PHD MEETING
SEČ 2019

October 29 – 31, 2019

CONTENT

THE ORGANIZING COMMITTEE:

Ph.D. students:

Alice Abbondanza
Nikhil Ahuja
Vendula Čečmanová
Šárka Danačíková
Jiří Funda
Jaroslav Hrdlička
Veronika Kalendová
Lucie Leňková
Lucie Máčiková
Karolína Šuchmanová

Head of the Ph.D programme:
Martin Balaštík, Ph.D.

Secretariat of the Institute:
Diana Moosová

PROGRAM

29.10.2019

09:30	*Departure from Prague*	
11:30	*Arrival to Seč*	
12:00	**Lunch**	
13:00 – 13:20	**Opening speech: Jan Kopecký**	
	Introduction: Martin Balaštík	
13:20 – 14:00	**Session 1**	Chairs: *Funda, Leňková*
		Máčiková, Weissová, Kolcheva, Kalendová
14:00 – 14:40	**Session 2**	Chairs: *Abbondanza, Danačíková*
		Oseeva, Čečmanová, Sistilli, Funda
15:00 – 15:30	*Accommodation*	
15:30	**Coffee break**	
16:00 – 17:30	**Poster session 1**	
17:30 – 19:00	**Invited speaker: Jason Hwang**	
19:00	**Dinner**	
20:00 – 21:00	**Workshop: Jason Hwang**	
20:30	*Free evening/ Karaoke*	

30.10.2019

09:00 – 10:10	**Session 3**	Chairs: *Čečmanová, Šuchmanová*
		Korandová, Sivčev, Krajčovič, Doubková, Sinica,
		Pavluch, Janíková
10,30	**Coffee break**	
10:10 – 12:00	**Poster session 2**	
12:00	**Lunch**	
14:00 – 15:10	**Session 4**	Chairs: *Ahuja, Kalendová*
		Cechová, Kysilov, Malenínská, Chvojka,
		Šuchmanová, Benák, Balounová
15,10	**Coffee break**	
15:30 – 17:20		Chairs: *Máčiková, Kudláček*
	Invited speaker: Michaela Tencerová	
	Invited speaker: Helena Janíčková	
17:20 – 17:40	Awards announcement	
19:00	**Dinner**	
19:30	*Septolete Band*	

31.10.2019

09:00 – 09:20	**Traveling options: Viktor Kratochvíl**
09:20 – 09:40	**Popularization in: Olga Zimmermannová**
09:40 – 09:55	Students Questionare results

ORAL PRESENTATIONS

AFFECT OF AGING AND NEOPLASTIC TRANSFORMATION ON DIURNAL EXPRESSION OF THE CELL CYCLE GENES IN THE COLON

K. Balounova[1,2], M. Sotak[3], P. Ergang[1], M. Vodicka[1,2], K. Vagnerova[1], P. Kvapilova[1] and J. Pacha[1,2]

[1]Institute of Physiology, Czech Academy of Sciences, Prague, Czech Republic; [2]Faculty of Science, Charles University, Prague, Czech Republic; [3]Cardiovascular and Metabolic Diseases, Innovative Medicines and Early Development Biotech Unit, AstraZeneca, Mölndal, Sweden

The increasing risk of colon cancer is associated with aging or with disruption of the circadian clock. One of the main regulatory pathway - cell cycle - can be viewed as an endogenous oscillation and together with the circadian oscillation it creates a link whose disruption appears to be involved in aging and tumorigenesis. In our study, we focused on the impact of aging and tumorigenesis on the 24-hr mRNA expression profile of cell cycle genes, clock-output genes and clock genes. We compared their diurnal oscillations in the healthy colon of young mice and in the older mice with and without colorectal tumors. The circadian rhythms of clock genes (*Bmal1*, *Per2*, *Rev-erbα*, *Dec1/2*) and clock-output genes (*Dbp*, *Hlf*, *Tef*) were mostly undisturbed by age with the exception of lost rhythmicity of *Dec1/2* in older mice. *Rev-erbα* was the most affected clock and clock-output gene by tumorigenesis. Others maintained their oscillation with dampened amplitude. On the contrary, the effect of age was very obvious in cell cycle genes. Genes encoding cyclins (*Ccnd1-3*, *Ccne2*, *Ccna2*, *Ccnb1/2*), their kinases (*Cdk1*, *Cdk2*) and other regulators (*p21*, *E2f1/2/7/8*, *Wee1*, *Myt1*, *Cdc25a/b/c*) were arhythmic in the older colon, except of *Cdc25b/c*. The results suggest that both aging and tumorigenesis have an impact on the diurnal oscilations in the colon. The impact is markedly greater in the cell cycle genes and that supports theory of uncouple cell cycle from the circadian clock control.

THE ROLE OF RNA DEMETHYLASE FTO IN THE MYOCARDIUM

D. Benak[1], D. Sotakova[1], M. Cyprova[1], K. Holzerova[1], P. Telensky[2], Z. Bendova[2], J. Neckar[1], F. Kolar[1] and M. Hlavackova[1]

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Increased tolerance to ischemia/reperfusion injury can be induced by adaptation to chronic hypoxia. One of the adaptative changes is a shift in cellular energy metabolism. This alteration may be regulated by recently discovered fat mass and obesity associated (FTO) protein, an RNA-based epigenetic regulator (N-6 methyladenosine demethylase), known to affect energy metabolism. This project aimed to study the role of FTO in the chronically hypoxic hearts. Adult male Sprague-Dawley rats were adapted to continuous normobaric hypoxia (CNH; 10% or 12% O_2; 3 weeks). CNH reduced the size of myocardial infarction by 20% in these animals. The FTO protein level was measured in the tissues of left (LV) and right (RV) heart ventricles and moreover in the liver and the cerebrum. Under the normoxic conditions, the level of FTO in the RV was by 50% higher than in the LV. No circadian rhythmicity of FTO protein in the heart was observed. In the liver and the cerebrum, the levels of FTO were 6-times and 11-times higher than in the LV. CNH led to a significant increase of FTO protein level by 21% in the LV and by 27% in the RV. In the liver and the cerebrum, the levels were unaffected by CNH. Inhibition of FTO activity in isolated adult rat cardiomyocytes exposed to anoxia/reoxygenation increased the cellular injury measured by lactate dehydrogenase release. Our results show that CNH increases level of the FTO in the heart, which may potentially participate in the ischemia-resistant phenotype.

SINGLE-MOLECULE PHOTOGATE IMAGING REVEALED THAT κ-OPIOID RECEPTORS FORM DIMERS, WHEREAS μ-, AND δ-OPIOID RECEPTORS ARE MONOMERS

K. Cechova[1,2], M.H. Ulbrich[3,4] and P. Svoboda[1]

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Heroin and morphine are well-known substances used and misused for their effects like pain relief and euphoria. They process these effects via opioid receptors (OR), members of class A of G-protein coupled receptors. Three main types of OR were identified based on their genes: μ-, δ-, κ-OR. However, more subtypes were suggested as pharmacological properties of these OR are more diverse. One possible explanation is that OR form homo-oligomers and/or hetero-oligomers. In general, oligomerization is an important feature to understand the function of the given type of receptor. In the case of OR, the incidence of oligomerization is controversial, and the results are contradictory. Here, we decided to zoom in to single molecules to look at the homo-oligomerization of opioid receptors. In CHO-K1 cells, we expressed OR modified with fluorescent tags - red-colored SNAP-tag or green GFP. First, we used dual-color Total Internal Reflection Fluorescent Microscopy (TIRFM) to image cells with single-molecule expression level (<5 spots/μm^2). In these low densities, all three types of OR are predominantly monomers. Because physiological expression of OR is higher than density suitable for most single-molecule imaging configurations, we used dual-color PhotoGate in TIRFM set-up to overcome this issue. This technique allowed us to visualize single molecules, yet the overall expression is much higher (10-120 spots/ μm^2). Based on results from PhotoGate experiments, we conclude that κ-opioid receptors form dimers in higher densities (>30 spots/μm^2), whereas μ-OR and δ-OR remain monomers.

THE ROLE OF GLUCOCORTICOIDS IN THE ONTOGENESIS OF THE CIRCADIAN CLOCK

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Synchronization of the circadian clock with external environment is crucial for proper function of almost all metabolic and behavioural processes within the organism. The principal clock is located in suprachiasmatic nuclei (SCN) of the hypothalamus. Due to mutual connections between neurons, the SCN generate a robust rhythmic signal, which synchronizes the organism with daily changes in light conditions. SCN are developed gradually during the ontogenesis, neurogenesis is completed during the embryonic stage, but synaptogenesis is finished postnatally. Notably, the rhythmic expression of so called clock genes responsible for the molecular mechanism of the circadian clock is detected already at the late embryonic stage. The pups are born with the SCN clocks synchronized with the maternal circadian system but the mechanism of entrainment is unknown. To study the development of circadian clock, we have established the *in vitro* model of long-term cultivated organotypic explants of the embryonic SCN and placenta from transgenic Per2::LUC mice. This model allows monitoring of expression of one of the clock proteins PER2 by bioluminescence recordings in real time. The use of explants of both the fetal SCN and placenta provides a complex view on the development of the fetal circadian clock. *In vitro* results demonstrate that the rhythmic expression of clock genes in the SCN is developed gradually and spontaneously a few days before birth and individually within the litter. The data further show that two parts of placenta differ in the level of PER2 expression – the maternal part manifests the rhythmic expression of PER2 protein whereas the fetal part does not exhibit circadian oscillations. We examined the role of glucocorticoids, one of the maternal rhythmic cues, as a potential synchronizing signal for fetal clock. Our results show that glucocorticoids can synchronize clock in both fetal SCN and placenta.

