PHD MEETING
PRAGUE 2022

November 1st - 2nd, 2022

CONTENT

THE ORGANIZING COMMITEE

Ph.D. students:

Felipe Martínez-Ramírez
Alice Abbondanza
Milica Drapšin
Klevinda Fili
Šárka Danačíková
Pavla Průchová
Maria Jose Ruiz Estrada
Michaela Rusková
Rimjhim Tomar
Maria José Saucedo

Head of the Ph.D programme:

Martin Balaštík, Ph.D.

Secretariat of the Institute:

Diana Moosová

PROGRAMME

November 1st, 2022

8:30 – 9:00	Registration
9:00 – 9:10	**Opening speech and welcoming MUDr. Jan Kopecký, DrSc.**
9:10 – 9:15	**Introduction to the PhD meeting: Martin Balaštík, Ph.D.**
9:15 – 9:35	1st year Ph.D. students' presentations
9:40 – 10:45	**Session 1** – Students' oral presentations
	Chair: *María José Ruiz-Estrada*
	1. **Marko Mitrović** - Effect of a single bout of exercise on PAHSA lipokine levels in the circulation.
	2. **Sara Stanić** - Fibroblast growth factor 21 and CL 316,243 synergistically ameliorate metabolic abnormalities in dietary obese mouse
	3. **Guillermo Puertas-Frias** - Metabolic rewiring as an adaptive mechanism in CIV null cells
	4. **Zuzana Korandová** - Oxygen consumption measurements of cryopreserved PBMCs as a new diagnostic tool for mitochondrial diseases
	5. **Felipe Martínez-Ramírez** - Screening and identifying FAHFAs in edible mushrooms by Liquid chromatography – Tandem Mass Spectrometry (LC-MS/MS)
	6. **Andrea Beňová** - Novel TDZ analog MSDC-0602K manifests a different impact on bone and mesenchymal stem cell properties compared to classical TZDs
10:45 – 11-15	Coffee break
11:15 – 12:30	**Session 2** - Students' oral presentations
	Chair: *Milica Drapšin*
	1. **Karolína Hrůzová** - Early disruption of social memory in a TgF344-AD rat model of Alzheimer's disease
	2. **Daniela Černotová** - Anxiety and social-like deficits in Alzheimer's disease in the TgF344-AD rat
	3. **Sofiia Moraresku** - Timing of allocentric and egocentric spatial coding in human intracranial EEG
	4. **Daniela Kunčická** - Poly(I:C) molecular weight causes sex-dependent deficits in neurodevelopment and adult behavior in offspring in the maternal immune activation model
	5. **Anna Gunia** - Visuospatial perspective-taking brain dynamics captured by iEEG
	6. **Michaela Rusková** - Effect of microtubule detyrosination and tyrosination on CRMP2 and neuronal development
12:30 – 14:00	Lunch break
14:00 – 14:15	**"Influence of pipette technical specification to your pipetting results"** by Sipoch Gilson **Moderator**: *Pavla Průchová*
14:15 – 16:00	**Scientific Talk + Workshop: "Stress Management"** by Mgr. Anna-Marie Pospíšilová **Moderators:** *Rimjhim Tomar & Šárka Danačíková*
16:00 – 18:00	Coffee break & **Poster session 1**
18:00 – 20:00	Networking + Snacks

November 2nd, 2022

8:30 – 9:00	Registration
9:00 – 10:00	**Session 3** – Students' oral presentations
	Chair: *Felipe Martínez-Ramírez*
	1. **Antonín Sedlář** - Galectin-3 as a potential target for reversing vascular remodeling in hypoxic pulmonary hypertension
	2. **Rohit Ashok Joshi** - Nedd4-2 binding to 14-3-3 modulates the accessibility of its catalytic site and WW domains
	3. **Marine Morvan** – Chiral and aging proteomics applied to bovine and rat collagen
	4. **Milica Drapšin** - Circadian clocks in choroid plexus and its sensitivity to chronodisruption
	5. **Rimjhim Tomar** - Odor background increases the pheromone coding efficiency in moth olfactory neurons
	6. **Bazila Bazila** - Mitochondrial morphology and metabolism in pancreatic β – cell from IF1 KO mice
10:00 – 10:30	Coffee break
10:30 – 12:00	**Discussion panel: "Women in Science"** **Moderators:** *Alice Abbondanza & Klevinda Fili*
12:00 – 13:30	Lunch break
13:30 – 14:45	**Session 4** – Students' oral presentations
	Chair: *Michaela Rusková*
	1. **Jana Cimická** - Role of extracellular ATP and P2X receptors in hypothalamic paraventricular nucleus
	2. **Jakub Slepička** - Modulation of synaptic transmission in chemotherapeutics induced neuropathic pain
	3. **Monica Ponteraso** - The role of macrophage migration inhibitory factor (MIF) in neuropathic pain
	4. **Nikolai Chetverikov** - Molecular mechanisms of steroid effects on function of muscarinic receptors
	5. **Vera Abramová** - Creation and primary behavioural characterization of *Danio rerio Grin2ab* NMDA receptor subunit knockout by CRISPR/Cas genome editing
	6. **Yeva Prysiazhniuk** - Clinical translation of arterial spin labeling MRI in diagnostics of epilepsy, brain tumors, and cerebrovascular disease
	7. **Šárka Danačíková** - Development of *in vitro* methods for personalized diagnostics and treatment of Epilepsy
14:45 – 15:15	Coffee break
15:15 – 16:45	**Poster session 2**
16:45 – 17:00	IPHYS Popularization by Ing. Olga Zimmermannová, Ph.D.
17:00 – 17:30	**Awards announcement and Students questionnaire results**
17:30 – 18:00	**Closing speech**
18:00 – 20:00	Dinner
20:00 – 22:00	Live music

Poster Session - November 1st, 2022

No.	Main author	Poster title
1	Dobrovolskii, M.	*De novo* mutations and rare variants occurring in NMDA receptors: structure-function characterization and pharmacological screen of novel therapeutically relevant NMDA receptor modulators
2	Kysilov, B.	Role of C-terminal domains of the NMDAR receptor in the control of sensitivity to potentiating neurosteroids
3	Kuchtiak, Viktor	Functional analysis of genetic variations in the cytosolic domain of NMDA receptors
4	Fili, K.	Functional assessment of mice carrying a *de novo* missense *Grin2b* mutation associated with Autism Spectrum Disorder (ASD)
5	Boroš, A.	Effects of Acetylcholinesterase inhibition with Pyridostigmine on cardiovascular system in spontaneously hypertensive rats
6	Opletalová, B.	HIF-1α: an essential element for cardioprotection and proper mitochondrial function during adaptation to chronic hypoxia
7	Doubková, M.	Towards an *in vitro* model of clubfoot fibrosis: Enhancing extracellular matrix production with macromolecular crowding
8	Pohl, P.	14-3-3-protein regulates Nedd4-2 by modulating interactions between HECT and WW domains
9	Marković, A.	Searching for function of TMEM70, TMEM242 and c15orf61 – Recently identified interactors of subunit C from mammalian F_oF_1 ATP synthase
10	Heleš, M.	Mu-opioid receptor desensitization in the spinal cord dorsal horn is reduced by the endogenous TRPV1 agonist N-oleoyldopamine
11	Vasconcelos, D.	Role of the KCTD16 protein in modulation of $GABA_B$ receptors under pathological neuropathic pain condition
12	Shekhar, N.	The role of RF-amide peptide receptors GPR10 and NPFFR2 in energy homeostasis and diet-induced obesity
13	Kalendová, V.	Metabolic effects of n-3 Fatty acids as Calanus oil in transgenic mice with modified PPARα expression
14	Haasová, E.	Effect of ambient temperature on skeletal muscles non-shivering thermogenesis in mice differing in propensity to obesity

Poster Session - November 2nd, 2022

No.	Main author	Poster title
1	Búran, P.	Maternal auto-antibodies and their role in autism spectrum disorder
2	Ruiz-Estrada, M. J.	TRAK1 modulation of mitochondrial dynamics in neurons
3	Weissová, R.	Prolyl isomerase fkbp12 binds to crmp2a and regulates microtubule dynamics
4	Gotvaldová, K.	Role of BCAA in oxidative metabolism and lipid droplet biogenesis in pancreatic cancer cells
5	Průchová, P.	Antioxidant role and cardiolipin remodeling by redox-activated mitochondrial Ca^{2+-} independent phospholipase A2γ in the brain
6	Nelic, D.	Biased agonists of muscarinic receptors
7	Abbondanza, A.	Deletion of beta2* nicotinic acetylcholine receptors in striatal interneurons inhibit striatal activity and control striatal-dependent behaviors.
8	Masaryk, J.	The main potassium importer in yeast responds to decrease in intracellular potassium concentration by increasing affinity and maximum velocity
9	Domanská, V.	The role of peroxiredoxin 6 in biosynthesis of anti-diabetic and anti-inflammatory FAHFA
10	Rakušanová, S.	LC–MS workflow (LIMeX) for untargeted metabolomics and lipidomics analysis of mouse plasma, feces, and cecum-content
11	Dočkal, T.	Deciphering the impact of the reversed restricted feeding on the circadian clock in choroid plexus
12	Krajčovič, B.	Modulation of immediate-early gene expression in the hippocampal CA1 by long-term and short-term behavioral experience
13	Malenínská, K.	Chemogenetically induced brain-wide reduction of Parvalbumin interneurons activity: Electrophysiological preliminary study
14	Radostová, D	A novel one-trial association task relevant to episodic-like memory in rats

1st year PhD students – Short informal presentation

Name	Department
Pražák Šimon	Laboratory of Biomaterials and Tissue Engineering
Yu-Chieh Wu	Laboratory of Biomaterials and Tissue Engineering
Havelková Jarmila	Laboratory of Biomaterials and Tissue Engineering
Janošev Maša	Laboratory of Structural Biology of Signaling Proteins
Saba Selvi	Laboratory of Biological Rhythms
Alexandra Ptakova	Laboratory of Cellular Neurophysiology
Zavbi Jus	Laboratory of Molecular Neurobiology
Monika Křivonosková	Laboratory of Mitochondrial Physiology

Invited speaker – "Stress management"

Mgr. Anna-Marie Pospíšilová is a psychologist working with students and employees of Charles University. She helps dealing with challenging life situations by helping people understand their individual needs and then finding coping mechanisms for them. She is experienced in topics of high sensitivity, work/study-life balance and self-care.

Research suggests that chronic stress contributes e.g. to high blood pressure or causes brain changes that may contribute to anxiety, depression, and addiction. In this workshop we will dive into understanding the stress response (known as fight-flight-freeze) and how to cope (and prevent) effects of long term stress. One of the main focuses will be on mindfulness, which is considered as one of the most impactful and protecting tools in stress coping. Studies have shown that stress-related health problems like anxiety and depression might be treatable with regular practice of mindfulness.

Invited discussion panelists – "Women in Science"

Dr. Michaela Tencerová is the head of the Laboratory of Molecular Physiology of the bone at the Institute of Physiology of the Czech Academy of Sciences in Prague. She obtained her PhD in 2010 at Charles University in Prague. Through two abroad Postdoctoral trainings in Prof. Michael Czech`s lab (UMASS Medical School, USA) and Prof. Moustapha Kassem`s lab (SDU, Odense, Denmark) she moved her research interest from immunometabolism in obesity and diabetes to studying bone marrow adiposity and interactions between bone and stem cells metabolism in relation to metabolic diseases. Her lab is focused on studying the role of bone marrow adiposity in the regulation of bone and whole-body metabolism. She is a member of Scientific board of the Bone Marrow Adiposity Society (BMAS), ABMR Women in Science committee and ECTS Basic science committee. She has obtained several international and national grants and young investigator awards, including The L'Oréal–UNESCO for Women in Science award 2021.

Dr. Lydie Plecitá-Hlavatá is a principal investigator in the Laboratory of Mitochondrial Physiology at the Institute of Physiology of the Czech Academy of Sciences. She graduated from Charles University in Prague in 2004. She started her career studying oxidative stress and ageing in a yeast model in the laboratory of Prof. Nyström, at Göteborg University, Sweden, and in the laboratory of Prof. Breitenbach at the University of Salzburg, Austria. After joining Dr. Ježek in 2006, she focused on studies of mammalian cell metabolism with emphasis on mitochondria, energy metabolism, and redox signalling. She pioneered the study of mitochondrial morphology using super-resolution fluorescence microscopy. In 2011, she started a collaboration with Prof. Stenmark at the University of Denver, Colorado, USA, where she is still involved in studying the mechanism of pulmonary arterial hypertension. In the same year, she began her research on the role of redox homeostasis and signalling in pancreatic beta cells. She led the construction of a new mouse strain carrying a NOX4 conditional mutation for diabetic studies, which was recently described in the journal of Diabetes (2020). She currently searching for targets of NOX4 signaling in pancreatic beta cells.

