in the EPM test in mice. Our experiments show that ECS participates in the modulation of anxiety-related processes, especially those in which cholinergic pathways are implicated, suggesting the effective pharmacotherapy of diseases which are associated with cholinergic dysfunctions, such as AD.

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Ia EPSPs in rat motoneurons are potentiated after a 5-week whole-body vibration

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Whole-body vibration (WBV) has become an alternative treatment for the strength training and is often applied as a rehabilitation method preventing muscle force decrease. Its influence on motoneuron electrophysiological properties, motor unit function and myosin heavy chain composition in the rat medial

gastrocnemius has been determined in our previous studies. WBV induces changes in contractile parameters predominantly of fast motor units which can be recruited earlier and fast motoneurons are able to achieve higher firing rates at lower stimulus intensities compared with the control group. In this study we evaluated the influence of the WBV on Ia monosynaptic input from muscle spindles. The vibration training was performed on adult male Wistar rats, 5 days a week, for 5 weeks, and each daily session consisted of four 30-s runs of vibration at 50 Hz. Fast-type medial gastrocnemius motoneurons (the experimental group, n=34; the control group, n=32) were investigated intracellularly during experiments on deeply anesthetized animals. Monosynaptic Ia EPSPs were evoked by electrical stimulation of afferent fibers from the synergistic lateral gastrocnemius and soleus muscles at an intensity of 1.1-1.5 times threshold for the recruitment of the most excitable fibers in the nerve. The WBV induced a significant increase of the mean EPSP amplitude and a shortening of the EPSP rise time. This suggests that frequent activation of muscle spindles during WBV evokes changes in synaptic transmission and/or downregulation of Ia presynaptic inhibition, which may partly explain adaptive changes in motoneuron excitability.

The authors declare no conflicts of interest, financial or otherwise.

Emotional face processing and the N170 in Parkinson disease

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Parkinson's disease (PD) is associated with impaired emotional information processing. But it is still not clear how this impairment is related to the severity and phenotype of the disease.
In this study, we used the images of neutral and happy faces (NimStim Set of Facial Expressions) to elicit the N170 component of visual event-related potentials (ERP). 150 patients with PD (Hoehn-Yahr 2.0-3.0) and 20 control participants without neurological disorders participated in the experiment. To identify non-motor subtypes of PD patients we used the cluster analysis based on demographic and clinical data (Unified Parkinson's Disease Rating Scale, Non-Motor Symptoms Scale). 4 clusters were extracted (numbers 1 to 4 corresponds to an increase in UPDRS scale). Prominent non-motor symptoms were typical for clusters 2 and 4 only. There was no difference in N170 latency between all clusters and the control group. The amplitude of this component was significantly higher in controls compared to clusters 2 and 4 in right temporal and occipital sites for happy faces. A decrease in N170 amplitude was additionally registered in response to neutral stimuli in patients of cluster 4 (the most severe PD), and this difference was bilateral.
Thus, our results show that the face processing subsystem is mostly impaired in patients with higher severity of non-motor symptoms, and N170 amplitude in response to face images may be useful for PD non-motor subtypes classification.

The authors have no conflicts of interest to declare.

CNS myelination: a role for autophagic function

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Autophagy comprises a major lysosome-dependent degradation mechanism which engulfs, removes, and recycles unwanted cytoplasmic material, including damaged organelles and toxic protein aggregates. Although a few studies implicate autophagy in CNS demyelinating pathologies, its role, particularly in oligodendrocytes and CNS myelin, remains poorly studied.

In our study, we aim to shed light on this role, using both *in vitro* and *in vivo* approaches. *In vitro*, pharmacological and genetic inhibition of autophagy have revealed severe defects in myelin sheet formation, delayed maturation and altered cellular distribution of major myelin protein constituents. In parallel, we are currently examining the role of autophagy in vivo, utilizing a new conditional mutant mouse line, in which a core gene of autophagic machinery (*atg5*) is specifically ablated in the myelinating glial cells after tamoxifen administration. Biochemical and electron microscopy analysis of this mouse line has revealed differences in myelin protein levels as well as morphological alterations in cKO animals compared to age-matched controls.

Our data support the principle that the progression of myelination in the CNS requires the involvement of a fully functional autophagic machinery.

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Lack of PIANP alters gross brain morphology and cerebellar histoarchitecture in mice

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PIANP is a novel protein of primarily neuronal localisation which binds to the inhibitory immunoglobuline-like type 2 receptor PILRa as well as the N-terminal domain of GABA-B receptors [1]. A homozygous loss-of-function mutation of PIANP is associated with features of intellectual disability and global development delay in humans whilst PIANP knockout mice display heightened anxiety levels and autism-like behaviour [2]. To further investigate the neuronal role of PIANP, we analysed male homozygous knockout mice (PIANPcre) and compared them to both mutant mice floxed at the PIANP locus (PIANPflp)

and wildtype C57Bl6/N mice. PIANP knockouts showed altered gross brain morphology compared to wildtype mice. To investigate possible factors behind these results in detail, we performed in-depth histological analyses of different brain areas like the cerebellum and dentate gyrus. Following alterations in the thickness of layers in the hippocampus which we previously reported [2], we observed that PIANP knockouts displayed marked cytoarchitectural changes of the cerebellum, with both structural and cellular alterations present. Interestingly enough, we observed changes in all layers of the cerebellum with specific alterations for each individual layer present. These results shed light on the multifaceted neuronal role of PIANP and link the behavioural phenotype observed in knockout mice to both qualitative and quantitative alterations in the mouse cerebellum. Further analyses are necessary to understand the neuro-cellular interplay underneath the behavioural and cognitive symptoms observed in both humans and animal models of PIANP mutations.

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Neuroprotective effect of Urolithin A against rotenone-induced mitochondrial impairment in a rat model Parkinson's disease

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Urolithin A (UA) is a metabolic compound generated by intestinal bacteria from ellagitannins (ETs). Due to its antioxidative, anti-inflammatory, and autophagy-inducing activities [1], UA also demonstrates neuroprotective potential. Previously, we reported that the treatment with pomegranate juice, rich in ETs, provided neuroprotection in a rotenone (ROT) model of Parkinson's disease (PD). In addition, we provided evidence for the distribution of UA to the brain [2]. Therefore, we surmised that UA might contribute to the overall neuroprotective effects reported for pomegranate.

Since mitochondrial dysfunction is implicated in PD pathogenesis, in this study, we aimed to investigate UA's capability for counteracting the loss of nigral dopaminergic neurons and examine whether it is associated with protecting mitochondrial impairment. Rats exposed to prolonged, low-dose ROT due to sustained inhibition of complex I, related oxidative injury, and α-synuclein aggregation develop PD-like neurodegeneration [2,3]. To evaluate the neuroprotective mechanism of UA, Wistar rats were administered with UA (25 mg/kg b.w./day, *i.p.*) and injected with ROT (1.3 mg/kg b.w./day, *s.c.*) from the 11th day. The experiment lasted 45 days, including 10 days pre-treatment with UA and 35 days combined treatment with UA and ROT. After that, we assessed the density of TH^+ neurons in the SN and striatum, mitochondrial membrane potential, and mitochondrial aldehyde dehydrogenase activity in the midbrain and determined the UA content in the brains of rats.

Our findings provide the first evidence that UA treatment protects against ROT-induced mitochondrial impairment that correlated well with the enhancement of neuronal survival.

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Gliogly – the investigation of glycobiology of glioblastoma

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Glioblastoma (GBM) is the most malignant brain tumour hallmarked by the aggressive infiltration of tumour cells into neighbouring brain regions. Alterations in the glycobiology of cancer cells is closely associated with malignant properties, including invasiveness and metastatic potential. However, the glycobiology of glioblastoma remains poorly investigated. Here, we aim to explore the role of glycobiology in glioma and map sialylation levels using an organotypic neocortical slice model.

Visualization and quantitative analysis of newly synthesised sialic acid (terminal moiety of glycocalyx) confirmed that glioblastoma cells demonstrate high rate of *de novo* sialic acid synthesis. Spatial transcriptomics and RNA-sequencing results indicate high expression of sialidases, enzymes that cleave sialic acid from glycoconjugates, in both tumour-infiltrated patient samples and patient-derived GBM

cell lines, suggesting the metabolism of sialylation being potentially involved in tumour growth. Inhibition of sialic acid synthesis and desialylation affected the pattern of tumour growth and lead to alterations in glioblastoma cells network activity as the inter- and trans- cellular Ca^{2+} signalling was found to be strongly reduced upon inhibition of sialic acid biosynthesis. Additionally, we showed that human organotypic brain slice culture technique can be used as a robust framework for glycobiology research allowing metabolic labelling of sialic acid moieties and quantifying changes in glycocalyx.
In summary, our results provide new insights into underinvestigated role of sialic acid in the functional activity of GBM and highlight the importance of functional relationships and cellular migration. In the future, these interactions have the potential to be targeted therapeutically.

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Stimulation of the bed nucleus of stria terminalis in the treatment for treatment--resistant obsessive-compulsive disorder and depression

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Objective: The bed nucleus of stria terminalis (BNST), a tiny nucleus located in the ventral forebrain, plays a significant role in both anxiety and addiction; two highly prevalent and debilitating symptoms of all anxiety-related psychiatric disorders. The potential neuromodulatory role of BNST is discussed in the clinical application for patients suffering mostly from treatment-resistant obsessive-compulsive disorder (trOCD) and treatment-resistant depression (TRD).

Methods: The medical literature was reviewed using two medical databases: Medical Literature, Analysis and Retrieval System on-line (MEDLINE) and Cochrane Central Register of Controlled Trials (CENTRAL) on BNST DBS outcomes in trOCD or TRD.

Results: We have found 8 clinical studies of BNST DBS reporting a cumulative number of 60 patients. Six studies have reported the effects of BNST DBS on trOCD symptoms, while two studies for TRD. Only in two studies the electrodes were exclusively implanted in BNST, while in other studies the mixture of patients stimulated at BNST with co-stimulation with anterior limb of the internal capsule (ALIC), ventral capsule (VC), or nucleus accumbens (Nac) was reported. The mean YBOCS (Yale Brown Obsessive Compulsive Scale) scores reduction ranged from 27 % to 66 %.

Conclusions: The clinical experience of BNST DBS worldwide is limited to only a few studies. In most studies the primary indication was refractory trOCD and only in 2 studies TRD.The BNST may turn out

to be an effective target in the treatment of anxiety-related psychiatric disorders, but more clinical studies are still needed.

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Declarations of interest
The authors have no conflict of interest to disclose.

NTPDase1/CD39 and Ecto-5′-nucleotidase/CD73 are Upregulated in a Sex-specific fashion in the Rat Fetal Brain After Repeated Antenatal Dexamethasone Treatment

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To accelerate organ maturation and prevent complications due to preterm birth, antenatal treatment with synthetic glucocorticoids (GCs – dexamethasone or betamethasone) is usually given between the 24th and 34th week of pregnancy to women at risk of delivery within the next seven days [1]. Despite recommendations, repeat courses of antenatal GCs are frequently given, although excessive GC stimulation may exert adverse neurodevelopmental effects [1]. The purinergic system is essential for neurodevelopment [2]. Extracellular purine levels are regulated by ectonucleotidases, with ectonucleoside triphosphate diphosphohydrolase 1 (NTPDase1/CD39) and ecto-5′-nucleotidase (e5′NT/CD73), abundant in the CNS, which jointly hydrolyze ATP to adenosine. Both ectonucleotidases are also involved in cell adhesion and migration [3]. We aimed to explore the effects of antenatal dexamethasone (DEX) treatment on the expression and enzymatic activity of NTPDase1/e5′NT tandem in the rat fetal brain. Wistar rat dams were treated with 0.5 mg/kg DEX, at gestation day (GD) 16, 17, and 18. We found sex-specific male-biased upregulation of CD39 and CD73 mRNA and protein abundances, and an increase in the corresponding

enzymatic activities in the rat fetal brain at GD21, induced by antenatal DEX treatment. Observed changes indicate a possible decrease in P2, and an increase in P1 purinergic receptors-mediated signaling, as well as a potential decrease in migration of progenitor cells, particularly pronounced in the brain of male fetuses. Together, sex-dependent induction of CD39 and CD73 might interfere with neurodevelopmental processes, thus contributing to adverse effects of antenatal DEX treatment, especially in males.

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Neuroligin-2 regulates long-term GABAergic plasticity

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For decades, the research into synaptic plasticity was mainly focused on the excitatory synapses while GABAergic inhibitory transmission was thought to be devoid of complex long-term plasticity. This view changed recently when numerous plastic phenomena were discovered in GABAergic synapses (e.g. different forms of iLTP and iLTD). Nevertheless, our knowledge of adhesion proteins in GABAergic plasticity is still limited. Neuroligin-2 (NLG-2), locates specifically at the postsynaptic density, where it interacts with scaffold protein gephyrin and $GABA_AR$. Additionally, NLG-2 provides trans-synaptic adhesion through the binding of presynaptic neurexins. This study aimed to address the function of NLG-2 in GABAergic plasticity.

We recorded mIPSCs in CA1 pyramidal neurons in slices and induced iLTP using a short-term application of NMDA. Additionally, we used neurolide-2, a peptide that blocks the interaction between NLG-2 and neurexins.

Obtained results show that when interactions between NLG-2 and neurexins are disrupted, NMDA-iLTP is abolished (control with scrambled peptide: 116% of baseline; neurolide-2: 93%, n=5-6, $p<0.001$, 22 min after induction). Next, we asked about the time window of NLG-2 involvement in plasticity phenomenon.

We observed iLTP impairment when NLG-2 inhibitor was applied immediately after induction protocol (89%, n=6, p<0.001 vs. control). Interestingly, if neurolide-2 was present only during NMDA treatment, we observed unchanged iLTP (120%, n=6, p= 0.603 vs. control), suggesting that the rearrangement of adhesion takes place after iLTP induction. Taken together, these results demonstrate a crucial role of neuroligin-2 in the maintenance of iLTP, opening new avenues for studying adhesion proteins during plastic changes at GABAergic synapses.

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Integration and transformation of temporal information for vibrotactile sensation along the ascending neuraxis

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Perceiving substrate vibrations is a fundamental component of somatosensation [1]. In mammals, action potentials fired by Pacinian corpuscle afferents are known to reliably time lock to the cycles of a vibration. However, little is known about how this precise timing information is processed in the central nervous system. We recently found that neurons in the somatosensory cortex of mice encode vibration frequency with a rate code tuned to a preferred value [2]. Such surprising feature-selective rate coding raises an important question: how are cyclically entrained action potentials in peripheral mechanoreceptors transformed along the ascending neuraxis into a rate code in the cortex? We traced this transformation with electrophysiological recordings along all stages of the ascending somatosensory pathway: primary sensory afferents (mechanoreceptors), dorsal root ganglia, dorsal column nuclei, thalamus and cortex. Recordings from nerve fibers of primary sensory neurons in lightly anesthetized mice showed that rapidly adapting mechanosensitive units (RAII) display phase-locked spiking for vibrations up to 2000 Hz and some can respond to vibrations over 3000 Hz. This precise temporal code was also found in dorsal column nuclei, but not in thalamus. Diverse V-shaped vibrotactile sensitivity curves could be derived from RAII responses and their combination closely resembled perceptual threshold curves obtained with behavioral experiments. By comparing the neural codes at different stages of the somatosensory pathway, we discovered that major neural signal transformations occur between dorsal column nuclei and thalamus. This finding allows modeling the underlying computational principle to reveal novel features of vibrotactile sensation.

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Visual information processing in the RHOP$^{23H/WT}$ mouse model of retinal degeneration: more robust activity doesn't mean better stimulus selectivity

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Photoreceptor degeneration is a prominent cause of blindness in the western world. Even if single mutations that cause the death of photoreceptors are known, the physiological changes happening to the retina and visual system are poorly understood. The worsening of visual perception and shrinking of the visual field are obvious symptoms resulting from photoreceptor loss. While the degeneration process unfolds, some compensatory mechanisms in the remaining photoreceptors also can affect visual processing. This project investigated how mechanisms occurring in the retina of young RHO$^{P23H/WT}$ mice impact higher-level visual processing in the primary visual cortex (V1), which shows significantly elevated visual responses and may result from overexcitability of cones before degeneration. We used drifting gratings to study fine differences between healthy mice and two age groups of RHO$^{P23H/WT}$ mice: 1 and 3 months of age. In the V1 of younger RHO$^{P23H/WT}$ mice, both visually evoked responses and spontaneous activity were higher than in the wild-type mice, whereas stimulus selectivity was poorer. Two months later, this phenomenon disappeared, and visually evoked potentials in 3 month-old RHO$^{P23H/WT}$ mice became significantly reduced compared to WT mice. The single-cell selectivity to certain stimulus parameters such as orientation selectivity and preferred size became even worse than in 1 month-old RHO$^{P23H/WT}$ mice. These results show that lower visual acuity also happens at the beginning of neurodegenerative eye disease, and such defects can be easily missed because of elevated global responses. We suggest that such global phenomena could be linked to cone pathway hyperexcitability in younger mutant mice.

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Anxiety-free human values engage limbic-related brain regions: fMRI study

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Human values differ in their relation to anxiety, and they motivate different behaviours. Pursuit of values such as *"conformity"* or "*power''* serves to cope with anxiety and uncertainty, and these values protect the self. Other values such as "honesty" or "creativity" express anxiety-free motivations, and contribute to the expansion of the self and growth. We aimed to examine the neural basis of Anxiety--free vs Anxiety-based values. Due to emotion-related aspects of the latter, involvement of limbic structures could be expected. To achieve our aim we asked volunteers to rate different human values as their personal guiding principles in life, while brain activity was recorded with an fMRI scanner. BOLD signals during the rating of Anxiety-free and Anxiety-based values were compared (t-test between conditions, cluster defining threshold of 0.001; cluster-size thresholding: Monte Carlo simulations, BrainVoyager). Anxiety-free values were associated with greater activity in the ventromedial cortex (BA10), and anterior (BA32) and posterior cingulate cortices (BA24/23). The observed higher activity in brain regions connected with the limbic system may suggest stronger emotional components in Anxiety-free than Anxiety-based values, which is surprising. On the other hand, higher cingulate activation for Anxiety-free values might also reflect less de-activation of the default mode network during stimulus presentation, as suggested by beta weights corresponding to relative activation levels in both conditions. Further studies are advisable to identify the complex nature of values which motivate behaviours free from and based on anxiety.

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Relation between white matter integrity and performance in the complex real-time strategy video game

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Playing video games has become one of the most popular ways of spending free time for people in all age ranges. Recent research shows that playing video games is associated with a wide range of cognitive processes such as perception abilities or attention. People have the capacity to learn new complex abilities, but it is still not clear how cognitive and neural predispositions may accompany this process [2].

The presented study is focused on investigating whether the pre-training measures of brain white matter integrity (fractional anisotropy, FA) are correlated with indicators of cognitive–motor abilities extracted from real-time strategy game (StarCraft 2, SC2) replay data. Using a region of interest (ROI-based approach), we extracted white matter characteristics from FA maps and correlated them with obtained values of motor and cognitive measures: perception action cycles (PACs) per minute, average hotkey usage, and actions per minute (APMs) from the game.

Results revealed a positive correlation between the external capsule and average total score and with the average total score only for won SC2 matches. The left cingulum at the level of the hippocampus correlated with the average total score and PACs per minute. We have also found a relation between the anterior part of the internal capsule and motor-cognitive measures from the game (APMs and hotkey usage). Study shows that the higher FA value in chosen regions of the brain the better SC2 performance. This indicates that people with specific white matter integrity may be predisposed for more efficient complex skills acquiring.

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Perivascular fibroblasts activity precedes the onset of ALS neurodegeneration with high plasma SPP1 associated with short patient survival

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Apart from the well-defined neuron-centric factors, few reports consider that variability of sporadic ALS progression can depend on the less-defined contributions from non-neuronal cell types including glia and blood vessels. Nonetheless, inaccurate survival prognosis continues to confound clinical trial design and effective treatments will likely remain elusive unless we better understand how non-neuronal cells contribute to ALS aetiology. Here we report that perivascular fibroblast cell gene activity during presymptomatic disease stage remodels blood vessel matrix and provides distinct plasma protein biomarker that can independently predict short ALS patient survival at diagnosis[1]. We inferred cell activity in ALS spinal cord transcriptomes using single-cell guided profiling. We determined that sporadic ALS patients present cellular changes consistent with two mouse models in which gene expression patterns from vascular cells precede the blood- brain barrier dysfunction and microglial response. Notably, perivascular fibroblast cells elicited the strongest pre-onset gene enrichments and their marker proteins SPP1 and COL6A1 accumulated in enlarged perivascular spaces in sporadic ALS patients. Moreover, in 574 ALS patients from four independent cohorts, increased plasma levels of SPP1 at disease diagnosis repeatedly predicted shorter survival with a stronger effect than established indications of bulbar onset or neurofilament levels in cerebrospinal fluid. We propose that the activity of the recently-discovered perivascular fibroblast can predict ALS patient survival and provide a novel conceptual framework to re-evaluate definitions of ALS aetiology.

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Changes in the Brain in Temporal Lobe Epilepsy with Unilateral Hippocampal Sclerosis

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Background: Temporal lobe epilepsy (TLE) is a network disorder of the brain; network disorders predominately involve dysregulation of hippocampal function caused by neuronal hyperexcitability. However, the relationship between the macro- and microscopic changes in specific brain regions is uncertain. In this study, the pattern of brain atrophy in patients with TLE and hippocampal sclerosis (HS) was investigated using volumetry, and microscopic changes in specific lesions were observed to examine the anatomical correspondence with specific target lesions using diffusion tensor imaging (DTI) with statistical parametric mapping (SPM).

Methods: This retrospective cross-sectional study enrolled 17 patients with TLE and HS. We manually measured the volumes of the hippocampus (HC), amygdala (AMG), entorhinal cortex, fornix, and thalamus (TH) bilaterally. The mean diffusivity and fractional anisotropy of each patient were then quantified and analyzed by a voxel-based statistical correlation method using SPM8.

Results: In right TLE with HS, there was no evidence of any abnormal diffusion properties associated with the volume reduction in specific brain regions. In left TLE with HS, there were significant changes in the volumes of the AMG, HC, and TH.

Conclusions: Chronic left TLE with HS causes structural changes in the AMG, HC, and TH, unlike right TLE with HS.

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TCF7L2 – a master regulator of thalamic fate and function

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The thalamus is a hub for the integration of sensory information and selection of behavioural responses. Anatomical and functional abnormalities in the thalamus and its axonal connections are often identified in neuropsychiatric disorders, such as schizophrenia and autism. Improper development of thalamic nuclei, aberrant growth of thalamocortical axons, or the emergence of atypical electrophysiological properties of thalamic neurons all could potentially contribute to the aetiology of said disorders. Unfortunately, our understanding of the thalamic development and its adult homeostasis was limited, as the molecular mechanisms which govern these processes were poorly characterized. Our studies shown that both depend on the transcription factor TCF7L2, which directly regulates the expression of many genes critical for thalamic development [1].

Our goal was to determine the role of TCF7L2 in the embryonic development and postnatal maturation of the thalamus. To this end we examined mouse embryos with a total knockout of *Tcf7l2* and adolescent/adult mice with thalamus-specific, postnatal knockout of *Tcf7l2*. Anatomy, gene expression patterns, and axon fibres were visualised using Nissl staining, *in situ* hybridization, immunohistochemistry or DiI tracing. RNA-seq and ChIP-seq analyses were performed on thalami of both strains. Postnatal TCF7L2-deficent mice were used for behavioural tests and their brain slices were used for *in vitro* patch-clamp analysis.

We show that the development of proper anatomy/cytoarchitecture of the thalamus, molecular identity of its neurons and growth of thalamocortical connections are all regulated by TCF7L2. TCF7L2 is further required for the functional maturation of thalamic neurons, and TCF7L2-deficent mice exhibit abnormal social behaviour.

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Proteomic analysis of mitochondrial proteome after induction of early and delayed remote postconditioning

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Cerebral ischemia can cause irreversible brain tissue damage and is one of the most common diseases occurred worldwide. It was shown that activation of ischemic tolerance is very beneficial and decrease process of neurodegeneration. Recent data revealed crucial role of mitochondria in regulation of ischemic tolerance. In our experiment, changes in the proteome of CA1 hippocampus (as one of the most sensitive areas of the brain) was analyzed after treated with 2 types of remote postconditioning in animal model with the help of mass spectrometry. Three cohorts with three-days of survival were designed: negative control of global cerebral ischemia, sham with early remote postconditioning (ETQ), and sham with delayed remote postconditioning (DTQ). During remote postcondictioning, the blood flow occlusion on the pelvic limb for 20 minutes was applied. Totally, 350 proteins were identified in all tested groups. In both, ETQ and DTQ samples, proteins associated with mitochondrial specific pathways were identified: ATP synthetase or aconitate hydratase and antiapoptotic proteins such as elongation factor Tu. In the ETQ sample, the presence of acute damage-reducing proteins and of heat-shock family proteins was revealed. The DTQ induce expression of proteins regulating the proper function of mitochondrial pathways and protein synyhesis - malate dehydrogenase, mitochondrial fission factor and citrate synthase. Overall, the influence of postconditioning affecting the mitochondrial proteome was observed. These results contribute to the project identifying complex proteomic cascades activated after induction of the ischemic tolerance.

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Long lasting antidepressant effect of combined administration of psilocybin and mGluR2 antagonist (LY341495) in the TST in mice

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Current clinical trials with psychedelic substances show promising results in the treatment of drug--resistant depression, anxiety and many other psychiatric disorders. However, further clinical and preclinical studies are needed to fully understand the topic of psychedelic research. Hallucinogens, such as psilocybin, show the greatest affinity for the 5-HT2A serotonin receptors. However, it is likely that the

mechanism of action of hallucinogens requires the co-activation of glutamate receptors, in particular metabotropic glutamate receptor (mGluR) 2, which show spatial colocalization towards the previously mentioned serotonin receptors in the prefrontal cortex.

In our experiment, psilocybin in the conjunction with AMN082 (mGluR7 agonist) and LY341495 (mGluR2 antagonist) was used to evaluate the short and long lasting antidepressant-like effects in the Tail Suspension Test (TST) in mice. Additionally, the influence of the used substances on the spontaneous locomotor activity of mice was assessed.

Obtained results indicated no changes in the immobility time of mice subjected to acute combined psilocybin (0.5 mg/kg) and AMN082 (1 mg/kg) treatment in the TST. However a statistically significant decrease in the immobility time of mice in the TST was found for the acute, joint treatment of low dose of psilocybin (0.5 mg/kg) and LY341495 (0.3 mg/kg). No changes in the locomotor activity of the psilocybin +LY341495 was observed. Moreover, this antidepressant-like effect was still present 72 hrs after acute psylocibin+LY341495 administration.

It is possible that combined psilocybin+ LY341495 treatment in low inactive doses achieve the desired therapeutic effects while reducing psilocybin induced hallucinogenic side effects.

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The influence of temperature on contractile properties of motor units in rat medial gastrocnemius

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The influence of temperature on mammalian muscle physiology has been extensively studied and these experiments showed variable effects dependent on muscle architecture and metabolism [3]. The aim of the study was an assessment of temperature sensibility of motor units (MUs) contractile properties in rat medial gastrocnemius muscle containing fast-twitch fatigable (FF), fast-twitch resistant (FR), and slow-twitch (S) MUs. This approach was applied for the very first time and allowed the elimination of the influence of differences in muscle architecture on the results.