PATHOLOGY OF EXTRACELLULAR MATRIX PROTEINS AND FIBROBLAST BEHAVIOUR IN IDIOPATHIC CLUBFOOT TISSUE CONTRACTURE

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Idiopathic clubfoot (CF, *talipes equinovarus*) is the second most common congenital orthopaedic deformity, affecting the musculoskeletal system of a lower limb. Stiff, contracted tissue is formed between the *medial malleolus*, *sustenaculum tali* and *os naviculare*, rotating the foot inwards and downwards. Current treatment consists of physiotherapy, repeated cast fixations and Achilles tenotomy (the Ponseti). Even though various hypotheses have been proposed the etiology of CF is not clearly understood. The mass of a contracted tissue on CF medial side closely resemble tissues from other fibroproliferative disorders (e.g. Dupuytren's contracture), showing signs of fibrosis in histologically stained samples. High amount of extracellular matrix (ECM) proteins (e.g. collagen type I, III, VI) and profibrotic cytokines (TGFβ, PDGF) in CF tissue was also confirmed by our proteomic studies. In order to investigate the ECM composition more closely we have established and characterized primary cell cultures isolated from tissues of relapsed patients undergoing surgery (n=19). The affected tissue is subjected to mechanical tension. Predominantly fibroblasts are present, but also myofibroblasts – the cells related to tissue remodeling and fibrosis, which are known to react to mechanical stimuli. Therefore we are studying the cells in static conditions as well as in a dynamic culture system to emulate mechanical stress the cells are exposed to *in vivo*. Our main goal is to identify possible targets for antifibrotic treatment, to choose and test suitable substances which could impact the stability and stiffness of the ECM and thus reduce the contracture. This research can provide valuable information that might contribute to the development of adjuvant pharmacological therapy for use alongside the standard Ponseti treatment of relapsed CF and lessen the number of patients in need of a surgery.

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ALTERATIONS OF LIPID METABOLISM IN THE LIVER AND ADIPOSE TISSUE OF GPR-10 DEFICIENT MICE

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GPR-10 is a G-protein-coupled receptor expressed in parts of the brain involved in the regulation of body weight and food consumption. We aimed to characterize the effects of GPR-10 gene disruption on energy homeostasis on both whole body and organ levels. The deletion of GPR-10 gene (GPR-10 KO) was performed on mice with C57BL/6 genetic background. Both males and females were used in the study. Indirect calorimetry and an oral glucose tolerance test (OGTT) were performed and the levels of gene expression were determined in the liver and in adipose tissue using qPCR. GPR-10 KO males displayed higher body weight in comparison with wild-type littermates probably due to increased adipose tissue mass. GPR-10 KO females displayed elevated expression of genes engaged in the regulation of lipid metabolism in the liver, epididymal and subcutaneous white adipose tissue depots (eWAT/scWAT) in comparison with wild-type littermates. The expression of genes involved in lipogenesis was supressed in scWAT of GPR-10 KO males. Indirect calorimetry did not reveal any differences in energy expenditure. GPR-10 KO mice displayed higher levels of insulin in plasma 30 minutes after glucose administration in comparison with wild-type littermates. In addition, decline in plasmatic non-esterified fatty acids levels 30 minutes after glucose administration was less pronounced in GPR-10 KO mice, suggesting a defect in insulin-mediated supression of lipolysis. This was supported by higher triglyceride content in the liver of GPR-10 KO mice of both genders. In conclusion, GPR-10 gene deletion resulted in alterations of lipid metabolism in mice of both genders. Increase in adipose tissue mass observed only in GPR-10 KO males was possibly prevented in GPR-10 KO females owing to compensatory increase in the expression of metabolic genes. Altered response to OGTT in GPR-10 KO mice suggested impaired insulin sensitivity.

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STATE-DEPENDENT EFFECT OF INTERICTAL

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The impact of interictal epileptiform discharges (IEDs) on ictogenesis is not well understood. Previous studies demonstrated that IEDs are able to promote transition to seizure but also the presence of IEDs may delay or prevent seizure genesis. Using computer modeling, it was observed that the pro-ictogenic or anti-ictogenic nature of IEDs depends on the state of stability of the ictogenic network. In this study, we have explored this theory *in vitro*. Isolated CA1 slices were perfused with high potassium (>8 mM) aCSF. Field potentials from the hippocampal CA1 were recorded using extracellular electrodes. Interictal perturbations mimicking IEDs were delivered by stimulation of Schaffer collaterals. The CA1 network was perturbed with 1 Hz stimuli initiated after the end of the seizure. This stimulation delayed seizure onset (>50% increase, n=41/7 stimulations/slices). Prolongation of interictal period positively correlated with the duration of the stimulation (r = 0,97, 95% CI [0,62 0,99]). To evaluate the hypothesis that pro-seizure effect of IED occurs when the neural network is unstable, we have delivered a single stimulus either early after previous seizure or just before the next seizure. Only 38 % of early stimulations with the intensity of 300 µA were able to induce seizure (n=3/8 stimulations). In contrary, preictal stimulation with the intensity of 200 µA and 300 µA induced seizure in all cases (6/6 and 4/4 stimulations respectively). IEDs display anti-seizure effect if delivered in stable network state, pro-ictogenic effect occurs if the network exists in a less stable state. Results may have implications for design and optimization of brain stimulation therapy.

EFFECT OF ANTIGLUTAMATERGIC AGENTS ON COGNITIVE DEFICIT IN ANIMAL MODELS OF OBSESSIVE-COMPULSIVE DISORDER

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Obsessive-compulsive disorder is chronic neuropsychiatric disorder with lifetime prevalence of 1.6-2.5%. It is characterized by presence of obsessions or compulsions, usually both. Commonly found neuropathology in OCD patients is dysfunction within cortico-striato-thalamo-cortical circuits. Those alterations are hypothesized to be caused by hypoactivity of serotoninergic transmission, which leads to dopaminergic and glutamatergic hyperactivity. Because only 40-85% of patients respond to established SSRIs treatment, novel drug approaches that would target also dopaminergic and glutamatergic neurotransmission could be very beneficial. In our research we focused on effect of two antiglutamatergic agents, memantine and riluzole, in quinpirole (QNP) and 8-OH-DPAT sensitization animal models of OCD. Active place avoidance task in rotating arena with unmarked to-be avoided shock sector was used. During habituation phase, rats were subcutaneously injected with QNP/8-OH-DPAT (0.25 mg/kg) or saline solution and placed into rotating carousel arena without foot shock for 50-min free exploration. During acquisition phase rats were injected with memantine, riluzole (1 mg/kg) or saline 30 minutes before arena and with QNP/8-OH-DPAT or saline solution immediately before being placed into rotating arena with invisible shock sector. We found that both QNP and 8-OH-DPAT sensitization produced robust deficit in place learning and hyperlocomotion. Riluzole significantly decreased hyperlocomotion in 8-OH-DPAT model, however neither drug was significantly effective in decreasing spatial learning impairment measured by number of entrances into the shock sector.

METABOLIC EFFECTS OF N-3 FATTY ACIDS AS CALANUS OIL IN TRANSGENIC MICE WITH MODIFIED PPAR 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 metabolism genes. We aimed to determine how modifications in the PPARα expression affect metabolic effects of Calanus oil (CO), which is a novel lipid form of EPA/DHA (i.e. wax esters) isolated from marine zooplankton *Calanus finmarchicus*. 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 ~16 % 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.

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LECTINS MODULATE THE FUNCTIONAL PROPERTIES OF GLUN1/GLUN3 CONTAINING NMDA RECEPTORS

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N-methyl-D-aspartate receptors (NMDARs) play a key role in mediating excitatory neurotransmission within the mammalian central nervous system (CNS). NMDARs are heteromultimers containing GluN1, GluN2, and/or GluN3 subunits, thus giving rise to a wide variety of subunit combinations, each with unique functional and pharmacological properties. Importantly, GluN1/GluN3A and GluN1/GluN3B receptors form glycine-gated receptors. Here, we used electrophysiology in transfected HEK293 cells to examine the putative roles that *N*-glycosylation and a panel of lectins play in regulating the functional properties of GluN1/GluN3 receptors. We found that removing specific *N*-glycosylation sites alters the functional properties of GluN1/GluN3B receptors. Moreover, we found that the functional properties of both GluN1/GluN3A and GluN1/GluN3B receptors are modulated by a variety of lectins, including ConA, WGA, and AAL, and this effect is likely mediated by a reduction in GluN1 subunit-mediated desensitization. We also found that AAL has the most profound effect on GluN1/GluN3 receptors, and this effect is mediated partly by a single *N*-glycosylation site on the GluN3 subunit (specifically, N565 on GluN3A and N465 on GluN3B). Finally, we found that lectins mediate their effect only when applied to non-activated receptors and have no effect when applied in the continuous presence of glycine. These findings provide further evidence to distinguish GluN1/GluN3 receptors from the canonical GluN1/GluN2 receptors and offer insight into how GluN1/GluN3 receptors may be regulated in the mammalian CNS.