Invited discussion panelists – "Women in Science"

Dr. Helena Janíčková is a scientist in the laboratory of Neurochemistry at the Institute of Physiology CAS. Dr. Janičkova completed her PhD in neuroscience at the Institute of Physiology of the Czech Academy of Sciences and she spent 4 years of her postdoctoral training at the University of Western Ontario in Canada. Her scientific research tries to understand how acetylcholine receptors in different brain regions control behavior and neural functions. Currently, she is mainly focusing on the function of nicotinic receptors expressed by different types of GABAergic interneurons in the striatum and the prefrontal cortex. In addition, she recently started testing polymer nanoparticles to see if they can be used for targeted delivery of compounds to specific neuronal populations. She has active collaborations with laboratories at Sorbonne University in France and Ulster University in the United Kingdom. In addition, during her research career, she has been raising three children, mostly in close collaboration with her husband.

Dr. Hana Macíčková Cahová is a Junior research group leader of laboratory of Chemical Biology at the Institute of Organic Chemistry and Biochemistry CAS (IOCB) Prague. Hana completed her Ph.D. in organic chemistry in the group of prof. Michal Hocek at IOCB, where she developed techniques for enzymatic synthesis of modified DNA. Afterwards, she was awarded Alexander von Humboldt fellowship and she studied cofactor modified RNA and developed new type of photo-switchable DNA in the group of prof. Andres Jäschke at Heidelberg University. In 2016, she received The European Research Council (ERC CZ) funding and established her own independent group with focus on RNA modifications in viruses. In her career, she was awarded several prizes, namely, the Neuron Award for Young Scientists in Chemistry, Werner Siemens award for excellent female work, Otto Wichterle Award for Young Scientists and Alfred Bader Prize for Young Bioorganic Chemists. In 2022, she was awarded ERC StG to study non-canonical RNA caps as a cellular reaction to environment and stress. Besides her research career, she is the mother of three children.

ORAL PRESENTATION ABSTRACTS

EFFECT OF A SINGLE BOUT OF EXERCISE ON PAHSA LIPOKINE LEVELS IN THE CIRCULATION

M. Mitrovic[1,2], M. Riecan[1,3], O. Horakova[1], O. Kuda[1], M. Rossmeisl[1]

[1]Institute of Physiology, Academy of Science of the Czech Republic, [2]The First Faculty of Medicine, Charles University, [3]The Faculty of Science, Charles University

Exercise is an important tool in the prevention and treatment of metabolic disorders. Beneficial effects of exercise are partly based on improving adipose tissue function. Adipose tissue is a source of novel lipokines, i.e. palmitic acid esters of hydroxy fatty acids (PAHSA), which possess potent anti-inflammatory and insulin-sensitizing properties. We have recently shown that regular exercise increases total PAHSA levels in adipose tissue and circulation in humans. However, it is unknown how a single bout of exercise regulates circulating levels of PAHSA and their regioisomers. Therefore, in lean male C57BL/6J mice exposed to treadmill running, we first tested several exercise protocols in terms of achieving the maximum lipolytic stimulus, and then measured plasma PAHSA levels by liquid chromatography/mass spectrometry. Running mice were compared with sedentary animals exposed to a treadmill without running. Low-intensity treadmill exercise (8 m/min for 40 min, then increasing by 0.5 m/min every 10 min until exhaustion) was most effective in terms of elevating plasma non-esterified fatty acids and glycerol levels immediately after exercise. This was accompanied by an increase in plasma levels of 9-PAHSA (running, 355.9 ± 35.5; sedentary, 153.2 ± 25.5 pmol/L; $P<0.001$) and 11-PAHSA (running, 72.1 ± 10.5; sedentary, 19.6 ± 5.6 pmol/L; $P<0.01$). Single bout of exercise is able to raise circulating PAHSA levels in lean mice, but the complete PAHSA profile in the post-exercise period and its dependence on age or obesity remain to be determined.

This project was supported by grant No. NU21-01-00469 from the Czech Health Research Council.

FIBROBLAST GROWTH FACTOR 21 AND CL 316,243 SYNERGISTICALLY AMELIORATE METABOLIC ABNORMALITIES IN DIETARY OBESE MOUSE

Sara Stanić [1, 2], Blanka Haberlova [1], Jan Kopecky [1], Petr Zouhar [1]

[1]Institute of Physiology, Academy of Science of the Czech Republic, [2]Department of Animal Physiology, Faculty of Science, Charles University

Obesity is accompanied by numerous metabolic complications. Fibroblast Growth Factor 21 (FGF21) is a potential therapeutic agent shown to improve insulin sensitivity and lower blood glucose in obese mice. CL 316,243 (CL) is a beta-3 adrenergic agonist that induces lipolysis and acutely lowers blood glucose in lean, but not in obese mice. So, a combination of FGF21 and CL could be a beneficial treatment of obesity-associated impairment of glucose and lipid metabolism. We tested the effects of such combination on chow-fed and dietary obese C57BL/6J mice. In both lean and obese mice, 35 μg CL (i.p.) acutely induced lipolysis leading to a rise of free fatty acids (FFA) in plasma. FFA are known to induce insulin secretion. As expected, the insulin surge led to an acute drop of blood glucose in lean but not in obese (insulin-resistant) mice. The 4-8 d pre-treatment with 47.25 μg FGF21 (2x daily s.c.) increased insulin sensitivity and lowered basal insulin levels in obese mice. Injection of CL to FGF21-treated obese mice resulted in a smaller rise of insulin but a significant decrease of blood glucose. Thus, FGF21 sensitized the obese mice to glucose-lowering effect of CL. In our model of dietary obese mice, we demonstrate that 24 h after CL injection the FGF21 pretreated mice exhibited lower FFA levels and were also protected from hepatic triglyceride accumulation. In conclusion, we demonstrate beneficial effects of combined FGF21 and CL treatment in obese mice. FGF21 sensitizes the obese mice to insulin and restores glucose-lowering effect of CL. Also, FGF21 modifies the lipolytic effect of CL preventing ectopic lipid accumulation in liver.

METABOLIC REWIRING AS AN ADAPTIVE MECHANISM IN CIV NULL CELLS

G. Puertas-Frias[1], K. Čunátová[1], P. Pecina[1], M. Vrbacký[1], J. Eliáš[1], L. Alán[1], T. Čajka[1], J. Houštěk[1], T. Mráček[1], A. Pecinová[1]

[1]*Institute of Physiology, Academy of Science of the Czech Republic*

Mitochondria are responsible for ATP provision, redox balance maintenance and the supply of anabolic intermediates. They conduct oxidative phosphorylation (OXPHOS) through the action of the electron transport chain (ETC, complexes CI-CIV) and ATP synthase (CV). Reduced cofactors in the matrix, produced mainly by the tricarboxylic acid (TCA) cycle, fuel the ETC, which transfers reducing equivalents to oxygen and allow oxidative metabolism to progress. Defects in OXPHOS can impair cellular metabolism and ATP provision, causing mitochondrial disease in human patients. Such impairments trigger the rewiring of metabolic pathways to bypass the deficiency and maintain homeostasis. We studied metabolic rearrangements in HEK293 cells lacking CIV subunit COX6b1, which leads to total loss of the assembled enzyme. The resulting impairment on ETC caused a decrease on NAD^+/NADH and absence of respiration, compensated by the activation of aerobic glycolysis to cover ATP demands. Expression of the alternative oxidase (AOX) partially recovered the phenotype, but aerobic glycolysis was maintained. Metabolic tracing experiments of $U^{13}C$-glucose and $U^{13}C$-glutamine uncovered a severe impairment of glucose oxidation in mitochondria, which is mostly converted to lactate to support the higher glycolytic rate. Reductive carboxylation of glutamine was found to be an important pathway necessary to replenish the TCA cycle metabolite pools, accumulating succinate through both the oxidative and the reductive branches. This rearrangement results in the production of cytosolic acetyl-CoA from the breakdown of citrate, which can enter *de novo* lipogenesis. Interestingly, we found an accumulation of triglycerides containing glutamine-derived acyl chains in the KO cells, which was normalised by the expression of AOX. We propose the use of triglycerides as alternative electron stores in these cells.

The project is supported by Grant Agency of the Czech Republic (21-18993S).

OXYGEN CONSUMPTION MEASUREMENTS OF CRYOPRESERVED PBMCs AS A NEW DIAGNOSTIC TOOL FOR MITOCHONDRIAL DISEASES

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Mitochondrial diseases are severe inherited metabolic disorders affecting paediatric population. They are caused by defects of mitochondrial biogenesis or by mutations in the structural subunits of oxidative phosphorylation (OXPHOS) apparatus. Because mutations in mtDNA are responsible for only 25% of mitochondrial disorders and other mutations could not be detected by next-generation sequencing, thus biochemical analysis demonstrates a powerful tool in diagnostics. Instead of invasive sample collecting such as muscle or skin biopsy for derivation of a fibroblast culture, we can identify a mitochondrial defect manifested in peripheral blood lymphocytes. We developed a protocol for peripheral blood mononuclear cells (PBMCs) isolation from 7 ml of children's blood using Ficoll centrifugation and optimised freezing conditions to achieve high viability of PBMCs required for functional measurements. Development of standard and reproducible protocol for the isolation and freezing of PBMCs is a very important step for collaboration of any paediatric diagnostic centre and biochemical laboratories. To establish this approach as an alternative diagnostic tool, we adapted and optimized highly sensitive oxygraphy of intact and digitonin-permeabilized PBMCs for analysis of the function of mitochondrial respiratory chain and glycolysis. Based on our previous results, we purposed the best respiratory parameters and their ratios predictive for defects of OXPHOS. Respiratory rates of coupled and uncoupled respiration, uncoupling control ratio (UCR) and relative respiratory contribution of complex I, II and GDPH could be indicative for different OXPHOS defects. Samples were obtained from more than 30 patients aged from 3 months to 60 years. Patients with known mutations were analysed as well as suspected mitochondrial patients.
Our study demonstrates sensitive, fast and non-invasive approaches for diagnostics of different types of mitochondrial disorders, especially of nuclear genetic origin manifesting in paediatric patients

This project is supported by the Ministry of Health of the Czech Republic (AZV NV19-07-00149).

SCREENING AND IDENTIFYING FAHFAS IN EDIBLE MUSHROOMS BY LIQUID CHROMATOGRAPHY - TANDEM MASS SPECTROMETRY (LC-MS/MS)

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Different species of edible mushrooms are popular in central and Eastern Europe. Credible evaluations of their nutritional value are limited due to incomplete knowledge of their composition and the availability of their constituents. Fatty acids (FAs) make up a significant portion of the lipid content in mushrooms despite the overall amount of lipids is small. Branched fatty acid esters of hydroxy fatty acids (FAHFAs) are a unique class of bioactive lipids that have favorable effects on human health. FAHFAs are frequently found in human food, including fish, rice, and mushrooms. Although nutritional studies have analyzed the lipid composition of mushrooms, they have overlooked their FAHFAs composition. In this context, this study aims to characterize the FAHFA profiles of various edible mushrooms. We have identified more than 60 different fatty acids, including 34 FAHFAs. Saturated and unsaturated FAs, mainly long carbon chain acids, accounted for the total FAHFAs, being linoleic acid derivatives the most abundant. Some FAHFAs identified in Shiitake (*Lentinula edodes*) and Enokitake (*Flammulina velutipes*) mushrooms significantly differ between species. At the same time, other FAHFAs in Shimeji brown (*Hypsizygus tessulatus*) and Shimeji white (*Hypsizygus ulmarius*) hold high similarities in kind and quantity. Using the liquid chromatography-mass spectrometry (LC-MS)-based lipidomics approach and bioinformatics analysis, we aim to identify differences in lipid content and species-specific FAHFAs. By enhancing the knowledge of FAHFAs, several types of information will be uncovered, namely the biological roles of FAHFAs in edible mushrooms and their chemotaxonomic relevance, as well as their potential use as nutraceuticals.

Supported by the grant from the Czech Academy of Sciences [Lumina Quaeruntur LQ200111901].

NOVEL TZD ANALOG MSDC-0602K MANIFESTS A DIFFERENT IMPACT ON BONE AND MESENCHYMAL STEM CELL PROPERTIES COMPARED TO CLASSICAL TZDs

[1,2]Andrea Benova, 1Michaela Ferencakova, [3]Kristina Bardova, [3]Jiri Funda, [4]Jan Prochazka, [4]Frantisek Spoutil, [5]Tomas Cajka, [1]Glenda Alquicer, [1]Martina Dzubanova, [8]Tim Balcaen, [6,7,8]Greet Kerckhofs, [9]Alena Pecinova, [9]Tomas Mracek, ,[3]Martin Rossmeisl, [3]Jan Kopecky, [1]Michaela Tencerova

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Insulin-sensitizing effect of thiazolidinediones (TZD) has been shown to act via mitochondrial pyruvate carrier (MPC) inhibition and increasing glucose uptake in cells. On the other hand, the use of TZDs has side effects on bone quality and differentiation capacity of bone marrow mesenchymal stem cells (BM-MSCs). MSDC-0602K as a novel thiazolidinedione (TZD) analog has been developed to reduce adverse effects of TZD therapy. However, it is not known whether MSDC-0602K may differently affect bone quality and differentiation potential of BM-MSCs and cellular metabolism compared to typical TZD. Twelve-week-old C57BL/6N male mice were divided into 4 dietary groups (n= 8-10 per group): chow (ND), high-fat-diet (HFD), HFD supplemented with pioglitazone (HFD+P) and HFD with MSDC-0602K (HFD+M). After 8 weeks of treatment, microCT analyses revealed improved cortical porosity in L5 vertebrae in HFD+M compared to HFD group. Interestingly, BMAT evaluation in proximal tibia showed increased number of smaller adipocytes in HFD+M vs. HFD group. Mechanical testing of the femurs showed increased bone strength in HFD+M vs. HFD group and no change in HFD+P group. Cellular analyses of BM-MSCs showed decreased adipocyte and higher osteoblast differentiation of HFD+M compared to HFD and HFD+P groups. Interestingly, measurement of oxygen consumption rate (OCR) showed higher maximal respiration of HFD-M BM-MSC) compared to HFD-P suggesting use of different substrates contributing to cellular metabolism. Indeed, acute treatment of BM-MSCs with MSDC-0602K induced preferential utilization of glutamine than glucose compared to pioglitazone-treated BM□MSCs, which was contrary to metabolic response of adipose derived mesenchymal stem cells after the treatment with TZDs. Taken together, our data showed positive effect of MSDC-0602K on bone phenotype and differentiation potential of BM-MSCs compared to pioglitazone via increased glutamine metabolism and opposite effect of TZDs in periphery compared to bone marrow, which may help in better understanding of their effect in different organ systems.