The experiments were performed on functionally isolated MUs. Three groups of animals were tested at hypothermia (25±1°C), normothermia (37±1°C), and hyperthermia (41±1°C). Classification of the MUs was done on the basis of our previous works [1, 2].

Hypothermia prolonged twitch time parameters in all types of MUs, whereas hyperthermia reduced it. These changes considerably influences the force-frequency of stimulation relationship. Hypothermia reduced the twitch force of FF MUs, but increased this parameter for FR ones and had no influence on S MUs. Hyperthermia decreased the twitch force in both types of fast MUs and had no effects in S MUs. The tetanic force was decreased in all types of MUs at 25°C, and in fast MUs at 41°C, but hyperthermia

had not influenced this force in S MUs. The twitch-to-tetanus-ratio, an indicator of force regulation, increased in hypothermia and decreased in hyperthermia in the fast MUs, but was not affected in slow ones. Concluding, fast-twitch MUs appeared to be more sensitive to changes in temperature than slow ones.

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Modulation of the ionic channel activated by cold and menthol TRPM8 by the prostacyclin receptor

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TRPM8 (Transient Receptor Potential Melastatin member 8) is an ionic channel activated by cool temperatures, with a 25°C threshold, and also by an array of natural and synthetic agonists. This channel plays a role in thermoregulation, cold analgesia and, paradoxically, in cold hypersensitivity and cold pain. Although modulation of TRPM8 by inflammatory mediators is not yet completely understood, it has been shown that those which activate Gαq-coupled receptors inhibit TRPM8 [1]. The prostacyclin receptor IP is a GPCR (G-protein coupled receptor), preferentially activated by prostaglandin I2 (PGI2), involved in acute inflammation, inflammatory pain and a series of severe cardiovascular and pulmonary diseases. The IP receptor couples both with Gαs, in platelets and smooth muscle cells, and Gαq, in Dorsal Root Ganglia [2], [3]. Thus, our research aim was to investigate the signaling pathways involved in the modulation of the TRPM8 channel by a series of selective IP receptor agonists. For this purpose, we co-transfected HEK 293T cells with TRPM8 and the IP receptor and treated them with cicaprost and iloprost, two analogs of prostacyclin. Using non-ratiometric calcium microfluorimetry, we then monitored how the activation of the IP receptor affects the activation of TRPM8. Our results demonstrate that the signaling pathway triggered by the IP receptor depends on the specific agonist and this effect leads to differential modulation of TRPM8.

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Effects of heme precursors ALA and PBG on Nav1.7 and Nav1.8 ion channels

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Acute porphyrias define a subfamily of diseases characterized by enzyme deficits in the early steps of the heme pathway, leading to accumulation of heme precursors. Acute intermittent porphyria is caused by loss-of-function mutations in porphobilinogen deaminase, generating an increase in δ-aminolevulinic acid (ALA) and porphobilinogen (PBG) in blood plasma and tissues. Since abdominal pain is the most frequent complaint of acute porphyrias, our aim was to investigate the role of two voltage-gated sodium channels known to be involved in pain signaling, $Na_v1.7$ and $Na_v1.8$, in mediating this symptom. The activity of human isoforms $Na_v1.7$ and $Na_v1.8$ was examined in heterologous expression systems (HEK293t cells stably expressing $Na_v1.7$ and ND7-23 cells transiently transfected with $Na_v1.8$) using the patch clamp technique in the whole-cell configuration, in control conditions and in the presence of ALA or PBG. ALA 100μM applied in the intracellular solution increased the current density of $Na_v1.7$ compared to control and PBG 100μM applied intracellularly shifted the voltage dependence of inactivation of $Na_v1.7$ to more depolarized potentials. ALA and PBG, both 100μM, applied in the extracellular or intracellular solution did not affect the biophysical parameters of $Na_v1.8$. In conclusion, ALA and PBG modify the current density and shift the inactivation V_{50} of $Na_v1.7$ to more depolarized potentials, permitting the channel to stay open for a longer period, which may explain the neuronal depolarization and hyperexcitability of nociceptors, triggering the pain in acute porphyria attacks. Further experiments on nociceptors should be performed to investigate the effect of ALA and PBG.

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Effects of background sounds on cognitive performance: modulatory role of personality traits

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Aims: Background sounds influence cognitive performance and this effect can be modulated by personal and environmental variables. No previous research has evaluated effects of music or noise on executive functions considering the modulatory role of personality traits such as extraversion or neuroticism. Our main aims were to evaluate effects of exposure to different sounds on attention, memory and executive function as well as to explore the influence of the individual's personality and anxiety traits on the cognitive effects of these background conditions.

Methods: 30 university students randomly allocated to one of three background conditions (silence, music or noise) while performing different cognitive tests: Rey Complex Figure Test (RCFT), Trail Making Test (TMT) and Tower of London (TOL). Personality measures were taken using NEO-Five Factor Inventory (NEO-FFI) and anxiety state was measured with State-Trait Anxiety Inventory (STAI).

Results: Regarding personality factors, there were positive correlations between openness to experience and short ($p<0.01$) and long-term ($p<0.05$) visuospatial memory in RCFT; and between anxiety state and total rule violations in TOL ($p<0.05$). There was also a negative relationship between consciousness and total initiation time in TOL test ($p<0.01$).

Conclusions: Our results suggest that personality factors and anxiety state may have a modulatory effect on cognitive performance under background sounds conditions. In fact, high levels of openness to experience implied better cognitive performance on a memory task; high levels of anxiety were associated to more rule violations on a task measuring planning proficiency; and high scores in consciousness implied taking less time in starting a cognitive task.

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Conflicts of interests: None.

The manifestation of summation processes in the illusion of length with a different arrangement of contextual distractors

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Aim:

The aim of the study was to further develop a quantitative model of the filled-space illusion and test it to account for the effects caused by stimuli containing distracting line-segments of various lengths and positions.

Methods:

Illusion was studied as a function of the length of distracting lines arranged differently relative to lateral terminator of the three-dot stimulus. Data obtained in three different series were fitted with relevant functions of the model.

Results:

It was shown that the model satisfactorily describes all changes in the illusion magnitude for stimuli with a distracting line located either outside or inside the interval, as well as for a stimulus with two lines located symmetrically relative to the lateral terminator. In addition, the model was successfully applied to fit the experimental data previously obtained for conventional Oppel-Kundt stimuli.

Conclusions:

A good correspondence between the experimental and theoretical results supports the suggestion that the context-evoked augmentation of neural excitation can determine the occurrence of the filled-space illusion.

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Exposure to vicarious repeated social defeat is effective in increasing depressive symptomatology in female mice

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A previous study has demonstrated that exposure to vicarious repeated social defeat on 10 consecutive days induces depression-like behavior in females [1]. In the present study we tested the effects of a different schedule of exposure to vicarious social defeat (four episodes separated by intervals of 72 hours) in the Tail Suspension and Splash tests. On PND 47, 50, 53 and 56, female C57BL/6 mice witnessed an episode of social defeat of a male of the same strain by an aggressive opponent mouse of the OF1 strain. A control group of females spent the same period of time in an empty cage. Forty-eight hours after the last episode of repeated social defeat, both groups of females were tested in the tail suspension and splash tests. In comparison with the control group, female witnesses of vicarious social defeat showed higher immobility in the tail suspension test and displayed a higher latency of grooming in the splash test. However, no differences were observed between control and stressed females in the time spent or frequency of grooming in the splash test. These results indicate that intermittent vicarious social defeat induces stress and increases depression levels in female mice. These results are similar to those observed previously with male mice exposed to repeated social defeat [2]. Thus, we can confirm that exposure to vicarious social defeat is effective in inducing stress in females and endorse this procedure for studying the effects of social stress on different behaviors and resilience to said effects.

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Without conflicts of interest

Responses of lateral habenula neurons to an aversive stimulus across alternating brain states of urethane anaesthetised rat

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The lateral habenula (LHb) is a glutamatergic epithalamic structure which, through its participation in signalling aversive events, plays an important role in processes related to motivation and learning. In response to aversive stimuli (AS), LHb indirectly inhibits dopaminergic neurons in the ventral tegmental area (VTA) via direct excitation of GABAergic neurons in VTA or rostro-medial tegmental nucleus. Based on the responses to AS, LHb neurons were divided into two subpopulations, one excited and other, less numerous, inhibited by AS. Differences in the response of LHb neurons to AS are correlated with the pattern and level of basal activity as well as their spatial location, i.e. AS inhibited neurons display fast, regular firing and are clustered in the medial portion of LHb, whereas excited neurons are slow, irregular firing and are uniformly distributed throughout the LHb. We decided to check whether part of this heterogeneity of the LHb neuronal populations is due to the modulating effect of ongoing brain states. Therefore, we performed *in vivo*, extracellular recordings of LHb neurons' activity and their responses to the electrical footshock during alternating brain states in urethane anesthetized rats. We have observed that in addition to neurons with stable response to AS, there is a hitherto undescribed subpopulation of LHb neurons that changes the direction of the response to AS during REM-like and nREM-like brain states. Moreover, baseline activity of LHb neurons is also modulated by these brain states. This study shows one source of heterogeneity of AS-induced and spontaneous LHb neuron activity.

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State related hyperbolic dependence of breathing rate on the pulse respiration quotient – possible impact for artificially ventilated patients

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Pulse respiration quotient (PRQ, number of heart beats per breathing cycle) [1] expresses the relation of cardiorespiratory coupling (CRC) and reflects the autonomic regulation patterns, important for general health [1,2]. PRQ dependence on breathing rate (BR, 1/min) was investigated so far only on simulated data [1]. Supine position with slow 0.1 Hz BR (Supin01) and active standing (Stand) represent the states of maximal cardiac vagal respectively sympathetic modulation in physiological quiescence; standing with 0.1 Hz BR (Stand01) is characterized by a qualitatively specific CRC pattern [3]. Our aim was to investigate the BR-PRQ correlations in 4 states: supine position with spontaneous BR (Supin), Stand, Supin01 and Stand01. ECG and respiratory signal were simultaneously recorded in 20 healthy human subjects in four conditions, acquisitioned/processed as described in [3]. PRQ signals with exact calculation of its temporal variations and hyperbolic data fitting were obtained by advanced matlab algorithms. Change of posture and BR induced increase of hyperbola parameter (0.000-Supin-Stand, 0.000-Supin-Stand01, 0.000-Supin01-Stand01) and hyperbolic fitting error (0.028-Supin-Stand, 0.034-Supin--Stand01). These results indicate on posture and BR related modification inducement of the parameters of hyperbolas fitted to the relations of PRQ to BR. In general, more sympathetic predominance (Stand, Stand01) is reflected in a reduced PRQ-BR hyperbolic dependence and a higher fitting error compared to vagal predominance (Supin, Supin 01). Postural change and slow BR could be beneficial in the ICU for re-training of untuned cardiorespiratory PRQ regulating autonomic networks, which have been shown important for successful cardiorespiratory recovery after arteficial breathing [1,3].

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Survival and changes in brain of wild type and *swiss cheese (sws)* mutants *Drosophila melanogaster* after exposure to neurotoxic organophosphates

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The effects of organophosphates found in pesticides and insecticides can cause in human organophosphates-induced delayed neuropathy (OPIDN). The target of action is Neuropathy target esterase (NTE) encoded by the *PNPLA6* gene, whose ortholog in *Drosophila* is called *sws* [1].

The studies were performed on wild-type *Oregon-R* control individuals and *sws mutants.* 5-days old flies were kept for 24 hours on a medium with organophosphates 32 mg/ml ToCP or 0.0015 mg/ml diazinon, then the flies were kept on the standard medium. Survival curves were made using 100 males from each study group. To assess brain tissue, histological sections of at least 20 males were made. Quantitative analysis of the degeneration zones was carried out using ImageJ software. GraphPadPrism7 software was used for data processing.

The survival of all individuals after the exposure of both organophosphates was significantly reduced ($p<0.0001$), but *sws* mutants were more sensitive. Also, the effect of ToCP was delayed – mass extinction began on day 7, while diazinon had both instant toxic effect and delayed. Degenerative changes occurred after the ToCP exposure in the brain tissue of 10% of the control flies; the penetrance of the *sws* phenotype remained unchanged (82%). However, quantitative analysis revealed a significant increase in the size of brain degeneration zones in the lamina by 4% ($p=0.016$).

The obtained data suggest that combining of wild type and *sws* mutants is a perfect model system for studying the mechanisms of neurotoxic organophosphates influence. Our data require the further research of molecular and cellular mechanisms of OPIDN.

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Aberrant social behaviour of group housed BTBR T+Iptr3tf/J mice

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The ever-growing incidence of Autism Spectrum Disorder (ASD) diagnosis makes it the most common neurodevelopmental disorder worldwide. Genetic heterogeneity of patients hinders the development of uniform therapeutic strategies and points to a need for tailored therapies [1], the formulation of which relies heavily on the use of animal models. Mouse models of ASD require constant validation as their usefulness for preclinical tests depends on the accuracy with which they replicate symptoms of the spectrum observed in human population. Most reports of impaired sociability and communication skills in these models are currently based on recordings of short dyadic interactions of non-familiar social partners placed in a novel environment. Such stressful and often variable conditions decrease the replicability, and as such, clinical value, of the results. As an alternative, we proposed a more ethologically relevant testing environment, the Eco-HAB system [2]. Here we will present data showing that, in comparison to the normo-social c57BL/6J mouse strain, Eco-HAB group-housed BTBR T+Iptr3tf/J mice (a mouse model of idiopathic ASD) display abnormal approach to social cues, exaggerated changes in incohort sociability related to introduction of the cue, as well as instability of social networks and status over time.

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Effect of cobalt chloride-induced chemical hypoxia on blood platelets function and metabolism of neuronal cells

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Abstract

In early stages of hypoxia cranial blood flow is increased to oxygenate the brain and to protect it against severe damage or death. We investigated the effect of cobalt chloride-induced chemical hypoxia on blood platelets metabolism and function as well as metabolism of neuronal cells (SHSY-5Y).

The experiments were carried out on blood platelets isolated from buffy coats from the local blood bank, and on neuronal cells of the SHSY-5Y line, with neuroma. Blood platelets were incubated (120min) with cobalt chloride ($CoCl_2$) (0.01-2 mM) to assess thrombin-induced aggregation, platelets glycylotic metabolism as lactate dehydrogenase (LDH) activity and mitochondrial activity as dehydrogenase succinate (SDH) activity by MTT test. Also, cytotoxicity of $CoCl_2$ was measured by LDH activity in the extra-platelet space. SHSY-5Y cells were cultured with $CoCl_2$ (0.01-1mM) (24h at 37°C) and LDH, pyruvate dehydrogenase (PDH), iso-citrate dehydrogenase (ICDH) and aconitase activites were analyzed.

Platelet exposure to $CoCl_2$ significantly increased their aggregation. Whereas $CoCl_2$ inhibited platelet succinate dehydrogenase by over 50% and did not cause any LDH release to extra-platelet compartment. Exposure of SHSY-5Y line cells to $CoCl_2$ caused a significant increase in LDH activity and a concentration-dependent decrease in PDH, ICDH and aconitase activities

$CoCl_2$-induced hypoxia of SHSY-5Y cells reduced oxidative metabolism, with a simultaneous compensatory increase in glycolysis to prevent neuronal energy deficiency. At the same time, $CoCl_2$-dependent hypoxia inhibited platelet oxidative metabolism, enhancing aggregation, probably through a compensatory increase in glycolysis and ATP production. Thus, hypoxia may induce platelet hyperactivity contributing to thrombotic complications.

There is no conflict of interests.

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Characterisation of novel CD73 inhibitors in primary astrocyte culture

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Concomitant up-regulation of ecto-5'-nucleotidase (CD73) and adenosine $A_{2A}R$ receptor signaling by microglia and astrocytes, are the hallmark of almost every chronic neuroinflammatory pathology studied so far. Thus, concomitant CD73/$A_{2A}R$ blockade may have potential therapeutic value [1]. In the present study we have tested the potency of several novel CD73 inhibitors - purine derivates MRS4598, MRS4552, MRS4602 [2] and dual CD73/$A_{2A}R$ blocker (DB-12) [3]. We have applied a range of concentration (10^{-9} – 10^{-4} M) in rat primary cortical astrocyte cultures and tested cell viability, metabolic activity and CD73 activity *in vitro*. Bright-field and confocal images demonstrated unusual cell morphology, cell aggregation and detachment in the presence of >10 μM DB-12. Cell shape alteration was also induced by MRS 4552, in concentrations >50 μM, while MRS4598 and MRS4602 did not affect cell morphology in any applied concentration. MTT assay showed that applied inhibitors in a concentration bellow 10 μM and the dual blocker in a concentration bellow 1 μM did not affect metabolic activity of primary astrocytes. The efficacy of the applied compounds as CD73 inhibitors was tested in 5'-phosphohydrolase Malachite green assay and expressed as IC50 values. All applied purine derivates have IC50 values in the low micromolar range with a following descendant order of potency: MRS4598, MRS4602, MRS4552, while the dual-blocker DB-12 showed only partial ability to inhibit CD73 in the concentrations >10 μM. The study further aims to optimize the application of novel CD73 inhibitors in several neuroinflammation models.

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No conflicts of interest

Proenkephalin is the precursor of Leu-enkephalin in the basal ganglia of the brain

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Enkephalins are the primary ligands of mu and delta opioid receptors in the central nervous system. The two main peptides belonging to this group are Met- and Leu-enkephalin, which are produced by proteolytic cleavage of proenkephalin. The Leu-enkephalin-encoding sequence is also present in the prodynorphin gene, however, whether prodynorphin does serve as a precursor remains unclear [1, 2]. To verify the hypothesis we generated mice (Mus musculus L) with germline deletion of the proenkephalin gene (*Penk* KO). Verification of the *Penk* gene's deletion was performed by genotyping, immunofluorescence staining on brain slices with the usage of antibodies directed against proenkephalin, and analysis of relative expression of *Penk* mRNA transcripts in the heart and the brain's striatum. Initial behavioral characterization revealed no obvious mutation effects, normal activity in the open field and intact preference of sweet taste. We examined the levels of Leu- and Met-enkephalin in the basal ganglia using nano-flow liquid chromatography combined with tandem mass spectrometry (nano-LC-MS). In the chromatogram of the control animals, two distinct peaks were observed, at m/z of 574.2 ± 0.2 and 556.2 ± 0.2 g/mol. Analysis of the collision induced fragmentation spectra of individual ions confirmed, that the observed peptides are Leu-enkephalin (YGGFL) and Met-enkephalin (YGGFM) respectively. Thus, deletion of the proenkephalin gene caused absence of both enkephalin pentapeptides in the brain areas analyzed, which indicates that proenkephalin is the major or possibly the sole precursor of Leu-enkephalin.

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Maternal high-sugar diet disrupts the NMDA receptor subunits expression and DLC2-GKAP proteins interaction in the adolescent offspring

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Literature data indicate that maternal unbalanced diet might induce in the offspring long-term negative effects such as depression, anxiety, autism spectrum disorder or learning deficits [1], [2]. Disruption of NMDA receptors is often involved in the pathogenesis of mentioned disorders [3].

In this study, we evaluated the influence of maternal high-sugar diet during pregnancy and lactation on the expression of the NMDA receptor subunits in the offspring. Additionally, we assessed DLC2-GKAP proteins interaction, which is important for NMDA receptor proper functioning.

Female Wistar rats were divided into 2 groups and fed standard or high-sugar diet. Offspring were separated on postnatal day 21 and fed only standard diet. On postnatal day 28, the offspring were sacrificed, and brains were collected. The expression of NMDA receptor subunits: GluN1, GluN2A, GluN2B was determined in the hippocampus and prefrontal cortex using the Western blot method and these results were confirmed on brain sections using immunohistofluorescence staining. DLC2-GKAP interaction was assessed using proximity ligation assay.

Maternal high-sugar diet decreased the expression of all analyzed NMDA receptor subunits in the prefrontal cortex in both female and male offspring, while no changes were found in whole hippocampus. However immunohistofluorescence staining revealed reduced expression of analyzed subunits in the CA1 and dentate gyrus regions of the male offspring and proximity ligation assay revealed reduced DLC2-GKAP interaction. Similar trend of impaired interaction was found in female offspring. Results indicate that maternal high-sugar diet may affect NMDA receptor composition and regulation. This may result in behavioral, metabolic and psychological disturbances.

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Idiosyncratic functions of active touch strategies in shape perception.

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The motivation for this work emerged from the following gap. Even though hand movements are essential for tactile perception of objects, the specific motion strategies chosen by each participant to perceive, their kinematics and idiosyncratic selection, is unclear and understudied. In a sequence of practice sessions, we have used a high-resolution high-speed system to track hand movements during tactile recognition of planar (2D) shapes, to gain insights into idiosyncratic selection of active touch strategies in shape perception. We measured variability and consistency in idiosyncratic physiological thresholds, thresholds that are indicative of the participant's spatial resolution abilities and her or his effective adaptation time. We examined the advantages of the participants' idiosyncratic motion strategies in the context of adaptive perceptual control. Two dominant hand movements strategies were identified: Contour-following movements, either tangential to the contour or oscillating perpendicular to it, and exploration by scanning movements, crossing between distant parts of the shapes' contour. Both strategies exhibited non-uniform coverage of the shapes' contours. Idiosyncratic movement patterns were specific to the sensed object and could be explained in part by spatial and temporal tactile thresholds of the participant. Using simulations, we show how some strategy choices may affect receptors activation. These results suggest that motion strategies of active touch adapt to both the sensed object and to the perceiver's physiological parameters.

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Temporal information processing on milli- and suprasecond levels

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Temporal information processing (TIP) sets a frame for human cognitive functions. Previous studies indicated two distinct levels of TIP, i.e., milli- and suprasecond ones. Nevertheless, relations between these domains remain unclear. The aim of this study was to test whether the performance on millisecond level is linked to that on suprasecond level. 77 young adults (M_{age} = 23) participated in this study. They underwent two tasks: 1) the Temporal Order Judgement task devoted millisecond domain, and 2) subjective accentuation (SA) task addressed suprasecond level. The former task measured the Tempo-

ral Order Threshold – defined as a minimum gap between two sounds presented in rapid succession necessary to reproduce their order correctly. The SA task relied on a subjective accentuation and creating individual rhythmic patterns during listening to metronome beats presented at various frequencies. The length of intervals in which subjects could integrate successive beats into an individual perceptual unit was measured. Participants were classified into two groups characterised by high (N = 38, M_{age} = 23) or low (N = 39, M_{age} = 23) TIP efficiency on millisecond level. These groups differed in the integration strategy applied in SA task, but only for beats presented at higher metronome frequencies. The group more skilled in TOJ, relied more on the constant time in SA. On the contrary, the less skilled group applied also the mental counting in SA. These results indicated that better performance on millisecond domain corresponds to different performance on suprasecond level.

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Cortico-thalamic synaptic input effects on thalamocortical network for slow oscillations: A computational study

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Cortical slow oscillations (SOs) and thalamo-cortical sleep spindles are two predominant EEG rhythms of slow wave sleep. Whereas SOs are cortical in origin, sleep spindles require thalamic activity. The thalamocortical system plays a vital role in synchronizing SOs and concurrent spindle activity. To examine this interaction and eventually for future modelling of neuromodulation, we developed a thalamocortical network model [1]. The cortical network contains pyramidal cells (PY) and interneurons (IN), both with two compartments (dendritic and axo-somatic). In the thalamic network the thalamocortical (TC) and reticular (RE) cells are presented by one (somatic) compartment. The intrinsic currents of all cells are simulated by Hudgkin-Huxely kinetics. Synaptic currents of AMPA, NMDA, and GABAA receptors are simulated by a first order kinetic activation scheme [2], whereas GABAB receptors have a complex activation scheme. The depolarization SO up state is initiated by mini synaptic currents and terminated through short-term synaptic depression in cortical cells. Weight changes of AMPA receptors are used to vary synaptic conductances between cortical and thalamic networks. Results reveal that augmentation of the excitatory cortico-thalamic connection reduces spindle activity in the thalamic network. Subsequently, cortical cells receive weaker thalamic synaptic input, and at a considerable delay (~450 ms after active/up state initiation), corresponding to the end of the up state. Strong cortical glutamatergic inputs to the thalamic network, on the other hand, appear to reduce spindle activity in the thalamocortical network and thereby disturb the temporal coordination between SO and spindles.

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Retrograde Adenosine/A_{2A} receptor signaling mediates presynaptic LTP in the hippocampus

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The dentate gyrus (DG) of the hippocampus contains two types of excitatory neurons: granule cells (GCs) and mossy cells (MCs). These neurons are reciprocally connected, thereby forming an associative circuit that has been implicated in DG-dependent memory and temporal lobe epilepsy. Remarkably, a single MC contacts about 30,000 GCs. We previously found that MC-GC synapses express a robust form of presynaptic long-term potentiation (MC-GC LTP) that can increase DG output. Moreover, initial epileptic seizures trigger broad MC-GC LTP *in vivo*, which can promote further seizures. It is therefore crucial to understand the molecular processes underlying MC-GC synaptic strengthening. MC-GC LTP requires both *post*synaptic TrkB activation and *pre*synaptic cAMP/PKA signaling, indicating the involvement of a retrograde messenger, whose identity remains unknown. Here, we address this knowledge gap using a combination of complementary approaches. Immunoelectron microscopy revealed the presence of Gs-coupled adenosine A_{2A} receptors (A_{2A}Rs) at MC axon boutons. Activation of A_{2A}Rs was necessary and sufficient to induce MC-GC LTP in acute rodent hippocampal slices. Using a genetically encoded adenosine sensor, we found that LTP induction was associated with a transient, TrkB-dependent increase in extracellular adenosine concentration. Interfering with adenosine release from GCs abolished LTP. By expressing the adenosine sensor in MC axons, we also detected adenosine release *in vivo* during acutely induced epileptic seizures. By enhancing DG information flow, A_{2A}R-mediated MC-GC LTP may contribute to memory formation and epilepsy. Our findings reveal adenosine/A_{2A}R emerges as a novel retrograde signaling mechanism that may help develop new strategies for early intervention in epilepsy.

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Alzheimer's disease and DNA methylation of candidate genes

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Alzheimer's disease (AD) is a slow, progressive, and irreversible neurodegenerative disorder with complex and multifactorial nature. Central risk factors for AD are older age, genetic predisposition, gender, cardiovascular factors and presence of the mild cognitive impairment (MCI). MCI is characterized by slight changes and disruptions in mental abilities such as memory, the ability to think and to remember. A great percent of subjects with MCI (50 % - 65 %) later develop some form of dementia, especially AD. Clinical diagnosis of (probable) AD is established thorough a combination of clinical symptoms, cognitive screening tests, detailed neuropsychological testing and imaging techniques. Molecular-genetic tests are in their infancy, and are currently relevant only after the disease has already made a considerable progress. We applied contemporary methods (next generation sequencing, droplet digital PCR) and determined methylation status of AD candidate genes, COMT and BDNF, in white blood cells and circulating cell-free DNA (cfDNA) from plasma in clinically well-defined AD patients and subjects with MCI. Differences between blood cells and cfDNA methylation, representing a pool of DNA from distant tissues, could lead to identification of peripheral markers reflecting broader picture of the organ status, including the status of otherwise unobtainable tissue, like the brain.