MOLECULAR MECHANISM OF PATHOGENESIS IN PATIENTS WITH MUTATION IN MITOCHONDRIAL RNA POLYMERASE

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Mitochondrial RNA polymerase (POLRMT) is nuclear encoded enzyme – DNA dependent RNA polymerase responsible for transcription of the mitochondrial genome. Besides mitochondrial mRNA synthesis, POLRMT is also required for replication of the mitochondrial genome, since it synthesizes replicative RNA primers. Previously, function of POLRMT was studied in animal models, where absence of POLRMT gene led to embryonic lethality. Recently, whole exome and genome sequencing approaches identified sequential variants of POLRMT in a group of patients with suspected mitochondrial disease. Here we performed functional validation at the biochemical level in those cases, where patients' fibroblasts were available. Mitochondrial oxygen consumption analysis revealed combined defect in mitochondrial respiratory chain, as can be expected for mutations in POLRMT. While we did not observe profound differences in the amount of mitochondrial RNAs, steady state content of most of mtDNA encoded proteins was significantly lower. However, this was not accompanied by the decrease in mitochondrial proteosynthesis. Content of mitochondrial DNA amount was significantly increased in patients' fibroblasts, probably reflecting compensatory mechanism. The overall aim of presented project is unequivocal functional validation of POLRMT as a gene responsible for disease development and revelation of molecular mechanisms contributing towards the pathogenesis of this rare hereditary disease. Altogether, the obtained results may provide new insights into mitochondrial rare disease diagnosis and contribute to targeted therapies.

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HIPPOCAMPAL ENSEMBLE MECHANISMS OF DISORGANIZED COGNITION IN ANIMAL MODELS OF PSYCHOSIS AND SCHIZOPHRENIA

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Psychosis and schizophrenia (SZ) are marked by loose associations and delusions. Based on the convergence of our observations that MK-801 animal model of psychosis displays altered overlap of hippocampal (HPC) ensembles representing two distinct behavioral episodes and the emerging literature on the neural mechanisms of memory linking (Sehgal et al. 2018), we propose a novel hypothesis of HPC ensemble overlap as a substrate of excessive associations and cognitive disorganization in SZ. Both excessive CA1 ensemble overlap between distinct behavioral episodes and dimnished overlap between similar episodes might promote contextually inappropriate associations/cognitive linking that supports delusion formation. Examine CA1 ensemble overlap in acute and chronic/developmental models of psychosis/SZ. If altered ensemble overlap is present, then test for contextually inappropriate memory linking on behavioral level using fear conditioning. Here we report partial results as the project is ongoing. To assess similarity of ensembles representing two 5 min exploratory episodes separated by 20 min in homecage we used *Arc/Homer1a* catFISH (two-timepoint RNA *in situ* immediate-early gene imaging). Subjects, male Long-Evans rats, explored either twice the same environment (A/A) or two distinct environments (A/B). MK-801 0.15mg/kg or saline was i.p. injected 30-40 min prior to the first exploratory episode. Controls displayed higher ensemble similarity (ES) in A/A compared to A/B condition (ES≈40% and 20%). MK-801 substantially increased CA1 ensemble overlap in A/B – no stat. signf. difference in ES between A/A and A/B conditions (ES≈35%). In an independent replication the contextual specificity of ensembles was again eliminated. However, in this replication study MK-801 substantially decreased ensemble overlap in A/A to the level of no stat. signf. difference compared to A/B condition (ES≈20%). This effect might be related to drug pharmacokinetics and timing of behavioral episodes. Our data show that altered linking of CA1 ensembles representing distinct behavioral episodes is present in the acute MK-801 model. To test cognitive linking, chronic/developmental models are needed as acute NMDAR blockage by MK-801 disrupts long-term memory which is necessary for behavioral testing.

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PREGNANE BASED STEROIDS: NOVEL POSITIVE ALOSTERIC MODULATORS OF N-METHYL-D-ASPARTATE RECEPTORS

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The hypofunction of N-methyl-D-aspartate receptors (NMDARs) is implicated in neuropsychiatric disorders like autism, intellectual disability, and schizophrenia. Positive modulators of NMDARs, such as some neurosteroids, may have a favorable effect in these diseases. Experimental data from several laboratories indicate that steroids with a flat structure at the A/B ring – like an endogenous neurosteroid pregnenolone sulfate (PE-S) – potentiate NMDAR responses, while steroids with a "bent" structure – like an endogenous neurosteroid pregnanolone sulfate (PA-S) - inhibit them. We aimed to characterize the structure-activity relationship underlying the modulatory effect for newly synthetized analogs of PA-S. We prepared a set of PA analogs in which the ester bond was substituted by a C-C bond (ω5β-pregnan-3α-yl derivatives of carboxylic acids) and assessed their activity at recombinant GluN1/GluN2B receptors expressed in HEK293 cells using the patch-clamp technique. Our experiments showed that analogs with short C3 residues (e.g. PA-Acetate) inhibit NMDAR responses. However, derivatives with elongated aliphatic chain at C3 (e.g. PA-Butyrate; PA-But) potentiate the responses, which is unexpected due to their "bent" structure. Subsequent experiments indicated that PA-But act at the transmembrane domain (TMD) of NMDARs. Alanine scanning mutagenesis of the amino acid residues within outer segments of the TMDs of GluN1 and GluN2B subunits showed that residues which affect PA-But potentiation are located at the interface between the TM1 and TM4 of both subunits. Our results can contribute to the development of new therapeutics to treat the neuropsychiatric disorders associated with hypofunction of NMDARs.

CYTOPLASMIC INTER-SUBUNIT INTERFACE CONTROLS USE-DEPENDENCE OF THERMAL ACTIVATION OF TRPV3 CHANNEL

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Precise detection of environmental temperature and sensitization to potentially harmful heat is essential for any organism, human included, to avoid tissue damage. The vanilloid transient receptor potential ion channel TRPV3 is a putative molecular thermosensor most prominently expressed in the skin. Its initial activation requires high temperatures above 50 °C and repeated stimulation progressively shifts the activation threshold to physiological temperatures. This functional characteristic does not occur in the related heat-sensitive TRPV1 channel which activation threshold is retained above 40 °C during activations. What further makes the TRPV3 channel different from TRPV1 is a longer loop, called finger 3, between repeats 3 and 4 of its cytoplasmic N-terminal ankyrin repeat domain. We show that the chimeric replacement of the TRPV3 finger 3 tip with the homologous residues of TRPV1 resulted in channels that similarly to TRPV1 exhibited a lowered thermal threshold, were sensitized and failed to close completely after intense stimulation. Since finger 3 makes a contact with the β sheet of the adjacent subunit, the mutations in finger 3 might exert their effects through altering the β sheet-finger 3 inter-subunit interface. In support, cysteine substitutions of two residues, F259 and V385, crosslink the interface and lock the channel in the open state. The β sheet-finger 3 inter-subunit interface is therefore critical for thermal sensitization and represents a key element of TRPV3 regulation by temperature.

SPATIAL AND TEMPORAL DISRUPTIONS IN ANIMAL MODEL OF SCHIZOPHRENIA INDUCED BY DIZOCILPINE (MK-801)

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Temporal and spatial cognition constitute basic elements of the cognitive function. Perception of time, unlike spatial navigation and memory, is less explored. Impairments occur in many neurodegenerative and neuropsychiatric disorders and is evident that timing is impaired in patients with schizophrenia, but the results are still ambiguous. In our experiment, we tested temporal and spatial strategies using modified active place avoidance task on rotating arena in rat model of psychosis induced by dizocilpine. In this task carefully-titrated doses of dizocilpine disrupted timing strategy but not performance based on spatial orientation. Rats were not able to avoid the aversive sector in parts testing timing strategies and had higher number of entrances into it. The neural circuit which is affected in patients with schizophrenia is the same which should be the main in interval timing and our results support this concept. This modified task can also serve as a useful tool for testing other animal models.

OMEGA-3 INDEX IN THE CZECH REPUBLIC

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Omega-3 index is considered to be a marker of omega-3 fatty acids intake. It reflects the relative amount of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in red blood cells. Dietary intake of these essential fatty acids is an important component of healthy lifestyle. Their low consumption may increase the risk of cardiovascular and Alzheimer's diseases, dementia, poor fetal development and other problems. Our aim was to evaluate omega-3 index of people who live in the Czech Republic. For this purpose, red blood cells were obtained from volunteers and homogenized in a mixture of water, methanol and tert-butyl methyl ether to extract lipids. To convert esterified acyl chains into fatty acid methyl esters (FAMEs), we used sodium methoxide as a catalyst. FAMEs profile was measured by comprehensive two-dimensional gas chromatography coupled to time-of-flight mass analyzer. We compared omega-3 index of people from urban (n=487) and rural (n=387) areas and evaluated the general FAME profiles with respect to self-reported intake of omega-3 fatty acid supplements and fish consumption.