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EARLY DISRUPTION OF SOCIAL MEMORY IN A TGF344-AD RAT MODEL OF ALZHEIMER'S DISEASE

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Social memory dysfunction is a frequent yet neglected hallmark of Alzheimer's disease (AD). The hippocampus, particularly CA2 region, is implicated in social memory. We used 6-month-old TgF344-AD rats, an animal model of AD, to investigate their social behaviour and memory and electrophysiological properties of their hippocampal CA2 region. We tested TgF344-AD male and female rats in a modified 5-trial social memory test (5-TSMT). This task consists of four trials presenting the first rat intruder in the subject's home cage, followed by the fifth trial showing the second rat intruder. Compared to control F344 rats, TgF344-AD rats were less interested in the first intruders, indicating reduced sociability. Moreover, we found a diminished preference for social novelty in the TgF344-AD rats when the second intruder was presented. Next, we asked if AD is reflected in the electrophysiological properties of the CA2 in freely behaving TgF344-AD rats during the 5-TSMT and in the following memory consolidation during sleep. Analysis of electrophysiological local-field potential (LFP) recordings from bilaterally implanted electrodes in CA2 is currently underway. We aim to analyze the power of theta and gamma frequency bands and sharp-wave ripples distribution during the consolidation period

This project is supported by START/MED/099 grant, GACR grant GF21-16667K and GACR 22-08045S

ANXIETY AND SOCIAL-LIKE DEFICITS IN ALZHEIMER'S DISEASE IN THE TGF344-AD RATS

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Transgenic animal models in research of neuropsychiatric diseases consist primarily of mice. However, rats display a more complex behavioural repertoire, and their physiological and genetic background is more similar to humans. Therefore, studying Alzheimer's disease (AD) could benefit more from using rats rather than mice. We performed behavioural tasks assessing anxiety, spatial memory, and social interactions in 10- and 14-month-old TgF344-AD male and female rats and compared them to the control F344 rats. Our results showed that spatial acquisition and reversal in the Morris Water Maze task were surprisingly unaffected. In contrast, 10-month-old TgF344-AD male rats displayed an anxiety-like phenotype in the open field and elevated plus-maze. Interest in social interactions was significantly decreased in the TgF344-AD rats of both ages and sexes. We did not observe a worsening of the behavioural deficits with age. As the next step, we aim to analyse histopathological changes and their possible individual impact on performance.

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TIMING OF ALLOCENTRIC AND EGOCENTRIC SPATIAL CODING IN HUMAN INTRACRANIAL EEG

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Spatial reference frames (RFs) play a key role in spatial cognition, especially in perception, spatial memory, and navigation. There are two main types of RFs, which we routinely use and switch between: egocentric (self-centered) and allocentric (object-centered) RFs. Although many fMRI studies examined the neural correlates of egocentric and allocentric RFs, they did not sample the fast temporal dynamics of the underlying cognitive processes. Therefore, the interaction and timing between these two RFs remain unclear. Taking advantage of the high temporal resolution of intracranial EEG (iEEG), we aimed to determine the timing of egocentric and allocentric information processing and their relationships and describe the brain areas involved. In the first experiment, we recorded iEEG and analyzed broad gamma activity (50-150 Hz) in 37 epilepsy patients performing a spatial judgment task in a three-dimensional circular virtual arena. We found overlapping activation in response to egocentric and allocentric conditions in many brain regions, with the highest number being in the frontal cortex. In most cases, the egocentric response showed an earlier activation than the allocentric response. However, we also identified several brain regions with most of the channels selective to egocentric and allocentric conditions. Egocentric-selective channels were prevalent in the medial occipito-temporal region and in the supramarginal gyrus. In contrast, allocentric-selective channels prevailed around the intraparietal sulcus, and in the occipital and lateral temporal cortex. We found the earliest activations of the egocentric selective response, followed by the allocentric selective response in the parietal, occipital, and lateral temporal cortex. Our findings favor the hypothesis that egocentric spatial coding is a more primary process, and allocentric representations may be derived from egocentric ones.

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POLY(I:C) MOLECULAR WEIGHT CAUSES SEX-DEPENDENT DEFICITS IN NEURODEVELOPMENT AND ADULT BEHAVIOR IN OFFSPRING IN THE MATERNAL IMMUNE ACTIVATION MODEL

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Maternal immune activation (MIA) by poly(I:C) models are widely used to explore the link between exposure to infection during pregnancy and subsequent changes in offspring's neurodevelopment. However, the reproducibility of the behavioral phenotypes in rodent MIA by poly(I:C) varies despite the same dosage, strain, application route, and insult timing. Recently, it has been reported that poly(I:C) salt obtained from major vendors contains a random mixture of high (HMW) and low molecular weight (LMW) poly(I:C). The molecular weight of poly(I:C) may be one of the sources of variability, as it has been shown that HMW and LMW poly (I:C) affects maternal immune response and pregnancy outcomes differently. Here we explored the effect of MIA by HMW and LMW poly(I:C) in Wistar rats at gestational day 14 on the offspring's neurodevelopment, social behavior, anxiety, cognition, and schizophrenia-like behavior using a battery of behavioral tasks. Our pilot results show that poly(I:C) HMW and LMW disrupt the offspring's neurodevelopment and adult behavior differently and in a sex-depending manner. Poly(I:C) HMW significantly disrupted the neurodevelopment of the male pups and increased anxiety-like behavior in adult male offspring. Poly(I:C) LMW disrupted the cognitive abilities of adult female rats, as shown by a significantly decreased performance of adult female rats in rewarded T-maze alternation task. Our results indicate that poly(I:C) molecular weight may be one of the major confounding factors in MIA by poly(I:C) models. Stating the LMW and HMW poly(I:C) ratio in the research methods may improve the transparency and replica ability of the model.

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VISUOSPATIAL PERSPECTIVE-TAKING BRAIN DYNAMICS CAPTURED BY iEEG

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Visuospatial perspective-taking (VPT) is imagining a scene from another position than during self-perspective judgments (SPJ). We use VPT when trying to understand how others see the environment. VPT requires overcoming self-perspective, which is challenged during autism and schizophrenia. However, brain areas involved in VPT are not well differentiated from SPJ-related areas as spatiotemporal features of the responses only to VPT but not to SPJ are understudied. Therefore, we aimed to spatiotemporally distinguish the brain responses domain-specific to VPT (VPT-specific), self-perspective (Self-specific), and domain-general responses to both perspectives (General). Hierarchical processing theory suggests that in the sensory system, stimulus-specific responses emerge over time. Next, we aimed to test whether VPT-specific and Self-specific responses start later than General. The temporoparietal junction (TPJ) is a frequently reported area in VPT; however, its role is controversial. Finally, we aimed to elucidate the role of the TPJ in VPT.
We recorded iEEG data from 30 patients with epilepsy. The patients performed a VPT task requiring laterality judgments from self or another perspective. We spatiotemporally analyzed the responses in the broad gamma band (50-150 Hz).
We found VPT-specific processing in a larger brain network than Self-specific. Their dynamics were similar but started later and lasted shorter than General responses. Responses from the TPJ captured the difference between self-other representations. Our results anatomically distinguish VPT-specific from Self-specific processing. Also, we temporally differentiate between domain-specific and domain-general processes within and outside the sensory system. Finally, we show the role of the TPJ in the self-other distinction process.

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EFFECT OF MICROTUBULE DETYROSINATION AND TYROSINATION ON CRMP2 AND NEURONAL DEVELOPMENT

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Microtubules are cell components important for development, growth, transport, and migration. Deregulation of microtubules has been associated with neurodevelopmental and neurodegenerative disorders. The function of microtubules is regulated by microtubule-associated proteins and recently highly investigated tubulin code, collection of tubulin isotypes, and posttranslational modifications. The combined effect of tubulin posttranslational modifications and associated proteins enable proper neurodevelopment. We have previously demonstrated the role of microtubule-associated protein CRMP2 in axon guidance and cortical development. Here we analyze the effect of specific posttranslational modifications of tubulin, e.g. detyrosination on CRMP2 binding to microtubules in developing neurons. Using primary cortical neuron cultures we analyze posttranslational modifications of tubulin in neurons and demonstrate its effect on CRMP2 localization in cells. Using TIRF microscopy we analyze the binding of CRMP2 to tyrosinated and detyrosinated microtubules.

GALECTIN-3 AS A POTENTIAL TARGET FOR REVERSING VASCULAR REMODELING IN HYPOXIC PULMONARY HYPERTENSION

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Pulmonary hypertension is a life-threatening disease, which affects the pulmonary blood vessels and the heart. Its pathophysiology is characterized by progressively increasing pulmonary vascular resistance and remodeling, leading to right ventricular hypertrophy and eventually to heart failure. Chronic hypoxia is an important pathogenetic factor in the development and progression of pulmonary hypertension. Long-term and/or repeated hypoxia leads to the remodeling of blood vessels, i.e. thickening and increased muscularity of their walls. Galectin-3 (Gal-3) is a β-galactosyl-binding protein that interacts specifically with carbohydrate ligands on other molecules. This pleiotropic molecule has a wide range of positive and negative effects *in vitro* and *in vivo*. It is also involved in the onset and progression of cardiovascular diseases, namely heart failure, atherosclerosis, and systemic and pulmonary hypertension. Moreover, Gal-3 has been shown to be a strong and independent prognostic biomarker of pulmonary hypertension regardless of etiology. However, the available data regarding its potential role in this devastating disease are limited. We therefore aim to elucidate the effect of Gal-3 and hypoxia on vascular smooth muscle cell (VSMC) behavior *in vitro*. The experiments will also aim to develop new potent Gal-3 inhibitors with the ability to attenuate the biological effect of Gal-3. The inhibitors will then be administered to a rat model of hypoxic pulmonary hypertension.

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NEDD4-2 BINDING TO 14-3-3 MODULATES THE ACCESSIBILITY OF ITS CATALYTIC SITE AND WW DOMAINS

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Neural precursor cells expressed developmentally downregulated protein 4-2 (Nedd4-2), a homologous to the E6-AP Carboxyl Terminus (HECT) ubiquitin ligase, triggers the endocytosis and degradation of its downstream target molecules by regulating signal transduction through interactions with other targets, including 14-3-3 proteins. In our previous study, we found that 14- 3-3 binding induces a structural rearrangement of Nedd4-2 by inhibiting interactions between its structured domains. Here, we used time-resolved fluorescence intensity and anisotropy decay measurements together with fluorescence quenching and mass spectrometry to further characterize interactions between Nedd4-2 and 14-3-3 proteins. The results showed that 14-3-3 binding affects the emission properties of AEDANS-labelled WW3, WW4 and, to a lesser extent, WW2 domains and reduces their mobility, but not those of the WW1 domain, which remains mobile. In contrast, 14-3-3 binding has the opposite effect on the active site of the HECT domain, which is more solvent exposed and mobile in the complexed form than in the apo-form of Nedd4-2. Overall, our results suggest that steric hindrance of the WW3 and WW4 domains combined with conformational changes in the catalytic domain may account for the 14-3-3 binding-mediated regulation of Nedd4-2.

1. Pohl P et al. (2021) *Commun Biol.*, 4, 899.
2. Joshi R et al. (2022), *Biophys. J.*, 121 (7), 1299-1311

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CHIRAL AND AGING PROTEOMICS APPLIED TO BOVINE AND RAT COLLAGENS

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In nature, proteins are made up of a multitude of amino acids linked together by peptide bonds, thus forming a long chain. These proteinogenic amino acids are found in their L-form. However, during the aging process, a natural amino acid racemization can take place. Indeed, the detection of D-amino acids in aging proteins has been shown to correlate with age and be linked to aging diseases.
In this work, the amino acids racemization rate was determined in bovine and rat collagens at different ages. The exact positions of D-amino acids totally racemized were identified in the collagen sequences. Then, post-translational modifications were also studied according to age. The number of hydrophobic post-translational modifications increases in aging collagens and can explain their partial insolubility. Finally, the effects of aging on collagen sequences were studied. After successive enzymatic digestions to remove specific peptide bonds on aging and recent collagens, the resulting peptides from aging collagen were identified and compared to those of recent collagens. Peptides that are not common were identified as peptides resulting from age-related degradations. The number of these peptides increases with age.