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Post-weaning social isolation alters ventral hippocampus plasticity and increases anxiety-like behavior in the Wistar rats

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Lack of social contact in adolescence has become a growing problem due to the COVID-19 pandemic, while the adolescent brain is especially vulnerable to adverse experiences. Here we examined the effects of adolescent social isolation on plasticity in the ventral hippocampus, which participates in emotional regulation, and assessed anxiety-like behavior in male Wistar rats. The isolation procedure started immediately after weaning at postnatal days 23-25 and lasted for 4-5 weeks. After that, rats were assigned to either behavioral or electrophysiology studies. Anxiety-like behavior was assessed in the elevated plus-maze test (EPM). For plasticity studies, acute hippocampal slices were prepared from the ventral hippocampus (VH), and field excitatory postsynaptic potentials were recorded from the stratum radiatum of the hippocampal CA1 region after stimulation of the Schaffer collateral. Long-term potentiation (LTP) was elicited by high-frequency tetanic stimulation (HFS). Short-term potentiation (STP) was defined as a period between 2-10 minutes after HFS, and LTP was evaluated at 30-40 minutes after HFS. We found that STP in the VH of isolated rats was increased comparing to group-housed controls (166.4 ± 12.4 % vs. 137.1 ± 5.2 %, $p < 0.05$), while LTP elevation didn't reach the level of significance (122.0 ± 5.6 % vs. 110.9 ± 4.6 %). Moreover, isolated rats showed decreased time spent in the open arms of the EPM (2.7 ± 1.1 s vs. 6.0 ± 1.2 s, $p < 0.05$). Taken together, our data suggest that post-weaning social isolation leads to plastic changes in the ventral hippocampus associated with emotional dysregulation.

The authors declare no competing interests.

Increased dopamine signaling in thalamus of adolescent rats neonatally exposed to mild normobaric hypoxia

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Mild hypoxic events during an early mid-gestation may cause subtle motor, sensory and behavioral deficits that become detectable only later in life, unabling so an early intervention. In order to search for early behavioral, structural, neurochemical, and molecular markers of mild hypoxia, we have developed a corresponding rat model by exposure of 1-day-old pups (PND1) to hypoxic conditions (8% O2, 92% N2) in a normobaric chamber for 2 hours. In adolescent rats, we have previously observed altered ex-

ploratory behavior, paralleled by midbrain dopamine (DA) increase, which was interpreted as possible difficulties in hippocampus (Hc)-related spatial mapping or thalamus (Th)-related somatosensory processing. This study examined possible alterations in DA signaling in the mentioned regions receiving inputs from the midbrain DA-neurons. By using qPCR, relative mRNA expression of D1 and D2 receptors, and their down-stream targets protein kinase A (PKA) and dopamine- and cAMP-regulated neuronal phosphoprotein (DARP-32), was measured in 16 hypoxia-exposed and 15 control rats sacrificed on PND50. In comparison to controls, relative D1 and D2 mRNA levels were unchanged in hippocampi but were significantly increased in thalami of the treated animals. Increased thalamic expression of D1 was accompanied by highly correlated upregulation of mRNA for PKA (regulatory subunit 2A) and DARP-32, suggesting that one of the consequences of the neonatal exposure to hypoxia might be a long-lasting increase in the thalamic DA signaling. Further research on the thalamo-cortical signaling and somatosensory processing should reveal the contribution of this neuronal path to the observed behavioral changes.

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Are neurons in the foveal-V1 getting peripheral properties upon transient narrowing visual field?

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In V1, objects appearing at the center of the visual field are processed in detail by densely packed foveal neurons with small receptive fields (RFs), while neurons with large RFs at the periphery of the visual field are sensitive to motion. Measuring simultaneously the central visual acuity and peripheral motion sensitivity by acuity-motion task reveals that the peripheral negative contrast high velocity motion stimulation significantly impairs central acuity thresholds [1]. To gain insight into dynamics of the functional readjustments upon transient removal of peripheral stimulation, we restricted visual field by goggles to 10deg for 15 minutes. We performed in 20 normal-sighted participants a fMRI scanning for a population receptive field mapping (pRF) and motion-acuity task outside the scanner. For nested general linear model on pRF size and eccentricity analyses the eccentricities were divided in 2deg bins. In foveal-V1, (2deg, central), upon removal of peripheries we revealed an increase of the pRF sizes and shift towards higher eccentricities. Further from center (2-8deg) within the cortical visual field representation pRFs still increase, but the shift toward higher eccentricities is not preserved. Individually, bigger pRFs and higher eccentricities at foveal-V1 were positively correlated with better motion-acuity for fast-velocity in negative contrast and negatively with worst motion-acuity for fast-velocity in positive contrast. We can

hypothesize that the transient loss of the peripheries brings foveal-V1 in a new peripheral state by getting larger pRFs shifted to higher eccentricities, which correlates at the individual level with behavioural performance associated with peripheral sensitivity as shown by acuity-motion thresholds.

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Sex and strategy effects on brain activation during a 3D-navigation task

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Sex differences in navigation have often been attributed to the use of different navigation strategies in men and women. However, only few studies have investigated sex differences in the neural correlates of navigation and no study so far has investigated sex differences in the brain networks supporting different navigation strategies. To address this issue, we employed a 3D-navigation task during functional MRI in 36 men and 36 women, all scanned thrice, and modelled navigation strategies by instructions requiring an allocentric vs. egocentric reference frame on the one hand, as well as landmark-based vs. Euclidian strategies on the other hand. We found distinct brain networks supporting different perspectives/strategies. Men showed stronger activation of frontal areas, whereas women showed stronger activation of posterior brain regions. The left inferior frontal gyrus was more strongly recruited during landmark-based navigation in men. The hippocampus showed stronger connectivity with left-lateralized frontal areas in women and stronger connectivity with superior parietal areas in men. We discuss these findings in the light of a stronger recruitment of verbal networks supporting a more verbal strategy in women compared to a stronger recruitment of spatial networks supporting a more spatial strategy use in men. In summary, this study provides evidence that different navigation strategies activate different brain areas in men and women.

The authors declare no conflict of interest or competing interests.

The role of AMPA receptor surface mobility in high-frequency short-term synaptic plasticity

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Activity-dependent plasticity of synaptic transmission is one of the key mechanisms underlying learning and memory. During high-frequency short-term synaptic plasticity (HF-STP) the amplitude of synaptic responses changes in time upon pre-synaptic stimulation on a timescale of seconds. HF-STP is important for information processing in the brain, serving particularly for temporal integration. It is generally accepted that HF-STP is regulated majorly by pre-synaptic mechanisms and the paired-pulse ratio of response is widely used as a proxy of changes in pre-synaptic processes. However, post-synaptic mechanisms have been shown to regulate HF-STP as well. Here, we study the functional role of AMPAR surface diffusion in HF-STP in organotypic hippocampal slices (OHS) and *ex vivo* brain slices. We developed the APGluA2KI mouse model where GluA2 subunits can be specifically biotinylated when co-expressed with a biotin ligase BirA. This approach allowed us to show that immobilization of endogenous AMPARs in high release probability conditions modulates HF-STP by decreasing synaptic facilitation in the Schaffer collateral-CA1 synapse. This effect was reversed when a desensitization blocker CX546 was applied. Next, we examined the effect of AMPAR immobilization in synapses made onto granule cells of the dentate gyrus by the medial and lateral perforant path, that display paired-pulse depression and facilitation, respectively. Overall, we have shown that post-synaptic mobility of AMPARs can modulate HF-STP by regulating the replacement of desensitized receptors. This is a step forward to a better understanding of the implications of receptor surface diffusion and post-synaptic mechanisms in the regulation of HF-STP.

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The effect of RAGE and its ligands on the progression of ALS in SOD1 G93A transgenic mice

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Amyotrophic lateral sclerosis *(ALS)* is a devastating neurodegenerative disease characterized by a progressive loss of both upper and lower motor neurons resulting in paralysis and muscle atrophy. The pathogenesis of ALS is still not elucidated. One of the most prospective hypothesis on the ALS pathogenesis suggests that oxidative stress, excessive inflammation and protein aggregation play key role in the development of this disease [1].

RAGE, receptor for advanced glycation end-products, via its pro-inflammatory and pro-oxidative stress ligands such as HMGB1, S100B and CML participates in these processes, likely contributing to ALS pathogenesis [2, 3].

Here, in our study we examined by qRT-PCR and immunoblotting changes in expression of RAGE and its ligands during the progression of the disease from the onset until the terminal stage.

The results of qRT-PCR showed increased expression of RAGE and HMGB1 mRNA throughout all studied time points as compared to controls, contrary to S100B mRNA, that peaked at the disease onset and declined throughout remaining time points as compared to controls. Immunoblotting demonstrated increased protein expression of RAGE at the disease terminal stage, contrary to HMGB1 and S100B, that had the highest level at the disease onset and high, but not statistically significant at remaining time points as compared to controls.

These results contribute to understanding the implication of RAGE and its ligands in pathogenesis of ALS and highlight the potential use of RAGE ligands as biomarkers in early ALS diagnosis and potential targeted therapeutic interventions at the early stage of this devastating disease.

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Chemogenetic study of thalamic neurons related to maternal behavior

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The posterior intralaminar thalamic nucleus (PIL) has been suggested to play an important role in the regulation of maternal behavior. Fos expression was demonstrated in response to suckling in calbindin expressing neurons in the PIL. Our aim was a chemogenetic investigation of maternal function of PIL neurons with parallel assessment of anxiety-and depression-like behaviors.

For behavioral studies, we injected an adeno-associated virus into the PIL of Calbindin-Cre mice for Cre-dependent expression of designer receptors exclusively activated by designer drugs (DREADDs). Behavioral tests demonstrated that injection of clozapine-N-oxide (CNO), the designer ligand of DREADD, resulted in changes in maternal, anxiety-related and depression-like behaviors.

We also mapped the projections of calbindin neurons from the PIL and found labelled fibers in a variety of different brain regions. In addition, we examined Fos activity in response to CNO. CNO injection in mice expressing stimulatory DREADDS resulted in Fos expression in DREADD containing cells of the PIL, and also in other brain regions, where the PIL calbindin neurons projects, such as the medial preoptic area, lateral septum, periaqueductal gray. In turn, administration of CNO in inhibitory-virus injected mice reduced Fos expression evoked by pup exposure in several brain regions.

In conclusion, selective modulation of PIL calbindin neurons changed maternal behavior of mice suggesting that these neurons are involved in maternal control of behavior.

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Analysis of Synapse-enriched Circular RNAs For Functional Analysis in Primary Cortical Neurons

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In the recent years non-coding RNAs have emerged as a potent regulatory molecules in versatile biological processes, both in health and disease. Among them, circular RNAs (circRNAs) were discovered and have been shown to be particularly highly abundant in the central nervous system. A subset of circRNAs

was shown to be enriched in the synpatoneurosomes in comparison to the cytoplasmic fraction in the brain [1, 2]. However, currently there is little information regarding circRNA function in neurons. In this study, we sought out to investigate the biology of these synapse-enriched circular RNAs. We selected a list of potentially interesting candidate circRNAs based on high-throughput RNA sequencing data from mouse cortical neurons, mouse whole cortex and previously published synaptoneurosomes [2]. The selection criteria involved, among others, conservation between rodents and human, higher expression ratio in synaptoneurosomes and overall high expression score in cortex compared to other mouse tissues (as liver and heart). Expression levels of our circRNA candidates were examined in primary cortical neuron cultures and compared to primary mouse astrocytes and brain tissues (qRT-PCR). Further, we confirmed their circular structure by performing RNaseR treatment followed by qRT-PCR and Sanger sequencing of the back-splice junction sites which are unique to the particular circRNAs. In the subsequent studies we want to knockdown some of the validated candidates to study the impact of their loss in primary neurons and/or astrocytes. Understanding the role of selected circular RNAs will shed more light on the function of regulatory RNAs in the fundamental processes in neurons.

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Vocal, heart-rate and motor responses to ultrasonic playback are influenced by prior stress and single rearing in rats

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Rats emit 50-kHz ultrasonic vocalizations in appetitive states and 22-kHz ones in aversive states. Both affective states influence heart rate. We introduced a behavioral model employing exposure to pre-recorded playbacks in home-cage-like conditions. Effects of social context and prior stress were also investigated. We showed that: i. rats moved faster during 50-kHz playback and slowed down after 22-kHz playback; ii. they all approached the speaker, which was more pronounced during and following 50-kHz playback than 22-kHz playback; iii. 50-kHz playback caused heart rate (HR) increase; 22-kHz playback caused HR decrease; iv. the rats vocalized more often during and following 50-kHz playback than 22-kHz playback. Observed effects were more pronounced in singly housed rats compared to the paired housed animals. Pre-shocked rats showed higher locomotor activity during 50-kHz playback and a more significant decrease in activity following 22-kHz playback; they vocalized more often, their USV

were longer and at a higher frequency than in control animals. These last two observations could point to hypervigilance, a symptom of post-traumatic stress disorder (PTSD) in human patients. Therefore, the increased vocalization may be a valuable measure of hypervigilance used for PTSD modeling. Some of the above-mentioned results have been already published[1].

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Eye tracker results in watching photographs related to experience in photography

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Area of interest: Eye trackers can show eye fixations and their durations in AOI (area of interest). The aim of the study is to find the difference in fixated attention areas in eye tracker results in different photography experience level groups.
Basic assumptions: The AOI scores are expected to be different in the experienced photographers group in relation to less experienced or unexperienced groups.
Methods: Tobii Pro X2-60 eye tracker measurements were made during watching 10 photography contest photographs. The study groups were doyens and academicians of photography as the experienced group and photography students as less experienced group. Control groups were two groups with same in the same level educated people in the same age as in the first two groups without any special interest in photography. Ten photographs about “Istanbul” were shown to the subjects in a row each for five seconds. The viewers had been given the instruction to decide whether the photograph should be eliminated or not. The eye movements at the judging period were recorded with the eye tracker. Area of interest (AOI) areas are determined and compared between the groups.
Results: There were no statistically significant AOI fixation differences between the study and control groups in all photographs except in one. Photography doyans were significantly quicker in negative decisions than in positive. The decision making took slightly longer in lower experience groups.
Conclusion: The eye tracking results in watching photographs seem to be similar in different photography experience levels.

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Light-responsive neurons in the suprachiasmatic area of the diurnal murid rodent *Rhabdomys pumilio*

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Photic sensitivity of neurons in the hypothalamic suprachiasmatic nuclei (SCN), site of the master circadian clock, has been documented in several species, but most of these have been nocturnal rodents. Those studies have shown that SCN neurons are excited by light stimulation and great majority of them respond to extended light pulses with maintained increase in firing, the magnitude of which is defined by stimulus irradiance. That type of 'irradiance response' is considered to reflect the SCN's use of retinal input to track the daily change in ambient light. In contrast, far less attention was paid to the effects of retinal illumination on firing rates of SCN neurons in diurnal mammals. We set out to examine these issues, by using *in vivo* acute multielectrode recordings from the hypothalamus of the diurnal murid rodent *Rhabdomys pumilio*. We find that, as in nocturnal rodents, firing pattern of many hypothalamic neurons was influenced by light presentation, however inducing more diverse and faster responses. Both transient and sustained responses were observed and cells were either excited or suppressed by light. Surprisingly, only ~15% of neurons showed classical sustained responses to the presence of light, indicative of irradiance coding. In fact, the most common response phenotypes were transient responses to the appearance and/or disappearance of light, which are universally associated with rods and cones signals. Thus, our data suggest that *R.pumilio* SCN neurons not only read the time of the day by coding irradiance but also might be specialized in processing more sophisticated visual information.

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Antidepressant activity of two new heteroarylcarbonyloxyaminopropanols in mice

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Depression is a major prevalent neuropsychiatric problem with symptoms not only manifested at the behavioral, but also at the physiological level. The current therapies have many limitations. As a consequence, there is a clear need to develop more efficient and safer drugs as alternative and/or complimen-

tary therapy for depression. Insufficient amounts of monoamine neurotransmitters e.g. serotonin (5-HT) have been long related to pathogenesis of the disorder. 5-HT is a neurotransmiter within the central and peripheral nervous systems, which exerts its actions through the interaction with seven distinct receptor. The 5-HT_{1A} receptor is widely recognized as a relevant therapeutic target for several psychiatric disorders, such as anxiety and depression [1].

Two new heteroarylcarbonyloxyaminopropanols (TP25B and TP38B) with higher selectivity profile towards 5-HT_{1A} have been evaluated by behavioural tests (e.g. locomotion and forced swimming tests – FST) to determine functional activity.

The experiments were performed on male albino Swiss mice. FST was used to evaluate the antidepressant-like activity. (±)-8-hydroxy-2-dipropylaminotetralin hydrobromide (8-OH-DPAT; the 5-HT_{1A} receptor agonist) was used as a reference drug [2].

TP25B and TP38B at the same doses 7.5, 15 and 30 mg/kg significantly decreased the immobility time in the FST, without producing locomotor alterations.

The results showed that two new heteroarylcarbonyloxyaminopropanols exert antidepressant-like effects in FST in mice.

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Mechanism of MMP-9-1562C/T single nucleotide polymorphism-dependent regulation of MMP-9 (Matrix Metaloproteinase-9) expression in human neurons

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The -1562C/T functional polymorphism of *MMP-9* promoter modulates *MMP-9* expression in *MMP-9*--dependent human diseases generally leading to increased MMP-9 mRNA expression with the T allele. However, molecular mechanism responsible for this phenomenon is unknown in mammals.

Our study was carried out in differentiated neurons derived from the SH-SY5Y human neuroblastoma cell line. We found that transcriptional activity of the T allele of MMP-9 gene promoter is higher than the C allele in human neurons. We also found the allele-specific formation of nuclear protein complexes in human neurons by EMSA.

Using magnetic beads coated with the C or T human allele, we pulled down human neuronal nuclear proteins binding specifically to the alleles. Then, we analyzed the identity of these proteins by mass spectrometry. We identified numerous transcriptional regulators which bound specifically to the alleles and we selected those of them which seemed to have especially high probability to regulate MMP-9. Then, we checked by EMSA *supershift* whether they could bind to the alleles in neurons and we identified ZNF384 and HDAC1 as the –1562C/T binders. They associated stronger to the C allele that to the T allele *in vitro* in human neurons. The silencing of ZNF384 or HDAC1 upregulated MMP-9 mRNA expression significantly with the T allele and much less with the C allele in human neurons *in vivo*. Altogether, our results show that the MMP-9-1562C/T exerts its differential influence on MMP-9 expression *in vivo* in human neurons due to its distinct binding to MMP-9 transcriptional repressors ZNF384 and HDAC1.

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Intravenous infusion of neuroectodermal stem cells exerts beneficial effect on tissue sparing and functional recovery after traumatic thoracic spinal cord injury

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Traumatic spinal cord injury is characterized by an acute mechanical insult followed by a series of secondary lesional events including acute vascular disruption, cell death, ischemia, inflammation and demyelination. In this study we investigated the effect of intravenous neuroectodermal stem cell therapy which could reduce the severity of secondary injury and enhance tissue preservation and ultimately promote functional recovery.

A moderately severe thoracic contusion injury was induced at T5 spinal level in adult female Sprague-Dawley rats, followed by an intravenous tail vein infusion of NE-GFP-4C stem cells 30 min or 1 week after the injury. Control animals underwent contusion injury without intravenous stem cell administration. Functional tests (Basso, Beattie, Bresnahan, and kinematic analysis) and detailed morphological analysis (quantification of retrograde labelling and immunohistochemistry) were performed to evaluate the effects of grafted cells.

NE-GFP-4C cell infused rats displayed significantly improved functional recovery compared to controls. Morphologically, the contusion cavity was significantly smaller, and the amount of spared tissue was significantly greater in grafted animals than in controls. Retrograde tracing studies showed a statistically significant increase in the number of retrogradely labelled neurons in different segments of the spinal cord, the brainstem and the sensorimotor cortex of stem cell treated animals. The extent of functional

improvement was inversely related to the astrocytic reactions in the injured segment. Intravenous stem cell treatment promoted preservation/sprouting of serotonergic fibers caudally to the injury.
Taken together, we demonstrate selective long-term functional recovery alongside histological improvements with NE-GFP-4C cell infusion in a clinically relevant model.

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The authors declare no competing interests.

The *stim2a*$^{-/-}$ gene deletion effect on transcriptional landscape of zebrafish and its impact on zebrafish behavior

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Calcium is ubiquitous messenger in the cell known to regulate numerous and diverse processes. We focus on the impact of calcium signaling on the functioning of neurons. All the cellular machinery that is part of calcium signaling was defined as calcium toolkit (CaTK) or else calciomics. We were the first to characterize the CaTK in zebrafish [1], further focusing on Stim2 [2,3], which was shown to have different from Stim1 functions, including regulation and fine tuning of calcium signaling.
We characterized the role of stim2 at the level of organism [2, 3]. For that we utilized CRISPR-derived mutants of zebrafish. In our previous studies we have revealed the behavioral abnormalities caused by deletion of *stim2* genes and changes in calcium oscillations in larval brain [2,3]. This points to the dysregulation of CaTK in the absence of these genes. In our published work we focused on transcripts with known function. However, the biggest differences between transcriptomics of mutant and wild-type zebrafish fall into uncharacterized transcripts (10- to 100-fold change, for reference see NCBI SRA database, accession number PRJNA635784). Here we aim to describe the novel transcripts that are highly up-/downregulated in *stim2a*$^{-/-}$ line. We reached for bioinformatics methods to predict function of these transcripts and speculate about possible connection with the phenotype observed.

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Late sustained potential reflects common mechanism of conflict monitoring

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Existing fMRI data support the hypothesis of common conflict monitoring mechanism, localized in the anterior cingulate cortex (ACC) and fronto-parietal network [1]. However, EEG studies provide a more complex picture reporting different ERP waves depending on the employed conflict task [2].

To address this issue we recorded EEG during an extended Multi-Source Interference Task (MSIT) in which Simon and flanker conflicts are presented concurrently and in isolation [3]. We used microstate analysis to identify time windows of semi-stable between-conditions differences and employed a Linearly Constrained Minimum Variance (LCMV) beamformer to identify their brain sources. If these two conflicts are managed independently, their concurrent presentation should result in an additive effect, while if they share a common mechanism, they will affect each other resulting in the interaction.

In line with the latter scenario, during concurrent conflicts presentation we observed lower amplitude of late sustained potential (LSP, ~524-733 ms after stimulus onset; $p<0.001$) than expected from the simple addition of Simon and flanker effects. The amplitude of LSP was increased in response to both flanker- and Simon-only trials (both $p<0.01$ in comparison with no-conflict condition). However, when the two conflicts were presented concurrently, LSP amplitude did not differ from the control condition ($p=0.192$).

LCMV indicated putative sources of this effect in the medial frontal cortex (supplementary motor area and ACC constituting 60% and 29% of cluster mass (6386 voxels), respectively), with some input from more posterior regions (precuneus, superior parietal lobule, paracentral lobule; 3463 voxels) and the hippocampal area (1610 voxels).

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Cholinergic system plays a modulatory role in the neuronal network of the intergeniculate leaflet nucleus of the rat

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The intergeniculate leaflet (IGL) of the thalamus is an important structure of the mammalian biological clock. The well-known function of the IGL is integration of photic and non-photic stimuli and sending this information to the master biological clock – suprachiasmatic nuclei. One of the main nonspecific brain projections that transmit non-photic information to this structure is the cholinergic system with the source in pedunculopontine and laterodorsal tegmental nuclei. Cholinergic system, which is a part of the ascending reticular activating system (ARAS), is responsible for many brain functions such as arousal, sleep phase and attention. The aim of this study was to examine the influence of cholinergic agonist on the processing of the photic information within the IGL.

First step of our research was to verify if carbachol and nonspecific agonists of muscarinic (Oxo) and nicotinic (Nic) receptors have a day/night variation in affecting the IGL network. For this purpose we used multielectrode array (MEA) *ex vivo* recordings, performed both during the day and at night. This experiments indicated that only the effect of nicotine is dependent on the day/night cycle. Finally we performed MEA recording with optogenetic stimulation of the axonal endings from retina. In this study we observed that carbachol can increase the response to optogenetic stimulation but can also in some cases decrease this response. To sum up, our results prove that cholinergic system modulates photic information incoming to the IGL and this modulation is dependent on day/night cycle.

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Microglia activation in the midbrain of the human neonate: the effect of perinatal hypoxic- ischemic injury

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Perinatal hypoxic-ischemic injury (PHI) is a major risk factor for the later development of neuropsychiatric deficits. After prolonged PHI a dramatic reduction of tyrosine hydroxylase (TH) in the substantia nigra (SN) of the human neonate was observed[1], without extensive neuronal degeneration[2]. Since microglia activation could precede neuronal death, we investigated two microglia activation markers-ionized calcium-binding adapter molecule 1 (Iba1) and Cd68- in the midbrain of 20 autopsied neonates (corrected age: 25.5- 46.5 weeks) obtained after written parental consent. In the SN, morphological variety of microglial phenotypes was revealed by Iba1, indicating differential microglial activation among the cases. Cd68 was extensively expressed only at very severe/prolonged PHI. Quantitative morphometry showed the highest Iba1 expression in severe/abrupt PHI, and the lowest in moderate/prolonged PHI. Very activated microglia expressing intense Iba1 was found in close attachment with TH neurons in cases with very severe/prolonged PHI, indicating early signs of SN degeneration in this group. Moreover, females appear to express more Iba1 than males, suggesting a sex difference in microglia maturation and immune reactivity. PHI-induced microglial "priming" during this sensitive for brain development perinatal/neonatal period, combined with genetic or other epigenetic factors, could mediate the induction of gender-related neuropsychiatric disorders later in life[3].

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No conflict of interest.

Guanfacine inhibits interictal epileptiform discharges and action potentials in prefrontal cortex pyramidal neurons

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Guanfacine (an alpha-2 receptor agonist) is a commonly used drug with recognized efficacy in the treatment of Attention Deficit Hyperactivity Disorder (ADHD). Short-lasting epileptic discharges (interictal activity) in cortical neurons have been associated with symptoms of ADHD and autism spectrum disorder [1]. Interictal discharges do not cause seizures. This study aimed to assess the effect of guanfacine on prefrontal cortex neurons' excitability and interictal activity.

We conducted current-clamp recordings in prefrontal cortex pyramidal neurons in slices obtained from young rats. Epileptiform events were evoked in zero magnesium proepileptic extracellular solution with elevated potassium concentration. Epileptiform discharges were spontaneous depolarizations which triggered action potentials. We regarded epileptic events as interictal because of their short duration (less than 3 seconds) [2]. Guanfacine 100 μM very potently inhibited the frequency of epileptiform discharges. It was possible to obtain wash-out. The effect of guanfacine on interictal events persisted in the presence of alpha-2 receptor antagonist idazoxan 1 μM.

Moreover, we recorded action potentials in physiological extracellular solution. Guanfacine 100 μM potently inhibited neuronal excitability which was defined as the number of action potentials per depolarization step. It was possible to obtain wash-out. Guanfacine 100 μM slightly hyperpolarized the membrane potential.

Inhibition of interictal discharges by guanfacine suggests a novel mechanism in which this drug may exert its central nervous system effects. We hypothesize that this mechanism could be independent of alpha-2 adrenergic receptors.