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A ROLE OF TRANSCRIPTION FACTOR NKX6.1 IN INSULIN SECRETION AND MITOCHONDRIAL BIOGENESIS IN PANCREATIC B-CELL LINE INS-1E

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Nkx6.1 is transcription factor specific for β cells in pancreatic islets. It controls a gene regulatory network and it is necessary for β cell maintenance and development. Nkx6.1 functions as a repressor for Arx, key factor for α-cell determination. The competition between Nkx6.1 and Arx determines the β or α cell identity, respectively. Our previous results showed, that diabetic Goto Kakizaki rats contain a lower number of mtDNA per nucleus, mitochondrial nucleoids specific proteins and that they have reduced amount of Nkx6.1 vs Wistar rats at the level of mRNA and protein. Because ATP is necessary for insulin secretion and mtDNA codes for one subunit of ATP synthase, we hypothesized, that Nkx6.1 affects transcription of some nucleoids specific factors and indirectly influences insulin secretion. Using CRISPR technology we edited genome of pancreatic β cell line INS-1E and prepared Nkx6.1 KO cell line from single cell colony. qPCR analysis revealed increase in transcription of insulin genes INS1 and INS2. On the contrary, insulin secretion were diminished as measured by ELISA. Main factor for mitochondrial biogenesis, PGC1α, was up-regulated. Nucleoid packaging protein TFAM and mtDNA polymerase γ were down-regulated. Our results can contribute to understanding of complicated β cell metabolism and heterogeneity.

BOTH NOXIOUS COLD AND HEAT ACTIVATION AND VOLTAGE-DEPENDENT MODULATION OF HUMAN AND MOUSE TRPA1

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Transient Receptor Potential Ankyrin 1 channel (TRPA1) serves as a key sensor for reactive electrophilic compounds across all species. Its sensitivity to temperature, on the other hand, differs among species, which has been attributed to an evolutionary divergence. Mouse TRPA1 was initially implicated in noxious cold detection but later on also identified as one of the prime noxious heat sensors. Moreover, human TRPA1, originally considered to be temperature-insensitive, turned to act as an intrinsic bidirectional thermosensor capable of sensing both cold and heat. Recently, a modular allosteric model has been proposed potentially capable of reconciling these disparate findings, predicting that activation of cold-activated channel could be achieved by a heat-activated temperature sensor. Here, we compare the temperature sensitivity of human and mouse TRPA1 and demonstrate that both orthologs are activated by noxious heat at negative and positive membrane potentials, and their kinetically distinct components of voltage-dependent gating are differentially modulated by cold and heat temperatures. We attribute the divergent thermal sensitivities of human and mouse TRPA1 to differences in intrinsic gating equilibrium and in allosteric coupling between the temperature- and voltage-sensor modules.

EFFICACY OF MARINE OMEGA-3 PHOSPHOLIPIDS IN ALLEVIATING DIFFERENT STAGES OF NAFLD IN DIETARY OBESE MICE

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Non-alcoholic fatty liver disease (NAFLD) encompasses a spectrum of diseases ranging from accumulation of fat in liver (steatosis), which can develop in steatohepatitis (NASH), if inflammation occurs. Regarding alleviation of steatosis, preclinical studies indicate higher efficacy of omega-3 fatty acids (ω-3), in particular EPA and DHA, when administered to rodents in form of marine phospholipids (PLs) compared to triacylglycerol (TAGs). However, the effects of ω-3 PLs on NASH are unknown. Therefore, we compared the efficacy of ω-3 PLs and TAGs in terms of alleviating liver steatosis/NASH using a new mouse model of NAFLD based on high-fat feeding at 30°C. Five groups of male C57BL6/N mice were reared at 30°C and fed for 24 weeks: 1) Chow, 2) lard-based high-fat diet (LHF), or 3) LHF supplemented with ω-3 PLs as Krill oil (ω3-PL; 30 mg EPA+DHA/g diet). Mice in the additional groups, i.e. 4) ω3-PL-R and 5) LHF supplemented with ω-3 TAGs (ω3-TG-R; 30 mg EPA+DHA/g), were fed LHF during the first 8 weeks of the study (R=reversal). Plasma markers of liver injury and hepatic TAGs were measured using biochemical methods, liver gene expression by quantitative PCR, and insulin sensitivity by hyperinsulinemic-euglycemic clamps. Compared to LHF, ω3-PL reduced body weight gain to 91%; liver TAGs were reduced to 54% as were plasma levels of AST (66%) and ALT (32%). Lipogenic genes expression was markedly down-regulated in ω3-PL. The changes in NAFLD phenotypes in ω3-PL-R were comparable to ω3-PL, while effects on inflammation- and collagen remodelling-related genes were even stronger. No significant changes were detected in ω3-TG-R. Finally, the effects of ω-3 PLs occurred in a situation of improved whole-body insulin sensitivity. Our data confirm the superior efficacy of ω-3 PLs on NAFLD-related phenotypes under stringent experimental conditions provided by the appropriate murine NAFLD model.

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ALLOSTERIC MODULATION OF P2X RECEPTORS BY NEUROSTEROIDS

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Purinergic receptors (P2XRs) are unevenly distributed in central and peripheral nervous systems and other tissues. Endogenous molecules such as divalent cations and phospholipids can allosterically modulate P2XRs. However, little is known about modulation by neurosteroids. We tested several steroids, neurosteroids and their derivatives (testosterone, bioamid- allosteric modulators of NMDA receptors, bile acids) for their capability to modulate P2XRs. Several analogues were newly synthesized to increase their activity. The effect of drugs was examined electrophysiologically in HEK293 cells expressing recombinant P2X2R, P2X4R and P2X7R, and pituitary cells endogenously expressing these receptors. Our measurements revealed that numerous steroids modulate positively 1μM ATP-evoked currents in cells expressing the P2X4Rs and P2X2Rs. Only derivatives of secondary bile acids, particularly lithocholic acid, exhibited inhibitory effect on P2X2R currents. The potentiating effect of steroids was concentration dependent and was also observed on endogenously expressed P2XRs in pituitary cells. Synthetic testosterone derivatives increase receptor sensitivity to ATP, reduce rate of P2X4R desensitization and accelerate resensitization. However, pretreatment with lithocholic acid exhibited significantly lower effect on P2X4R desensitization and resensitization. Both testosterone derivatives and lithocholic acid antagonize the deactivation effect of ivermectin, the P2X4R-specific modulator, in a concentration-dependent manner, suggesting these drugs bind to a position related to ivermectin binding site within transmembrane domain. These results provide evidence for allosteric modulation of particular subtypes of P2XRs by steroids, potential role of ivermectin binding site in steroid binding, and plasticity in the mechanism by which P2XRs respond to allosteric signals by steroidal compounds.

SYNCHRONIZATION OF THE HIPPOCAMPAL CIRCADIAN CLOCK

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The daily regulation of behavioral and physiological processes, including memory formation, is maintained by the circadian system, consisting of principal clock in the suprachiasmatic nuclei of the hypothalamus, and peripheral clocks. Peripheral circadian clock has also been found in the hippocampus, where it temporally regulates processes related to memory formation. However, little is still known about mechanisms synchronizing the clock with external cues. Therefore, our aim was to ascertain possible role of glucocorticoid hormones (GCs) in this process. The GCs are likely candidates because hippocampus is involved in their regulation and contains high levels of glucocorticoid receptors. To achieve this, we first investigated the impact of GCs absence on the hippocampal clock in vivo in Wistar rats. Expression profiles of clock genes *Per1, Per2, Rev-erbα* and *Bmal1* were examined using RT-PCR. We found that absence of GCs abolished rhythmical clock gene expression in the hippocampus, and administration of GCs analog dexamethasone partly reversed this effect. Next, we measured responses of the clock to the GC stimulation in vitro using long-term cultivated organotypic hippocampal explants from Per2::LUC mice. The GC signaling directly influenced circadian rhythmicity of PER2 protein *in vitro*. The effect was dependent on time of GCs pathway stimulation and was inhibited by glucocorticoid receptor antagonist. These findings favor the role of GCs as hippocampal clock synchronizers. Our results provide insight into plausible mechanisms of hippocampal circadian clock dysfunction in neuropsychiatric disorders accompanied by abnormal glucocorticoid levels and memory impairment and should also be taken into consideration when administering systemic GC therapy.

PROLYL ISOMERASE FKBP12 BINDS CRMP2A, REGULATES ITS PHOSPHORYLATION, MICROTUBULE DYNAMICS AND NEURAL DEVELOPMENT

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CRMP2 (Collapsin response mediator protein 2) is a microtubule associated protein which is enriched in neurons and plays an important role in microtubule dynamics and neural development. Its function is regulated by phosphorylation by kinases CDK5 and GSK3beta on several phosphorylation sites. CRMP2 exists in two isoforms, CRMP2B and longer CRMP2A, but mechanisms regulating CRMP2 in an isoform-specific way are still poorly understood. We have previously shown that CRMP2A is enriched in axons and that it is upon phosphorylation at its N-terminal Ser27 bound and regulated by prolyl isomerase PIN1. Now we found that other prolyl isomerase, FKBP12, binds specifically to unphosphorylated CRMP2A. Moreover, we show that downregulation of FKBP12 affects CRMP2A phosphorylation at Ser27 and that knockdown of FKBP12 leads to changes in microtubule dynamics in IMCD3 cells as well as in cortical neuron migration. Our data thus indicate that conformational changes catalysed by prolyl isomerase FKBP12 represent a specific regulatory mechanism controlling phosphorylation of CRMP2A, microtubule dynamics and neural development.