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CIRCADIAN CLOCKS IN CHOROID PLEXUS AND ITS SENSITIVITY TO CHRONODISRUPTION

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Circadian rhythms regulate most of the physiological and behavioral processes with periodicity of approximately 24 hours. In mammals, the key players in this system are the master clock located in the suprachiasmatic nuclei (SCN), and the peripheral clocks that are distributed throughout the brain and other peripheral tissues. Recently, circadian clock was found also in epithelial cells of the choroid plexus (ChP). Despite significant advances in chronobiology field regarding the brain peripheral circadian clocks, the mechanism, regulation and entrainment for a plethora of clocks are still elusive. The main aim of the study was to elucidate impact of the disruptions in the circadian system on the circadian clocks in various brain regions, namely ChP, SCN, hippocampus (HIP), prefrontal cortex and cerebellum. Chronodisruption was achieved via disruptions ¬¬¬in the regular light/dark (LD12:12) cycle. Transgenic PER2::LUC mice were divided into 3 groups which were 1) exposed to constant light, 2) exposed to weekly 6 hours shifts in LD cycle, and 3) left untreated as controls. The effect on the circadian clocks was studied in vitro by real-time monitoring of PER2-driven bioluminescence rhythms in organotypic explants of the brain regions (ChP from the fourth ventricle and SCN) at the tissue and cellular levels using Lumicycle and LV200 microscope, respectively. Clock gene transcript levels were detected in vivo by RT qPCR in laser-dissected brain regions. The in vitro data revealed that the the clock in ChP and SCN differed in their sensitivity to chronodisruption, suggesting different pathways entraining these clocks. The pathways may involve effect of glucocorticoids because chronodisruption affected their receptors differently in the specific brain regions; it caused their downregulation in the ChP and HIP, but not in the other peripheral circadian clocks. These findings could provide a novel insight into the sensitivity of the brain clocks to chronodisruption and suggest involvement of different sensitivity of the circadian clocks to glucocorticoids as a plausible mechanism.

ODOR BACKGROUND INCREASES THE PHEROMONE CODING EFFICIENCY IN MOTH OLFACTORY NEURONS

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Insects detect odorants with olfactory receptor neurons (ORNs), located on their antennae. Male moths specifically depend on pheromone-responding neurons (Phe-ORNs) to find females for reproduction purposes, using sex pheromones emitted by female moths. These sex pheromones are a small part of a complex olfactory world and some of the volatile plant compounds (VPCs) found in the environment interfere with the Phe-ORNs. Male moths use VPCs to locate food sources and potential habitats of female moths. Insect behavior generally results from the integration of multiple odor sources, however the effects of VPCs as they naturally appear in the environment have not been studied extensively yet. To this end, we stimulated the ORNs of male Agrotis ipsilon with short puffs of pheromone against VPC backgrounds of different concentration, to mimic the natural environment. We found that the Phe-ORNs have a variable response to different concentrations of the VPC (Z)-3-hexenyl acetate. Of particular interest is a high concentration of the VPC, where we observe an improved coding efficiency per spike of the Phe-ORNs. We confirmed, using regression and other statistical methods, that the accuracy of the stimulus prediction is consistently higher with VPC background. It has been frequently observed that VPC background often suppress the Phe-ORNs reaction when pheromone is introduced, our research shows that certain VPC concentrations increase the information transmission in Phe-ORNs.

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MITOCHONDRIAL MORPHOLOGY AND METABOLISM IN PANCREATIC β-CELL FROM IF1 KO MICE

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Inhibitory factor 1(IF1) is an endogenous regulator of ATP synthase. Previous studies have established that IF1 binds and inhibits ATP synthase upon a large decrease in membrane potential (□□) when ATP synthase reverses and hydrolyses ATP, preventing total ATP depletion. What is yet to be established is how IF1 regulates ATP synthesis under normal physiological conditions when the enzyme works in the regular forward mode. We have previously shown that IF1 regulates cellular respiration, ATP levels and glucose-stimulated insulin secretion in model pancreatic β-cells (INS-1E) under normal physiological conditions.

The aim of this study was to investigate cellular metabolism and mitochondrial morphology in pancreatic beta cells in-situ in pancreatic islets isolated from IF1 KO mice. Therefore, we studied ATP levels in pancreatic islets treated with low and high glucose concentrations with inactive/active glucose stimulated insulin secretion (GSIS). Insulin and glucagon expression was also studied by immunohistochemistry and compared between pancreatic islets of IF1 KO and WT mice while also considering their age and gender. Moreover, using super-resolution microscopy, mitochondrial morphology was successfully visualised in pancreatic islets under different metabolic conditions. Altogether the obtained data provided further insight into the emerging role of IF1 in the metabolism of pancreatic beta cells.

ROLE OF EXTRACELLULAR ATP AND P2X RECEPTORS IN HYPOTHALAMIC PARAVENTRICULAR NUCLEUS

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The aim of this project is to use electrophysiological methods to elucidate the function of P2X receptors in various types of paraventricular nucleus neurons in the anterior hypothalamus of the rat and to study the mechanism by which these receptors regulate synaptic transmission and electrical activity of neurons. The properties of ionotropic purinergic P2X receptors detectable by the presence of ATP (100 μM) -evoked currents are electrophysiologically investigated on identified oxytocin and vasopressin neurons in brain sections of a transgenic rat. In parallel, a mapping experiment takes place, which will result in a 3D construct showing the spatial distribution of vasopressin and oxytocin neurons in the anterior hypothalamus, their connections, number and size. The study is performed on double-labelled transgenic rats that have genetically labelled magnocellular neurons: oxytocin neurons with red fluorescent protein and vasopressin neurons with green fluorescent protein. Another part of the project is the detection of P2X receptors and their characterization in parvocellular neurons of the paraventricular nucleus, which produce a number of other hormones, such as corticotropin-releasing hormone (CRH). Parvocellular neurons are identified by the absence of fluorescent protein and by electrophysiological characteristics. The obtained data should provide the first direct evidence for the specific expression of P2X receptors and their role in the modulation of synaptic transmission in different types of paraventricular nucleus neurons.

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MODULATION OF SYNAPTIC TRANSMISSION IN CHEMOTHERAPEUTICS INDUCED NEUROPATHIC PAIN

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Chemotherapeutics used for cancer therapy usually produce serious side effects that may prevent patients from finishing their treatment. About 1/4 of patients with chemotherapy□induced peripheral neuropathy (CIPN) suffer from chronic pain that has no satisfactory treatment. Our work focuses on mechanisms of neuropathic pain development with commonly used chemotherapeutics, paclitaxel and bortezomib, and their modulatory effects on nociceptive transmission in spinal cord dorsal horn (SCDH).

In our previous publications, we showed that mechanical hyperalgesia in rodents is partially mediated by sensitization of presynaptic TRPV1 receptors in the SCDH and this effect is dependent on PI3K activation[1]. Using behavioral testing and electrophysiological recordings from identified dorsal horn neurons in spinal cord slices we have studied the role of glia activation in this process. Our data show that chronic inhibition of glial activation by minocycline prevents TRPV1 receptors sensitization following paclitaxel application. Recently we have developed a model of chemotherapy-induced pain using an application of bortezomib, a proteasome inhibitor used to treat hematological malignancies. We have demonstrated a robust increase of mechanical sensitivity in mice (mechanical allodynia) that persists for at least 21 days. In further experiments, we will use electrophysiological whole□cell patch clamp recordings of SCDH from transgenic mice with optional specific activation of inhibitory interneurons and immunohistochemical analyses to study the changes leading to the neuropathic pain conditions. Our goal is to suggest new effective ways of prevention or treatment of this neuropathic pain condition.

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THE ROLE OF MACROPHAGE MIGRATION INHIBITORY FACTOR (MIF) IN NEUROPATHIC PAIN

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Neuroinflammation is a proinflammatory cytokine-mediated process often associated with direct injury to the nervous system. It occurs most commonly with injury to peripheral nerves and involves neural-immune interactions that activate immune cells, glial cells and neurons and can lead to the debilitating pain state known as neuropathic pain. Therapy is problematic, but new trials targeting cytokines provide hope for future success in treating neuropathic pain. Macrophage migration inhibitory factor (MIF) exerts its biological functions mainly through binding to the putative membrane receptor CD74. In our experiments, we used a MIF tautomerase inhibitor (S,R)-3(4-hydroxyphenyl)-4,5-dihydro-5-isoxazole acetic acid methyl ester (ISO-1) that binds the MIF active site and blocks the MIF-CD74 binding.

Our aim was to examine whether systemic treatment with ISO-1 alleviates the hypersensitivity induced by peripheral neuropathy and modulates synaptic transmission at the spinal cord level. Peripheral neuropathy was induced by chronic constriction injury (CCI) of the sciatic nerve. Electronic Von Frey test to assess mechanical sensitivity and patch-clamp recordings of excitatory and inhibitory postsynaptic currents (EPSCs, IPSCs) from superficial dorsal horn neurons in acute spinal cord slices were used. Inhibitory currents were evoked by optical stimulation of channelrhodopsin expressing inhibitory neurons. Mechanical allodynia induced by CCI was prevented with the ISO-1 treatment. CCI also significantly increased the spontaneous (s) EPSCs frequency and decreased the amplitude of the light-evoked IPSCs in excitatory dorsal horn neurons. The systemic ISO-1 treatment largely diminished these pathological effects on nociceptive synaptic transmission induced by the CCI. Our results suggest that ISO-1 treatment reduces the development of hypersensitivity after nerve injury. One of the underlying mechanisms of the ISO-1 effect could be balancing the CCI-induced excitatory and inhibitory synaptic transmission changes in the spinal cord dorsal horn.

MOLECULAR MECHANISMS OF STEROID EFFECTS ON FUNCTION OF MUSCARINIC RECEPTORS

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Muscarinic acetylcholine receptors are G-protein coupled receptors (GPCRs) located in the plasma membrane of many cell types of various tissues. This receptor family consists of 5 distinct subtypes, denoted M1 to M5. Alterations in signalling via muscarinic receptors play an important role in several neurological and psychiatric disorders. Cholesterol has been found to co-crystallize with a number of GPCRs and it was shown that membrane cholesterol specifically binds to muscarinic receptors and slows down their activation. Cholesterol is the precursor of many steroid compounds (e. g. sex hormones, corticosteroids). Recently, steroid compounds have been shown to allostericaly modulate muscarinic receptors. Aim of the project is to delineate the molecular mechanisms of cholesterol and steroid action at muscarinic receptors and to identify possible differences among different subtypes of muscarinic receptors. We anticipate to get hints for cholesterol and neurosteroids binding sites by site-directed mutagenesis. We will use these hints for comparative modelling of activation of the receptor with bound modulator and modulator free receptor. We expect to find differences in interaction networks in the presence and absence of modulators. Mutants aimed to alter action of modulators will be designed based on outcomes of molecular modelling. Also, the binding of cholesterol and steroid-based compounds will be inferred from a fluorescence resonance energy transfer (FRET) between neurosteroids labelled with fluorescent probe and muscarinic receptors fused with cyan fluorescent protein (CFP) at different positions.

CREATION AND PRIMARY BEHAVIOURAL CHARACTERIZATION OF DANIO RERIO GRIN2AB NMDA RECEPTOR SUBUNIT KNOCKOUT BY CRISPR/CAS GENOME EDITING

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De novo mutations in genes encoding NMDA receptor subunits (GluN1, GluN2A-D, GluN3A-B) may underlie neuropsychiatric disorders. *Danio rerio* (zebrafish) is an attractive model organism to study behavioral consequences of mutations in the NMDA receptor. There are two genes (paralogs) for each NMDA receptor subunit in a zebrafish genome. We aimed to characterize the behavioral consequences of single and double knockout mutations in *grin2aa* and *grin2ab* genes. *Grin2ab* KO mutant was created by injecting Cas9 protein and sgRNA (designed to target exon 4 of the *grin2ab*) into fertilized eggs. The mutant has a premature stop codon resulting from 16bp deletion in exon 4. Open field experiments on 6 dpf *grin2ab*$^{-/-}$ larvae showed that total swimming distance was longer (~ 2-fold) and the number of swimming bouts was higher (~2.5-fold), but the mean length of individual swim bouts, acceleration, and mean speed were similar to that found in the wild-type larvae. G*rin2ab*$^{+/-}$ larvae had swim characteristics similar to wild-type larvae of the same age. In contrast to *grin2ab*$^{-/-}$ larvae, behavioral examination of *grin2aa*$^{-/-}$ and *grin2aa*$^{+/-}$ in an open field showed no quantitative differences from control wild-type larvae.In further steps, we aim to use *grin2ab* $^{-/-}$ larvae to study the behavioral consequences of negative allosteric modulators of NMDA receptors on the increased zebrafish swimming.