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Lipocalin 2 as an immune system protein with an important role in the regulation of brain development

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Epidemiological studies indicate that maternal infection during pregnancy is a risk factor for neurodevelopmental disorders. However, the mechanisms underlying this phenomenon remain unclear. One of the highly expressed proteins in the adult brain in response to infection is Lipocalin 2 (Lcn2), an innate immune response protein. The aim of our studies is to characterize the role of Lcn2 in the regulation of brain development, especially upon prenatal infection.

To mimic maternal infection the pregnant mice received three *i.p.* injections of lipopolysaccharide or saline on E16, 17, and 18, representing infection in the second-trimester pregnancy in humans. To evaluate Lcn2 mRNA expression in the fetal brain we performed qRT-PCR on fetal cortex and hippocampus isolated 24 hours after the last injection. To address how lack of Lcn2 during prenatal infection may influence intrinsic electrophysiological properties of neurons in the adult brain, we performed excitability recordings from hippocampal CA1 pyramidal cells on acute brain slices from Lcn2 WT and KO offspring after the LPS challenge.

Our results indicate that Lcn2 mRNA is significantly upregulated during maternal infection-induced fetal brain inflammation. We also observed that the absence of Lcn2 in the developing brain results in higher intrinsic excitability of hippocampal neurons in the adult brain, but only in mice exposed to prenatal infection. These results suggest that Lipocalin 2 could be a promising link between immune response and brain development, however, to answer questions regarding its role in neurodevelopmental disorders more studies are required.

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Changes in types of ultrasonic vocalisations in a poly I:C rat model of autism in the social interaction test.

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One of the core symptoms of autism spectrum disorder (ASD) is a persistent deficit in social communication, visible since early childhood. Recent studies provided evidence for the link between maternal infection during pregnancy and increased risk of developing ASD in the offspring. Here the viral mimic,

polyinosinic:polycytidylic acid (poly (I:C)), was used to induce maternal immune activation in rat dams on GD 15. The control group mothers were injected with saline on a corresponding day of pregnancy. To evaluate social deficits, animals from both groups were tested in the social interaction test in early adulthood. Rodents communicate with conspecifics emitting calls within the ultrasonic spectrum. Such ultrasonic vocalisations (USVs) can be divided into appetitive and alarm calls. Appetitive calls (~50kHz) are much more diversified than alarms and can be further assigned to various categories.
We have previously shown a higher peak frequency of poly (I:C) males' calls. Detailed analysis of USVs' types provided information about more specific changes in ultrasonic vocalisations of these rats. Poly (I:C) males emitted more flat and short calls. Moreover, their trills had altered acoustic characteristics. The peak frequency of emitted calls was higher in poly (I:C) males in all categories. Most of the observed changes were present only in male animals, which corresponds with a higher prevalence of ASD in boys.

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Development of a new method for purification of modified Rabies Virus

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Millions of people worldwide suffer from vision defects associated with degenerative diseases of the retina, characterized by loss of cones and rods, which leads to total blindness. Regardless of promising research, there is still no effective way to restore visual functions in affected individuals [1].
Rabies virus (RV) is a (-)RNA rhabdovirus that has the ability to infect neurons via retrograde transport. RV is an attractive candidate for gene delivery due to the high expression of proteins of interest, which can be potentially used in gene therapy of degenerative diseases. However, the production of modified RV is a multistep, time-consuming, and expensive process that requires ultracentrifugation of viral particles in a density gradient, limiting production efficiency [2].
Therefore, this study aimed to develop a new method of purification of modified RV using Ni-charged immobilized metal-affinity chromatography.
To this purpose, gene encoding RV surface glycoprotein (G) was cloned into pET28a(+) plasmid to generate N-terminal fusions with a His_{tag} in the surface domain of G protein sequence (G_{His}). Additionally, due to potential steric clashes and obscured tag, an additional linker (GGSGG) was introduced between His_{tag} and N-termini (GL_{His}). Next, both proteins (G_{His} and GL_{His}) were produced recombinantly and purified using Ni-charged immobilized metal-affinity chromatography. Performed SDS-PAGE analysis revealed that both modified proteins, G_{His} and GL_{His}, could bind the Ni-charged resin; however, a more significant yield was observed for GL_{His}. Therefore, the obtained results indicate one possible alternative way to purify modified RV with modified G, using metal-affinity chromatography.

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We have no conflict of interest to declare.

Effect of neonatal treatment with the NMDA receptor antagonist, MK-801, during different temporal windows of postnatal period in adult prefrontal cortical and hippocampal function

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The neonatal MK-801 model of schizophrenia has been developed based on the neurodevelopmental and NMDA receptor hypofunction hypotheses of schizophrenia. This animal model is generated with the use of the NMDA receptor antagonist, MK-801, during different temporal windows of postnatal life of rodents leading to behavioral defects in adulthood. However, no studies have examined the role of specific postnatal time periods in the neonatal MK-801 (nMK-801) rodent model and the resulting behavioral and neurobiological effects. Thus, the goal of this study is to systematically investigate the role of NMDA hypofunction, during specific temporal windows in postnatal life on different cognitive and social behavioral paradigms, as well as various neurobiological effects during adulthood. Both female and male mice were injected intraperitoneally (*i.p.*) with MK-801 during postnatal days 7-14 (p7-14) or 11-15 (p11-15). Control mice were injected with saline during the respective time period. In adulthood, mice were tested in various cognitive and social behavioral tasks. Mice nMK-801-treated on p7-14 show impaired performance in the novel object, object-to-place and temporal order object recognition tasks, the sociability test and contextual fear extinction. Mice nMK-801-treated on p11-15 only affects performance in the temporal order object recognition task, the social memory test and contextual fear extinction. No differences were identified in the expression of NMDA receptor subunits, the synapsin or PSD-95 proteins, either in the prefrontal cortex (PFC) or the hippocampus (HPC), brain regions significantly affected in schizophrenia. The number of parvalbumin (PV)-expressing cells is significantly reduced in the PFC, but not in the HPC, of nMK-801-treated mice on p7-p14 compared to their controls. No differences in PV-expressing cells (PFC or HPC) were identified in nMK-801-treated mice on p11-15. We further examined PFC function by recording spontaneous activity in a solution that allows up state generation.

We find that the frequency of up states is significantly reduced in both nMK-801-treated mice on p7-14 and p11-15 compared to saline-treated mice. Furthermore, we find adaptations in the gamma and high gamma activity in nMK-801-treated mice. In conclusion, our results show that MK-801 treatment during specific postnatal temporal windows has differential effects on cognitive and social behaviors, as well as on underlying neurobiological substrates.

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Conflicts of interests.

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The role of prestimulus oscillatory brain activity with respect to sound localization performance in cochlear implant users.

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The ability to locate the source of a sound is critical to survival. A further aspect in which spatial hearing is relevant is human communication. Consequently, impairments and loss of hearing affect orientation in space, and communication. This study aimed to investigate the role of prestimulus oscillatory activity, encompassing a wider range of frequencies, including the alpha band. To examine the characteristics and topographic distribution of prestimulus oscillatory brain activity, in spatial sound localization, the oscillatory brain activity in cochlear implant patients had been recorded during the performance of specially designed sound localization task. The objective of the present study was not only to investigate the particular accounts of oscillatory activity function but also to provide anatomical specificity that may unravel the neural correlates of spatial sound localization. No significant differences were found in the prestimulus power spectrum in the frequency band of interest (2-30 Hz, in the step of 2 Hz) between trials, where spatial sound localization task was performed correctly and those in which was done incorrectly. Hence, this study failed to account for showing the role of the prestimulus oscillatory power in the spatial sound localization. However, active in the prestimulus period sources correlating with the ability to localize sound in space were found in several unilateral cortical regions in the right hemisphere at 4 Hz, i.e. the precuneus, postcentral gyrus, cingulate cortex, posterior parietal cortex. It can thus be reasonably assumed that these cortical regions account for spatial sound localization.

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The authors declare no conflict of interest.

Electrocorticogram changes following claustrum stimulation during two types of anesthetics in adult rats

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Introduction

The claustrum is a controversial subcortical structure, regarding its roles, being connected with many structures of the brain. The aim of this study is to investigate the impact of the claustrum electrical stimulation on the anesthetic depth, using different stimulation protocols and under different types of anesthesia.

Material and methods

Materials and methodsIn this study we used two lots of five adult rats. For each lot, the experiment was conducted under a different anesthetic: chloral hydrate and ketamine-xylazin respectively. After bein mounted in the stereotaxic frame, three epidural electrodes were placed on the left frontal and parietal lobes and on the olfactory cortex (as a reference electrode) in order to perform the electrocorticogram (ECoG) recording. A bipolar tungsten electrode was stereotaxically inserted at the coordinates of the left claustrum. The claustrum was electrically stimulated by applying 10 stimuli of 5 second duration each, at interstimulus interval of 5 seconds under chloral hydrate anesthesia and ketamine-xylazin anesthesia. ECoG analysis using median frequency, spectral edge frequency and burst count were performed for the assessment of anesthesia depth changes during claustrum stimulation.

Results

Stimulation of the claustrum produced a deepening of anesthesia expressed by the Burst Supression (BS) pattern, both in the Chloral Hydrate and the Ketamin-Xylazin. BS was maintained during the whole time of stimulation.

Conclusion

The occurrence of BS pattern during Chloral Hydrate and Xylazin-ketamin slow waves anesthesia after electrically stimulating the claustrum could serve as an indicator of the its contribution to the anesthesia depth.

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Carol Davila University of Medicine and Pharmacy, Bucharest, Romania.

Conflict of interest

There is no conflict of interest

The course of neonatal vocalization development in the animal model of schizophrenia.

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Schizophrenia is typically diagnosed during late adolescence or early adulthood, but subtle deficits in communication and sociability are often evident from early infancy. Despite this, socio-communicative deficits are poorly investigated in animals at an early stage of development in schizophrenia-like models. These impairments can be experimentally modelled using rodents' ultrasonic vocalizations (USVs). Specifically, USVs emitted by pups separated from mother/nest serve as a useful tool to reveal reduced attachment with mother.

Using a neurodevelopmental model of schizophrenia, based on the prenatal injection of methylazoxymethanol acetate (MAM; 22 mg/kg; embryonic day 17), we assessed early communicative behavior by maternal-separation induced USVs in pups. To study the course of vocal development, pups were recorded at 3-time points: at 6, 9, and 12 postnatal day (PND).

The results show that MAM male and female pups vocalize comparable to control animals at 6 and 9 PND. However, at 12 PND, MAM rats produced fewer USVs of longer duration than control animals did. Call types distribution did not differ between treatment groups on all experimental days.

The present study demonstrates that MAM-exposed pups have impaired social communication (reduced number and extended duration of USVs).

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The authors declare that there is no conflict of interest.

Superior colliculus controls the activity of the substantia nigra pars compacta and ventral tegmental area in an asymmetrical manner

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Dopaminergic (DA) neurons of the substantia nigra pars compacta (SNc) and ventral tegmental area (VTA) control animals' orienting and approach toward relevant external stimuli. To optimise the choice of motor actions these neurons perform calculations based on the information from various sources. The information about the environmental stimuli is provided predominantly by the superior colliculus (SC),

a brain region processing sensory information from the contralateral body side. Since the direction of animal's movement is strongly dependent on the difference in dopamine release in the left and right striatum, we aimed to investigate the lateralisation of the connection between SC and midbrain DA system. To study the anatomical connections between the aforementioned brain regions we used viral--based transsynaptic tracing methods. To examine the physiology of this circuit we performed both *in vivo* single unit and *ex vivo* multielectrode array recordings combined with optogenetic stimulation of either ipsilateral or contralateral SC or SC terminals, respectively. DA neurons were identified optogenetically during *in vivo*, and pharmacologically during *ex vivo* recordings. Anatomical observations revealed that SC innervates predominantly ipsilateral DA system with the emphasis on SNc. *In vivo* recordings indicated a slight tendency towards prevalence of DA neurons' responses to ipsilateral SC stimulation, whereas *ex vivo* recordings showed clear ipsilateral lateralisation of the SC to SNc/VTA connection. Observed anatomical and physiological lateralisation within the studied brain circuit suggests its involvement in orienting and approach behaviours based on the direction of incoming sensory stimuli.

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Non-invasive vagus nerve stimulation enhances cognitive functions

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Vagus nerve stimulation (VNS) as a modern effective method of neuromodulation produces therapeutic effects for the treatment of neuralgia, psychiatric disorders, heart failure, and others. Relaxing effects (reducing anxiety, alleviating stress) also improve cognitive functioning. The aim of the current study was to reveal a causal relation between VNS and a related cognitive function. 6 right-handed male volunteers aged 18-22 years participated in this study. We used the combination of pleasant meditative classical music and a slow bi-polar wave (0.1-0.2 Hz) of electrical non-invasive transcutaneous auricular vagus nerve stimulation for 5 minutes. The set of 4 VNS was performed at intervals of 3 days. To determine the development of short-term memory we used 2 computer subtests. The results of the test indicate the improving effect of the set of VNS on short-term memory. The observed increase in the theta-Fz/alpha-Pz ratio reflects an enhancement of the activation level, amount of brain resources involved in processing the perceived information. A higher left frontal activity under cognitive test points to a positive emotional attitude towards the task. A focus of beta, gamma, and theta activities in the vertex (Cz) reflects the effect of VNS on learning at the level of the sensorimotor cortex. Activation of the right dorsolateral prefrontal cortex reflects the VNS effect on the attention processes. The observed

increase in the theta activity in the left occipital cortex indicates the involvement of the visual cortex. We may conclude that VNS has an activating effect on the processes of short-term memory, attention.

Key words: Vagus nerve stimulation, cognition, short-term memory, attention, EEG

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Condition of intramural nerve ganglia in experimental portal hypertension

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Portal hypertension is a condition that causes structural and functional changes in all organs and systems [1]. This requires careful study. The aim of the work is investigation of the intramural nerve ganglia two weeks after modeling experimental portal hypertension. The experimental study was performed on outbred dogs with all norms of bioethics. Simulation of portal hypertension was performed by means of a one-moment narrowing of the trunk of the liver portal vein [2, 3]. Histological, neurohistological, histochemical, histoenzymatic studies were performed after 14 days. Sections of the pulmonary circulation are characterized by reactive and destructive changes in the intramural nervous system. Neurons undergo changes in the form of edema, hyperargentophilia, chromatolysis, displacement of the nucleus. Nerve fibers mainly show signs of reactive changes: dyschromia, swollen, strongly tortuous. Receptors undergo structural changes in the form of even and uneven thickening along the fibers, hyperargentophilia, vacuolation in the preterminal departments. Content of RNA, the activity of acid phosphatase, AChEase and ATPase in the cell bodies of neurons decreases. Thus, after two weeks of portal hypertension modeling pronounced reactive and destructive changes develop in the intramural nervous system in the direction from the right ventricle to the left atrium.

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Artifact Cancellation for Same-Electrode Stimulation and Recording

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Stimulus artifacts restricts recording of the electrophysiological signals evoked by electrical stimulation. The artifacts often lead to saturation of the recording amplifier which prevents recording for several milliseconds following the stimulation pulse. The problem is even more challenging if the same microelectrode is meant to be used for both generation of the stimulation pulse and recording of the neuronal response. We have developed a new method for reduction of the stimulation artifact for same-electrode stimulation and recording configurations. The stimulation pulse is followed by precisely optimized correction signal, which cancels the artifact and enables recording of the neuronal response while the correction pulse is being generated. Based on measured impedance-vs-frequency curve for the electrode, the artifact after the end of the stimulation pulse is estimated and the optimal shape of the correction pulse is calculated. The correction pulse amplitude is dozens times lower than the amplitude of the stimulation pulse, therefore the neuronal response is not affected but the artifact is greatly reduced. The method requires realistic modelling of the electrode impedance [1] and dedicated electronics that generates the stimulation signals with high amplitude resolution and enables impedance spectroscopy [2,3]. According to numerical simulations the method allows for recording of the neuronal signals at the stimulating electrode as soon as 0.1ms after the stimulation pulse. Results of the experimental validation are currently under analysis and will be presented at the conference.

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Na+, K+-ATPase submembrane localization and activity reflects redistribution of gangliosides and cholesterol following thermally induced membrane reorganization

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Na^+/K^+-ATPase (NKA), an enzyme expressed by all mammalian cells, is a membrane protein and its positioning as well as different functions of active and inactive pools depend on interactions with neighboring membrane lipids. Ganglioside and cholesterol enriched lipid rafts (LR) are housing active, pumping pool, while the bulk membrane (non-LR) contains the non-pumping pool [1]. Aim of this study was to assess NKA activity and submembrane localization after multiple cycles of thermally induced membrane reorganization. Homogenates from mouse brain cortices were subjected to multiple freeze-thaw cycles. NKA activity was measured spectrophotometrically, followed by Western blot quantification of protein amount for each corresponding cycle. Submembrane localization of NKA was analyzed in time points with largest difference in activity after LR isolation utilizing ultracentrifugation in discontinous sucrose density gradients. Gangliosides from LR and non-LR fractions were purified by organic extraction, DEAE exchange chromatography, gel-filtration, and analyzed by high performance thin layered chromatography and immunoblot. Cholesterol concentration in submembrane fractions was determined spectrophotometrically. Our results show oscillations in NKA activity in different lipid environments, even though the total amount of NKA is constant through all cycles. Membrane subfractionation showed that NKA shifts within the membrane plane, with different cycles having distinct distribution of NKA between LR and non-LR fractions. Analysis of ganglioside composition and cholesterol content in membrane fractions isolated from murine cortical tissue reveals that particular lipid environment affects NKA catalytic activity and aids NKA positioning within the membrane enabling optimal function of the pump.

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The influence of genetically induced loss of noradrenergic neurons on the pro- and anti-inflammatory markers in the SN/VTA neurons of mice.

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Parkinson's disease (PD) is accompanied by immune response due to neurodegenerative process in the substantia nigra and ventral tegmental area (SN/VTA), but also locus coeruleus (LC) region. Pre-clinical data indicate that chemical lesion of LC exacerbates the dopaminergic cells loss in rodent PD models, while enhancement of noradrenergic transmission has beneficial influence on the dopaminergic cells survival.

The aim of our study was to investigate whether progressive degeneration of noradrenergic cells induced by loss of TIF-IA factor in LC has impact on the expression of inflammatory markers in the SN/VTA.

RT-PCR studies revealed a 2-fold increase in the expression of IL10 mRNA, but no changes in mRNA for IL1 β and IL6 in the mutant animals. On the other hand, immunoblotting revealed decrease by 32% in IL6 protein level, without significant changes in IL1 β or IL10. Semi-quantitative antibody array for 40 cytokines showed increased levels of pro-inflammatory cytokines and chemokines, like: GM-CSF, IFNγ, IL-13, I-TAC etc. On the other hand, increased levels of neuroprotective proteins TIMP1 and TIMP2 in mutant animals were found.

The degeneration of noradrenergic neurons in TIF-IA^{DbhCre} mice evokes weak inflammatory state in the SN/VTA, as shown by the increased level of proinflammatory cytokines. Moreover, increased expression of IL10 mRNA or increased level of TIMP1 and TIMP2 suggest neuroprotective process. More than 12 weeks of observation would be required to understand the influence of these cytokines on the survival of dopaminergic neurons in TIF-IA^{DbhCre} mice, however, it was impossible due to short lifespan of these animals.

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No conflict of interest.

Effect of antidepressants treatment on zinc and zinc homeostasis-regulating protein levels in the brain of rats subjected to olfactory bulbectomy and zinc deficiency animal model of depression

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Zinc (Zn) is an essential trance element. Preclinical and clinical data reported the key role of Zn in the pathology and treatment of depression. The aim of the study was to investigate the significance of proteins regulating Zn homeostasis and the Zn level in the rodent model of depression.
The removal of olfactory bulbs (OB) was performed as described previously. In control rats the bulbs were left intact. 7 days following surgery, rats were fed zinc deficient diet (3mg Zn/kg) or zinc adequate diet (50mg Zn/kg) for 3 weeks. Then, escitalopram (ESC), venlafaxine (VEN) 10 mg/kg, *i.p.* or combined ESC/VEN (inactive dose 1 mg/kg, *i.p.*) and Zn (5 mg/kg) treatment begun. Following 3 weeks of drug administration the behaviour of rats was examined. 24h after the test, rats were decapitated and prefrontal cortex (PFC) and hippocampus (Hp) was collected for Mass Spectrometry analysis and staining. Proteomic analysis showed changes in the zinc transporters level, which confirms the disturbed Zn homeostasis in the brain of rats subjected to the OB + ZnD model. On the other hand chronic administration of ESC, VEN and combined administration of ESC with Zn increased the level of intracellular and synaptic Zn in PFC of rats.
The present study suggests that alterations in Zn transport proteins may contribute to the pathophysiology of depression. One of the mechanisms of action of the antidepressant drugs is the regulation of Zn homeostasis in the brain.

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Effect of synchronization of firings of different motor unit types on the force variability in a model of the rat medial gastrocnemius muscle

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This study investigated the effects of synchronizing the firings within three types of motor units (MUs) (slow - S, fast resistant to fatigue – FR, and fast fatigable – FF) on the force production using a model

of the rat medial gastrocnemius based on the actual proportion and physiological properties of MUs and motoneurons innervating the muscle. The isometric muscle and MU forces were simulated predicting non-synchronized firing of a pool of 57 MUs (8 S, 23 FR, and 26 FF) to ascertain a maximum excitatory signal when all MUs were recruited. The mean firing frequency of each MU depended upon the twitch contraction time, whereas the recruitment order was determined according to increasing forces (the size principle). The synchronization of firings of individual MUs was simulated using four modes and inducing the synchronization of firings within three time windows (± 2, ± 4, and ± 6 ms) for four different combinations of MUs. The four scenarios of synchronization increased the values of the root-mean-square, range, and maximum force in correlation with the increase of the time window. Greater synchronization index values resulted in higher root-mean-square, range, and maximum of force outcomes for all MU types as well as for the whole muscle output; however the mean force remained nearly unchanged. The range of variability and the root-mean-square of forces were higher for fast MUs than for slow MUs; meanwhile, the relative values of these parameters were highest for slow MUs, indicating their important contribution to muscle tremor, especially during weak contractions.

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Effects of uroguanylin's signaling pathways on ischemic lesion size

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Stroke is one of the leading causes of mortality and disability in industrialized countries. Guanylate cyclase (GC) A activation has a neuroprotective effect after ischemic stroke [1] therefore the aim of this study is to determine if agonists of GC-C have similar effects. Uroguanylin (UGN) activates guanylate cyclase C (GC-C) and a Ca^{2+}-dependent signaling pathway [2]. In this study, middle cerebral artery occlusion (MCAO) was performed on wild type (WT), GC-C KO and UGN KO mice. Before and 24h after MCAO MR images were taken. 48h following MCAO brain slices were isolated and Ca^{2+} response to UGN stimulation was recorded. Immunohistochemical staining was performed with GC-C, NeuN, and GFAP antibodies. WT and UGN KO animals exhibit a stronger Ca^{2+} response to UGN stimulation in astrocytes of the ischemic penumbra in cerebral cortex but not in the unaffected hemisphere. This stronger activation is gone in GC-C KO animals which results in development of smaller ischemic lesions in GC-C KO mice compared to their WT littermates. Considering the fact that GC-C becomes expressed on penumbral astrocytes following ischemia, while in normoxic conditions it is expressed only in cortical neurons, effects of GC-C on intracellular Ca^{2+} concentration could be due to activation of cGMP-dependent Ca^{2+} channels in penumbral astrocytes [3]. Stronger activation of the Ca^{2+}-dependent

signaling pathway could lead to the development of larger ischemic lesions, possibly through upregulation of Na^+/H^+ exchanger, tissue acidification, and neuronal death.

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Methamphetamine and cocaine induce distinct behavioral responses in *Drosophila*

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Methamphetamine (METH) and cocaine (COC) are psychoactive substances with highly addictive potential. They activate reward system in the brain leading to increased drug-taking and cause motor-activating effects. These behavioral effects can be quantified in *Drosophila* by measuring two phenotypes: locomotor sensitization (LS) and self-administration (SA).

To test COC and METH induced behaviors we used FlyBong and FlyCafe, two high-throughput methods we developed in our lab. FlyBong measures changes in the locomotor activity after administration of volatilized psychostimulants (vCOC or vMETH), while FlyCafe precisely quantifies amount of self-administered psychostimulant. Our results show that both COC and METH induce LS and preferential consumption, although the temporal profiles differ significantly. Flies develop LS to the second administration of vCOC after 6-hour interval[1], and after 10-hour interval to vMETH[2]. Exposure to vCOC leads to sharp increase in locomotor activity within 5 minutes after administration, while locomotor activity of flies exposed to vMETH stays elevated for at least 10 min and shows slow decline, especially after the second administration. The temporal dynamics of rewarding effects of COC and METH are also distinct. Flies show high METH preference on the first day[2], while preference for COC is positive on the first day and continues to increase over subsequent days.

Our studies show that FlyBong and FlyCafe are versatile behavioral tests that precisely measure number of behavioral parameters induced by COC and METH exposure. The difference in temporal dynamics can be used for genetic dissection of underlying molecular mechanisms.

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Cortisol and autonomic responses to a novel stress test in virtual reality that adjusts challenge to individual's performance

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Laboratory stressors are an essential tool to study the human stress response. However, most do not adapt to individual abilities and require dedicated personnel. Virtual reality (VR) stress-induction tests usually come with the cost of lower stress responses. Here, we present a novel 10 minutes VR stress test that adapts to individual's performance and contains important elements of stress elicitation, such as social-evaluative threat and uncontrollability. Specifically, participants are simultaneously exposed to mental and environmental challenges, with intense visual and auditory stimulation, while having to rapidly respond to arithmetic calculations. Failure to respond accurately is penalized with negative feedback and aversive stimulation. We aimed at validating and characterizing the stress response to our test in one-hundred-and-eighteen participants, split into stress (N=57) and control (N=61) groups, similar in age (21.1±4.83), cognitive ability, trait and social anxiety. Besides cortisol, which was significantly elevated in the stress group, a total of 50 autonomic nervous system (ANS) variables were computed in 2.5 minutes blocks from each participant's electrocardiogram, electrodermal activity and respiration. In the two last blocks, more than 50% of all variables showed significant differences from the control group, in pairwise comparisons using independent samples t-tests corrected for multiple comparisons, consistent with known stress induced ANS activations. These were used to develop a machine learning model that successfully identifies stress blocks, producing a stress score associated with cortisol responses. In summary, our new VR stress test is a potent method to induce robust changes in endocrine and cardiovascular variables while mitigating known limitations.

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Visual involvement precedes motor dysfunction in experimental autoimmune encephalomyelitis: evidence from non-invasive *in vivo* electrophysiology and imaging

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Background and objective: Experimental autoimmune encephalomyelitis (EAE) is associated with abnormalities in motor (MEP) and visual (VEP) evoked potentials and neuroretinal thinning at optical coherence tomography (OCT), consistently with clinical symptoms observed in multiple sclerosis. Understanding the time course of these abnormalities is pivotal for the translational testing of novel therapeutic strategies.