POSTERS

DELETION OF β2* NICOTINIC ACETYLCHOLINE RECEPTORS IN STRIATAL GABAERGIC INTERNEURONS LEADS TO ALTERATIONS OF BEHAVIOR IN MICE

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While the function of nicotinic acetylcholine receptors (nAChRs) expressed by nerve terminals projecting to the striatum have been extensively studied, less is known about the nAChRs expressed by striatal neurons. Striatal projecting neurons (SPNs) express very low levels of nAChRs as they are mainly expressed by striatal interneurons. Electrophysiological studies have shown that specific types of GABAergic interneurons (GABAINs) are activated by nicotine and they inhibit SPNs as a result of this activation. Hence, nAChRs expressed by GABAINs may be important in modulation of striatal output and behavioral control.

To determine the function of nAChRs expressed by GABAINs, we deleted β2 nicotinic subunit by injecting Cre-expressing AAV viral vector into the dorsal striatum of β2-flox/flox mice. The resulting mice were tested in a battery of behavioral tasks focused on striatal-based behavior including open field test, grooming test, forced swimming test and social preference test. In addition, we measured activation of striatal neurons after acute amphetamine injection using the neuronal activity marker c-Fos.

We confirmed, by immunofluorescence and RT-qPCR, that the deletion is limited to the striatum and the viral vector is not retrogradely transported to other brain regions.

Initial behavioral tests suggested the β2 deletion leads to impairment of goal-directed behavior, motivation and sociability while it induces hyperactivity and axiety.

We conclude that nAChRs expressed by GABAINs in the striatum have a functional role in the control of striatal-based behavior.

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COLD INDUCED UCP1-MEDIATED THERMOGENESIS DISSOCIATES FROM THE ACTIVATION OF LIPID METABOLISM IN WHITE FAT: A STUDY OF TWO MURINE STRAINS DIFFERING IN PROPENSITY TO OBESITY

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Triacylglycerols (TAG) synthesis, *de novo* lipogenesis and lipolysis in epididymal white adipose tissue (eWAT) was shown to be stimulated by cold exposure (CE) with a greater effect in obesity-resistant A/JOlaHsD (AJ) than obesity-prone C57BL/6J (B6) mice. The aim of this study was to learn whether the strain-specific activation of WAT lipid metabolism is related to differential induction of UCP1-mediated thermogenesis. Two-month-old male B6 and AJ mice fed a standard chow diet were maintained at 30°C or exposed to cold (6°C) for 7 days. The capacity of UCP1-mediated thermogenesis was evaluated by norepinephrine-induced whole-body oxygen consumption in anaesthetised mice (NE-max). UCP1 in interscapular brown fat (iBAT) was quantified using Western blotting (WB) and MS-based label-free approach (MS-LFQ). Uptake of 18-FDG into BAT was determined by PET-CT 2 hours after CL 316 243 injection.NE-max increase induced by CE was more pronounced in B6 (~1.8-fold) mice than in AJ (~1.2-fold). In iBAT, WB as well as MS-LFQ revealed greater specific UCP1 content in response to CE (per mg homogenate protein; WB: AJ, ~2.8-fold; B6, ~3.7-fold; MS-LFQ: AJ, ~2.2-fold; B6, ~4.3-fold) documenting a stronger effect in B6 mice. Evaluation of the MS-LFQ data indicated strain-specific modulation of iBAT metabolism by CE. 18-FDG uptake into BAT was augmented more in B6 (~5.8-fold) than AJ (~2.3-fold) mice by CE. Our results demonstrated a lower induction of UCP1-mediated thermogenesis in AJ than B6 mice by CE. In conclusion, involvement of UCP1-independent lipid catabolism is very likely in the differential response to both cold and obesity in the two strains.

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QUINPIROLE SENSITIZATION RAT MODEL OF OCD IS RELATED TO INCREASED ACTIVITY OF ORBITOFRONTAL CORTEX BUT NO CHANGES IN ACTIVITY OF ANTERIOR CINGULATE CORTEX.

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The aim of this study was to determine the changes in neuronal activity of anterior cingulate cortex (ACC), orbitofrontal cortex (OFC) and medial prefrontal cortex (MPC) in quinpirole (QNP) sensitization rat model of obsessive-compulsive disorder (OCD). Twenty-two adult male rats were used for the experiment. The half of the rats was sensitized to quinpirole by receiving daily subcutaneous injections of QNP (0,5 mg/kg) while the other half received saline. Both groups were habituated for ten days to enriched open-field arena. On the eleventh day, the part of saline and quinpirole treated groups explored the arena for 5 min while the other subgroups were left as cage-controls. Immediately after the end of experiment, all rats were sacrificed, and the extracted brains were cryopreserved. To determine the changes in neuronal activity of selected brain regions, fluorescence *in situ* hybridization of immediate early gene *Arc* was conducted on the brain tissue and the fractions of neurons expressing *Arc* mRNA were calculated using semi-automated software. There was a significant increase in proportion of *Arc* mRNA expressing neurons in OFC of quinpirole sensitized rats. There were no significant differences in proportions of *Arc* expressing neurons in MPC or ACC in quinpirole sensitized animals. CONCLUSIONS: The results of this study suggest that QNP sensitization display hyperactivation activity of OFC, a region implicated in OCD.

THE EFFECT OF INHIBITION ON RATE CODE EFFICIENCY INDICATORS

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Neurons communicate by firing action potentials, which can be considered as all-or-none events. The classical rate coding hypothesis states that neurons communicate the information about stimulus intensity by altering their firing frequency. Cortical neurons typically receive a signal from many different neurons, which, depending on the synapse type, either depolarize (excitatory input) or hyperpolarize (inhibitory input) the neural membrane. We use a neural model with excitatory and inhibitory synaptic conductances to reproduce in-vivo like activity and investigate how the intensity of presynaptic inhibitory activity affects the neuron's ability to transmit information through rate code. We reach a counter-intuitive result that increase in inhibition improves the signal-to-noise ratio of the neural response, despite introducing additional noise to the input signal. On the other hand, inhibition also limits the neuronal output range. However, in the end, the actual amount of information transmitted (in bits per energy expended) is remarkably robust to the inhibition level present in the system. Our approach also yields predictions in the form of post-synaptic firing rate histograms, which can be compared with in-vivo recordings.

ADENOSINERGIC MODULATORY INHIBITORY SYSTEM - MOLECULAR BIOLOGY AND FUNCTION DURING POSTNATAL DEVELOPMENT

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A1 adenosine receptor (A1R) is one of the adenosine G protein-coupled receptor (GPCR). A1R is expressed at a high level in the central nervous system (CNS) and is known by its inhibitory effect which among other functions controls epileptic seizures. However, the distribution and expression of the A1R in the brain during its development remains still enigmatic. Therefore, the expression of the A1R during brain development in rats is analyzed at different ages (between 12 and 60-days-old) under normal physiological conditions and after status epilepticus induced at the 12th day in selected structures (cortex, hippocampus, and cerebellum). After RNA isolation and transcription into cDNA, the droplet digital PCR (ddPCR) is used to analyze the expression of A1R in selected brain tissues. Partial results suggest that the concentration of the A1R mRNA in the hippocampus varies with age under physiological conditions. The analyses of the expression of the A1R at RNA level in the cortex and cerebellum will be also performed. In the perspective, the studies will be enlarged to the expression of the A1R at the protein level, which will allow us to compare the expression and the distribution of the A1R in different tissues at the two levels.

BONE MARROW ADIPOSE TISSUE (BMAT) AS A NOVEL ADIPOSE DEPOT IN THE REGULATION OF THE WHOLE BODY METABOLISM AND BONE HOMEOSTASIS.

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In obesity, impairment of peripheral adipose tissue (AT) expandibility leads to ectopic fat accumulation in non-adipose organs such as liver, muscle or cardiovascular system. Recent studies have shown that bones are also affected, leading to enhanced adipocyte formation in bone marrow (BMAT). Higher BMAT volume is often associated with a increased risk for bone fractures and osteoporosis, overlooked complications affecting the quality of life in obese and diabetic subjects. Opposite to peripheral AT, there is limited information on a physiological role of BMAT in relation to bone and a whole body energy metabolism. Our recent findings showed that bone fragility in obese mice is caused by BMAT infiltration induced by enhanced insulin signaling of bone marrow skeletal stem cells, which leads to creation of senescence microenvironment affecting bone homeostasis. Thus, the major aim of this project will be to investigate the functional significance of metabolic changes in BMAT phenotype in metabolic complications and whether we can prevent these changes using pharmaco-nutrition intervention applying high-throughput methods such as metabolomics, lipidomics, RNA sequencing and bioenergetics. The project will employ murine and human cellular systems and animal models studying molecular differences in metabolic responses between extramedullary adipose tissue and BMAT. Further, this study will contribute to better understanding of bone and fat metabolism in the regulation of whole body metabolism.