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CLINICAL TRANSLATION OF ARTERIAL SPIN LABELING MRI IN DIAGNOSTICS OF EPILEPSY, BRAIN TUMORS, AND CEREBROVASCULAR DISEASE

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Perfusion imaging provides information about cerebral blood flow (CBF) and volume and can be used in clinical practice to indicate pathological areas in the brain, distinguished by hypo- or hyperperfusion. There are two main implementations of perfusion MR imaging: based on the administration of gadolinium contrast agent (Dynamic Susceptibility Contrast (DSC) and Dynamic Contrast Enhancement (DCE)) and labeling of endogenous particles (Arterial Spin Labeling (ASL)). Whereas DSC and DCE rely on the signal change induced by paramagnetic gadolinium chelates (T2/T2* and T1 shortening respectively) as they perfuse in the brain, ASL uses spatially selective magnetic pulses to label endogenous water molecules of inflowing blood to acquire an image of their cerebral perfusion. Due to the invasive and potentially toxic nature of exogenous-contrast-based MRI methods, there is an increasing interest in using contrast-free methods, especially in pediatric subjects or subjects with renal impairment. Though ASL is non-invasive, easier to implement, and provides absolute quantification of CBF, it suffers from a lower signal-to-noise ratio. ASL is not fully validated and thus waiting to be a part of the standard clinical pipeline. In our project, we aim to derive and optimize clinical diagnostic protocols with the use of ASL in epilepsy, brain tumors, and cerebrovascular disease at Motol University Hospital. In addition to perfusion quantification, we use ASL MRI to measure cerebrovascular reactivity (CVR) in the acetazolamide challenge test. The experimental protocol is already being tested and initial results are to be presented.

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DEVELOPMENT OF *IN VITRO* METHODS FOR PERSONALIZED DIAGNOSTICS AND TREATMENT OF EPILEPSY

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Epilepsy is the most common chronic neurological disease that affects approximately 1-2 % of the world's population. It is accompanied by metabolic changes, both at the cellular level and the brain tissue level, by alternating ictal hypermetabolic and interictal hypometabolic states. There are many causes of epilepsy, including genetic factors, birth complications, brain injury, infectious diseases, and brain tumors. Due to the multifactorial nature of epilepsy, the current pharmacological treatment is symptomatic and it is mainly based on seizure suppression. However, the treatment is inefficient in approximately 30 % of patients. A personalized approach for epilepsy diagnosis and treatment is now increasingly being used to identify disease-causing mutations of epilepsy-related genes. To test the effect of potential pathological mutations, we use a suitable model system of induced pluripotent stem cells (iPSCs), which we further differentiate into neurons. First, we characterize the cell system in terms of altered morphology and changes in the expression of pluripotent, neural, and metabolic markers. We further characterize metabolic parameters using the Oxygraph instrument and Fluorescence lifetime imaging microscopy (FLIM). Our preliminary results show that beta III tubulin and MAP2 expression levels increase during neuronal differentiation. In differentiating neurons, there is an increase in the maximal capacity of oxidative phosphorylation. In collaboration with Motol University Hospital, we selected potential pathological mutations in genes e.g. PIK3CA and mTOR. In addition, using the CRISPR/Cas9 system or viral transduction, we will introduce a particular mutation found in a patient into neural progenitors and monitor the effect of the mutation mainly on cell metabolism. We expect that the development of a reliable *in vitro* cell system will contribute to the elucidation of the role of particular mutations in the pathophysiology of epilepsy.

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POSTER ABSTRACTS

DE NOVO MUTATIONS AND RARE VARIANTS OCCURRING IN NMDA RECEPTORS: STRUCTURE-FUNCTION CHARACTERIZATION AND PHARMACOLOGICAL SCREEN OF NOVEL THERAPEUTICALLY RELEVANT NMDA RECEPTOR MODULATORS

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N-methyl-D-aspartate receptors (NMDARs) are a class of ionotropic glutamate receptors that play a fundamental role in synaptic plasticity. Mutations in the GRIN gene family encoding NMDARs have been associated with a wide range of neuropsychiatric disorders, including Alzheimer's disease, Parkinson's disease, Autism spectrum disorder, developmental delay, and epilepsy. To reveal the underlying structural consequences of NMDAR mutations and propose possible treatment strategies, we use the single-molecule FRET (smFRET) technique. In smFRET two sites on a single receptor are labelled with fluorophores, one of which transfers its excitation energy to the other. The latter emits light with intensity depending on the distance between the fluorophores. This allows us to precisely monitor the distance between the sites over time and thus detect individual conformational states and transitions in the course of receptor activation. In our project, we firstly compared conformational dynamics of the amino-terminal domain in wild-type and several human mutant NMDARs. We have detected significant differences in the activation pathway for the GluN1/GluN2B_I150V mutant. We further compiled a list of mutations associated with neurological disorders and will continue to perform experiments with human mutated receptors prepared recently. Secondly, we have tested over a dozen of new prospective inter-domain labelling sites to expand our readout of receptor conformational space. We found several promising candidates in the ligand-binding and transmembrane domains. We also developed a new labelling and pull-down strategy for intra-domain labelling. Last but not least, we significantly improved our transfection protocol to be three times more efficient and ten times cheaper.

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ROLE OF C-TERMINAL DOMAINS OF THE NMDAR RECEPTOR IN THE CONTROL OF SENSITIVITY TO POTENTIATING NEUROSTEROIDS

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N-methyl-D-aspartate-receptors (NMDARs) are glutamate-gated ionotropic receptors that are highly permeable to Ca^{2+} and play a key role in excitatory synaptic transmission. NMDAR-mediated Ca^{2+} influx regulates numerous functions in the cell, including posttranslational modifications, such as palmitoylation, glycosylation, and phosphorylation. Our recent study showed that Ca^{2+} -regulated palmitoylation of the NMDAR CTDs controls receptor sensitivity to inhibitory neurosteroids. To test the implication of the CTDs in the control of sensitivity to potentiating neurosteroids, we electrophysiologically assessed the effect of endogenous pregnenolone sulfate (PE-S) and synthetic neurosteroid 4-(20-oxo-5□-pregnan-3□-yl) butanoic acid (EPA-But) at recombinant GluN1/GluN2B receptors with truncated CTDs. GluN1/GluN2B-R847* receptors showed profoundly decreased potentiation by PE-S and EPA-But. Subsequent analysis of NMDARs containing the GluN2B CTD truncated to different lengths showed that the lack of the terminal region of the CTD (GluN2B-T888*; GluN2B-I925*; GluN2B-Y1004*) did not significantly alter the degree of PE-S and EPA-But potentiation. However, the truncations of the proximal part of the CTD (GluN2B-F864*; GluN2B-E878*) diminished PE-S and EPA-But potentiation to the same extent as GluN2B-R847*. The proximal part of the GluN2B CTD contains several posttranslational-modification sites, including cysteine cluster I which is known as a site of palmitoylation. To test whether potentiation by PE-S and EPA-But is affected by palmitoylation of cysteine cluster I, we generated a triple mutant of the GluN2B subunit where all three cysteines (C849, C854, and C871) were mutated to non-palmitoylable alanines (GluN2B-AAA). The GluN2B-AAA mutation mimicked the effect of GluN2B-R847* on PE-S and EPA-But potentiation, implying that the CTDs palmitoylation plays an important role in the control of the sensitivity of NMDAR to potentiating neurosteroids
Our results indicate new possibilities for the development of neurosteroid-like drugs to treat disorders associated with NMDAR malfunction.

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FUNCTIONAL ANALYSIS OF GENETIC VARIATIONS IN THE CYTOSOLIC DOMAIN OF NMDA RECEPTORS

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The expression and activity of ionotropic glutamate receptors control signal transduction at the excitatory synapses in the CNS. The major role is played by the calcium-permeable NMDA receptors (NMDARs) that are represented by three types of subunits: GluN1, GluN2A-D, and GluN3A-B. Each subunit consists of four domains, with the intracellular C-terminal domain (CTD) representing up to half of the entire NMDAR subunit (GluN2A/B). The CTD of the NMDAR is crucial for trafficking of the receptor to synapses, endocytosis, and subsequent degradation of the receptor. The direct effect of altered CTD on the functional properties of the NMDAR ion channel has also been shown. CTD interacts with several intracellular proteins, such as cytoskeletal proteins, scaffold proteins, or proteins involved in intracellular signaling. Amino acid mutations in the cytosolic part of NMDAR subunits have been identified in individuals with various neurological impairments. We employed electrophysiological and microscopy techniques to study the impact of four (P1386L, N1076K, T1064A, V967L) disease-associated mutations (schizophrenia or epilepsy) in the CTD of the GluN2A subunit, which we identified in our NGS data set from patients. We analyzed the NMDAR ion channel properties, their changes in surface expression, and their trafficking to synapses. Our findings suggest that the mutations investigated affect ion channel properties and significantly reduce NMDAR surface expression. Furthermore, mutation P1386L found in schizophrenia patients, shows a defect in synaptic localization. We found that decreased synaptic localization of P1386L mutated NMDAR is associated with decreased affinity for the scaffold protein PSD-95. In summary, we showed that genetic changes in the CTD of the NMDA receptor altered its functions, impairing its delivery to the cell surface and synaptic localization, which might contribute to the emergence of neurological disorders.

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FUNCTIONAL ASSESSMENT OF MICE CARRYING *A DE NOVO* MISSENSE *GRIN2B* MUTATION ASSOCIATED WITH AUTISM SPECTRUM DISORDER (ASD)

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Based on epidemiologic data, nearly a sixth of the world's population suffers from a neurological disorder, and one in 100 children worldwide has Autism Spectrum Disorder. Sequencing data for neurological and psychiatric patients indicate numerous mutations in genes encoding N-methyl-D-aspartate receptor (NMDAR) subunits. NMDA receptors are glutamate-gated ion channels that mediate signaling at most excitatory synapses in the nervous system. We have created and evaluated a transgenic mouse carrying a missense mutation (L825V) in the *Grin2b* gene, associated with Autism Spectrum Disorder (ASD). To characterize the impact of this mutation, we used a combination of methods, including patch-clamp recording immunochemical methods and behavioral tests. Using the recombinant wild-type and mutated subunits, we assessed the NMDAR channel open probability (Po). The Po of the wild-type diheteromeric receptors was determined to be 9.9 ± 1.0%, the Po of the triheteromeric receptors with a mutation in one GluN2B subunit was decreased to 4.9 ± 0.8% and in diheteromeric receptors with a mutation in both subunits to 1.0 ± 0.1%. Subsequently, we used primary hippocampal neurons prepared from WT and *Grin2b*$^{WT/L825V}$ mice to characterize the density of whole-cell and synaptic currents mediated by the NMDAR. The whole-cell NMDAR current densities, but not AMPAR current densities, were reduced in neurons prepared from *Grin2b*$^{WT/L825V}$ compared to WT mice. Interestingly, the sensitivity to ifenprodil, a GluN2B antagonist, was decreased in neurons from heterozygous mice. At synapses, the deactivation rate of NMDAR was significantly accelerated in *Grin2b*$^{WT/L825V}$ compared to WT, and the peak current density was not changed.The behavioral tests indicated differences in certain cognitive tasks between WT and *Grin2b*$^{WT/L825V}$ mice. The *Grin2b*$^{WT/L825V}$ mice provide a relevant model of ASD, that we plan to use in subsequent experiments to rectify the deficits through genetic and pharmacological treatments.

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EFFECTS OF ACETYLCHOLINESTERASE INHIBITION WITH PYRIDOSTIGMINE ON CARDIOVASCULAR SYSTEM IN SPONTANEOUSLY HYPERTENSIVE RATS

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Introduction: Autonomic nervous system (ANS) imbalance resulting in sympathetic overactivity and parasympathetic deficiency is a characteristic of cardiovascular diseases including hypertension and heart failure. The spontaneously hypertensive rats (SHR) are one of the most studied animal models of essential hypertension and are characterized by ANS imbalance. SHR are a suitable model for examining the effects of chronic cholinesterase inhibition to restore ANS balance.

Objectives: To determine if chronic inhibition of acetylcholinesterase with pyridostigmine (PYR), can improve sympatho-vagal balance (SVB) in SHR, reduce blood pressure (BP) and heart rate (HR), affect heart rate variability (HRV) and systolic blood pressure variability (SBPV).

Materials & Methods: Adult male (5-months-old) SHR and Wistar-Kyoto (WKY) rats were treated by PYR (25 mg/kg/day) in drinking water for 2 weeks. The rats were implanted with telemetric transducers to measure BP, HR, activity, and body temperature. HRV and SBPV was determined by power spectral analysis. SVB was evaluated pharmacologically in a separate set of rats, and noradrenaline plasma level was determined by ELISA assay.

Results: SHR have elevated BP, increased sympathetic nerve activity (LF-SBPV), and reduced HRV compared to WKY. PYR significantly reduced HR in treated rats of both strains (during both dark and light phase) and BP during dark (active) phase compared to untreated controls. Acetylcholinesterase activity in plasma was significantly reduced by PYR treatment in both strains (SHR 53±3%, WKY 46±3%). SVB examination showed reduced cardiac vagal tone in SHR as evidenced by blunted HR response to the muscarinic receptor blocker methylatropine and increased cardiac sympathetic tone as evidenced by elevated HR response to the β-blocker propranolol. PYR increased cardiac vagal tone and decreased cardiac sympathetic tone in SHR to untreated WKY levels. PYR increased HRV and reduced LF-SBPV in SHR showing restoration of ANS balance. Noradrenaline plasma levels were reduced in PYR-treated rats of both strains.