Methods: We performed VEP, MEP and OCT in 30 C57BL/6 mice immunized with MOG 35-55 (*vs* 10 controls), at 7, 14 and 31 days post-immunization-dpi (10 mice sacrificed at each timepoint-dpi). We report electrophysiological and OCT data.

Results: Compared with controls, EAE mice had delayed VEPs at all consecutive time points (p=.00009, p=.014, p<.001, respectively; Student's t test) and reduced neuroretinal thickness at 7 (p=.003) and 31 dpi (p=.013). EAE MEPs did not significantly differ from controls at 7 and 14 dpi, while at 31 dpi, MEPs were delayed in 5 hindlimbs from 4 mice (1 bilateral, 3 unilateral) and absent in 10 from 6 mice (4 bilateral, 2 unilateral).

Abnormal VEPs were more frequent *vs* MEPs at 7 dpi (56.7% eyes *vs* 6,7% hindlimbs, p<.001, McNemar's test), at 14 dpi (35% *vs* 10%; p =.031), and at clinical onset (13, 15 ±0.95 dpi, 42,5% *vs* 10.5%, p=.004); with no significant group difference at 31 dpi (55.6% *vs* 83.3%; p=.227).

Conclusions: VEPs abnormalities appear before electrophysiological or clinical motor involvement, pointing to the relevance of electrophysiological measures to detect early, subclinical demyelination as a potential target of novel therapeutic approaches targeting inflammation, demyelination and neuroaxonal loss.

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Disclosure of interests: None

Two strategies of neural plasticity enhancement in aged mouse somatosensory cortex

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Brain aging is associated with progressive functional losses in perception, cognition, and memory, however, in many brain regions aging brain retains a considerable functional plasticity. The mechanisms governing functional fear-learning-induced plasticity in mice somatosensory cortex get weaker with age. Here we used two experimental strategies: environment enrichment and taurine supplementation, to enhance fear-learning-induced plasticity in aged mouse somatosensory cortex.

Aged C57BL/6 mice (1-year-old) were placed in an enriched environment or received 5% solution of taurine in drinking water for 6 weeks. Plasticity was induced using fear conditioning protocol with tactile conditional stimulus applied to the vibrissae and electric unconditional aversive stimulus applied to the tail. Plastic changes were visualized with 2-DG mapping of brain metabolic activity. The level of several amino acids, including taurine, glutamate, glutamine and GABA in somatosensory cortex was detected using HPLC.

Both strategies were effective in stimulating plasticity in aging somatosensory cortex since we observed an enlargement of conditioned vibrissae representation after short 3-days lasting conditioning protocol – the effect that cannot be observed in control aged mice. However, results of HPLC analysis revealed, that each approach used to stimulate plasticity acts via different mechanisms.

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The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Short-term fasting does not affect hypothalamic insulin expression in female rats

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We previously reported that six-hour fasting increased insulin expression in the hypothalamus of male rats [1]. Given the gender-related differences in response to metabolic stressors [2], the goal of this study was to examine whether short-term fasting also affects hypothalamic insulin expression in female rats.

The female rats in proestrus or diestrus were either exposed to the six-hour fasting or had *ad libitum* access to food. Proestrus and diestrus were chosen due to the highest and the lowest circulating levels of sex hormones, respectively. The insulin concentration in serum, cerebrospinal fluid, as well as the

hypothalamic protein isolates was determined using RIA. Quantitative PCR was performed on cDNA transcribed from the total hypothalamic RNA.

Following six hours of fasting, circulating insulin concentration was decreased. However, both the cerebrospinal fluid and hypothalamic insulin levels remained unchanged regardless of the estrus cycle phase. Moreover, hypothalamic insulin mRNA expression was unaffected by fasting in both examined phases of the estrus cycle.

The aforementioned results suggest that, contrary to the findings in male rats, fasting affects neither insulin mRNA expression nor insulin content in the hypothalamus of female rats in proestrus and diestrus.

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The Effect of Long-Term Supplementation with Aluminum or Selenium on Antioxidant Enzymes Activities

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In our previous studies, we evaluated the effect of acute exposure to aluminum (Al) on oxidative stress and the capacity of the antioxidant system in mouse organs [1, 2]. The aim of this study was investigated the effects of aluminum or selenium (Se) on the "primary" antioxidant defense system enzymes (superoxide dismutase (SOD), catalase (CAT), and glutathione reductase (GR)) in cells of mice brain and liver after long-term (8-week) exposure to drinking water supplemented with $AlCl_3$ (50 mg or 100 mg Al/L of drinking water) or Na_2SeO_3 (0.2 mg or 0.4 mg Se/L of drinking water). Results have shown that high-dose of Se increased the activities of SOD and CAT in mouse brain and liver. Exposure to low-dose of Se resulted in an increase of CAT activity in mouse brain, but did not show any statistically significant changes of SOD activity in both organs. Meanwhile, the administration of both doses of Al caused no changes in activities of these enzymes in mouse brain and liver. The greatest sensitivity to the effect of Al or Se was exhibited by GR. Exposure to both doses of Al or Se resulted in statistically significant increase of GR activity in both brain and liver. It was concluded that 8-week exposure to Se caused statistically significant increase of SOD, CAT and GR activities in mouse brain and/or liver, however,

these changes was dependent on the used dose. The exposure to both Al doses caused statistically significant increase only in GR activity of both organs.

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HBK-15, a 5-HT1A receptor ligand, which shows a fast antidepressant-like effect, preferentially activates ß-arrestin signaling

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Our previous experiments showed 1-[(2-chloro-6-methylphenoxy)ethoxyethyl]-4-(2-methoxyphenyl)piperazine hydrochloride (HBK-15) displayed antidepressant-like, and memory-enhancing properties in rodents. Here, we investigated the possible functional selectivity of HBK-15, a compound with a high affinity for 5-HT1A receptors, and the antidepressant-like activity of a single administration of HBK-15 in the mouse model of depression.

We used various cell-based functional assays to determine the intrinsic activity of HBK-15 at the 5-HT1A receptor. To determine the antidepressant-like activity of a single compound's administration, we used the unpredictable chronic mild stress with the forced swim test, sucrose preference test and locomotor activity test as behavioral endpoints. After the procedure, stressed mice were injected intraperitoneally with saline, HBK-15, fluoxetine, or ketamine. Next, we collected prefrontal cortices to determine the levels of BDNF, p-CREB, p-CaMKIV, p-PKA, and p-ERK1/2 using ELISA method.

The compound showed functional selectivity at the 5-HT1A receptor, i.e., it preferentially activated ß--arrestin signaling. HBK-15 presented antidepressant-like activity in a disease model – it reversed an increase in immobility and reduced preference for sucrose in the stressed mice. HBK-15 upregulated the decreased levels of BDNF p-CREB, p-CaMKIV, p-PKA, p-ERK1/2 in the prefrontal cortex of the stressed mice.

We found that a single administration of HBK-15 – a 5-HT1A biased agonist – reversed depression--like behaviors and regulated decreased BDNF and p-CREB levels in the prefrontal cortex in stressed mice. The transcription factor CREB was activated via several pathways, i.e., p-CaMKIV, p-PKA and

p-ERK1/2. Our results suggest that the activation of ß-arrestin signaling might accelerate the antidepressant response.

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The authors declare that there is no conflict of interest.

MiRNA in extracellular vesicles from the cerebrospinal fluid of suicide victims

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Suicide is a global public health problem with more than 800,000 deaths every year. With around 400 suicides every year, Slovenia ranks among the countries with the highest suicide rate (number of suicide victims/100,000 citizens) in Europe. Numerous factors affect suicide, and besides social and economic factors, it has been shown that biological factors also importantly influence suicidal behavior. Among biological factors, there is a special interest in the field of epigenetics. The term epigenetics includes several different ways of regulating gene expression: DNA methylation, posttranslational modifications of histone tails, and noncoding RNAs. Differences at the level of epigenetics are happening all the time and are reflecting external stimuli. Micro RNAs (miRNAs), the noncoding RNAs, are regulating gene transcript by complementary binding. Around 70 % of known mature miRNAs are transcribed in the central nervous system, where they regulate gene expression during neurogenesis and neuroplasticity. MiRNAs are especially enriched in exosomes (the smallest type of extracellular vesicles) which are secreted by most eukaryotic cells and found in body fluids. Exosomes can be found also in cerebrospinal fluid (CSF), which is in direct contact with the central nervous system. We are going to isolate miRNAs from exosomes that are isolated from CSF with ultracentrifugation. Selection of tested miRNA associated with suicide will be made with an algorithm made by our group, that includes data from five miRNA prediction databases. The best targets will be tested on samples of suicide victims and control group.

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In relation to this presentation, I declare that there are no conflicts of interest.

Apoptosis and Necrosis Mechanisms May Be Downregulated in Dementia with Lewy Bodies

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Dementia with Lewy bodies (DLB) is a progressive neurodegenerative disease characterized by changes in sleep, behavior, cognition, movement, and autonomic bodily functions. However, cellular mechanism of transformation into DLB is not accurately understood yet. The aim of this study was to detect possible pathophysiological factors in DLB on examining the expression levels of genes by using bioinformatics tools. For this purpose, GSE28094 dataset [1,2] downloaded from Gene Expression Omnibus (GEO) database was re-examined for this research. In the dataset, total DNA samples from brain tissues of the patients with DLB (n=13) and non-demented controls (n=6) are recruited. After the gene expression levels in the dataset were re-analysed in the R program, gene set enrichment analyses were performed in Gene Ontology (GO) and ENRICHR tools. Based on Benjamini-Hochberg correction, adjusted p-values <0.05 were accepted as significant. Gene expression levels indicated that caspase 8 and 10 (CASP8, CASP10), fas cell surface death receptor (FAS), BCL2 related protein A1 (BCL2A1), kirsten rat sarcoma viral oncogene (KRAS) [responsible for apoptosis]; tumor necrosis factor and related genes (TNF, TNFSF1A, TNFRSF8, TNFSF10, TNFRSF1B, TNFRSF10A, TNFRSF10C, TNFRSF10D), lymphotoxin-alpha (LTA), myosin light chain kinase (MYLK), interleukin 1B, 6 and 10 (IL1B, IL6, IL10), interferon gamma (IFNG) [responsible for necrosis] genes downregulated ($p<0.05$) in DLB group, compared with non-demented group. Results from this in silico analysis indicate down--regulation in the expression levels of genes known to be involved in apoptotic and necrotic process in the pathophysiological mechanisms of DLB.

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There is no conflict of interest to declare in this study.

NPAS3 aggregation, implicated in schizophrenia, is common in *post mortem* brain samples and can be induced by oxidative stress in neuroblastoma cells

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Chronic mental illnesses are complex conditions, that include various genetic and non-genetic aspects. Recent research suggests the existence of insoluble protein aggregates in the brains of patients. The onset of protein aggregation could be due to disruptions in protein homeostasis or incorrect protein folding, similar to neurodegenerative disorders, such as Alzheimer's and Parkinson's diseases [1]. One of the proteins implicated in schizophrenia is Neuronal PAS domain protein 3 (NPAS3). NPAS3, a member of the bHLH-PAS superfamily of transcription factors, is involved in the regulation of many processes, such as neurogenesis, metabolism, and circadian rhythms. NPAS3 has been associated with schizophrenia through genetics, including a familial translocation [2], and more recently a V304I mutation, which causes aggregation, was found in a family [3].

We performed a Western Blot analysis of *post mortem* brain samples obtained from the Human Brain Tissue Bank, Semmelweis University, Budapest. Additionally, we investigated overexpressed NPAS3 vectors in neuroblastoma cells, inducing after oxidative stress, induced by sodium arsenite treatment. Insoluble NPAS3 insolubility was seen in 74 % of brains examined, which is far more than can be explained by the V304I mutation, and suggesting other mechanisms to exist. In cell systems, NPAS3 aggregation did not depend on V304I mutation, with both wild-type and mutant NPAS3 showed cytoplasmic localization after stress inducement. Based on our research, it appears that NPAS3 aggregation in the brain is a more widespread phenomenon than first predicted. We are now investigating its relevance to clinical diagnosis.

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A disclosure of conflicts of interest.

Dissecting neuronal pathways underlying theta rhythmogenesis – evidence for cell-specific involvement of the nucleus incertus-medial septum axis

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Theta oscillations are a behaviour-specific brain rhythm that underlie learning and memory, spatial navigation, and anxiety-related behaviours. The neural connection between the nucleus incertus (NI) and the medial septum (MS) plays an important role in theta rhythm generation [1], but the precise electrophysiological and neurochemical characteristics of the NI neurons innervating MS are not known. In the rat brain, the NI is the primary source of relaxin-3 (RLN3), and a source of cholecystokinin (CCK), two neuropeptides involved in learning and memory-related processes. In light of the importance of the NI–MS connection in theta rhythm generation [2], we examined the nature of this neural loop. We confirmed that the connection between NI and MS is direct and monosynaptic, using combined optogenetic activation of NI neurons innervating the MS, with whole-cell, patch clamp electrophysiological recordings. We identified that NI neurons monosynaptically innervating MS belong to the distinct electrophysiological type of neurons (type I NI cells [3]). Moreover, in neural tract-tracing studies, we demonstrated that both RLN3 and CCK neurons innervate MS, and that neurons synthesising RLN3 and CCK belong to largely non-overlapping populations. Finally, we found that RLN3 and CCK cells are type I NI neurons. These studies have characterised the neurochemical and electrophysiological nature of distinct NI neuronal populations that innervate MS, and suggest their specific involvement in the control of hippocampal theta rhythm generation.

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The authors declare that there is no conflict of interest.

Disruption of the circadian clock in the Rat Dorsomedial Hypothalamus under high-fat diet

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Obesity and the impairment of circadian rhythmicity are causally related in a bidirectional fashion. Among the network of brain structures involved in the regulation of the circadian rhythms, the Dorsomedial Hypothalamus (DMH) is specifically associated with the rhythmicity of food intake and metabolism. In this study we aimed at exploring the changes in the rhythmical processes occurring in the DMH in rats fed high-fat diet (HFD). In order to investigate the possible causes, rather than results of obesity, we performed experiments after 3-4 weeks on a diet, before the HFD-fed rats became overweight. These included *in situ* hybridisation for *Per2* mRNA and Multi Electrode Array (MEA) extracellular recordings, performed both during the day and at night. On top of that, we also performed long-term (~30h) MEA recordings in order to pinpoint the time of DMH highest neuronal activity independently of incoming stimuli.

Our results revealed daily differences in both *Per2* expression and the frequency of action potential generation, with high values during the night and low during the day. However, this effect was only observed for rats fed control diet, whereas for the HFD-fed group this daily variation was lost. MEA long--term recordings showed the DMH neuronal activity to peak at night-time in both control and HFD-fed animals, but HFD delayed this rhythm by 3h compared to control.

These results present a dysregulation of circadian rhythms in the DMH during the development of obesity in a way of both blunting the day-to-night differences and evoking a phase delay in the electrical activity.

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Impact of biological sex in neonatal and juvenile behavior of mouse model of neurofibromatosis type 1: focus on male susceptibility to autism spectrum disorder

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Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by social- communication impairments and repetitive behavior. ASD displays a strong male bias with a ratio of approximately 4:1 male to female, yet the reason for this remains unclear. The diagnosis of autism is difficult to achieve before age 3 and is even more complex in females. Understanding severity of ASD symptoms between male and females is an important step to find new sex-targeted therapies.

Our work aimed to characterize differential behavioral impairments between male and female in a well-stablished animal model of ASD, neurofibromatosis type 1 (*Nf1*$^{+/-}$) mouse. Here, we performed a longitudinal study from perinatal period (postnatal day 4-14) to juvenile phase (postnatal day 30). Developmental milestones were performed during perinatal period, focusing on motor and sensory systems. We observed that *Nf1*$^{+/-}$ males were more susceptible to early developmental delay, specifically in tasks involving strength and coordination. Maternal-induced separation ultrasonic vocalizations (USVs) were also recorded between postnatal day 6 and 10 and we found that *Nf1*$^{+/-}$ males have a less complex vocal repertoire. Juvenile mice were subjected to a social empathy test (with an anesthetized animal) and transgenic females show less interest in stranger animal when compared to male *Nf1*$^{+/-}$.

Overall, our data demonstrated that *Nf1*$^{+/-}$ males show ASD symptoms early-on, whereas in *Nf1*$^{+/-}$ females they emerge further into their development, highlighting the importance of understanding sex-dependent impacts of ASD during brain development.

These findings could be important to defining sex-specific therapies in order to improve ASD core symptoms.

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The role of transient potential receptors in development of acute respiratory distress syndrome

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ARDS (acute respiratory distress syndrome) is a direct cause of death due to lung lesions of various origins including SARS-CoV-2 infection. Most lung and respiratory diseases are characterized by inflammation. Understanding the pathological processes involved in the regulation of the immune response may lead to the discovery of new mechanisms that support or suppress inflammatory processes in the lungs and respiratory tract. Most researches devoted the pathogenesis of this syndrome focus on immune mechanisms, vascular permeability disorders, hypoxia etc. Despite there is detailed information about the innervation of the airways the role of the nervous system in this pathology is not well studied [1]. It is known that nerve endings are a source of many biologically active substances that affect various lung cells. In particular, sensory neurons have been shown to suppress the immune response in bacterial infections [2].

Using experimental model of ARDS, which induced by precision pulmonary hyperventilation and intratracheal administration of poly I:C, which reproduces the body's response to viral infection (mimic viral infection), we estimated impact of TRPV channels in development of ARDS. Electrophysiological registration of vagal nerve potentiation shows a significant increase of spike frequency due to development of ARDS. Application of TRPV1 agonists provides modulation of vagal nerve activation and augmentation of its activity during ARDS induction. TRPV1 channels releases neuropeptides, in particular: calcitonin-gene-linked peptide (CGRP), substance P and somatostatin, which regulate inflammation through the NF-kB protein complex that controls DNA transcription. We show that activation of TRPV1 activity leads to additional anti-inflammatory effect.

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Depression, Stress, and Anxiety Among Medical Students During Online Learning in COVID-19 Pandemic

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Background. The COVID-19 pandemic has produced new challenges for humanity. Medical students are being negatively affected by this issue [1].

Objective. To examine the level of depression, stress, and anxiety in medical students during online learning in COVID-19 pandemic.

Methods. In this cross-sectional study the main dependent variables were levels of depression, stress, and anxiety, measured using the Depression, Anxiety, and Stress Scale (DASS-21) questionnaire [2]. The DASS-21 and sociodemographic questionnaires were distributed online to medical students (MD1--MD5 semesters) with one cut-off point (May 2021). Nonparametric tests were used for data analysis.

Results. A total of 45.6% of the students completed the surveys. The prevalence of depression was 55% (25% of students with mild (DASS-21 score 10-13), 20% - moderate (DASS-21 score 14-20), 10% - extremely severe (DASS-21 score 28+) depression). The prevalence of stress was 45% (5% - mild (DASS-21 score 15-18), 25% - moderate (DASS-21 score 19-25), 10% - severe (DASS-21 score 26-33), 5% - extremely severe (DASS-21 score 34+) stress). The prevalence of anxiety was 55% (15% - mild (DASS-21 score 8-9), 25% - moderate (DASS-21 score 10-14), 10% - severe (DASS-21 score 15-19), 5% - extremely severe (DASS-21 score 20+) anxiety). The determinants of depression and anxiety included individual, social, economic, and environmental factors. The determinants of stress were limited to individual factors.

Conclusion. COVID-19 pandemic had a negative influence on the level of depression, stress, and anxiety in medical students. Implementation of effective strategies to support students' educational, mental, and professional well-being is recommended.

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Assessing the salience of the subject's own name by default EEG reactivity

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The brain is constantly exposed to a plethora of stimuli arriving through multiple sensory channels. At any given time, some stimuli capture attention and "pop-out" from other stimuli. We recently introduced a method and apparatus to compare the attentional impact (salience) of complex sensory stimuli (EP3646784B1) by measuring the reactive changes in the electrical activity of the brain recorded by electroencephalography (EEG). Specifically, we proposed to measure the extent to which a stimulation protocol suppresses the resting network activity by calculating the default EEG reactivity (DER), numerically ranging from 0 (no reactivity) to 100%. It remained unclear the extent to which DER reflects conscious or unconscious attention mechanisms. Here we measured in a group of 13 healthy control adult volunteers the DER to a very salient auditory stimulus, the subjects own name (SON) by comparison with the name in reverse (rSON), which carries an equivalent acoustic energy. To minimize the emotional dimension of the stimulus, we focused on SON stimuli generated by a voice synthesizer. We found that the DER was larger for SON than for rSON. The difference in DER, referred to as the salient EEG reactivity (SER) was 10.47 ± 2.6 % (Mean ± SEM). In contrast, SER was abolished to 0.56 ± 1.8% in a group of 14 post-stroke comatose patients. These data suggest that DER reflects, at least in part, conscious processes.

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Relationship between STIM proteins and NMDA receptor and their influence on the process of NMDA receptor endocytosis

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STIM proteins (STIM1 and STIM2) are calcium (Ca^{2+}) sensors localized in the endoplasmic reticulum (ER). They participate in Store-Operated Ca^{2+} Entry (SOCE), which is described as Ca^{2+} influx from the extracellular milieu into the cytoplasm in response to the ER Ca^{2+} depletion. Although the main partner of STIMs is Orai, we showed that under physiological conditions STIMs can also interact with NMDAR2 [1]. Functional NMDARs are tetramers assembled from two obligatory GluN1 subunits and two GluN2 or GluN3 subunits. While physiological activation of NMDAR is essential in neuronal plasticity, learning and memory, its overstimulation and subsequent endocytosis contribute to neurodegeneration in acute neuropathologies (cerebral ischemia, traumatic brain injury) and chronic neurodegenerative diseases (Alzheimer's disease, Parkinson's disease) [2]. Therefore, we decided to investigate NMDAR endocytosis after excessive NMDA and glycine stimulation and the impact of STIMs on this process in rat cortical neurons *in vitro*. Using subcellular fractionation and biotinylation assays, we demonstrate that NMDAR endocytosis results in decreased immunoreactivity of the GluN1, GluN2B and STIMs in the plasma membrane. Co-immunoprecipitation studies show that there are no interactions between STIMs and GluN1, however STIMs associate with GluN2B and these interactions are increased after NMDAR endocytosis. Currently, we are knocking down STIMs using shRNA and lentiviruses to determine the effect of STIM1 and STIM2 on NMDAR endocytosis. Our preliminary data show that the knockdown of STIMs results in decreased immunoreactivity of pCaMKII and PSD-95. Further investigation of the STIM-NMDAR interactions under excitotoxic conditions may help to understand basic processes underlying neurodegenerative diseases.

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Role of the perirhinal cortex and postrhinal cortex in visuospatial information encoding

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The perirhinal cortex (PRC) plays a role in object recognition memory and the postrhinal cortex (POR) is involved in the integration of information for spatial navigation [1]. Both structures are directly and indirectly connected with the hippocampus, thus they can transfer information from cortical and subcortical regions to the hippocampus [2]. The exploration of novel spatial cues with distinct dimensions and functional context triggers subfield-specific neuronal encoding in the hippocampus [3], but it is still unclear how these aspects of items in space are encoded in the POR and PRC. We assessed the immediate early gene Arc as biomarker to examine the effects of item-place learning along the rostro-caudal axis of the PRC and POR. Using fluorescence in situ hybridization, analysis of layer II of the POR and of area 35 and 36 of the PRC was conducted. We observed that the exposure to a constellation of large, landmark cues elevated somatic Arc mRNA expression only in the caudal part of the PRC area 35 and POR, suggesting that this type of learning is processed in specific compartments of PRC and POR. By contrast, exploration of small cues had no effect on Arc mRNA expression in layer II of each of the analysed compartments. We therefore conclude that item-place encoding in the PRC and POR is influenced by the dimensions and representation of items in space.

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Conflicts of interest: none.

N2 and P3 components during auditory equiprobable Go/NoGo task in Problematic Internet Use in a sample of non-clinical Internet users

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Impairments of response inhibition are one of the key characteristics of addiction, including alcoholism, gaming, and Problematic Internet Use (PIU). However, PIU is highly comorbid with mental disorders, like depression or ADHD. Little is known about response inhibition in non-clinical Problematic internet users. We aimed to evaluate the response inhibition reflected by N2 and P3 components during Go/NoGo task in a sample of non-clinical Internet users. 58 young adults (25.14 ± 0.55 years, 28 male) were enrolled. Subjects were divided into two groups based on the results of short version of Problematic Internet Use Questionnaire (PIUQ-9). A set of tests was used to monitor the presence of comorbidities: Barratt Impulsiveness Scale (BIS-II), Beck Depression Inventory (BDI-II), Beck Anxiety Inventory (BAI) and Clark-Beck Obsessive-Compulsive Inventory (CBOCI). EEG data were recorded during the auditory equiprobable Go/NoGo task and three electrodes (Fz, Cz, Pz) were selected for further investigation. PIU group demonstrated higher scores on Impulsiveness Scale and Obsessive-Compulsive Inventory. PIUQ-9 scores correlated with impulsivity, and obsessions-compulsions. PIU had higher P3 amplitudes observed during both Go and NoGo, and lower N2 in NoGo.
Problematic Internet Use was associated with impulsivity and obsessions-compulsions. PIU group showed decreased N2 in NoGo and increased P3 amplitudes in both Go and NoGo.

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Hidrox® roles in neuroprotection: biochemical links between traumatic brain injury and Alzheimer's disease

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Traumatic brain injuries (TBI) are a serious public-health problem. Furthermore, subsequent TBI events can compromise these patients' quality of life. TBI is linked to a number of long and short-term complications like cerebral atrophy, risk of developing dementia and Alzheimer Disease (AD). Following the direct TBI damage the oxidative stress and inflammatory response lead to tissue injury neurodegenerative process that are characteristic of TBI-induced secondary damage. Hidrox® showed positive

effects in preclinical models of toxic oxidative stress and neuroinflammation, thus the aim of this study was to evaluate the effect of Hidrox® administration on TBI-induced secondary injury and the propagation of the AD-like neuropathology. Hidrox® treatment reduced histological damage after controlled cortical impact. Form the molec-ular point of view hydroxytyrosol is able to preserve the cellular redox balance and protein homeostasis by activating the Nrf2 pathway and increasing the expression phase II detoxifying en-zymes such as HO-1, SOD, Catalase and GSH, counteracting the neurodegenerative damage. Additionally, Hidrox® showed anti-inflammatory effects by reducing the activation of the NFkB pathway and the related cytokines overexpression. From the behavioral point of view Hidrox® treatment ameliorated cognitive dysfunction and memory impairment TBI-induced. Addition-ally, Hidrox® showed a significant increased number of hippocampal neurons in CA3 region which were reduced post-TBI. In particular, Hidrox® decrease AD-like phenotypic markers as ß-amyloid accumulation and APP and p-Tau overexpression. These findings indicate that Hidrox® would be a valuable treatment for TBI-induced secondary injury and AD-like pathological features.

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Electrophysiological characteristics of neurons putatively involved in the generation of the posterior hypothalamic theta rhythm.