MATERNAL RELATED AUTOANTIBODIES AND ITS ROLE IN THE PATHOGENESIS OF AUTISM SPECTRUM DISORDER (ASD)

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Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterised by difficulties in behaviour, social interaction, communication and sensory sensitivities. The current understanding derived from the past decades of research shows these behavioural abnormalities stem from disruption of highly important processes during neurodevelopment such as cortical lamination, neurogenesis or synaptic pruning. Mounting evidence points to the maternal immune system as one of the main factors responsible for the disruption of fine-tuned neurodevelopment in non-genetic cases of ASD. More precisely, maternal antibodies (MAB) reactive to the fetal brain proteins are suggested to play a key role in the pathogenesis of ASD. CRMP1 and CRMP2 are two of the few proteins targeted by maternal immune system associated with ASD. In this work, we examine the prevalence of MAB reactive to CRMP1 and CRMP2 in the blood sera samples from mothers with a child diagnosed with ASD compared with the control maternal sera. We further describe the pathogenic effects of antibodies reactive to CRMP1 or CRMP2 on neurodevelopment in mouse model *in vivo* and *in vitro* using various techniques.

THE ROLE OF CYTOCHROME C OXIDASE SUBUNIT 4 ISOFORMS IN HEALTH AND DISEASE.

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Oxidative phosphorylation (OXPHOS) is responsible for production of majority of ATP in mammalian organisms. This process is partially regulated by nuclear-encoded subunits of cytochrome c oxidase (COX). One of its regulatory subunits, Cox4, is an early-assembling COX component essential for the formation of catalytically functional enzyme. Moreover, regulated expression of its two isoforms (COX4i1, COX4i2) is hypothesized to optimize respiratory chain function according to oxygen supply. However, details of functional alterations between the two variants have not yet been described, as well as the impact of pathogenic substitutions in COX4i1 and COX4i2 genes identified in rare OXPHOS defects. We established HEK293 cell line-based model with complete absence of subunit Cox4 (knock-out, KO), and characterized its impact on OXPHOS. Knock-out of both isoforms (COX4i1/4i2 KO) resulted in total absence of COX holoenzyme, making cells fully reliant on OXPHOS-independent ATP production. COX4i1/4i2 KO were subsequently utilized for knock-in of COX4i1 or COX4i2 isoform. The content of COX as well as its ability to incorporate into supercomplexes were comparable in COX4i1 and COX4i2 expressing cells. Respiratory rates and COX capacity were not distinguishable between cells expressing either isoform of COX4. However, significant changes in COX oxygen kinetics indicate decreased oxygen affinity of COX4i2-containing enzyme. Interestingly, we observed COX4 isoform dependent modulation of reactive oxygen species (ROS) production - COX4i2 KI clones manifested decreased mitochondrial ROS generation. Besides, we successfully utilized our COX4i1/4i2 KO cells for expression of COX4i1 and COX4i2 variants harboring pathogenic Lys101Gln and Glu138Lys substitutions, respectively. This research indicates suitability of our cells for modelling inherited OXPHOS defects, thus alleviating the demands for analyses on bioptic material from patients.

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DEVELOPMENT OF *IN VITRO* METHODS FOR PERSONALISED DIAGNOSTICS OF NEUROPSYCHIATRIC DISORDERS

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Epilepsy is the most common chronic neurological disease, characterized by recurrent spontaneous seizures caused by an excessive and synchronous activity of neurons in the brain. There are many causes of epilepsy, including genetic factors, delivery complications, brain injuries, infectious diseases, brain tumors and metabolic impairments. Because of multifactorial nature of epilepsy, the current pharmacological treatment is symptomatic and 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 with the aim to identify disease-causing mutations of epilepsy-related genes. In cooperation with the Motol University Hospital, we selected potential pathological mutations in the selected genes of epileptic patients using next the generation sequencing technique. To test the effect of potential pathological mutations *in vitro*, we will employ either the method of transdifferentiation of human fibroblasts into neural progenitors or reprogramming fibroblasts into induced pluripotent stem cells. The obtained cells will be differentiated towards the neural line. In addition, using the CRISPR/Cas9 system, we will introduce a particular mutation found in a patient into neural progenitors. Subsequently, we will monitor the effect of the mutation on morphological properties of neural cells, change in the representation of binding partners or electrophysiological properties of the cell membrane. We expect that development of a reliable *in vitro* cell system will contribute to elucidation the role of particular mutations in pathophysiology of epilepsy. In long-term the method will allow reprogramming or transdifferentiating of fibroblasts obtained from patient with epilepsy and their subsequent characterization and functional or pharmacological testing.

CIRCADIAN CLOCKS IN BRAIN AND THEIR SENSITIVITY TO CHRONODISRUPTION AND NEUROINFLAMMTION

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Circadian rhythms regulate various biochemical, physiological, and behavioral processes with a periodicity of 24 hours, enhancing the efficiency and survival of organisms by allowing them to anticipate and adapt to changing environmental conditions. The suprachiasmatic nucleus (SCN) is considered to be hierarchically at the top of all body clocks and it is named master clock. It acts as a central pacemaker to synchronize the circadian clocks outside the SCN that are named peripheral clocks. The PhD project puts focus on the peripheral clocks that are localized in specific brain areas that are mostly involved with memory and mood. Techniques such as qRT PCR in laser dissected brain areas, in situ hybridization, immunohistochemistry, Western blot, and recording of gene expression in real time in organotypic explants are being used to describe the properties of extra-SCN clocks and to test their sensitivity to various external cues, such as misaligned feeding regime, aging, photoperiod and sleep deprivation. The results may contribute to advancing therapies of the neuropsychiatric disorders.

METABOLIC CHANGES ASSOCIATED WITH CACHEXIA IN PANCREATIC CANCER

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Pancreatic ductal adenocarcinoma (PDAC) is a cancer with one of the worst prognosis within oncological diseases and rising incidence in the Czech Republic and worldwide. PDAC is often associated with cancer cachexia, which is a systemic condition leading to the depletion of skeletal muscle, and general substrate reserves of the patients. Importantly, it is not known, what factors trigger cancer cachexia and if there is any link between metabolic phenotype of the tumour and occurrence of cancer cachexia.

The presented project aims to identify key metabolic pathways of the tumour related cachexia phenotype. We use primary cell cultures derived from the patients, which were previously diagnosed for presence/absence of cancer cachexia. First, we aim to optimize primary ex-vivo cultures to obtain significant amount of biological material for subsequent biochemical analyses. To this end, we use matrigel-embedded cancer-derived samples to produce organoid cultures, which should be optimal to maintain cancer phenotype similar to in vivo conditions. Subsequently, we will perform extensive biochemical analyses, focused on glutamine and branched-chain amino acids metabolism, and the interference of their degradation pathways. GC-MS and LC-MS-based analytical approaches will be used to determine steady state metabolic levels and to measure stable isotope incorporation into mitochondrial metabolites. Our results should describe metabolic phenotype of PDAC and possibly identify potential metabolic pathways associated with cancer cachexia.

MEASURING PERSPECTIVE TAKING ABILITIES WITH iEEG

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Perspective taking is defined as the ability to imagine what a scene looks like from different viewpoints. Having this ability intact is important for processing spatial scenes that we encounter in everyday life.

The given study observes perspective taking abilities using intracranial EEG (iEEG) brain-imaging technology with patients undergoing treatment for epilepsy. The study aims at identifying brain regions responsible for the scene stimuli processing and to measure the time course of activation.

The study employs Modified Arena perspective taking task to measure the perspective taking ability while processing spatial scenes. The accomplishment of the task is based on decisions about the visible goal position in four different categories of trials. These decisions are made either relative to subjective position or towards visible orientation mark while the scene is presented in 2D or in 3D view.

Consequently, iEEG data from 20 patients was analyzed in the broad gamma band frequency domain (50-150 Hz). Results indicate that particular brain regions, namely, temporal cortex, posterior cortex and frontal brain areas respond in the spectrum of broad gamma in self-perspective or perspective taking conditions. Some brain regions, e.g. lingual gyrus, fusiform gyrus and occipital areas showed increased activity in case of first-person and 2D view conditions in comparison to perspective taking and 3D view conditions.

THE EFFECT OF CHOLESTEROL SYNTHESIS INHIBITION ON GLUTAMATERGIC SYNAPTIC TRANSMISSION

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Cholesterol is an essential structural component in the brain, important for the physiology of neuronal membrane, the maintenance and morphology of synapses and synaptic vesicles. Impairment of cholesterol metabolism leads to neurodegenerative symptoms of Smith-Lemli-Opitz syndrome (SLOS).

SLOS is a human autosomal recessive multiple malformation syndrome caused by an error of cholesterol synthesis, characterized by mutations in the gene which encodes the enzyme 7-dehydrocholesterol (7DHC) reductase (DHCR7). In the complex biochemical pathway of cholesterol synthesis DHCR7 catalyzes the reduction of 7DHC to cholesterol. A consequence of impaired cholesterol synthesis is an accumulation of 7DHC which has multiple toxic effects in cells. Neurophysiological studies on DHCR7 knock-out mice showed an impaired response of frontal cortex neurons to glutamate. The aim of the present study is to test the influence of inhibited cholesterol synthesis, possibly accompanied by an increased concentration of 7DHC, on glutamatergic synaptic transmission.