Conclusions: PYR is effective in improving ANS balance by increasing cardiac vagal tone and reducing sympathetic tone. Such results prompt further research into the effects of PYR as a possible therapeutic strategy for the treatment of cardiovascular diseases, mainly by augmentation of parasympathetic tone.

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HIF-1α: AN ESSENTIAL ELEMENT FOR CARDIOPROTECTION AND PROPER MITOCHONDRIAL FUNCTION DURING ADAPTATION TO CHRONIC HYPOXIA

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Adaptation to chronic hypoxia (CH), a phenomenon increasing cardiac ischemic tolerance, stabilizes the transcription of hypoxia-inducible factor-1α (HIF-1α). This study aimed to examine the role of HIF-1α in cardioprotection with special respect to mitochondria using a model of heterozygous Hif1-α knockout (Hif1α$^{+/-}$) mice. Adult male wild type (wt) and Hif1α$^{+/-}$ mice were adapted to CH or kept in normoxia. Physiological responses to CH were assessed and myocardial infarction was induced in isolated perfused hearts. Expression analyses, mitochondrial respiration, and electron microscopy were performed to evaluate mitochondrial characteristics. Our results showed decreased infarct size in chronically hypoxic wt mice compared to normoxic counterparts. In contrast, this protective effect of CH was absent in Hif1α$^{+/-}$ mice. Moreover, Hif1α haploinsufficiency resulted in changes in mtDNA content, mitochondrial size, respiration, protein expressions, and citrate synthase activity. Our data suggested that HIF-1α is crucial for CH-induced myocardial protection against ischemia/reperfusion injury likely by altering mitochondrial function.

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TOWARDS AN IN VITRO MODEL OF CLUBFOOT FIBROSIS: ENHANCING EXTRACELLULAR MATRIX PRODUCTION WITH MACROMOLECULAR CROWDING

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Idiopathic clubfoot is the second most common congenital orthopedic deformity of a lower limb. Mass of contracted tissue formed on medial side of affected foot resembles other fibroproliferative disorders. There are increased levels of profibrotic cytokines and growth factors, upregulation of collagen types I, III, VI and also an increase in collagen crosslinking. We have established primary fibroblast-like cell culture from the tissue obtained during surgeries of relapsed clubfoot. We evaluated extracellular matrix (ECM) production while subjecting the cells to anti-fibrotic substances in the past to assess their therapeutic potential as an adjuvant treatment. However, ECM production is limited under conventional culture conditions and animal model is not available to further our research. To improve experimental conditions we utilized the phenomenon of macromolecular crowding (MMC). MMC induces an excluded volume effect, which effectively emulate dense in vivo extracellular space. Such biomimetic microenvironment, created by addition of inert water soluble macromolecules in culture media, can enhance cellular signalization, gene expression, enzymatic activity, and natural ECM deposition. We tested different concentrations of Ficoll and Polyvinylpyrrolidone to mimic the molecular crowding of the physiological environments corresponding to the fractions of volume occupied by the macromolecules in the cultivation media (4-54%). We assessed the changes in ECM production under MMC conditions in culture of clubfoot cells and human dermal fibroblasts cultivated in a media supplemented with 5 or 10% of fetal bovine serum and ascorbic acid by metabolic assays, immunofluorescence staining, Second Harmonic Generation imaging, and collagen deposition assay.

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14-3-3-PROTEIN REGULATES NEDD4-2 BY MODULATING INTERACTIONS BETWEEN HECT AND WW DOMAINS

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Neural precursor cell expressed developmentally down-regulated 4 ligase (Nedd4-2) is an E3 ubiquitin ligase that targets proteins for ubiquitination and endocytosis, thereby regulating numerous ion channels, membrane receptors and tumor suppressors. In turn, Nedd4-2 activity is regulated by autoinhibition, calcium binding, oxidative stress, substrate binding (through its WW domains), phosphorylation and 14-3-3 protein binding. However, the structural basis of 14-3-3-mediated Nedd4-2 regulation remains poorly understood. Here, we combined several techniques of integrative structural biology to characterize Nedd4-2 and its complex with 14-3-3. The results from our binding affinity and crystallographic analyses demonstrate that phosphorylated Ser342 and Ser448 are the key residues that facilitate 14-3-3 protein binding to Nedd4-2 and that Ser448 is the dominant site. Moreover, 14-3-3 protein binding induces a structural rearrangement of Nedd4-2 by inhibiting interactions between its structured domains, including the N- and C-lobes of the catalytic HECT domain. Overall, our findings provide the first structural glimpse into the 14-3-3-mediated Nedd4-2 regulation and highlight the potential of the Nedd4-2:14-3-3 complex as a pharmacological target for Nedd4-2-associated diseases such as hypertension, epilepsy, kidney disease and cancer.

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SEARCHING FOR FUNCTION OF TMEM70, TMEM242 AND c15orf61 – RECENTLY IDENTIFIED INTERACTORS OF SUBUNIT C FROM MAMMALIAN FoF1 ATP SYNTHASE

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Most of the cellular energy in eukaryotes is produced by mitochondrial F1Fo ATP synthase, the key enzyme of the oxidative phosphorylation (OXPHOS) system. The F1Fo ATP synthase is a multisubunit complex consisting of two domains – the membrane-embedded Fo, and the globular F1 domain. While the composition and biogenesis of F1 domain is conserved throughout evolution, the Fo region underwent significant changes during evolution and in metazoans requires additional assembly factors.

Searching for such factors, we performed a screen for interactors of subunit c. Using MS affinity enrichment analysis, we have identified three candidates: TMEM70, TMEM242 and c15orf61. Therefore, using CRISPR-Cas9 knockout approach we generated knockout models in HEK293 cells to study their role. We confirmed that both TMEM70 and TMEM242 protein deficiency lead to decrease in assembled ATP synthase as well as accumulation of dissociated F1, indicating the role of this protein involved in biogenesis of subunit c-oligomer and/or its incorporation in the enzyme. While we did not observe drop in the content of other OXPHOS complexes in TMEM70 KO cells, TMEM242 deficiency was accompanied with downregulation of complexes I and IV.

In contrast, lack of c15orf61 did not cause defect in the assembly of ATP synthase and subunit c content remained at control levels. Interestingly, we show that c15orf61 binds to assembled ATP synthase and comigrates with both monomers and dimers, pointing to its role in regulation of enzyme function.

Ultimately, we investigated possible role of all three proteins in regulation of mPTP opening. Significant differences were found only in case of TMEM242 deficient cells, where decrease in the cyclosporin A sensitive portion of calcium retention capacity was observed.

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MU-OPIOID RECEPTOR DESENSITIZATION IN THE SPINAL CORD DORSAL HORN IS REDUCED BY THE ENDOGENOUS TRPV1 AGONIST N-OLEOYLDOPAMINE.

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Opioid receptors are expressed throughout the afferent pain pathway, including central terminals of primary afferent neurons in the spinal cord dorsal horn (SCDH), where opioids exert inhibitory control of the nociceptive transmission. At these presynaptic endings opioid receptors co-express at the first nociceptive synapse with the transient receptor potential vanilloid type 1 (TRPV1) channel, known for its role in the development of thermal and mechanical hyperalgesia during pathological states. Our study focused on the interaction of TRPV1 and μ-opioid receptor (MOR) in agonist-induced desensitization of MOR. We used whole-cell patch-clamp recordings of miniature excitatory postsynaptic currents (mEPSC) from SCDH neurons in rat spinal cord slices. Short application of MOR agonist DAMGO (1 μM, 3 min) depressed mEPSC frequency to 62.3 ± 3.6 %. To induce robust MOR desensitization, we incubated slices with 1 μM DAMGO for 2 hours; acute application of DAMGO after the incubation failed to depress the mEPSC frequency. Addition of endogenous TRPV1 agonist N-oleoyldopamine (OLDA) to DAMGO during incubation prevented the MOR desensitization. Short DAMGO application in slices incubated with DAMGO+OLDA evoked a decrease of mEPSC frequency to 66.94 ± 9.6 %. Furthermore, results from *in vivo* experiments demonstrate how OLDA diminished chemokine-induced MOR desensitization. Our data reveal how endogenous TRPV1-mediated pathways may interact with MOR function, reduce MOR desensitization and thus promote the efficacy of opioids. Further study of the underlying mechanisms could contribute to improved opioid-mediated analgesia.

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ROLE OF THE KCTD16 PROTEIN IN MODULATION OF GABAB RECEPTORS UNDER PATHOLOGICAL NEUROPATHIC PAIN CONDITION

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Neuropathic pain is a serious debilitating condition affecting up to one-tenth of the adult population worldwide, thus representing a major public health problem. Poor diagnostic accuracy coupled with the lack of effective treatments makes this pathology particularly impactful to patients, caregivers and state economies. It was suggested before that depression of inhibitory mechanisms at the spinal cord level may be one of the causes of neuropathic pain development and maintenance. Gamma-aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the central nervous system and it's known that GABAergic neurons conduct pre- and postsynaptic inhibition in the spinal cord dorsal horn, thus, controlling the transmission of nociceptive information from the periphery. GABA inhibitory effects are mediated via anion-selective ionotropic GABAA receptors and metabotropic GABAB receptors. The GABAB receptors are G□protein coupled receptors consisting of hetero-multimers composed of principle subunits and auxiliary potassium channel tetramerization domain (KCTD) subunits. The KCTD subunits 8, 12, 12b and 16 are cytosolic clade F proteins that determine the kinetics of the GABAB receptor response. KCTD16 is highly expressed in a number of brain regions and in our experiments we found it to be also highly expressed in the spinal cord dorsal horn and dorsal root ganglia (DRG). KCTD16 has been demonstrated to influence neuronal excitability by regulating GABAB receptor□mediated gating of postsynaptic ion channels. In this study we aim to uncover the role of KCTD16 in neuropathic pain development and maintenance by comparing WT and KCTD16 -/- mice under control, neuroinflammatory and neuropathic pain conditions. Behavioral, electrophysiological EPSC and IPSC recordings from the dorsal horn neurons in the spinal cord slices and calcium imaging recordings from DRG cultures were performed. Preliminary data will be presented.

THE ROLE OF RF-AMIDE PEPTIDE RECEPTORS GPR10 AND NPFFR2 IN ENERGY HOMEOSTASIS AND DIET-INDUCED OBESITY

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Energy homeostasis as a balance between energy intake and expenditure is controlled by brain centers, mainly hypothalamus. RF-amide peptides control numerous biological functions such as regulation of food intake. The aim of the study is to characterize an effect of deletion of two of their receptors, GPR10 and NPFFR2, on energy homeostasis. We are using double knockout (GPR10 x NPFFR2; dKO) mice on a hybrid B6J x B6N background and their non-littermate controls, both males and females. Mice are fed either standard (STD; Ssniff RMH) or high-fat diet (HFD; based on lard) for 16 weeks starting at 4 months of age. Dual-energy absorptiometry is used to quantify bone mineral content, fat and lean body mass, and indirect calorimetry is used to quantify energy expenditure, energy intake, substrate partitioning, and physical activity at the beginning and end of this study. The goal is to detect possible differences which might precede or be a consequence of increased diet-induced obesity in dKO as compared to control mice. In the subsequent experiment, we will characterize the impact of an analog of a natural ligand of both receptors on body weight reduction. This experiment on mice after 16 weeks of high-fat-diet feeding will include 2-week intervention with application of either analog or control solution and will be performed along with a continuous measurement on indirect calorimetry. Both experiments are currently in progress with a planned end at the end of December. Consequently, data analysis together with the analysis of collected samples will be performed.

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METABOLIC EFFECTS OF N-3 FATTY ACIDS AS CALANUS OIL IN TRANSGENIC MICE WITH MODIFIED PPARα EXPRESSION

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Obesity is associated with insulin resistance and disorders of glucose and lipid metabolism. Polyunsaturated fatty acids of n-3 series, namely eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), exert hypolipidemic effects while acting as endogenous ligands of the transcription factor peroxisome proliferator-activated receptor α (PPARα), which primarily regulates lipid catabolism genes. We aimed to determine whether modifications in the PPARα expression affect metabolic effects of n-3 fatty acids administered as Calanus oil (CO), which contains EPA and DHA primarily in wax esters. Male 129S1/SvImJ mice including wild-type animals (WT), those expressing the human form of PPARα (hPPARα), and mice completely lacking PPARα (PPARα-KO) were used. Twelve-week-old mice were either maintained on a low-fat chow or placed for 8 weeks on the following obesogenic diets: (i) corn oil-based high-fat diet (cHF; lipids ~32 % wt/wt), and (ii) the cHF diet, in which ~15 % of dietary lipids (corn oil) was replaced by CO (cHF+CO; ~6 g EPA+DHA/kg diet). Intraperitoneal glucose tolerance test was performed, and plasma levels of lipid metabolites and lipid content in the liver were assessed by biochemical methods. One-way ANOVA was used to determine statistical significance and $P<0.05$ was considered significant. Compared to cHF, CO supplementation in WT reduced body weight and adiposity, independently of energy intake, and ameliorated glucose intolerance while lowering plasma triacylglycerol and cholesterol levels. Although CO supplementation in hPPARα improved glucose tolerance, it did not affect the other measured parameters. Of note, also in PPARα-KO dietary supplementation with CO reduced fasting blood glucose and lipid content in the liver. The absence of body weight-reducing effects of CO supplementation in mice with humanized PPARα is in line with the known limited efficacy of n-3 fatty acids to reduce adiposity in humans.