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Theta oscillations of neuronal activity are thought to contribute to a number of cognitive and behavioral functions of the central nervous system, including, but not limited to, memory consolidation, sleep and exploratory activity. Apart from the known cortical and hippocampal sources of theta oscillations, several other subcortical brain regions are known to generate this type of rhythmic field activity. The posterior hypothalamic nuclei (PH) and the supramammilary nucleus (SuM) have recently been demonstrated to exhibit theta oscillations both *in vivo* and *ex vivo* in brain slices. In order to pinpoint the cellular correlates of PH and SuM theta, we performed whole-cell current clamp recordings to determine the intrinsic membrane properties of these neuronal populations. In addition, neurons were filled with biocytin which allowed to recover their morphology. It was found that SuM neurons had a more depolarized resting membrane potential compared to their PH counterparts, with other passive membrane properties such as input resistance and membrane capacitance largely overlapping between the two cell groups. Both regions contained spontaneously firing as well as quiescent cells. Notably, a small proportion of neurons in either region were found to exhibit subthreshold membrane potential oscillations (MPOs) in the

theta (3-12 Hz) frequency range, consistent with a phasic theta-on cell phenotype present in *in vivo* recordings. Taken together, these findings help explain the cellular mechanisms involved in theta rhythm generation in the posterior hypothalamus.

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Comparison Study of Taichi and Therapeutic Training Program on Cognitive Function and Life Satisfaction in Elderly Women

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This study aimed to compare the efficacy of Taichi with the balance and torso muscle training program from the aspect of cognitive function and life satisfaction. 39 women (age=71.8±5, BMI=27.5±3.5) were randomly divided into Taichi group and the balance and torso muscle training program (BTTP) group for 8 weeks training (2 times/week, 40 min/time). The evaluation tools included Six-item Cognitive Impairment Test (6-CIT), Cognitive Failures Questionnaire (CFQ), and Satisfaction with Life Scale (SWLS). Data analysis was based on Wilcoxon Signed-rank Test and Mann-Whitney U test in SPSS. The scores of 6-CIT reduced significantly in both Taichi group ($z=-2.8$, $p<0.05$) and BTTP group ($z=-2.4$, $p<0.05$) without a significant difference between groups. The scores of CFQ decreased significantly in Taichi group only ($z=-3.2$, $p<0.01$), and the decrease was significant comparing to BTTP group ($z=-2.2$, $p<0.05$). The scores of SWLS increased significantly only in Taichi group ($z=-2.3$, $p<0.05$), but the increase was not significant comparing to BTTP group. Both Taichi and BTTP improved participants' cognitive function on the cognitive impairment test, while Taichi group had larger improvement on the cognitive failures test. Taichi also improved participants' life satisfaction, but the influence was not significantly different from that of BTTP.

Authors have no conflict of interest to declare.

A degradation of words and pseudowords affects differently the reading performance in ASD and DD

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Understanding the factors that determine reading failure is important for successful identification of deficits that may vary in different developmental disorders. A condition of Developmental Dyslexia (DD) is most often associated with reading difficulties. Although the core deficit of Autism Spectrum Disorder (ASD) is in social domains, many children with ASD have diminished reading skills. The aim of our study was to evaluate the effect of visual factors on the reading performance in both disorders in comparison to typical development (TD). To this aim the reading speed and proportion of errors were compared in children and adolescents (8 – 16 years old) with DD, ASD or TD. In order to study the visual noise effect, test samples were degraded using positional noise produced by random Gaussian displacement of letter position below or above the horizontal line. The results obtained showed that the external visual noise affected reading of all groups of participants in a different way, with the strongest effect on the ASD group. A comparison between reading words and pseudowords at different noise levels suggests possibility for a transition between using lexical and sublexical pathways at high noise level. The relationship between the proportion of correctly read words and pseudowords and the reading time implies a strong link between the reading rate and accuracy, which is different for the groups with different development. Possible sources of external noise effect could be connected to the compromised ability to filter external visual noise, potentially increased neural variability, and crowding.

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The effect of the second generation of antipsychotic risperidone on aggressive behaviour in male CD-1 mice

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Aggression is a serious medical problem associated with several psychiatric disorders, causing impairment of skills that are important for social, academic success and quality of life [1]. Current treatments which target aggressive behaviour can induce sedation, affect mobility and induce idleness. In this study we aim to assess the effect of risperidone; an atypical antipsychotic used to treat schizophrenia [2] acting at several 5-HT (serotonin) receptor subtypes. Briefly, CD-1 male mice underwent 3 days of aggression screening by introducing C57BL/6J intruder to their home cage for a maximum of 180s and latency to attack was measured. Following, CD-1 aggression phenotype was evaluated, leading on to selecting only the most aggressive subjects. The CD-1 mice were then treated with vehicle or risperidone (0.05, 0.5 and 3 mg/kg *i.p.*). Thirty minutes post the injection mice underwent a single aggression screen followed by locomotory assessment in the open field. Both, high (3 mg/kg) and medium (0.5 mg/kg) doses of risperidone significantly decreased further attacks ($p<0.0001$), however at the same time, both doses significantly affected locomotor activity ($p<0.0001$). The low (0.05 mg/kg) dose of risperidone resulted in a significant reduction ($p<0.005$) of aggressive behaviour as measured by latency to attack and 70% of CD-1 mice did not initiate an attack post-administration. Taken together, this study successfully demonstrates the aggression inhibiting activity of the 0.05 mg/kg of risperidone in the CD-1 mice aggression screening paradigm. Thus, the 5-HT1A receptor may represent a therapeutic target for aggressive behaviour.

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Antagomirs given i.v. change the phenotype of C57BL/6J mice from stress-susceptible to stress-resilience

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In our previous study we have shown that C57BL/6J mice subjected to restraint stress (RS) display longer immobility time in the following forced swim test (FST), in contrast to other studied genotypes, SWR/J and NET-KO mice, which were RS-resilient. Further analyses indicated, that RS-resilient geno-

types were characterized by higher serum level of miR-1 and miR-155, which are involved in regulation of BDNF expression, and can be regarded as biomarkers of stress-resilience [1].
In the present study we show, that intraventricular (i.v) administration of the antagomirs directed against these miRNAs can alter the behavior of C57BL/6J mice in the FST. Additionally, the expression of miR-1 in the serum and cerebellum was pre-determined.
The Miracle mmu-miR-1a-3p and mmu-miR-155-5p antagomirs were administered i.v. (0.4 nmol), separately and jointly, to the C57BL/6J mice. The next day, the FST was carried out. Additionally, the cerebellum and blood were collected. The miRNAs were obtained from the serum and tissue and RT-qPCR reactions with TaqMan MicroRNA Assays were performed.
The obtained results indicated that i.v. administration of the studied antagomirs shortened immobility time in C57BL/6J mice subjected to FST, what indicates change from stress-susceptible to stress--resilient phenotype. Additionally, we confirmed the decrease of miR-1a expression after corresponding antagomir administration, both in the serum and in the cerebellum.

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The effect of a human mutant tau protein on intra-neuronal signaling in layer III frontal pyramidal neurons

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Transgenic rTg4510 mice express high levels of human tau protein with the P301L mutation that has been associated with frontotemporal dementia. By 9 months of age, these mice show pathological alteration similar to those in human tauopathies, including presence of hyperphosphorylated tau and neurofibrillary tangles in brain tissue, atrophy and loss of neurons and synapses, hyperexcitability of neurons as well as cognitive deficiencies. We investigated effects of the mutant tau protein on neuronal membrane, subthreshold dendritic signaling and synaptic input pattern recognition in layer III frontal pyramidal neurons of 9-month-old rTg4510 (TG) mice. These features were compared to characteristics of pyramidal neurons from age-matched, wild-type (WT) mice. Compartmental cable models of WT and TG neurons were created in the NEURON simulator by using 3D reconstructed morphology and electrophysiological data of these cells. Our computer simulations predict specific membrane resistance and capacitance to be unaffected by the mutant tau protein. Computer models of TG neurons showed

only modest alterations in somatopetal voltage- and current transfers along dendrites relative to WT control. In contrast, a consistent and statistically significant slow-down was detected in the speed of dendritic signal propagation in all regions of the dendritic surface of mutant neurons. Synaptic input pattern recognition was predicted to remain unaltered in TG neurons. This suggests that tau-pathology is primarily associated with failures/loss in synaptic connections rather than with altered intra-neuronal synaptic integration within neurons of affected networks. Overall morphofunctional comparison of these TG and WT neurons is presented in an accompanying poster.

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OB-induced oxidative stress and inflammation in the frontal cortex and hippocampus of rats are associated with changes in NMDA and AMPA receptors trafficking

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Numerous studies have implicated oxidative stress and (neuro)inflammation in the pathophysiology of depression. The links between these processes and glutamatergic system dysfunction are strong[1]. The receptor trafficking is the key elements in the control of neuronal activity[2].

In this study we first determined the levels of oxidative stress markers (TBARS and carbonylated proteins-CP) and Interleukin-1α/β in the frontal cortex (FCx) and hippocampus (Hp) of rats subjected to the olfactory bulbectomy (OB; model of depression;according to[3]) procedure. Next, we measured and compared the levels of NMDA and AMPA receptor subunits (GluN1,GluN2A,GluN2B and GluA1,GluA2, respectively) in whole tissue lysates (WTL) and synaptic fraction (S).

Our results showed an increased levels of TBARS in FCx (↑47%) and CP in Hp (↑56%) of OB rats *vs*. control group (Sham). Moreover, an increased levels of IL-1α(↑24%) in FCx and IL-1β in both brain structures (HP:↑62%;FCx:↑77%) of OB group were observed. We did not reveal any major changes in NMDA (except GluN2A:↑33% in FCx) and AMPA (except GluA2 in FCx:↑56% and HP:43↑) receptor subunit levels in WTL of OB rats. Simultaneously, significant increase in the levels of these proteins in S was noticed. Importantly, this effect was stronger in FCx (GluN1:↑116%;GluN2A:↑30%;GluN2B:↑81%; GluA1:↑49%;GluA2:83↑%) than in HP (GluN2B:↑92%) OB group.

Obtained finding confirm the role of oxidative stress and neuroinflamation in depression, which may be consequence of changes in the ionotropic glutamate receptors distribution (not expression) in the brain. FCx appears to be more sensitive (than HP) to OB-induced alterations in the function and signaling of NMDA/AMPA receptor complexes.

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Investigation of systemic amyloid deposition in the absence of endogenous PACAP and its receptor

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Introduction: PACAP (pituitary adenylate cyclase activating polypeptide) is a neuropeptide expressed in many organs that has been shown to have general cytoprotective, anti-inflammatory, and antiapoptotic effects. However, we only have little data on its role in the aging process. Our aim: In our previous experiments, we observed accelerated systemic senile amyloid deposition in PACAP KO mice. The aim of the present experiment was to investigate the effect of partial PACAP deficiency in PACAP heterozygous (HZ) mice and PACAP receptor inefficiency in PAC1 receptor KO animals on amyloid deposits.

Methods: In our experiment, we sampled more than 20 organs from PACAP HZ (n = 4) 12-18 months and 1-year-old PAC1 receptor wild (n = 9) and KO (n = 2) mice. Haematoxylin-eosin and Congo red staining were used to examine the amyloid deposits. The amyloid content of the organs was rated on a scale of 0 to 3 according to severity.

Results: In our histopathological analysis, the same or more severe deposits were observed in HZ mice with partial PACAP deficiency as in PACAP KO mice. The organs most severely affected were the kidneys, spleen, liver, skin, thyroid, trachea, esophagus, and intestines. No signs of amyloid deposits were found in any of the PAC1 receptor WT and KO mice.

Conclusions: Based on our results, we can say that systemic amyloidosis develops not only due to the complete but also partial absence of PACAP, in contrast, the lack of its receptor does not lead to pathological protein deposits.

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Repetitive transcranial magnetic stimulation improves cognitive dysfunction in trimethyltin-induced hippocampal neurodegeneration

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Repetitive transcranial magnetic stimulation (rTMS) is a non-invasive stimulation protocol that modulates excitability and activity of the stimulated brain area through the delivery of magnetic pulses with a predefined administration pattern. Studies have shown that rTMS induces clinical outcomes in neurological disorders, including Alzheimer's disease, and significant improvement of motor and cognitive functions in healthy subjects. Our study aimed to evaluate the effect of intermittent theta-burst stimulation (iTBS) on trimethyltin-induced hippocampal neurodegeneration. Trimethyltin (TMT) is a potent neurotoxin targeting the limbic lobe, particularly the hippocampus, causing cognitive impairment developing over the 3 weeks after intoxication. Two-month-old male Wistar rats were subjected to a single dose of TMT (8 mg/kg) and randomly divided into three experimental groups: the group receiving no stimulation protocol (TMT), the group subjected to iTBS two times per day for 21 days (TMT+iTBS) and the group receiving noise stimulation (sham). One group of naïve rats was used as intact control. Three weeks after intoxication, all animals were evaluated with an open field and novel object recognition test for

exploratory behavior and recognition memory, respectively. In contrast to TMT animals, which exhibited increased ambulation score with reduced activity in the central area ($p < 0.05$) and reduced recognition index in comparison to intact controls ($p < 0.05$), TMT+iTBS animals showed a surprisingly significant improvement in all examined parameters of both tests, reverting them to control levels ($p < 0.05$). Thus, we concluded that iTBS produces beneficial effects on cognition and behavior in rats with TMT-induced hippocampal neurodegeneration.

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Plasma membrane Ca2+ ATPase expression and activity in Toll-like receptor 2 deficient mice

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Toll-like receptor 2 (TLR2) deficient mice model is widely used for investigation of microglial role in regulating inflammatory response following ischemic brain lesion (1). Additional functions and broader cell-type expression of TLRs have been evidenced in brain tissue which makes TLRs knock-out mice (such as TLR2-KO) a promising model for investigating neuron-microglia interactions, and microglial regulation of plasticity and excitability (2). Plasma membrane Ca^{2+} ATPases (PMCAs) participate significantly in ion homeostasis maintenance and signal transduction in brain tissue, thus we aimed to examine PMCAs protein expression and catalytic activity across brain regions in TLR2-KO mice. Results acquired by Western blot analysis and spectrophotometric enzyme activity measurements exhibit strikingly different protein expression and enzyme activity in brain tissue samples derived from TLR2-KO when compared with wild-type (WT) mice. Observed abundance of PMCA expression across cortex, hippocampus and cerebellum suggests that ubiquitous isoforms, PMCA 1 and 4 are less affected by TLR2 deficiency than neuron specific isoforms, PMCA 2 and 3. Notably, higher expression of PMCA2 in hippocampus and lower in cerebellum of TLR2-KO coincide with catalytic PMCA activity variations, higher activity is detected in hippocampus and lower in cerebellum of TLR2-KO compared with WT controls. Here collected evidence suggests that TLR2 deficiency is accompanied by a disturbance of

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Zinc deficiency-induced depression-like behavior in rats is associated with changes in the synaptic distribution of AMPA/NMDA receptor complexes in the frontal cortex.

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Zinc is essential for the proper functioning of the brain and its deficit can lead to the development of various diseases, including depression [1]. The receptor movement within the neurons (receptor trafficking) is one of the key element in the control of neuronal activity. Zinc, by binding to various synaptic proteins (e.g. Shank2), may affect synaptic location, mobility and trans-synaptic signaling of ionotropic glutamate receptors [3].

In the presented study, we used two groups of Sprague-Dawley rats: fed a zinc-adequate (ZnA) diet of 50 mg Zn/kg or a zinc-deficient (ZnD) diet of 3 mg Zn/kg, for 4 weeks. After behavioral verification, we measured and compared the levels of NMDA and AMPA receptor subunits (GluN1,GluN2A,GluN2B and GluA1,GluA2, respectively) in the whole tissue lysates (WTL) and synaptic fraction (S) of the frontal cortex using commercially available ELISA kits.

Our results indicate that ZnD induces depression-like behavior, assessed by a decrease (*vs*. ZnA) in the total movement distance (↓21%;$p<0.0001$) and total rearing number (↓42%;$p<0.0002$) in the open field test. Biochemical analyzes did not reveal statistically significant changes (ZnD *vs*. ZnA) in the level of tested proteins in WTL, which is in line with our previous report [3]. At the same time, however, a significant alterations in the GluN2A (↑30%;$p<0.036$), GluN2B (↑78%;$p<0.002$), GluA1 (↑33%;$p<0.037$) and GluA2 (↑73%;$p<0.017$) levels in S of ZnD (*vs*. ZnA) group was observed.

The obtained findings indicate that ZnD-induced behavioral alterations may be a consequence of a changed synaptic location (not expression) of NMDA/AMPA receptor complexes in the frontal cortex.

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Minocycline affects peripheral blood lymphocyte numbers in the streptozotocin-induced model of Alzheimer's disease in rats

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Minocycline (MINO) has an anti-inflammatory effect in neurodegenerative diseases [1,2]. Here, we investigated effects of (MINO) at a dose of 35 mg/kg b.w., administered intraperitoneally (*i.p.*) for 7 consecutive days, on peripheral lymphocytes in rats with a streptozotocin (STZ)-induced model of sporadic form of Alzheimer's disease (sAD). Thirty male Wistar rats were divide into four groups and administered intracerebroventricularly (i.c.v.) with STZ or vehicle (VEH) and MINO or saline (SAL) (*i.p.*). Flow cytometry was used to identify percentages of peripheral blood T ($CD3^+$), B ($CD45RA^+$), NK ($CD161a^+$) lymphocytes and $TCD4^+$ and $TCD8^+$ lymphocyte subsets, according to the method previously described [3]. Then, absolute lymphocyte numbers were calculated basing on the absolute numbers of leukocytes. Data is presented as mean total number (No./μ) of cells ± SD. Significantly ($p<0.05$) increased T and NK lymphocytes in the STZMINO (T:1802.43 ± 242.15; NK: 155.89 ± 32.19) and VEHMINO (T:1873.83 ± 178.26; NK:159.16 ± 31.39) were observed as compared to the respective controls (T lymphocytes: 1343.13 ± 109.43, VEH: 1475.77 ± 91.99 and NK cells: 100.29 ± 10.17, VEH: 51.58 ± 16.51, for the STZ and VEH group, respectively). In the STZMINO rats, the higher $TCD4^+$ and lower $TCD8^+$ lymphocyte number ($TCD4^+$: 1879.78 ± 269.66, $TCD8^+$: 259.93 ± 22.60, $p<0.05$) rather than VEHMINO (TCD4+: 1273.31 ± 289.31, $TCD8^+$: 525.15 ± 96.38) group were noticed. Moreover, increased $TCD4^+$ and $TCD8^+$ lymphocyte numbers ($p<0.05$) after MINO injections were noticed. The results indicate that minocycline changes peripheral blood lymphocyte distribution, including $TCD4^+/TCD8^+$cell ratio, in the sAD model.

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The authors declare no conflicts of intrests.

Long-term multimodal recording with a transparent, thiolene-acrylate based electrode array

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Multimodal neuroimaging approaches are advantageous to map brain functionalities with high spatial and temporal resolution. To achieve long-term stability of the electrode array, softening material-based devices are beneficial. Our study represents a soft, thiolene-acrylate based transparent microECoG device that allows cortical signal recording and two-photon imaging of Ca^{2+} signals. The used material combination and the detailed micromachining scheme of the electrode array were previously presented [1] and the multimodal measurement's experimental design [2].

Electrophysiological measurements were capture from head-fixed mice once a week for 10 weeks. Ca^{2+} signal imaging was repeated after 9 weeks, in the case of another animal, this time was 8 and further 13 weeks. We calculated the signal-noise-ratio (SNR) of the electrode recording sites and the average relative intensity change of the Ca^{2+} signals from these long-term measurements. The signals from the two modalities were processed separately by a custom-made MATLAB-based program. After 10 weeks the SNR remains acceptable for signal measurement and we were able to record Ca^{2+} signals also after 13 weeks. These results suggest that our device is suitable for long-term multimodal recordings.

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Adaptive changes of NMDA receptor subunits after chronic administration of antidepressant drugs in rat brain structures

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Major depressive disorder (MDD) is the most common mental disorder in humans. Studies in this field focus on the development of fast-acting drugs with fewer side effects. Ketamine, ionotropic glutamatergic NMDA receptor antagonist, causes rapid antidepressant effect. The NMDA receptor subunits expression and composition determine the physiological and pathophysiological neuronal processes[1]. Those findings promoted research of cellular mechanisms underlying this phenomenon.

Brain structures that have previously been linked with MDD are located in the prefrontal cortex (PFC) and hippocampus (Hp). New neuroimaging studies suggest the role of the cerebellum in the pathophysiology of the disease[2].

Our experiment evaluated molecular adaptive changes in PFC, hippocampus and cerebellum, after chronic administration of antidepressant drugs. Imipramine, reboxetine and escitalopram were selected. Saline was used as the control group. Drugs were injected to rats (n=8/group) intraperitoneally for 21 days. Brain structures were isolated and protein expression was determined using Western blot analysis.

Adaptive changes were observed in all investigated structures. In rats administered with imipramine and reboxetine, a decrease in GluN2A expression in the hippocampus and its increase in the cerebral cortex were observed. The latter effect was confirmed in rats under the influence of escitalopram. All the investigated drugs reduced the expression of the GluN2A subunit in the cerebellum. An increase of the GluN2B subunit was observed in PFC and Hp after administration of imipramine, as well as in the PFC and cerebellum of animals that received reboxetine. Chronic injections of escitalopram caused an elevated expression of GluN2B protein exclusively in the cerebellum.

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Mesenchymal stem cells conditioned media obtained at different conditioning times affected astrocyte migration in scratch model of spinal cord injury

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Mesenchymal stem cells (MSC) stimulate endogenous protective and restorative responses by paracrine mechanisms. Recent studies with MSC conditioned medium (MSC-CM) have suggested the possibility of its use in regenerative medicine instead of whole cells, which could solve problems such as immune compatibility and tumorigenicity [1]. Many studies have demonstrated the beneficial effect of factors released by bone marrow derived MSC (BMMSC) on different models [2]. MSC-CM contains various molecules of a neuroregulatory nature that promote the survival of neuronal / glial cells and create an environment conducive to regenerative processes [3]. Our *in vitro* study demostrates the impact of rat BMMSC-CM on astrocyte migration activity using an *in vitro* scratch model of spinal cord injury. We first prepared CM by conditioning BMMSC for 24, 48 and 72 hours in serum-free medium (CM24, CM48, CM72). After scratching astrocytes were cultured in CM24, CM48, CM72 at time intervals 5hours (DIV1), 24hours (DIV2) and 48 hours (DIV3). The paracrine effect on migration activity was evaluated by measuring the distance of the linear cell-free area (formed after scratch) occupied by astrocytes from one edge to the other. Our results showed significant impact of BMMSC-CM on astrocytes migration activity manifested by gradual overgrowth of cell free area compared to control. The most signicant effect was observed at DIV2 with CM24 and at DIV3 with CM24 and CM72. Our data suggest a different effect of BMMSC-CM on astrocytes migration depending on the time of CM collection as well as the time of cultivation.

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Dopaminergic D1 receptor signaling in the midbrain interpeduncular nucleus – possible involvement in control of novelty preference expression

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Discrimination between novel and familiar stimuli and adequate responses to novelty are crucial not only for proper functioning but also survival. Importantly, many neuropsychiatric disorders (such as anxiety, autism, schizophrenia and ADHD) manifest in an atypical reaction to novelty. Midbrain interpeduncular nucleus (IPN) was shown to play a role in novelty/familiarity signaling, preference toward novelty and behavioural inhibition. At the same time, neurotransmitter dopamine (DA) has an established role in motivation-related processes, and studies in mice showed that ventral tegmental area dopaminergic neurons innervate IPN and modulate novelty preference expression [1]. However, the nature of D1 receptor (D1R) signaling, D1R expression pattern as well as the source of IPN dopaminergic innervation in the rat remain largely unknown.

Ex vivo whole-cell patch-clamp recordings revealed that activation of D1R using its agonist SKF81297 (10uM) has excitatory action in majority of recorded IPN neurons. Recorded increase in whole-cell inward current persisted in the presence of tetrodotoxin and GABAergic and glutamatergic blockers, indicating that IPN neurons express D1R postsynaptically. Moreover, analysis of synaptic currents showed increase in frequency of spontaneous excitatory postsynaptic currents upon D1R activation. *Ex vivo* multielectrode array recordings revealed concentration-dependent D1R agonist mediated excitation and inhibition of IPN neurons. Using RNAscope *in situ* hybridization, D1R co-expression with vGAT1 was found and viral based tract-tracing showed that, among others, dopaminergic neurons of ventral tegmental area and substantia nigra pars compacta innervate IPN.

Together this data show that DA/D1R signaling in the IPN may influence novelty preference expression by exciting IPN GABAergic neurons.

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Empathic reaction for pain in highly sensitive person – facial electromyography study

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Highly sensitive person (HSP) is characterized by high sensitivity and strength of response to any type of stimuli, coming from both external and internal environments and processing of information at a deep level and higher level of emotional empathy. To date, very little is known about empathic response for pain in the HSP group.

The present study aimed to examine how the activity of *corrugator supercilli* (CS) and *orbiculator oculli* (OO) would change in response to different videos showing pain stimuli in the HSP group compared to lowly sensitivity person.

29 LSP and 28 HSP watched stimuli of the pain while the EMG from CS and OO were recorded. The stimuli consisted of videos with 3 levels of pain intensity, no pain, low, and high pain. Each video was divided into 4 scenes: (1) showing the target's neutral facial expression, (2) the action leading to the pain, (3) the actual pain stimulus, (4) the target's facial expression of pain).

The results of EMG analysis revealed a significant main effect of pain and video scenes, and interaction between pain intensity x group x video scene. HSP revealed stronger electrical activation of muscles than LSP group: for OO in scene 3 and 4 for high expression of pain, and CS in scene 4 for low and high expression of pain. Moreover, the HSP group revealed lower activation in scene 1 for CS than LSP for all intensity of pain.

HSP stronger responds to empathy for the pain of others than LSP.

The following author declared no potential conflicts of interest.

Individual gamma frequency based neurofeedback: a pilot study

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The neurofeedback (NFB), based on the restoration of neuroplasticity by operant learning to control brain activity, i.e. training brain functions by providing a timely congruent feedback, is encouraging in regard of improvement of aberrant cognitive and perceptual processes in neuropsychiatric disorders. Gamma activity is related to cognitive processes and is frequently impaired in these disorders. The gamma-activity based neurofeedback procedures showed a potential previously; however, they lack individualized approach.

The study is aiming at the development and pilot testing of a neurofeedback system relying 1) on the non-invasive assessment of a unique physiological parameter – an individual gamma peak frequency and 2) the application of a unique feedback based on auditory steady-state response (ASSR).
To estimate the IGF, responses to chirp-based auditory stimulation are assessed and phase-locking is used as a target measure. During NBF training the phase-locking of gamma response is continuously extracted and converted to frequency of auditory stimulation and the size of a ball displayed on the screen. Subjects are instructed to increase the diameter of the ball. The control group receives feedback based on the other person's EEG. Backward digit span task is used as a behavioral control measure.
In this work we demonstrate a gamma-ASSR/IGF-based neurofeedback system and results of initial evaluation of the capabilities of the proposed NBF system to modulate IGFs.