We treated primary hippocampal mass cultures with BM15.766 (4-[2-[1-(4-chlorocinnamyl)piperazin-4-yl]ethyl]benzoic acid), an inhibitor of the enzyme DHCR7, and we evaluated the resulting changes in the plasma membrane cholesterol content with mass spectrometry. We observed a significant inhibitory effect of BM15.766 on cholesterol concentration after 7 days of incubation. Concurrently, the concentration of 7DHC was increased. Treatment of primary hippocampal micro-island culture with a lower concentration of BM15.766 as well as a shorter period of incubation had no significant effect on glutamatergic synaptic transmission. Therefore, we increased the duration of BM15.766 treatment of micro-island cultures to 7 days to measure the effect of decreased cholesterol synthesis on excitatory postsynaptic currents as well the function of AMPA and NMDA receptors.

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MOLECULAR MECHANISMS OF SIGNALLING BIAS AT MUSCARINIC RECEPTORS

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G-protein coupled receptors (GPCRs) are membrane proteins, that are targets of many drugs. When the ligand is bound to the receptor, it can activate G protein that is followed by an exchange of GDP for GTP on the α-subunit of G-protein. Many GPCRs activate multiple signalling pathways. Development of agonists biased to specific signalling pathway may prevent side effects mediated by other signalling pathway(s).

Muscarinic acetylcholine receptors (M1-M5) are GPCRs with the different pharmacological profile. M1,3,5 preferentially couple with Gq proteins that leads to the accumulation of inositol phosphates while M2 and M4 receptors couple with Gi proteins that leads to a decrease of cAMP. Some newly synthesized muscarinic agonists derived from tetrahydropyridin activate only Gi pathway, therefore they are functionally selective for M2 and M4 receptors.

To perform the detailed analysis of receptor coupling profile upon activation by these new compounds we will introduce baculoviral/insect cell expression system. Set of endogenous GPCRs and G-proteins of these cells is limited. Thus this system enables direct analysis of coupling of any combination of individual muscarinic subtypes with individual G-protein α-subunits without interference.

The molecular modelling revealed differences in interactions of tetrahydropyridine derivatives and classical muscarinic agonists in the orthosteric binding site of muscarinic receptors that lead to the engagement of different activation networks.

To identify the key ligand-receptor interactions responsible for ligand bias towards engagement with individual G-protein subtypes we will mutate muscarinic receptors in the orthosteric binding site, allosteric activation network and receptor G-protein interface.

The ability of tested compounds to activate muscarinic receptors will be evaluated in functional experiments by measuring the second messengers. Direct coupling of receptor and G-protein will be evaluated using GTPgammaS binding assay.

HYPOXIA INDUCIBLE FACTOR-1 ALPHA MEDIATES INFARCT SIZE-LIMITING EFFECT AFFORDED BY EPOXYEICOSATRIENOIC ACID ANALOG IN RAT HEARTS

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Epoxyeicosatrienoic acids (EETs) decrease cardiac ischemia/reperfusion injury, however, the mechanism of this protective effect remains elusive. Here we investigated the cardioprotective action of a novel EET analog administered just prior to reperfusion and the role of hypoxia inducible factor-1 alpha (HIF-1α).

Adult male SD rats were subjected to 30-min left coronary artery occlusion and 2-h of reperfusion. The EET analog, EET-B (2.5 mg/kg iv) or the endogenous EET, 14,15-EET (2.5 mg/kg iv) were administered 5 min before reperfusion. In a separate set of experiments HIF-1α immunoreactivity was analyzed in ischemic area at risk (AR) and non-ischemic septa at the end of ischemia and after 20 min and 2 h of reperfusion.

EET-B and 14,15 EET reduced infarct size from 64.3±1.3% in control to 46.0±1.6% and 42.6±1.9%, respectively and their co-administration did not provide a stronger effect (45.9±1.4%). EET antagonist 14,15-EEZE (2.5 mg/kg iv) inhibited infarct size-limiting effect of EET-B (62.5±1.2%). HIF-1α inhibitors, 2-methoxyestradiol (2.5 mg/kg iv) and acriflavine (2.0 mg/kg iv) also completely abolished the cardioprotective effect of EET-B (63.3±1.6 and 63.6±1.2%, respectively).

HIF-1α immunogenic signal markedly increased in the AR compare to septum at the end of ischemia (9.3±1.1 vs 0.3±0.1%). After 20 min and 2 h of reperfusion, HIF-1α immunoreactivity in AR decreased to 2.4±0.5 % and 1.9±0.4%, respectively in the controls and EET-B administration blunted this decrease of HIF-1α immunoreactivity at both time-points of reperfusion.

EET-B provides a strong protection against myocardial infarction in rats that is mediated by increased level of HIF-1α.

C-TERMINUS OF GLUN2B MODULATES THE SENSITIVITY OF NMDA RECEPTORS TO INHIBITORY NEUROSTEROIDS

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N-methyl-D-aspartate receptors (NMDAR) belong to a group of ionotropic glutamate receptors that mediate excitatory synaptic transmission and play a key role in learning and memory. Dysfunction of NMDAR underlies neurological and psychiatric disorders. Neurosteroids modulate NMDAR and may prove to be clinically useful. The aim of this study was to elucidate the factors affecting NMDAR sensitivity to inhibitory neurosteroids. Using electrophysiological and molecular biology approach, we analyzed the naturally occurring neurosteroid pregnanolone sulfate as well as its synthetic analogs on recombinant and native NMDARs. We compared the effect of neurosteroid prior to (control) and after (test response) a Ca-challenge (NMDAR activation by 1 mM glutamate for 50 s in the presence of 2 mM $[Ca^{2+}]_o$). Test responses had up to 6-fold increase in the inhibitory effect of neurosteroids on NMDARs compared to control responses. The largest change in the inhibitory effect was observed for pregnanolone hemipimelate (PAhPim) on native NMDARs, where the control responses were largely unaffected by the steroid, whereas the test responses were potently inhibited (IC_{50} 25.1±0.4 μM). Next, we analyzed the effect of the deletion and shortening of the C-terminus of GluN1 and/or GluN2B subunit. Our data indicate that intracellular Ca^{2+} increases NMDAR sensitivity to inhibitory neurosteroids and this is dependent on the proximal region of the C-terminus of the GluN2B subunit. Deeper understanding of this new regulatory mechanism may help in finding new compounds with neuroprotective activity which could act preferentially under pathological conditions, without affecting physiological NMDAR activity.

POTENTIATION OF GABA RELEASE BY PRESYNAPTIC P2X RECEPTORS IS HIGHER IN VASOPRESSIN COMPARED TO OXYTOCIN NEURONS IN SUPRAOPTIC NUCLEUS

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The P2X receptors (P2X1-7Rs) are ATP-gated non-selective cationic channels which are permeable to Na^+, K^+ and Ca^{2+}. It has been shown previously that vasopressin (AVP), but not oxytocin (OT), neurons in the hypothalamic supraoptic nuclei (SON) express P2XRs on their neuronal cell bodies. In this work, we tested the hypothesis that AVP and OT neurons differ also in the P2XRs expression on their presynaptic nerve terminals. To characterize presynaptic P2XRs in identified SON neurons, we employed whole-cell patch-clamp electrophysiology on slices isolated from young (2 to 3 weeks) rats, and a newly developed transgenic rat model expressing an AVP-enhanced green fluorescent protein fusion gene (AVP-eGFP), and an OT-monomeric red fluorescent protein 1 (OT-mRFP1) fusion transgene. In both cell types, ATP application increased the frequency of miniature GABAergic (mIPSCs) or glutamatergic (mEPSCs) postsynaptic currents without changing their amplitude, indicating an involvement of presynaptic P2X receptors. Glutamatergic postsynaptic currents were observed in only about 30% of SON neurons, and were not studied further.

In 65% (17/26) of AVP neurons, the application of ATP increased the frequency of mIPSCs by 1198±202%, and the remaining 35% (9/26) of AVP cells showed ATP-evoked somatic current without ATP-induced increase in mIPSC frequency. OT neurons exhibited higher basal frequency of mIPSCs compared to AVP neurons, and ATP application increased mIPSC frequency only by 207±22% in 18 % of OT neuron (6/34). The remaining 82% (28/34) of OT neurons exhibited no effect of ATP on mIPSC frequency. These results indicate that extracellular AP potentiates differently GABA release in AVP and OT neurons, and activity of AVP neurons is highly subjected to purinergic modulation.