Supported by the Czech Science Foundation (17-11027S).

EFFECT OF AMBIENT TEMPERATURE ON SKELETAL MUSCLES NON-SHIVERING THERMOGENESIS IN MICE DIFFERING IN PROPENSITY TO OBESITY

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Non-shivering thermogenesis is an essential mechanism in mammals for the maintenance of the whole-body temperature at non-thermoneutral conditions. Apart from shivering, several mechanisms which can contribute to non-shivering thermogenesis in skeletal muscle (SM), including uncoupling of sarco(endo)plasmic reticulum calcium ATPases (SERCA) pump activity by sarcolipin (*Sln*), were described. Ambient temperature at birth and during early postnatal life can influence metabolism as well as non-shivering mechanisms in adults. The aim of this project is to study the effects of different ambient temperatures on the metabolism and mechanisms connected to non-shivering thermogenesis, mainly in SM. In this project, we are using two different strains of mice, obesity-prone C57BL/6J (B6) and obesity-resistant A/J, born and maintained at thermoneutrality (30°C) or at mild cold (20°C) till six weeks of age, when mice are moving at thermoneutrality. In the pilot study, we observed higher *Sln* gene expression in the skeletal muscle of A/J mice compared to B6 mice independent of ambient temperature. However, *Pdk4*, the gene of a key enzyme in the regulation of lipid metabolism, was unchanged between A/J and B6 maintained at 20°C but was significantly decreased in A/J mice maintained at 30°C. Currently, we are working on the collection of tissues connected to non-shivering thermogenesis, specifically different types of SN and brown adipose tissue from male mice dissected at different age stages of life. The next step will be to perform various analyses, such as measurement of plasma parameters, RNA sequencing of selected muscle samples, measurement of gene expression and protein content in SM and BAT.

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MATERNAL AUTO-ANTIBODIES AND THEIR ROLE IN AUTISM SPECTRUM DISORDER

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The maternal immune system was recognized as one of the major contributors to pathogenesis of autism spectrum disorder (ASD). Recent research provides a link between maternal auto-antibodies during pregnancy and various neurodevelopmental disorders including ASD. Collapsin response mediator proteins (CRMPs) are a family of microtubule-associated proteins identified as a major target of auto-antibodies. CRMPs are mediating repulsive Sema3A signaling during neurodevelopment and the latest research linked the deregulation of CRMP2 to various neuropsychiatric disorders such as autism spectrum disorder or schizophrenia. Despite extensive research in this area, the presence of autoantibodies targeting CRMP proteins in the context of the neurodevelopmental disorder remains unexplained. Using a combination of in vitro and in vivo methods, we demonstrate the effect of anti-CRMP2 antibodies on cortical lamination, neuronal migration, and axonal growth. Furthermore, we provide evidence for exogenous CRMP2 to affect the same processes with contrasting effects relative to anti-CRMP2 antibodies. Our data provide elucidation to the pathogenic mechanism of some maternal auto-antibodies during neurodevelopment with possible clinical relevance.

TRAK1 MODULATION OF MITOCHONDRIAL DYNAMICS IN NEURONS

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Mitochondrial dynamics, including fusion, fission and motility, are essential for neuronal function and survival. Deregulation of microtubule-based transport of mitochondria has been associated with several neurodevelopmental and neurodegenerative disorders; however, these processes are still not fully understood. Here we examined the role of TRAK1 - an adaptor protein that links mitochondria to microtubule-based molecular motors kinesins and dynein - in mitochondrial trafficking in neurons. Using confocal microscopy, live-cell imaging, we quantified mitochondrial motility in TRAK1 KO primary neuron cultures, or in WT neurons transfected with TRAK1 overexpressing or knock-down constructs. Our results indicate that TRAK1 downregulation affects particularly anterograde mitochondrial transport significantly decreasing the number of transported mitochondria, their velocity, traveled distance and time. However, retrograde transport is not significantly changed upon TRAK1 depletion. Furthermore, we found that deletion of the first 99 amino acids of the N-terminal domain (ΔN-TRAK1) induces mitochondrial fusion in transfected neurons and significantly interferes with the mitochondrial transport to the distal neurites. Together, these data suggest that TRAK1 is an essential regulator of mitochondrial dynamics in neurons. A more detail analysis of its deficiency in neurons will provide new information on how mitochondrial dynamics contribute to various neurological disorders.

PROLYL ISOMERASE FKBP12 BINDS TO CRMP2A AND REGULATES MICROTUBULE DYNAMICS

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Prolyl isomerases are enzymes catalyzing conformational changes of peptide bonds between proline and other amino acids in proteins and as such are important regulators of many processes in cells. We have shown that a significant substrate of phospho-specific prolyl isomerase PIN1 is Collapsin response mediator protein 2A (CRMP2A), which is a microtubule-associated protein highly expressed in neurons and particularly in axonal growth cones. The activity of CRMP2A is regulated by phosphorylation and in phosphorylated form, prolyl isomerase PIN1 can bind and stabilize the protein. If CRMP2A can be conformationally regulated also in its non-phosphorylated state is not known. Here, we show that prolyl isomerase FKBP12 is able to bind to CRMP2A specifically in its non-phosphorylated state and regulate its function. Using in vitro assays we show that CRMP2A is promoting microtubule growth and that this effect is abolished by the presence of FKBP12. To test the effect of FKBP12 on CRMP2A-mediated microtubule polymerization also in cells, we introduced the GFP-EB3 assay to track growing microtubule ends in the IMCD3 cells and observed the same effect as in vitro. Moreover, as CRMP2A is important mainly in neurons and growth cones, we tested the effect of FKBP12 on axonal growth. Overexpression of FKBP12 decreased the axon growth while knockdown had the opposite effect which is in agreement with the results obtained in microtubule dynamics assays. In conclusion, our data demonstrate that FKBP12 binds to CRMP2A and regulates microtubule dynamics and neural development.

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ROLE OF BCAA IN OXIDATIVE METABOLISM AND LIPID DROPLET BIOGENESIS IN PANCREATIC CANCER CELLS

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Branched-chain amino acid (BCAA) metabolism plays an important role in the development and spread of pancreatic cancer, particularly pancreatic ductal adenocarcinoma (PDAC). Patients who develop PDAC have elevated BCAA levels, but these levels are reduced in later stages of the disease. However, this association and the exact mechanism of the role of BCAAs in PDAC are not clearly understood. We have focused on the metabolism of BCAAs, whose products are particularly important for bioenergetics and fatty acid (FA) biosynthesis. We asked whether BCAAs serve as an oxidative substrate and as a factor in lipid droplet (LD) remodeling. In general, PDAC cell lines (PaTu-8902, MIA PaCa-2, and PANC-1) grown in a BCAA-free medium showed the greatest decrease in cell growth and lower respiration rates. To confirm the respiration results that BCAAs serve as an oxidative substrate, we measured TCA cycle metabolites that were lower in conditions without BCAA. Subsequent lipidomic analysis revealed a significant increase in triacylglycerols (TG) in BCAA-depleted media, which regulate FA uptake. We observed that LD enrichment in all three cell lines is correlated with increased TG synthesis. Branched-chain ketoacids were used for the rescue effect, and diacylglycerol acyltransferase inhibitors (DGATi) prevented the production of TG and LD. On the contrary, we confirmed that supplementation with etomoxir, a CPT1 inhibitor, increased LD production. Our results show a link between BCAA metabolism, FA import, and LD formation in PDAC cells. This may help to understand the role of BCAAs in PDAC and guide the target of anticancer therapy.

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ANTIOXIDANT ROLE AND CARDIOLIPIN REMODELING BY REDOX-ACTIVATED MITOCHONDRIAL CA^{2+}-INDEPENDENT PHOSPHOLIPASE A$_{2\gamma}$ IN THE BRAIN

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Redox-dependent regulations play an essential role in a wide range of biological activities. Mitochondria in numerous tissues represent a primary source of superoxide and subsequent downstream oxidants, notably H_2O_2 and lipid hydroperoxides. However, the understanding of the role of mitochondrial oxidant production in pathology and normal physiology is limited. Mitochondrial calcium-independent phospholipase $A_{2\gamma}$ (iPLA$_{2\gamma}$) belongs to a family of enzymes that participate in cellular signalling by simultaneously producing free fatty acids (FAs) and lysophospholipids. Here we hypothesize that redox activation of iPLA$_{2\gamma}$ has an antioxidant effect on the brain, as it contributes to FA-dependent H^+ transport, which leads to subsequent partial attenuation of mitochondrial oxidant production. We demonstrate the oxidant-induced activation of iPLA$_{2\gamma}$ by increasing respiration in brain mitochondria isolated from wild-type mice. The oxidant-induced increase in respiration was prevented by (1) *R*-bromoenol lactone (*R*-BEL), a selective inhibitor of iPLA$_{2\gamma}$, (2) carboxyatractyloside, an inhibitor of adenine nucleotide translocase (ANT), and (3) bovine serum albumin, the carrier of FAs. Oxidant-induced changes in respiration were absent in mitochondria isolated from iPLA$_{2\gamma}$-KO mice. Employing detailed lipidomic analysis, we also established a typical cleavage pattern for activated iPLA$_{2\gamma}$. The data show an increase in relative concentrations mainly of docosahexaenoic, arachidonic, and stearic acid which was prevented by *R*-BEL but not by ANT inhibitor. The acute antioxidant role of iPLA$_{2\gamma}$-released FAs is supported by monitoring both intramitochondrial superoxide and extramitochondrial H_2O_2 release. These results suggest that the oxidant-activated iPLA$_{2\gamma}$ releases free fatty acids, which promote ANT-dependent H^+ transport, leading to a decrease in the mitochondrial protonmotive force and subsequent attenuation of mitochondrial superoxide production.

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BIASED AGONISTS OF MUSCARINIC RECEPTORS

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The disruption of muscarinic signalling is frequently involved in various pathophysiological conditions, including neuropathic pain, neurological and psychiatric disorders, e.g., Alzheimer's disease or schizophrenia. To target these particular conditions, selective modulation of individual muscarinic subtypes to avoid undesired side effects is necessary. High homology of the orthosteric binding site among all muscarinic subtypes makes a finding of orthosteric agonists that bind selectively to individual muscarinic subtypes virtually unattainable. Selective targeting at a particular G-protein mediated signalling pathway by biased agonists, via agonist-specific conformation, can be the way to achieve functional selectivity among individual subtypes of muscarinic receptors. Specifically, the binding of an agonist to one or a subset of functional hot spots within the binding site results in activation of a subset of signalling pathways and thus in ligand-mediated signalling bias. An agonist relatively small in size has a greater chance to bind to a smaller number of functional hot spots than a larger agonist. We demonstrate that newly developed tetrahydropyridin based muscarinic agonists activate only subset of signalling pathways. They display agonist-specific activation profiles of individual G-protein isoforms, that differ among subtypes. Our results prove that selective activation of individual subtypes of muscarinic receptors by biased agonists can be achieved.

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DELETION OF BETA2* NICOTINIC ACETYLCHOLINE RECEPTORS IN STRIATAL INTERNEURONS INHIBIT STRIATAL ACTIVITY AND CONTROL STRIATAL-DEPENDENT BEHAVIORS.

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The importance of cholinergic signaling in the striatum for behavioral control and cognition has been extensively studied, but little is known about the functional role of nicotinic acetylcholine receptors (nAChRs) expressed by local neurons. The principal striatal neurons, the medium spiny neurons (MSNs), express only very low levels of nAChRs, hence the vast majority of nAChRs are expressed by striatal interneurons (INs), either cholinergic (CINs) or GABAergic (GABAINs), that overall represent less than 5% of the striatal population. We hypothesize that acetylcholine released by CINs activates nAChRs expressed by CINS themselves and GABAINs and that this activation is important for modulating striatal-based behavior.
At first, we used double fluorescent in situ hybridization (FISH) targeting the most prevalent nicotinic subunit, beta2, in combination with markers identifying CINs or GABAINs, to define which neuronal types express nAChRs, in the mouse striatum. To determine the functional role of strital nAChRs, we deleted beta2 nicotinic subunit by injecting Cre-expressing AAV viral vector into the dorsal striatum of beta2-flox/flox mice, which we tested in a battery of behavioral tasks. Mice with deletion of beta2* nAChRs showed increased anxiety-like behavior, together with a decrease in sociability ratio and a deficit in discrimination learning. As last task before sacrificing the animals, we tested the sensitivity to a stimulant drug, amphetamine, and analyzed the expression of a neural activity marker, c-Fos, in the dorsal striatum. The deletion of beta2* nAChRs increased amphetamine-induced hyperlocomotion along with c-Fos expression in MSNs and in striatal INs.
We conclude that beta2-containing nAChRs are primarily expressed by CINs in the striatum and that, even if in a small number, they modulate striatal signaling and striatal-based behavior.