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Two types of noradrenergic modulation of calcium currents in trigeminal ganglion neurons

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Sympatho-sensory interactions in the trigeminal pathways could be simulated by noradrenaline (NA) application on trigeminal ganglion neurons (TGN). The aim of our research was to analyze noradrenergic modulation of Ca^{2+} currents and signals in TGN. Noradrenaline (NA) in a dose-dependent manner inhibited Ca^{2+} currents with IC50 of 0.140 μM and the mean value of this effect was 38 ± 4% (n=12). Two electrophysiologically different types of the modulation were found: with no kinetic changes (62%, first type) and kinetic-slowing decreasing of the amplitude (29%, second type). The second type effect was partially relieved if predepolarization pulses were applied. This indicates direct interaction between Gβγ-subunit and Ca2+ channel in the modulation effect. Using yohimbine (selective antagonist of alpha2-adrenoceptors), it was shown that noradrenergic modulation of calcium currents partially (60%) mediated by α2-adrenoceptors activation.

Ca^{2+} signals were evoked by TGN stimulation with short (5 ms) pulses. The signal amplitude was linearly dependent on the number of stimuli with a mean slope of 17±2 nM/pulse (n=7). After stimulation offset the signals decayed exponentially with the mean time constant of 9.7±1.1 s. NA application dearesead the slope value to 7±3 nM/pulse (n=5) and other parameters were not significantly different. This suggests that NA affected Ca^{2+} influx and had no marked influence on Ca^{2+} basal level and extrusion.

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There is no conflict of interests of any kind. All the authors agree on this submission.

Kinetic of DNA damage repair induced in differentiated SH-SY5Y cells following proton radiation exposure

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DNA lesions are constantly produced in all the cells of the body, including non-dividing neuronal cells. Rapidly dividing cells, such as cancer cells, are believed to be particularly sensitive to DNA damage induction, and represents the main model for studying the repair mechanisms. Recent studies underline the importance of understanding the DNA damage response (DDR) of fully differentiated neuronal cells, as these cells can accumulate unrepaired DNA damage leading to cellular ageing/neurodegeneration.

Our aim was to evaluate the pathways of DDR signaling pathways in a neuronal model following particle irradiation exposure.

SH-SY5Y cells were differentiated by addition of retinoic acid to the media and reduction of serum. Neuronal differentiation was characterized by immunofluorescence and morphological observation.
DNA damage was induced by proton exposure at the dose of 2 Gy, previously selected by viability evaluation. Kinetic of DDR was evaluated by immunofluorescence and/or Western blot for proteins specific to DDR pathways such as CSB, 53BP1, γH2AX at several time points following irradiation (3, 6, 24 hours).
53BP1 and γH2AX DNA-damage foci were found starting shortly following irradiation (3h). Most of the 53BP1 accumulation are colocalized with γH2AX. However, γH2AX also formed individual unique foci. The number of foci diminished with time, but persistent foci can be seen at 24 hours following exposure suggesting that repair is not complete. Western Blot analysis confirmed upregulation of several proteins related to DDR pathways.
A future analysis will be used in order to distinguish if specific DNA repair mechanism are correlated with cellular phenotype.

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Authors have no conflict of interest to declare.

Effects of brain size on adult neurogenesis in shrews

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Shrews are small animals found in many different habitats. Like other mammals, adult neurogenesis occurs in the subventricular zone of the lateral ventricle (SVZ) and the dentate gyrus (DG) of the hippocampal formation. We asked whether the number of new generated cells is changed in shrews depending on the brain size. We examined Crocidura russula and Neomys fodiens weighing 10-22 g and Crocidura olivieri and Suncus murinus that weigh 3 times more. We found that the density of proliferated cells in the SVZ was approximately at the same level in all species. These cells migrated from the SVZ through the rostral migratory stream to the olfactory bulb (OB). In this pathway, a low level of neurogenesis occurred in C. olivieri compared to 3 other species. In the DG the rate of adult neurogenesis occurred differently. Specifically, the lowest density of newly generated neurons was observed in C. russula which had a substantial number of new neurons in the OB compared with C. olivieri. We suggest that the number of newly generated neurons in an adult shrew's brain is independent of the brain size and molecular mechanisms of neurogenesis appeared to be different in two neurogenic structures.

Conflict of interest: The authors declare that they have no conflict of interest.

Food restriction counteracts dexamethasone-induced downregulation of genes involved in cholesterol homeostasis during aging, in the cortex and hippocampus of rats

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Glucocorticoids are potent anti-inflammatory agents commonly used for the treatment of various inflammatory and autoimmune disorders. In addition, limited *in vivo* data are available to characterize the mechanism underlying their cognitive side effects and the occurrence of steroid psychosis. As cholesterol is important for brain plasticity and proper cognitive performance, we here present direct effects of a potent synthetic glucocorticoid dexamethasone on the expression of 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoAR), apolipoprotein E (ApoE) and cholesterol 24S-hydroxylase (CYP46A1) involved in cholesterol synthesis, metabolism, and excretion, respectively. Dexamethasone effects were examined during aging in the cortex and hippocampus of 6-, 12- and 18-month-old rats fed *ad libitum*

or following long-term food restriction (FR). The most prominent change observed was the age-related decrease in ApoE mRNA regardless of the food regimen applied. In animals kept on FR, this decrease was accompanied by an increase in the expression of HMG-CoAR and CYP46A1. In brief, food restriction reversed most of the dexamethasone-induced changes in the expression of genes involved in regulation of cholesterol homeostasis in aging rats, in a region-specific manner.

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The authors declare that there is no conflict of interest

Agmatine protects mitochondria in LPS-stimulated microglia

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Mitochondria play a key role in energy metabolism and regulate some of the principal cellular processes such as the production of ATP and reactive oxygen species, as well as a regulation of apoptotic cell death. Mitochondrial dysfunction and oxidative stress are common threads in most neurodegenerative disorders, which are also accompanied by chronic microglial activation. Agmatine, neuromodulatory polyamine, was shown to exhibit neuroprotective effects in oxidative stress conditions. Therefore, the goal of this study was to determine the ability of agmatine to preserve mitochondrial function and prevent apoptosis during neuroinflammation.

The effects of 100 μM agmatine on cellular energy status and cell death were examined in LPS-stimulated BV2 microglial cell line. To detect changes in mitochondrial membrane potential, TMRE fluorescent assay was performed, while the changes in intracellular ATP concentration were determined by bioluminescent assay, 6h, and 24h after LPS stimulation. The expression of apoptosis regulators Bax and Bcl2 was assessed by Western blot analysis and the Bax/Bcl2 ratio was determined.

Agmatine increases mitochondrial membrane potential, indicating its protective role during mitochondrial insult caused by LPS stimulation. LPS and agmatine administrated separately, increase intracellular ATP levels, however, agmatine treatment followed by LPS stimulation enhances ATP production even further, at both time points. Moreover, agmatine shows an antiapoptotic effect by reduction of Bax/Bcl2 ratio in comparison to LPS stimulation.

We conclude that the results of this study indicate the capacity of agmatine to protect mitochondrial function and suppress apoptosis, which may be beneficial in neurodegenerative disorders and neuroinflammation.

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Altered cerebellar output disrupts normal cortical oscillatory activity during movement and quiet wake

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The neural impulses responsible for accurate movement sequences are generated within the cerebello--thalamo-cortical motor circuit [1], that functionally group the structures involved in the motor act and present distinctive features depending on the motor behaviour. Here we aimed to identify the effect of altered cerebellar output on motor oscillations in the cortex during different motor states.

ECoG-EMG were bilaterally recorded on motor cortices and neck muscles, respectively, in Swiss albino mice. After a 90-min basal recording during a first day, recordings were done for 5 consecutive days, before (pre-kainate, 30-min) and after (post-kainate, 60-min) a daily kainic acid microinjection into the left cerebellar hemisphere, used to alter the cerebellar activity.

The epochs were classified by movement behaviour and dystonic phenotype. The results were computed into power spectral density (PSD) and coherence for each recording site.

The cortical PSD revealed a decrease in theta band after the cerebellar disturbance in both dynamic and quiet states, while beta power only dropped in the dystonic movements, suggesting that beta is sensitive to motor activity. The motor cortices coherence decreased in delta-theta and beta regardless of the motor behaviour. However, ECoG-EMG coherence revealed a decremental trend in beta and theta that was dependent to static behaviour, while motor activity triggered increased coherence in beta and low-gamma bands.

Our work showed that there are distinctive patterns of dystonic cortical oscillatory activity, which are dependent to motor behaviour, and others that are not sensitive to motor activity, suggesting a possible advance in describing dystonic ECoG patterns.

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The relationship between mentalization, mindfulness, working memory, and schizotypal personality traits in the general population

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Individuals with high schizotypal traits exhibit less ability to consciously observe, describe, and monitor feelings, thoughts, and experiences. However, the relationship between mindfulness, mentalization, working memory, and schizotypy has not been explored.

Three hundred individuals from the community completed questionnaires examining schizotypal traits, mindfulness, and mentalization. Participants also completed a set of working memory tasks. Linear regression analysis indicated that mentalization was a significant and common predictor of all schizotypal traits, including unusual experiences, cognitive disorganization, introverted anhedonia, and impulsive nonconformity, when the analysis was controlled for mindfulness and working memory. We also demonstrated a significant correlation between mindfulness and mentalization. Moreover, low mindfulness and mentalization abilities were both associated with high levels of schizotypal features. Working memory was only weakly associated with cognitive disorganization and introverted anhedonia. These findings indicate that weak mentalization is a common feature of schizotypy independent of mindfulness and working memory.

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Declaration of Conflicting Interests

The authors declared that there are no conflicts of interest.

The effect of modulation of glucocorticoid and mineralocorticoid receptors during lipopolysaccharide-induced neuroinflammation

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We studied the influence of agonist of glucocorticoid receptors (dexamethasone) and inhibitors of glucocorticoid (mifepristone) and mineralocorticoid (spironolactone) receptors on the neuroinflammation in the hippocampus which was induced by intrahippocampal injection of bacterial lipopolysaccharide (LPS). The compounds were bilaterally injected to dorsal hippocampi of the adult male Wistar rats. Phosphate buffered saline (PBS) was used as a vehicle. The experiment included 2 independent parts. The first part included 5 groups: (1) intact; (2) PBS; (3) LPS+PBS; (4) dexamethasone+PBS, (5) dexamethasone+LPS+PBS. The second part – 7 groups: (1) intact; (2) PBS; (3) LPS+PBS; (4) mifepristone+PBS; (5) spironolactone+PBS; (6) mifepristone+LPS+PBS; (7) spironolactone+LPS+PBS. Three days after the injection, the dorsal (DH) and ventral (VH) hippocampi were isolated. Total mRNA from samples was used to study gene expression of *Il1b*, *Il6* and *Tnf* by real-time PCR. In DH, LPS induced an increase in mRNA expression of *Il1b* and *Tnf* compared to the PBS group. During the LPS-induced neuroinflammation in DH, dexamethasone potentiated the expression of *Tnf,* mifepristone and spironolactone did not prevent an increase in the expression of *Il1b* and *Tnf* but induced a trend to a decrease in the LPS-induced increase in *Il6* expression. In VH, no changes in the expression of genes of interest were found. Thus, we found that modulation of activity of glucocorticoid and mineralocorticoid receptors by dexamethasone, mifepristone or spironolactone had minor effect on the inflammatory response suggesting that neuroinflammation induced by LPS is not dependent on these receptors.

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Modulation of Short-Term Neuronal Dynamics by beta-Adrenergic Receptors in the Dorsal and Ventral Hippocampus

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Noradrenergic transmission profoundly modulates neuronal activity in several brain areas including hippocampus, where beta-adrenergic receptors (β-ARs) strongly modulate long-term synaptic plasticity. Furthermore, recent evidence demonstrates that β-ARs facilitate long-term synaptic potentiation more in ventral than in dorsal hippocampus [1]. However, the action of β-ARs on short-term dynamics along the dorsal-ventral axis of the hippocampus remains unclear. Frequency-dependent short-term changes in synaptic transmission and neuronal excitation appear to play crucial roles in neural information pro-

cessing, and greatly differ along the longitudinal hippocampal axis [2,3]. In the present study, we used recordings of field potentials (i.e. excitatory synaptic potentials, fEPSPs and population spikes, PSs) from the CA1 field of hippocampal slices, and a frequency stimulation paradigm consisting of a train of ten pulses delivered at varying frequency to Schaffer collaterals. We show that activation of of β-ARs by their agonist isoproterenol facilitates neuronal output from the dorsal but not the ventral hippocampus. The β-AR-mediated facilitation of short-term dynamics in the dorsal hippocampus occurs at the frequency range of 3-40 Hz. Isoproterenol does not affect short-term synaptic plasticity in either segment of the hippocampus. Furthermore, isoproterenol increases basal synaptic transmission and neuronal excitation more in the dorsal than in the ventral hippocampus. These results suggest that β-AR-modulation of short-term neuronal dynamics differs along the longitudinal axis of the hippocampus. We propose that this action of β-ARs significantly contributes to diversify information processing along the longitudinal axis of the hippocampus.

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Do mitochondria mediate the beneficial effects of enriched environment in mice?

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Enriched environment, an environment that enhances locomotion, exploration and social interaction, has been reported to exert anxiolytic and neuroprotective effects in mice. Previous experiments in our lab have shown that mitochondria mediate anxiety and stress responses. Here we explored whether mitochondria modulate the beneficial effects of enriched environment in CD1 mice. Male and female mice were exposed to enriched environment conditions for 8 weeks. A battery of behavioral tests (Dark--Light, Open Field and Forced Swim Test) was performed to assess the impact of enriched environment

on anxiety- and depression- related phenotypes. Mitochondrial, metabolic and oxidative stress readouts were then assessed by biochemical and immuno- based methods in the prefrontal cortex. Our findings reveal that enriched environment has an impact on the behavioral phenotype which is mediated by changes in oxidative phosphorylation, antioxidant defense and mitochondrial import/transport. Taken together, we submit that mitochondrial regulation is implicated in the molecular mechanisms underlying the beneficial effects of enriched environment. Along these lines, mitochondria- targeted therapeutic strategies may hold great promise for alleviating stress and anxiety symptoms.

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Protective and pro-resolving impact of the formyl peptide receptor 2 treatment in the lipopolysaccharide-exposed primary microglia cells

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Neuroinflammation is a multicellular process that plays an important role in a variety of neuropsychiatric disorders. Microglia cells seem to play a crucial role in this process. Chronic activation of these cells leads to excess release of pro-inflammatory cytokines. The recent data indicate that resolution of inflammation (RoI) requires specialized pro-resolving mediators (SPMs) including endogenous lipoxin A4 (LXA4) and aspirin-triggered lipoxin (AT-LXA4) which exert their biological functions via formyl peptide receptor 2 (FPR2). Although endogenous SPMs cause RoI their unfavorable pharmacokinetic properties represent limitation of further studies. Therefore, the aim of the present study was to examine the pro-resolving properties of new exogenous FPR2 agonist MR-39 with ureidopropanamide scaffold.
Primary microglia cultures were obtained from 0-2 days old Sprague-Dawley rat pups. Microglia were pre-treated with: LXA4 (0,001-0,01μM), AT-LXA4 (0,001-0,5μM) and MR-39 (0,5-5μM). Then cultures were stimulated for 3 and 24h with lipopolysaccharide (LPS) (1μg/ml). To confirm the influence of FPR2 on ligands, antagonist WRW4 was also added into medium. Time-dependent realise of pro-inflammatory cytokines (TNF-α, IL-6, IL-1β) and level of the reactive oxygen species (ROS) were measured by ELISA and DCFH-DA test respectively.
Pre-treatment of microglia cells with SPMs demonstrated that LXA4 after short-term LPS stimulation inhibited the production of ROS and also diminished TNF-α protein levels. Moreover, MR-39 showed beneficial, long-lasting effects observed also after 24h of incubation by decreasing ROS, IL-1β, and TNF-α protein levels.

We postulate that compound MR-39 exerts a neuroprotective effect in LPS-activated microglia through FPR2, which may be new neuroprotective target in drugs development.

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Effect of memantine on behavioral reactions of rats in the active avoidance test

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Disorders of glutaminergic neurotransmitters play an important role in the manifestations of symptoms and progression of neurodegenerative dementia, especially with the participation of NMDA receptors. Memantine is an NMDA receptor antagonist that blocks the effects of abnormally elevated glutamate levels, which can lead to neuronal dysfunction and neurodegeneration. Also, experiments have shown the protective effect of memantine in damage to hippocampal cells by β-amyloid. This study aimed to investigate the possible direct positive effect of memantine hydrochloride on learning and memory processes in young rats. A classic variant of the experimental model of the avoidance reaction is the shuttle chamber. Installation “shuttle chamber” is a box divided into two halves by a partition with a hole in the middle. When the training procedure is repeated several times in the installation, the rat develops a conditioned avoidance reflex, which consists in the fact that the animal learns to move to the opposite side of the chamber after feeding the conditional stimulus (CS). A light and sound pulses at frequencies 2÷4 kHz were used as a CS. High-frequency signals in animals serve as an alarm signal, which coincides with the nature of the experimental setup, motivating the animal to perform the test task effectively. Transition reactions are a genetically determined form of response in rats, so it is possible to measure the ability to learn based on behavioral characteristics. In general, the experiment showed that animals treated with memantine hydrochloride performed active avoidance 1.3 times more than animals in the control group.

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Itaconic acid suppresses oxidative phosphorylation in brain cells by inhibiting mitochondrial respiratory chain complexes

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Itaconic acid (IA) is an anti-inflammatory metabolite produced in the central nervous system (CNS) by microglia and neurons upon pro- inflammatory stimulation such as LPS or viral infection. IA has been shown to inhibit mitochondrial respiration by targeting succinate dehydrogenase (SDH), however recent studies show that IA is able to inhibit succinate – independent respiration as well [1]. In this study we aimed to find the mechanism by which IA suppresses cellular respiration in the mitochondria of brain tissue. We used mitochondria, isolated from 2-3 months old male Wistar rat forebrains. Oxidative phosphorylation capacity was measured with complex I substrates (pyruvate + malate) using high resolution respirometry. Mitochondrial complex I and ATP synthase activities were measured spectrophotometrically while complex IV activity was measured using an oxygraphic method. We found that 1mM IA inhibits oxidative phosphorylation with complex I substrates in the brain mitochondria. 1mM IA had no effect on the enzymatic activity of complex I and ATP synthase, however significantly decreased the activity of complex IV. Raising the concentration of IA to 5mM caused a significantly higher inhibition of oxidative phosphorylation compared to 1mM group and significantly inhibited the activities of complex I, complex IV and ATP synthase. In conclusion, IA causes a dose- dependent inhibition of oxidative phosphorylation with complex I substrates by inhibiting complex IV at lower concentration and complexes I and IV and ATP synthase at higher concentrations.

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Neural cell adhesion molecule (NCAM) in the brain and blood plasma of rats with intracerebral hemorrhage

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Intracerebral hemorrhage (ICH) causes marked perihematomal edema formation and neurological deficits [1]. NCAMs are glycoproteins provided homophylic communication between cells [2]. The present study was aimed to evaluate changes of NCAM content in the blood plasma and different areas of brain of rats after intracerebral hemorrhage.

This research was performed on 18 Wistar rats divided into three groups (n = 6): 1 – intact, 2 – falsely operated (FO), 3 – experimental ICH. The experiment was conducted in accordance with the "Regulations on the use of animals in biomedical experiments". The total protein was measured due to Bradford in the cytosolic fraction obtained from differ brain areas and blood plasma. The NCAM content was measured with ELISA.

It was shown that the total protein content obtained from the brain of rats was significantly increased as in the FO and ICH group of animals compared with the intact rats depends on brain area. The total protein content in plasma tended to decrease in ICH rats. There was a significant increase of soluble and membrane NCAM level in the hippocampus and cerebellum of ICH group of animals with elevation of soluble NCAM content in the plasma of ICH rats for 3 days after ICH development.

Obtained data allow to suggest the soluble NCAM level in the blood plasma as a candidate of biomarker potentially associated with intracerebral hemorrhage outcomes.

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Visual and auditory responses of stable neurons in the rabbit amygdala

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Stable single-unit recording is a method of recording the same neurons for more than a single day [1]. In the reported study the microelectrode bundles were assembled manually [2]. 32 nichrome microelectrodes were implanted into amygdala of two adult rabbits under general anesthesia. The raw activity was filtered, neuronal spikes were detected by a threshold. Sorted action potentials were transferred to an automatic algorithm assessing stability of recordings [3]. Data were available for acquisition for 72 days in rabbit #1 and for 964 days in rabbit #2. We managed to test neurons' properties in different experimental conditions presenting visual or auditory stimuli to the animals due to stable recording. Neurons of the rabbit #1 were responsive to mostly to pure tones and neurons of the rabbit #2 responded to both visual and auditory stimuli although most neurons were indifferent to either stimulation.

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Effects of dorsal root avulsion injury on the spinal ganglia and spinal cord

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The changes in the ventral horn after ventral root injury are well-known, however, there are only a few studies investigating the effect of dorsal root avulsion (DRA). Here we examined the avulsion-induced changes in the affected cell populations of the dorsal root ganglia and the spinal cord.

The lumbar 4 and 5 (L4-5) dorsal roots were avulsed in deep ketamine-xylazin anaesthesia. The animals were perfused 3, 8, 21 days after the surgery. The injured and contralateral dorsal root ganglia along with the L4-5 spinal segments were removed. Immunohistochemical analysis carried out on cryostat sections included NF200 kDa protein, TrpV1 receptor and CGRP immunostainings and GSA-B4 histochemistry.

Three days following DRA a membrane-bound ring-like TrpV-1 expression could be observed in the large neurons of affected ganglia. At later time points the ring-like expression could not be detected in any of the affected neurons. Intensity of GSA-B4+ intracellular granules significantly increased in injured neurons 3 days after DRA compared with intact cells. Significant decrease of NF-200 kDa expression

could be found in the affected ganglia and in the ipsilateral gracile tract of the spinal cord 21 days after the injury. CGRP-positive fibers disappeared almost completely in the affected dorsal horn 3 days after the injury. In contrast, GSA-B4-positive fibers remained well preserved for at least 3 weeks after the injury. Our data suggest that DRA induces unique changes of expression pattern of the investigated markers in the injured dorsal root ganglia and results in the subsequent changes in the spinal cord, respectively.

There is no conflict of interest.

Beck's cognitive model of depression: critical appraisal and comprehensiveness of the model

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Aaron Beck was one of the first theorists to assemble an empirically based cognitive model to explain the psychological processes of depression, describing concepts such as the cognitive triad, biased information processing and dysfunctional beliefs. Since its initial conception in the 1960's [1], Beck's model has been favourably empirically supported and updated over time to incorporate evidence from multiple areas of research [2]. However, can Beck's model be considered a comprehensive cognitive theory of depression? A literature search of relevant articles supporting or confronting the different elements of the model was carried in Embase and Medline databases.

There is extensive proof that support the main aspects of the original model, being that the cognitive triad accompanies depression and that depressed people process information in a somewhat negative way. There is, however, little proof that dysfunctional beliefs cause depression, albeit this causal speculation can't be totally excused. The updates and neurobiological correlation of cognitive aspects of depression strengthen the hypothesis and could even be useful for purposes of depression prediction and prevention [3]. However, because of the current lack of longitudinal designs, only a putative association between these two areas of research can be suggested, and not direct association.

Beck's cognitive model of depression provides an exhaustive integration of cognitive aspects of the disorder, and its continuously expanding to integrate different theoretical perspectives, including neurobiological findings which account to further support the model, with the objective to become every time a more comprehensive framework to further understand and assess depression.

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Protective effects of dhea and dheas on sh-sy5y neuroblastoma cells and primary mouse neurons, exposed to aβ oligomers, hydrogen peroxide and oxygen-glucose deprivation, as *in vitro* models of dementia

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Neurosteroids dehydroepiandrosterone (DHEA) and its sulfate (DHEAS), the most abundant steroids in human blood, are also synthesized *de novo* in the brain, where they participate in varios functions such as neural plasticity, learning, memory and behavior [1]. Dementia is a syndrome of progressive cognitive decline, with Alzheimer´s disease (AD) and vascular dementia (VaD), as the most common forms. AD is neurodegenerative disorder characterized by the abnormal deposition of the amyloid β (Aβ) peptide and accumulation of neurofibrillary tangles, and is associated with multiple pathophysiologic mechanisms including apoptosis and oxidative stress [2]. VaD is due to the reduced blood flow, resulting in insufficient supply of nutrients and oxygen to the brain, leading to an impairment of memory and cognitive functions [3]. We have investigated potential neuroprotective effects of these neurosteroids in primary mouse neurons, as well as in the SH-SY5Y neuroblastoma cells. As *in vitro* models of AD and VaD, we have exposed these cells to the toxic Aβ oligomers, hydrogen peroxide (H_2O_2) inducing oxidative stress, or to the oxygen-glucose deprivation (OGD). The obtained results demonstrated the beneficial effects of DHEA and DHEAS treatment on the cell survival and viability, suggesting potential neuroprotective actions of these neurosteroids in AD and VaD.

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Beyond NMDA receptors – how cholinergic agonists influence pattern and level of firing in dopaminergic neurons - *in vivo* electrophysiological and pharmacological studies on NR1DATCreERT2 mice

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Bursting mode of activity of dopaminergic neurons results in phasic increase of dopamine release, which facilitates synaptic plasticity and supports learning processes. Basal level of this neurotransmitter is maintained by non-bursting firing of dopaminergic neurons. It was shown that functional NMDA receptors are crucial to evoke dopaminergic neurons' bursting activity, however whether other neurotransmitters can also evoke bursts remains an opened question. Therefore, aim of our research was to determine effect of cholinergic agonists on activity of dopaminergic neurons lacking functional NMDA receptor.

Genetically modified strain of mice with deletion of NR1 subunit of NMDA receptor selectively on dopaminergic neurons of adult animals was used. We performed single unit, extracellular recordings of midbrain dopaminergic neurons' activity combined with iontophoretic application of cholinergic receptors agonists under urethane anaesthesia.

After application of non-selective cholinergic agonist carbachol, majority of dopaminergic neurons increased their firing rate. Interestingly, in some of recorded cells, both in control and NR1DATCreERT2 mice, increase in firing was accompanied by development of slow, oscillatory changes in firing rate, which transformed into complex bursts of action potentials. Neurons tested with oxotremorine application responded with an increase of firing rate and similarly to carbachol iontophoresis - some of the recorded neurons developed complex bursts.

These results show that activation of cholinergic receptors alone, without the involvement of NMDA receptors, can switch subpopulation of dopaminergic neurons into bursting mode of firing. Furthermore our findings suggest that muscarinic receptors can be involved in this phenomenon.

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Loss-of-function variants in the schizophrenia risk gene *SETD1A* lead to altered neuronal network activity in human neurons.

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Heterozygous loss-of-function (LoF) mutations in *SETD1A*, which encodes a subunit of histone H3 lysine 4 methyltransferase, have been shown to cause a novel neurodevelopmental syndrome and increase the risk for schizophrenia. Recent rodent studies imply that *SETD1A* haploinsufficiency leads to dysregulated neuronal communication in cortical networks. However, the exact molecular and cellular mechanisms of how SETD1A deficiency causes neuronal network alterations, in particular in a human context, remains largely unknown. To study the effect of decreased SETD1A function in human cells, we generated excitatory/inhibitory neuronal networks from human induced pluripotent stem cells with a *SETD1A* heterozygous LoF mutation (*SETD1A*$^{+/-}$). Our data show that in human neuronal in-vitro excitatory/inhibitory networks, *SETD1A* haploinsufficiency resulted in altered neuronal network activity, which was predominantly defined by increased network burst frequency, whereas unchanged global firing activity. In individual neurons, this network phenotype was indicated by increased synchronized excitatory synaptic inputs and structurally by increased somatodendritic complexity in both glutamatergic and GABAergic neurons. The transcriptome in *SETD1A*-haploinsufficient neurons was perturbed in gene sets associated with several psychiatric and neurodevelopmental disorders, most prominently with schizophrenia, neuronal morphogenesis as well as glutamatergic synaptic function. In agreement with the molecular link to glutamatergic signaling, cultures only consisting of glutamatergic neurons recapitulated the excitatory/inhibitory network phenotype. In conclusion, we revealed key neuronal network and transcriptome alteration caused by SETD1A deficiency with glutamatergic neurons as the main contributor to the altered network phenotype.

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Changes in the activity of both serotonergic and glutamatergic systems after treatment 1MeTIQ tested in animal model of schizophrenia

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Both positive and negative symptoms of schizophrenia may be the result of dysregulation of different neurotransmitter systems. Ketamine act as an antagonist of NMDA receptors, and is able to block the reuptake of catecholamines by diminishing COMT activity [Koehntop et al., 1977]. Therefore, ketamine may induce schizophrenia-like symptoms. 1-Methyl-1,2,3,4-tetrahydroisoquinoline (1MeTIQ) is reversible MAO inhibitor and exhibits the neuroprotective, antidepressant- and anxiolytic-like effect [Wąsik et al. 2016; 2019].

The aim of the present study was to investigate the impact of acute 1MeTIQ administration on the release of serotonin (5-HT) and glutamate (GLU) disturbed by low dose of ketamine (10 mg/kg *i.p.*); 1MeTIQ (25 or 50 mg/kg *i.p.*) was administered 20 minutes before ketamine injection. The release of 5-HT was measured in the striatum (STR) while GLU was measured in the frontal cortex (FCX) using *in vivo* microdialysis study.

In vivo microdialysis study showed that combined treatment 1MeTIQ with ketamine increased the release of 5-HT (approx. 60%) and decreased the level of 5-HIAA in the rat's STR. Simultaneously, 1MeTIQ given combined with ketamine significantly increased the release of GLU (approx. 200%) in the FCX.

Our study indicated the ability of 1MeTIQ to reverse ketamine-induced disturbances in the activity of serotonergic system while enhancing the effects of ketamine on the glutamatergic system activity.

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Fingolimod ameliorates spatial and recognition memory deficits and improves deregulated gene expression of proteins engaged in amyloid metabolism in obese mice brain

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Obesity and type 2 diabetes mellitus (T2DM) represent a growing socioeconomic problem around the world. Recent data indicates that T2DM affects cognition and causes memory deficits and alterations of bioactive sphingolipids are evident during obesity. In present study we examined the effect of fingolimod (FTY720, sphingosine-1-phosphate receptors modulator) on spatial and recognition memory as well as mRNA levels of proteins involved in production and metabolism of amyloid beta (Aβ) in obese mice brain cortex and hippocampus.

10-12 week old male C57BL/6J mice were given high-fat diet (HFD) for 16-weeks. Control group received standard diet (SD). After 96 days on HFD, animals started receiving FTY720 (*i.p.* 1mg/kg) in NaCl for 2 weeks, then novel object recognition (NOR) test were performed and brain structures were isolated for qPCR analysis.

Our results indicate elevation of *Bace1, Psen2, Gsk3b, Ide* mRNAs that are engaged in regulation of Aβ levels in HFD mice brain cortex. Moreover, elevation of *Psen1* and reduction of pro-survival *Sirt1* mRNA levels were observed in hippocampus. During NOR test, recognition of obese mice was reduced compared to control mice. Administration of FTY720 to HFD mice downregulated mRNA levels of upregulated genes in brain cortex and improved spatial and recognition memory.

These results indicates that FTY720 modulates genes that encodes proteins involved in production of Aβ, phosphorylation of tau protein and degradation of insulin and thus may improve memory processes by amelioration of Aβ toxicity. FTY720 also improved spatial and recognition memory suggesting its potential in therapeutic strategy of diabetic brain disorders.

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Siponimod – a modulator of sphingosine-1-phosphate (S1P) receptors protects neuronal cells against ceramide toxicity.

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The disturbed homeostasis between pro-apoptotic ceramides and pro-survival sphingosine-1-phosphate (S1P) is characteristic for Alzheimer's Disease (AD) and other neurodegenerative disorders. Altered sphingolipid rheostat leads to ceramide accumulation and lower signal transduction mediated by S1P [1]. Since S1P exerts its action through binding to specific receptors ($S1PR_{1-5}$), numerous studies investigate neuroprotective properties of their modulators, e.g. siponimod (BAF312), a selective S1P receptor ($S1PR_{1,5}$) modulator approved for the treatment of multiple sclerosis [2]. Hence, the purpose of this study was to evaluate the potential neuroprotective activity of siponimod in neuroblastoma cell line (SH-SY5Y), used as a model of neuronal cells, exposed to C2-ceramide (C2-cer). Cells were treated with C2-cer (25 μM, concentration based on the previous studies), BAF312 (1 nM-10 μM), or in a combination of C2-cer with BAF312. Cell viability was assessed by MTT assay and by propidium iodide staining with flow cytometry analysis 24 h and 72 h after treatment. C2-cer at the concentration of 25 μM significantly reduced the viability of SH-SY5Y cells. Moreover, this effect increased with the time of incubation. Treatment with 1 μM BAF312 (24 h and 72 h) protected a significant pool of neuronal cells against the death evoked by C2-cer. Additionally, neuroprotective but insignificant effect of BAF312 was observed at 10 nM and 100 nM (72 h). However, it is worth noting that 10 μM BAF312 alone significantly decreased cell viability after 72 h. In summary, results of these studies confirmed the concentration-dependent neuroprotective action of siponimod in C2-ceramide toxicity.

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Activation of integrin β3 mediates the induction of long-term depression at inhibitory synapses between parvalbumin interneurons and pyramidal cells in hippocampus.

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During decades of research, long-term synaptic plasticity was thought to be limited only to excitatory synapses. However, recent years show that GABAergic synapses also exhibit various forms of long--term plasticity. Here, using electrophysiological recordings in hippocampal slices, we investigated the role of integrins, the adhesion proteins, in the regulation of the efficacy of GABAergic synapses. We have found that interference in β3 integrin-dependent adhesion with RGD peptide induced inhibitory long-term depression (iLTD; 90.6±3.0% of the baseline mIPSC amplitude, n=9; p=0.002, paired t-test). Similarly, the activation of β3 integrin with fibrinogen resulted in stable depression of the amplitude of mIPSC recorded in CA1 pyramidal cells (87.4±1.9% of the baseline mIPSC amplitude, n=9; p<0.001). To dissect the role of β3 integrin in defined inhibitory synapses we used optogenetic stimulation of presynaptic interneurons. Here also the activation of β3 integrin with fibrinogen decreased the efficacy of optogenetically stimulated inhibitory synapses between parvalbumin positive interneurons and CA1 pyramidal cells (78.3±7.8% of the baseline IPSC amplitude, n=9; p=0.01). Next, we asked about the molecular mechanism of discovered iLTD. Recordings in the presence of pharmacological inhibitors of different signaling proteins have shown, that the expression of β3 integrin dependent iLTD required the activity of calcineurin and postsynaptic endocytosis, but was independent on the activity of NMDA receptors, CaMKII, PKC or Src kinase. In conclusion, we present the first evidence that the interaction between integrins and extracellular matrix constituents operates as an endogenous modulator of GABAergic inhibition and plays a key role in inhibitory long-term plasticity.

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Analysis of selected gene expression in the hippocampus and the amygdala of rats exposed to chronic overcrowding stress

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Synapse is one of the elements of a neuronal network that undergo remodeling. Among factors significantly influencing synaptic plasticity is excessive stress. Effects of excessive stress on synapse

functioning are particularly apparent in the hippocampus and the amygdala – both critically involved in several neuropsychiatric disorders. Interestingly, opposite effects of stressful conditions have been shown in these structures. Whereas chronic stress impairs long-term potentiation in the hippocampus, in the amygdala, it promotes synaptogenesis. Despite major advances in understanding molecular mechanisms of stress-related neuroplasticity in these limbic regions, there is still a lot to learn.
We aimed to characterize an impact of chronic crowding stress on mRNA expression of proteins related to the synaptic organization in the amygdala and hippocampus, including members of the protein tyrosine kinases and phosphatases family.
Rats underwent overcrowding stress applied for 3, 7, and 14 days. The TaqMan arrays were used for simultaneous evaluation of 24 genes in a single sample, together with the RT-qPCR to assess mRNA expression of genes quantitively.
Our results demonstrate region-dependent alterations in mRNA expression of genes encoding for several intracellular proteins, including the non-receptor protein tyrosine kinase and phosphatase engaged in structural neuroplasticity. Chronic (14 days) overcrowding decreased mRNA expression of focal adhesion kinase and protein tyrosine phosphatase non-receptor type 5 in the hippocampus but not in the amygdala.
These results may help identify new destinations for future investigation of mechanisms of stress-related neuroplasticity and neuropsychiatric diseases and draw our attention to intracellular elements as potential targets for new pharmacotherapy strategies.

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Neural mechanisms of cognition in non-human apes and humans

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Comparative anatomy is a reputable field that has made substantial contributions to the neurosciences. Given the close phylogenetic relationship between non-human primates (NHPs) and humans, similarities have been observed in the anatomy, physiology and neurological characteristics of the brain across species in the order Primates. NHPs can therefore provide valuable insight into human brain function, organisation and evolution. They may also improve our understanding of neurodegenerative disorders

associated with cognitive dyfunctions in humans (prion and Alzheimer's disease), or motor deficits (Parkinson's disease). Despite the contributions of this type of research, the neuroanatomical basis of the ape brain has not been comprehensively examined in a comparative sense. For the current study, the anatomy of four neural systems – essential for modulating cognitive functions, such as learning, memory, and social complexity – were examined in the brain of the lar gibbon and chimpanzee, using immunohistochemical and stereological methods. More specifically, the cholinergic, catecholaminergic, serotonergic, and orexinergic systems were identified and extensively described. The main findings from this research reveal that the anatomical organisation is generally comparable to other mammals (Dell et al., 2010), including primates (Calvey et al., 2015), especially humans (Baker et al., 1990). Despite the similarities, unique features (e.g., in terms of neuronal morphology and distribution) were observed, which might be Hominoid- (lesser and greater apes, including humans) or primate-specific. This study therefore provides further insight into the anatomy and evolution of the neural mechanisms underlying important cognitive processes in apes, including humans.

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Involvement of D1-MSNs and D2-MSNs in processing of natural and addictive rewards in the nucleus accumbens

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Appetitive learning occurs after exposure to positive cues. It can be induced by naturally rewarding stimuli, such as sucrose consumption, or addictive stimuli, such as cocaine administration. It is not clear whether these two kinds of stimuli are processed by a common pathway in the nucleus accumbens (NAc). NAc is a region in the brain that has been previously shown to play a crucial role in reward-driven behaviour. There are two major neuronal subpopulations in NAc: dopamine receptor-1– and dopamine receptor-2–expressing medium spiny neurons (D1-MSNs and D2-MSNs). Their activation is marked by the increase in the levels of cFos and ARC proteins, which are markers of neuronal plasticity - ongoing adaptations of synaptic strength. Moreover, cocaine exposure generates silent synaptic connections, mainly on D1-MSNs. These are immature, excitatory synapses that represent the brain's increased

capacity to learn and can be detected with patch-clamp electrophysiology recordings.
The aim of the study was to compare the involvement of NAc D1-MSNs and D2-MSNs in processing of natural and addictive rewards.
Results indicate that sucrose and cocaine exposure have a partially overlapping effect on neuronal plasticity of D1-MSNs and D2-MSNs in NAc. This may suggest involvement of a common neuronal pathway in processing natural and addictive rewards.

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Different relations between autophagy and mitochondrial biogenesis during postischemic recovery in vulnerable and resistant hippocampal brain regions

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Ischemic episodes of the brain are one of the most common cause of disability and death worldwide and developing effective therapies against ischemia-reperfusion injury seems important. As mitochondria emerges as a key element determining cell fate, **the aim of this study is to evaluate the potential involvement of mitophagy, mitochondria biogenesis and mitochondria dynamics in neuronal survival after ischemia and reperfusion injury. In gerbil model of transient brain ischemia** (IR), in which the same stimulus, 5 min. common carotid arteries occlusion, causes different cell survival rate of hippocampal neurons in CA1 (more vulnerable) and CA2-4,DG regions (resistant to 5 min I/R), we evaluate a time course of autophagy (by immunodetection of LC3-I/II, p62, PINK1, Parkin) and mitochondria biogenesis (PGC1α, NRF1, TFAM) in a context of mitofusin 2 (Mfn2) role in mitophagy.
We observed diverse effect of IR on protein markers in CA1 *vs* CA2-4,DG indicating potentially different mitophagy and mitochondria biogenesis dynamics. Protein level of LC3-I/LC3-II was much more increased in CA1 pointing at autophagy induction. In CA2-4,DG increased TOM22 immunoreactivity followed by an increase in mitochondria biogenesis markers, especially TFAM were observed. Moreover, Mfn2 level decreased in CA1 and increased in CA2-4, DG after IR. Further research, concerning mitochondrial to nuclear ratio analysis and hematoxylin-eosin stain of brain sections, are conducted to confirm involvement of Mfn2 and this phenomena in endogenous neuroprotection of CA2-4,DG hippocampal neurons.

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Investigating Microglia Phagocytosis in Health and Disease using Human Stem Cell Technologies and Multi-Omic Organelle Profiling

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Microglia are the innate mononuclear phagocytes of the central nervous system that can phagocytose infectious particles, apoptotic cells, neurons, synapses and pathological protein aggregates. Microglia phagocytosis is crucial for normal brain development and brain tissue homeostasis. Impaired microglia phagocytosis is associated with numerous pathologies of the brain, including inflammatory and neurodegenerative diseases. However, only little is known of the composition of the microglia phagosomal machinery and the luminal phagosomal content in heathy and pathological conditions. Thus, we developed a method to rapidly isolate intact microglia phagosomes for proteomic and metabolomic profiling. Phagosomes were isolated from human pluripotent stem cell derived microglia in mono-culture, in neural co-cultures and from mouse brain microglia.

This approach allowed to detect dynamics in the microglia phagosomal machinery under different conditions. The experimental data suggests that the microglia phagosome is a signaling hub which can sense, integrate and control microglia behavior in response to changes in its microenvironment.

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Early alterations in transcription of genes coding NAD dependent- enzymes and mitochondria proteins in Alzheimer's disease animal model. Novel target(s) for neuroprotection

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Oxidative stress (OS) and disturbances of mitochondria are proposed to play a crucial role in pathogenesis of Alzheimer Disease (AD).-

In this study we analysed transcriptions of genes encoded enzymes of anti-oxidative defence including Sirtuins (Sirts) and also poly (ADP-ribose) polymerases (PARPs) in brain cortex at early stage of AD mice. Moreover, expression of genes related to mitochondria dynamic and function was analysed.

The study were carried out, using brain cortex from 3 and 6 months old AD transgenic mice FVB (APP+) with London mutation (V7171) and compared with control mice without transgene (APP-). Biochemical and qPCR methods, were applied.
Our data indicated downregulation of gene coding Sirt1, Sirt3 in 3 months AD Tg mice and alterations of gene coding PARP-1 in 3 and 6 months old AD Tg mice. Moreover, significant suppression of gene transcription for superoxide dismutase 2 (SOD2) was found. Then the significant up-regulation of mRNA levels of Fis1 and Drp1, crucial in mitochondrial fission in 3 and 6 months old AD Tg was demonstrated. Concomitantly mRNA levels of OPA1 - protein engaged in mitochondria fussion and Mitochondrial Transcription Factor A (TFAM) and also cytochrome C oxidase was up-regulated in 3 and 6 months old AD Tg. However, the expression of genes coding other subunits of Electron Transport Chain (ETC) complexes was slightly downregulated in AD Tg mice. Our data suggest that activator of Sirt1 and inhibitors of Drp1 or Fis1 may exert promising neuroprotective effects at early stage of AD.

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Judgement of Line Orientation and Contrast Sensitivity as Measures of Parkinson's Disease: A Systematic Review and Meta-Analysis

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Parkinson's Disease (PD) is best characterised by its motor signs, however, non-motor symptoms, including visuospatial deficits, commonly appear. Characterising these quantitatively could lead to the development of objective tools to monitor disease progression and predict further visual and cognitive decline. This study aimed to systematically review and meta-analyse the literature on two visuospatial measures, Contrast Sensitivity (CS) and Judgement of Line Orientation (JLO), to critically evaluate their utility as quantitative measures of PD.
The literature search was conducted using three databases (MEDLINE, EMBASE, PsycINFO). The search was completed on 15/12/2020. CS measured by the Pelli-Robson chart [1] and Benton's JLO test [2] allowed for statistical pooling of results. Pooled incidence and outcome measures were calculated through a random effects model utilizing an inverse variance DerSimonian-Laird estimator. Study bias was appraised through Egger's Test and Begg's Test. Analyses were performed in StataV16.
1880 articles were identified, 118 full texts screened with 60 studies (32 CS; 28 JLO) included in the qualitative synthesis and 22 (11 CS; 11 JLO) included in the meta-analysis. JLO (SMD = -0.73) and CS (SMD = -0.61) were significantly decreased ($p < 0.01$) in PD patients compared to healthy controls. Both were found to have low risk of publication bias.

This study confirmed CS and JLO as objective measures to distinguish between PD patients and healthy controls. However, the findings are limited by heterogeneity in disease duration and cognitive impairment. Further large-scale studies are needed to assess the utility of these metrics as objective biomarkers of PD.

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Effects of amyloid Aβ1-42 application on impulse activity of rat isolated hippocampal neurons

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An extracellular amyloid plaques formation plays a leading role in Alzheimer's disease development and also can affects the neuronal discharges in hippocampus. The common Aβ disturbances in the brain are mainly investigated. In contrast, the effect of Aβ at the single-cell level remain unexplored. Our study described the *in vitro* effect of amyloid Aβ1-42 on the spike generation in isolated neurons recorded from CA1 zone of the rat hippocampus using perforated patch-clamp technic and peptide concentrations from 200 nM to 10 μM in the external solution. To induce the spike activity, the intracellular stimulation by one second rectangular current pulses and ramp protocol were applied [1]. The amyloid Aβ1-42 produced a double effect on spike activity, and usually increased the neuronal firing. In the main group of neurons, amyloid application decreased the threshold current for action potential generation within a minute. In the second group of neurons, amyloid did not change the threshold but moderately increased the firing frequency by 15% with the same gain in generation frequencies at different input current levels. Our observations did not reveal the direct short-term harmful amyloid effect on neuronal firing. Also, Aβ1-42 increased the firing frequency and reduced the threshold required for action potential generation. Considering the above, we believe that long-lasting exposure to the elevated Aβ1-42 will cause synaptic dysfunctions due to a prolonged increase in neuronal firing.

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Insight into the structure of Disrupted in Schizophrenia 1 through integration of bioinformatics and empirical data

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Disrupted in Schizophrenia 1 (DISC1) is a multi-functional scaffolding protein crucial for neurodevelopment, which has been implicated in various mental illnesses. Although its roles in the brain have been extensively studied, research into its structure is lacking. Only recently, an empirical approach was employed to identify four distinct domains[1]. Protein domains represent structurally stable, folded regions and as such, any incomplete or misfolded domains would undergo proteasomal degradation. Here, we aim to test the stability of experimentally obtained domains by proteasome inhibition, as well as refine their boundaries using previously published bioinformatics data.

Previous bioinformatics predictions of DISC1 sequence repeats indicate the existence of conserved structures, in the form of α-helices connected by loops[2]. These structural loops are in overlap with some of the experimentally proposed domains. By expressing the constructs encoding different DISC1 regions in mammalian cell cultures, and inhibition of the proteasomal function, we have shown that the loops are indeed essential for the domain stability. Moreover, changing the boundaries of some experimental domains to match the end of the theoretical loops noticeably increased their expression, suggesting that current DISC1 domain boundaries should be redefined. Further testing of these redefined domains in neuroblastoma cells, through interactions with proteins involved in brain development, will serve as an additional confirmation of their stability. Thus, integration of these two approaches will enable further insight into DISC1 structure, which can help elucidate its inner workings in the context of neurodevelopment and mental illnesses.

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Towards the Application of Lanthanide-based Upconversion Nanoparticles in Drug Delivery and Optogenetics

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Nanoscale particles that exhibit photon upconversion are called upconversion nanoparticles. During photon upconversion, the incident low energy photons are absorbed and a photon with higher energy is emitted [1]. The incorporation of functional nanoparticles into implantable microdevices opens up new opportunities to go beyond the limitation of current neurotechnology, and this effort is envisioned to result in novel, integrated functionalities tailored by recent achievements of organic chemistry. In our approach lanthanide-based upconversion nanoparticles was immobilized on microfabricated neural implant surfaces. One half of the as-invented structure was further modified with fluorescence model drug molecules. Surface modification and functional groups was defined using SEM and ATR-FITR, respectively. Long term mechanical stability was tested using agar gel model, and drug release was also monitored using fluorescent microscopy. Our results indicate that the described complex system has the potential to be applied *in vivo* experiments, as precisely localized drug carrier with time-dependent drug release in a controllable manner. Furthermore, with the application of near-infrared light it is feasible to reach deeper regions in the brain and express less phototoxicity when controlling a neuron 's activity using upconverted UV light.

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Psychosocial crowding stress has a specific effect on gene expression in certain regions of the rat frontal cortex

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The hallmark of stress-related psychiatric disorders is disturbances in glutamate signal transduction within the frontal cortex (FC) evoked by stress exposure [1]. Our previous studies in rats revealed that chronic psychosocial crowding stress (CS) reduces glutamate receptor expression in FC and modulates synaptic transmission in the primary motor cortex (M1) [2]. Recently we showed that β-adrenergic receptors (βAR) blockade alleviated chronic stress effects on cerebral GTPases expression [3]. The current study aimed to assess the impact of the CS on the mRNA expression of β(1-3)AR, AMPA receptor GluA1,

GluA2 subunits, and their downstream effectors engaged in neuron remodeling after stress in two regions of the frontal cortex: mPFC and M1. We utilized the rat model of CS applied for 3, 7, and 14 days and RT-qPCR technique with custom TaqMan Array (TLDA) cards for simultaneous evaluation of 24 target genes in a single sample. Statistical analysis showed alterations in mRNA level dependent on the time of stress exposure and FC region. In M1, the expression of β3AR and GluA1 mRNAs was increased in the CS7d group *vs*. control, while in mPFC, β3AR decreased in CS3d, and CS14d GluA1 stayed unchanged. Among intracellular proteins, in M1, the mRNA level for kinase FAK, phosphatase STEP, GTPase Rac1, and RapGEF3 was increased while in mPFC decreased in CS14d group *vs*. control. Our results revealed differences in the CS effects on the intracellular signaling in two adjacent FC regions, which may help to understand biochemical mechanisms of stress-related synaptic plasticity and neuropsychiatric diseases.

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Diaph1 signaling in the development of diabetic neuropathy

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Diabetic neuropathy is the most common neurological complication of diabetes, affecting from 30 to 70% of all diabetic patients worldwide [1]. Although the exact mechanisms of diabetic neuropathy are not fully understood, growing evidence suggests that pathological changes might result from several

concomitant factors such as increased local inflammation, cellular oxidative stress, excessive protein glycation and axonal transport alteration [2]. Diaph1 is mainly known for its role in actin and cytoskeleton structural modifications, however over last decade it also gained attention as a RAGE binding partner. Studies indicate that Diaph1 expression might be tightly correlated with RAGE-triggered excessive protein glycation and inflammation, affecting axonal transport and contributing to the development of diabetic neuropathy [3]. Here, in our study we used electroneurography, morphometry, qRT-PCR and immunoblotting to show electrophysiological and structural alterations as well as changes in expression of Diaph1, RAGE and actin in peripheral nerve over the course of 6 months of pharmacologically induced diabetes in mice. Our study revealed slow decline in motor and sensory nerve conduction, increased latency and progressive loss of axons over the course of disease; in addition, we also observed progressive increase in mRNA expression of RAGE and Diaph1 and decrease in actin, partially corresponding to immunoblotting results with high protein level of RAGE but lower levels of Diaph1 and actin. Based on our results, we might speculate that both Diaph1-RAGE driven inflammatory signaling as well as actin related axonal transport impairment play a pivotal role in the development of diabetic neuropathy.

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Median Raphe controls acquisition of negative experience

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Acquisition of negative experience is essential for the survival. Negative stimuli immediately and simultaneously activate the main aversive centers, the lateral habenula (LHb) and medial ventral tegmental area (mVTA) and the memory processing septo-hippocampal system. However, it is still unknown, which neurons coordinate these processes during negative experience. Here, we found that the brainstem median raphe region (MRR) harbors a new type of excitatory population of neurons that expresses vesicular glutamate transporter 2 (vGluT2). With anatomical tracing experiments we found that these neurons innervate LHb, mVTA and the septo-hippocampal system and they receive inputs from negative experience-related brain centers. With *in vivo* optical tagging method we found that MRR

vGluT2 neurons are rapidly and selectively activated during aversive, but not rewarding events. Optogenetic stimulation of MRR vGluT2 neurons induced acute and conditioned place aversion, suppressed reward seeking behavior and created memory acquisition-promoting hippocampal theta-oscillations. By contrast, optogenetic inhibition of MRR vGluT2-neurons during an aversive foot-shock impaired both contextual and cued fear memory-formation and prevented fear generalization. Our results suggest that MRR vGluT2-neurons are both necessary and sufficient to acquire negative experience and they may play an important role in several types of mood disorders.

Conflict of interest: The authors have no conflict of interest.

The role of astrocytes in the progression of Alzheimer ‘s disease

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Alzheimer ‘s disease (AD) is the most common form of age-related neurodegenerative diseases [1]. The key neuropathological hallmarks of AD are extracellular plaques, consisting of aggregated Aβ, intracellular neurofibrillary tangles, consisting of phosphorylated tau, and chronic inflammation [2]. The inflammatory process changes astrocyte metabolism, so that the cells adapt and optimize their energy reservoirs, energy production, and mitochondrial dynamic to pathological conditions [3]. To investigate the processes behind AD– triggered astrocytic changes, iPSC-derived astrocytes were treated with sonicated Aβ fibrils for 7 days and then cultured for additional 0 to 12 days in Aβ-free media. Our data showed that to maintain cellular metabolism, Aβ-treated astrocytes initially display increased mitochondrial fusion, but at later time-points the Aβ-triggered pathology leads to the swelling and fission of mitochondria. Apart from changes in mitochondrial morphology, we noted disbalances in DRP-1 phosphorylation at serine 616 and 637. Confocal microscopy suggested secretion of these modified DRP-1 forms to media, which was confirmed by Western Blot studies of extracellular vesicles. Moreover, increased lipid droplet accumulation was noted in Aβ-treated astrocytes. Further studies confirmed a metabolic shift to peroxisomal-based fatty acid β-oxidation and glycolysis. Taken together, our studies showed that Aβ pathology profoundly affects astrocytes and changes the entire cell metabolism. However, an exact explanation of these processes will require further studies.

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