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THE MAIN POTASSIUM IMPORTER IN YEAST RESPONDS TO DECREASE IN INTRACELLULAR POTASSIUM CONCENTRATION BY INCREASING AFFINITY AND MAXIMUM VELOCITY

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One of the key prerequisites for yeast cell growth is uptake of essential compounds, such as potassium. Potassium is a vital cation and its sufficient intracellular concentration is crucial for various processes, for instance: regulation of membrane potential and cell turgor, enzymatic activity and protein synthesis. However, excess of internal potassium is potentially toxic for yeast cells and can lead to loss of membrane potential, deacidification of vacuoles and cell death. It is therefore of vital importance that the import of potassium is kept within precisely defined boundaries, regardless of its external concentrations. In yeast *Saccharomyces cerevisiae*, transporter protein Trk1 is considered a key player in potassium uptake. The most distinctive feature of Trk1 is its alleged ability to switch between two affinity modes (low and high-affinity mode), as a response to changes in external potassium concentration. The precise nature and regulation of the Trk1-mediated affinity changes remain unclear. In our study we focused on dependence of kinetic parameters of Trk1 on changes in external and internal potassium concentrations as well as characterization of specific mutations that abolish proper switching between affinity modes to elucidate, in more detail, the potential mechanism, dynamics and regulation of affinity changes of Trk1. We found a significant correlation between gradual loss of intracellular potassium and increase in both affinity and maximum velocity of Trk1-mediated transport, suggesting the possibility that Trk1 precisely adjusts its kinetic parameters as a response to changes in internal potassium content. Additionally, set of specific mutations within region of selectivity filter and potassium-binding site revealed a putative role of this region in the aforementioned changes in capacity of Trk1-mediated potassium uptake.

THE ROLE OF PEROXIREDOXIN 6 IN BIOSYNTHESIS OF ANTI-DIABETIC AND ANTI-INFLAMMATORY FAHFA

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Fatty acid esters of hydroxyl fatty acids (FAHFAs) represent a diverse group of recently discovered lipids with distinct biological activities - mainly anti-diabetic and anti-inflammatory. However, their metabolism has not been fully elucidated yet. Peroxiredoxin 6 (Prdx6), which belongs to a family of antioxidant enzymes, was shown to be involved in biosynthesis of FAHFA. Prdx6 is a multifunctional enzyme with ability to repair peroxidized membrane phospholids through phospholipid hydroperoxide GSH peroxidase (PHGPx), phospholipase A2 (PLA2) and lysophosphatidylcholine acyl transferase (LPCAT) activities. During this process, it generates precursors that could serve as a source of hydroxy fatty acid needed for FAHFA synthesis. In this study, we used three different mouse models with genetically altered Prdx6 to a certain extend and a wild-type group as a control. Collected tissues were extracted and subjected to lipidomic profiling together with targeted analysis of FAHFA using LC-MS platforms.

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LC-MS WORKFLOW (LIMeX) FOR UNTARGETED METABOLOMICS AND LIPIDOMICS ANALYSIS OF MOUSE PLASMA, FECES, AND CECUM-CONTENT

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Liquid chromatography-mass spectrometry (LC–MS) has become the most applied chromatography–MS tool for the analysis of both polar and nonpolar metabolites. The true breadth and scope of polar metabolites (metabolome), complex lipids (lipidome), and various exogenous compounds, such as drugs or food components (exposome), cannot be captured by a single extraction method or instrumental platform. Thus, the task is to achieve high metabolite coverage using as few platforms as possible while maintaining the requisite precision and accuracy. For mouse plasma, feces, and cecum-content, we have developed and validated an LC–MS workflow LIMeX for the simultaneous extraction of complex LIpids, polar Metabolites, and eXposome compounds (specifically antibiotics). The sub-groups of compounds are isolated using an 'all-in-one' extraction with a methanol/methyl tert-butyl ether mixture and water. Analysis of complex lipids is conducted using reversed-phase LC (RPLC) in positive and negative electrospray (ESI) mode while polar metabolites and exposome compounds are separated using hydrophilic interaction chromatography (HILIC) in ESI(+) and RPLC in ESI(-). Simultaneous acquisition of MS1 and MS/MS spectra in data-dependent mode is used for each platform. The acquired raw data files are processed using MS-DIAL 4 software. Overall, 500+ simple and complex lipids and 100+ polar metabolites can be reported based on matrix type.

DECIPHERING THE IMPACT OF THE REVERSED RESTRICTED FEEDING ON THE CIRCADIAN CLOCK IN CHOROID PLEXUS

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The epithelial cells of the choroid plexus (CP) in the brain ventricles produce cerebrospinal fluid and act as a blood-cerebrospinal fluid barrier. Importantly, the CP cells harbor a robust circadian oscillator that may control the function of CP. Recently, the oscillator has attracted attention primarily because it represents a non-neural clock in the brain that may play an important role in the circuitry between brain clocks. Therefore, understanding the mechanism of how the CP clock is synchronized is of utmost importance. The CP clock has been found to respond to glucocorticoids, but its sensitivity to other potential entraining signals has not been elucidated. In this study, we examined the effects of reverse restricted feeding (rRF) on the CP clock in mPer2^{Luc} mice. Mice either had unlimited access to food (ad libitum group) or received food for 6 hours in the middle of their resting period for 12 days (rRF group). The CP was collected from the 4th and lateral ventricles at 4-hour intervals around the clock to determine daily gene expression profiles by RT qPCR. We found that rRF has significant and ventricle-specific effects on the amplitude and phase of CP clocks. In addition, rRF affects the expression of selected CP function-related genes and modulates the expression of inflammatory genes. The results provide the first evidence that rRF strengthens and entrains the clock in CP, indicating the importance of the feeding regime for the non-neuronal brain clock.

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MODUTION OF IMMEDIATE-EARLY GENE EXPRESSION IN THE HIPPOCAMPAL CA1 BY LONG-TERM AND SHORT-TERM BEHAVIORAL EXPERIENCE

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Immediate-early genes (IEGs) are essential for memory formation, have been implicated in several neuropsychiatric disorders, and are used for mapping plasticity-related activity.

We applied the catFISH two-time point IEG RNA imaging to investigate how the expression of IEGs *Arc* and *Homer1a* in the left dorsal hippocampal CA1 is modulated by previous long-term behavioral experience and how the dynamics of expression change over the short-term.

48 male Long-Evans rats were either trained for 4 days in a task involving a wheeled robot in a circular open-field or served as cage-controls (CC). We used food restriction and foraging during training sessions to stimulate exploratory behavior. On the 5th day (test) we assessed the IEG expression. *Homer1a* marked the first and *Arc* marked the last 5 min of a 30 min test session. Experimental groups received training with either randomly moving (M) or stable (S) robot. If rats got into the vicinity of the robot they received a mild aversive foot-shock. On the test day, the rats experienced either the same (M/M, S/S) or alternative robot behavior (M/S, S/M) as in training or served as CC (M/CC, S/CC, CC/CC). The behavior of the robot did not change during the testing session, and the shocks were disabled.

Our preliminary results show that previous training did not change the percentage of IEG-positive neurons regardless of whether the condition on the test day was the same or different as in training. The IEG expression was decreased in all experimental animals at the end of the test session.

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CHEMOGENETICALLY INDUCED BRAIN-WIDE REDUCTION OF PARVALBUMIN INTERNEURONS ACTIVITY: ELECTROPHYSIOLOGICAL PRELIMINARY STUDY

Kristyna Maleninska[1,2], Dominika Radostova[1], Martina Janikova[1], David Levcik[1], Jan Svoboda[1], Hana Brozka[1], Ales Stuchlik[1]

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In schizophrenia, the power of gamma oscillation is abnormally increased during resting states and fails to upregulate during cognitively demanding tasks. As parvalbumin interneurons (PVIs) participates in generation of gamma oscillations, their altered function could therefore participate in development of schizophrenia. Also, changes in PVIs were confirmed in postmortem studies in schizophrenia patients. In our study we aimed to explore the effect of brain-wide chemogenetic inhibition of PVIs on brain wave oscillations and on behavioral in PV-cre mice. The animals were transduced by AAV PHP.eB capsids with double floxed hM4D(Gi) DREADD receptors coupled with mCherry via the left jugular vein. In two different mice cohorts we either studied changes of gamma and other band oscillations in prefrontal cortex and hippocampus or observed changes in schizophrenia-like behavior. The electrophysiological measurements revealed higher network excitability in the dorsal hippocampus in the alpha band in contrast with activity in the prefrontal cortex where the alpha activity was reduced. In preliminary behavior data, we found group and sex differences in elevated plus maze test, open-field and prepulse inhibition task. The animal model of schizophrenia based on chemogenetic disruptions of PVIs functions seems to be promising for further studies of various schizophrenia-like symptoms and disruptions in behavior and electrophysiology. To the future, detailed analysis with larger groups and more accurate dosing od AVV vectors can create useful animal model of schizophrenia.

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A NOVEL ONE-TRIAL ASSOCIATION TASK RELEVANT TO EPISODIC-LIKE MEMORY IN RATS

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In everyday life, episodic memories are acquired incidentally in a single-encounter fashion and are composed of sub-events separated by time gaps. We designed a behavioral task called One-Trial Trace Escape Reaction (OTTER), in which rats incidentally associated two temporally discontinuous stimuli. In OTTER, rats associate a neutral acoustic cue (conditioned stimulus, CS) with an aversive stimulus (unconditioned stimulus, US) which occurs two seconds later: we call this sequence CS-2s-US. In the first few sessions, rats are habituated to two similar environmental contexts (A and B); each context consists of interconnected dark and light sub-areas. Next, in the pairing session, rats experience CS-2s-US in the dark sub-area of one of the environmental contexts (either A or B). The US is terminated immediately after a rat escapes into the light sub-area. During the recall session 24 hours later, rats are presented with only the CS in the alternate environmental context (B or A) and their behavioral response is observed. Our results show that 50% of handled rats and 14% of non-handled rats responded to the CS by escaping to the light sub-area although they experienced only a single CS-2s-US pairing. The capacity to acquire a CS-2s-US association in a single CS-2s-US pairing indicates that rodents are able to form incidental temporal associations. The OTTER behavioral task offers a flexible high throughput tool to study memories acquired incidentally after a single experience.

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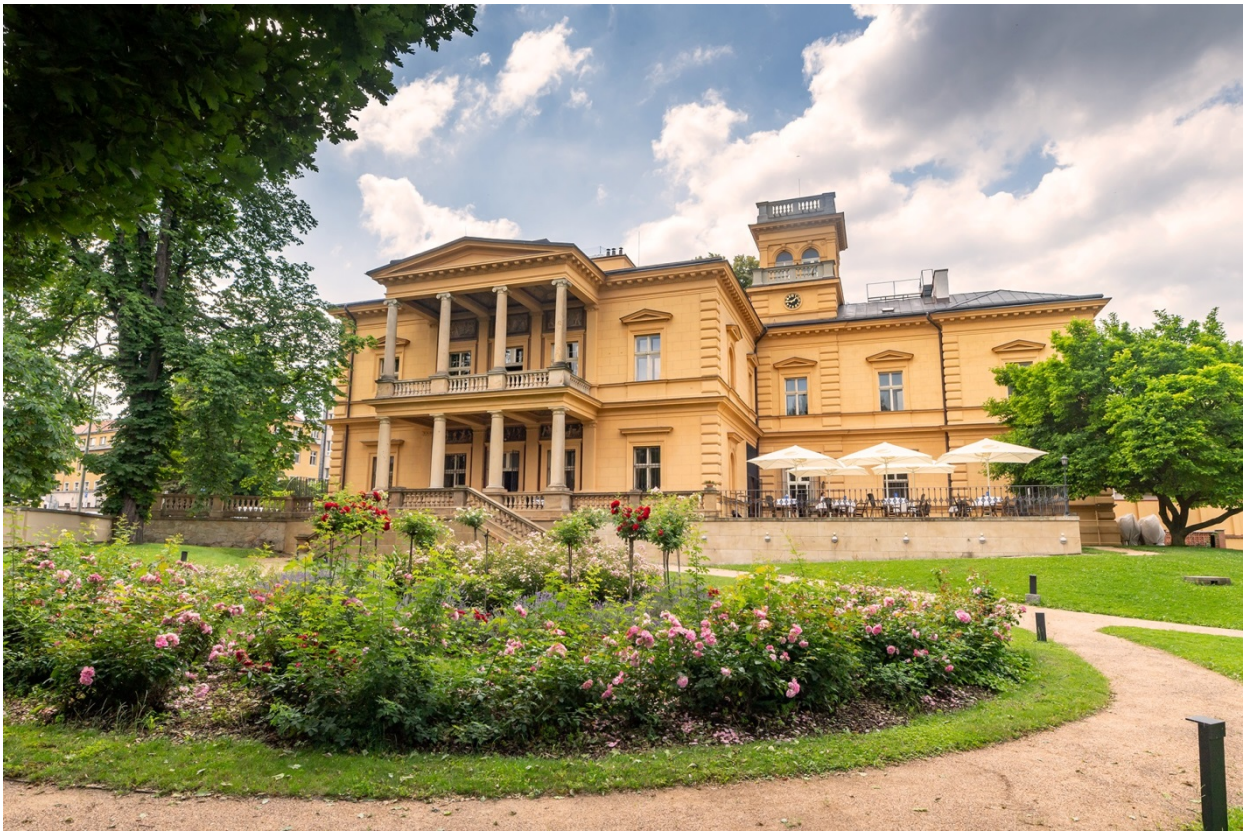

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