INHIBITORY FACTOR 1 (IF1): A NOVEL PLAYER IN REGULATION OF PANCREATIC β CELLS METABOLISM

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The majority of cellular ATP is produced by ATP synthase localized in the inner mitochondrial membrane. In pancreatic β cells, the ATP/ADP ratio is a key parameter of insulin secretion. Inhibitory Factor 1 (IF1) is a small, nuclear-encoded protein, which regulates ATP synthase. The well-established function of IF1 is to prevent total ATP depletion under conditions when the membrane potential is lost, such as during hypoxia/ischemia or starvation. Nevertheless, recent studies suggested that IF1 also regulates ATP synthesis. To study the role of IF1 in pancreatic β-cells metabolism, we established IF1 knockdown and IF1 overexpression with corresponding controls in INS-1E cells (model insulinoma cells). We observed a dramatic increase in all studied bioenergetic parameters (ATP production, insulin secretion or oxygen consumption) in IF1 knockdown cells. Accordingly, the cells stably overexpressing IF1 showed opposite effects. Further, we observed a higher amount and size of the insulin secretory granules in the cells overexpressing IF1. In conclusion, we propose that IF1 is a key player in control of metabolism of pancreatic β cells, and subsequent studies are required for a deeper understanding of its physiological function.

SEIZURE CLUSTERS IN TETANUS TOXIN MODEL OF EPILEPSY

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Introduction: The long-term fluctuations in seizure probability and seizure clustering were multiple times reported in both patients and animal models of epilepsy including the tetanus toxin model in rats. The aim of this study was to explore the mechanisms which govern cluster progression and cluster onset.

Methods: Epilepsy was induced in adult rats (n=6) by injection of 10 ng of tetanus toxin into the right dorsal hippocampus. Electrodes were implanted bilaterally into dorsal hippocampus and motor cortex. Animals were continuously video-EEG monitored for >2 weeks. Seizures were identified and classified as convulsive or non-convulsive. Signal power during seizures was analyzed in each studied brain area. Apart from that, we analyzed long-term evolution of epileptic bursts – few second long series of EEG spikes. We analyzed their rate, duration, power and channel cross-correlation.

Results: We analyzed one seizure cluster in each animal. Clusters lasted 2.3±0.2 days and contained 90±15 seizures. All clusters were characterized by progressive increase of ISI. The percentage of convulsive seizures progressively increased in all clusters (p=0.008) and so did the signal power in the motor cortices (p=0.008) whereas in the hippocampi a non-significant decrease of power was observed (p=0.74). Between the clusters, we observed increasing rate, power and spread of epileptic bursts whereas the duration produced a U-shape.

Conclusion: We have shown that during the course of a single cluster, the brain undergoes complex changes. We hypothesize that early non-convulsive seizures facilitate spreading of later seizures. In contrast, later generalized seizures have inhibitory effect which leads to reduction of seizure rate and finally to the cluster termination. Between the clusters, changes of the epileptic burst can be interpreted as increase of excitability and delayed recovery from internal perturbations which may serve as an early warning signal of impending transition to the next seizure cluster.

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FUNCTIONAL ANALYSIS OF GENETIC VARIATIONS IN THE PROMOTER AND CYTOSOLIC DOMAIN OF NMDARS

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Neurodevelopmental and neuropsychiatric disorders affect millions of people worldwide. Neurological disorders have a high degree of inheritance, however, their genetic background has not been satisfactorily explained yet. In the current decade, we have obtained a large amount of genetic data and uncovered many polymorphisms and mutations occurring in individual patients. Nevertheless, the functional consequences of these changes are mostly unknown. Common risk factors include genes involved in the formation and transmission of excitatory signaling at the synapses. Excitatory signaling is mediated by ionotropic glutamate receptors. NMDA receptors play a key role in the process of learning and memory formation in the CNS. Many of genetic changes found in patients occurred in less functionally analyzed parts of NMDA receptor; promoter region of the gene and the large intracellular domain of the receptor protein. We have focused on the structural and functional analysis of genetic changes in these regions found in patients affected by autism spectrum disorders (ASD), epilepsy (EPI) and schizophrenia (SCHZ). We sequenced the promoter region of the NMDA receptor subunit genes in patients suffering from SCHZ and ASD and analyzed genetic variations presented. Specific promoters containing different genetic variations were subcloned to reporter plasmid and subjected to the luciferase assay, which indicated on the case promoters with unique genotype exhibiting different expression activity compared to promoters with genotype occurring predominantly in control subjects. Moreover, electrophysiological measurements and microscopy analysis have shown that some SCHZ or EPI associated mutations found in the intracellular domain of receptor induce a different level of channel open probability, change surface expression, and trafficking to synapses. In summary, specific genetic changes in the promoter region of the gene or in the intracellular domain of the NMDA receptor alter gene expression or receptor function and may thus contribute to the emergence of neurological disorder.

DIFFERENCES OF SALIVA COMPOSITION IN RELATION TO TOOTH DECAY

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Most people worldwide suffer from dental caries. Only a small part of the population is caries-resistant and the reason for this resistance in unknown. Only a few studies compared the saliva protein composition of people with carious teeth and people with no caries. In this study, we compared the differences in the abundances of proteins in saliva between caries-resistant and caries-susceptible people by nano-liquid chromatography-tandem mass spectrometry (Label-Free Quantitative Proteomics). Our results demonstrate that the observed differences in the protein levels might have an influence on anti-caries resistance. We observed 14 proteins in the supernatant fraction with a significantly higher expression in the CF group.These significantly upregulated protein in CF group could play an important role in caries prevention.Newly detected potential protein markers of dental caries can be a good basis for further research and for possible future therapeutic use.

ACTIVATION OF LIPID METABOLISM IN EPICARDIAL ADIPOSE TISSUE OF PATIENTS WITH ADVANCED HEART FAILURE: MINOR IMPACT OF CARDIAC CACHEXIA

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Obesity predisposes heart failure (**HF**), however obese patients with already established HF have better long-term prognosis than those who are leaner. In turn, cachexia that develops in a subgroup of HF-patients is an independent mortality risk factor. Relatively little is known about immunometabolic changes in epicardial adipose tissue (**EAT**), which are associated with the development of HF in humans. Close proximity to myocardium and shared microvascular network suggest that EAT may affect cardiac functions. The aim of this study was to find novel therapeutic targets to ameliorate development of HF. EAT from patients undergoing heart transplantation for end-stage HF (HF-patients; n=52) were harvested from the anterior interventricular groove. Control samples were obtained from hearts of organ donors (**CTRL**; n=17). Patients with unintentional weight loss (> 7.5 %) during the last 6 months prior transplantation (**HF-cachexia**; n=17) were compared with weight-stable patients (**HF-BW stable**; n=35). Gene expression analysis was performed by quantitative real-time PCR (**qPCR**). Metabolomics and lipidomics approaches were combined with bioinformatic analysis (MetaboAnalyst 4.0). This study revealed pronounced differences between HF-patients and CTRL subjects in terms of the expression of selected genes in EAT and changes in tissue metabolome (i. e. 209 out of 710 analytes with mean levels differing significantly between these groups). The results seem to indicate a significant induction of lipid metabolism in EAT of HF-patients. In contrast, cardiac cachexia was associated with only minor changes in gene expression (NPRC, FAS, IL6) and EAT metabolome (41 statistically different analytes). To our knowledge, this is the first study to characterize EAT metabolome in HF-patients, as well as the impact of cardiac cachexia under these circumstances. The results may indicate that EAT of HF-patients is re-programmed to increase the export of energy fuels for a failing myocardium.

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NEW FACTORS REGULATING BIOGENESIS OF SUBUNIT C FROM MAMMALIAN F_OF_1 ATP SYNTHASE

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Mitochondrial ATP synthase is the key enzyme of the mitochondrial oxidative phosphorylation (OXPHOS) system and hence of the cellular energy provision. The human enzyme is a multisubunit protein complex that consists of two domains – the membrane-embedded F_o domain and the globular F_1 catalytic domain localized in the mitochondrial matrix. The biogenesis of mammalian ATP synthase holoenzyme involves formation of several assembly modules, one of them represented by octamer of c subunits. Despite long research on yeast model, detailed mechanisms behind membrane insertion of subunit c and c_8-oligomer formation remain unresolved. In our screen for subunit c interactors, we identified three proteins: TMEM70, TMEM242 and c15orf61. While TMEM70 is already known ATP synthase assembly factor, but without clear molecular mechanism of action, nothing is known about the remaining two. Interestingly, all three proteins represent metazoan innovation, without any true homologues present in lower eukaryotes In order to understand its potential modes of action we expressed c15orf61-eGFP construct in HEK293 cells and observed distribution compatible with mitochondrial localisation. TMEM242 has weak prediction for mitochondrial localisation, but we routinely see higher MS signal in mitochondria than in whole cells. We have analysed the effect of TMEM242 downregulation using siRNA approach. In HEK293 cells, we reduced TMEM242 mRNA expression levels to approximately 20% of wt. Such silencing led to decrease of subunit c content on SDS PAGE to 53±5 % compared to controls with no changes in the content of representative subunits of all OXPHOS complexes. When HEK293 siTMEM242 cells were analysed by hrCNE native electrophoresis, we observed decrease in the content of assembled ATP synthase as well as appearance of dissociated F_1 in siTMEM242 cells indicating defective biogenesis of ATP synthase.

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POSTTRANSLATIONAL REGULATION OF TRK1 ACTIVITY AND AFFINITY

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One of the key regulatory mechanisms of cellular ion homeostasis is a precisely adjusted transport across the plasma membrane that guarantees uptake and storage of essential, desired compounds, such as potassium. Potassium is a vital cation participating in fundamental processes, for instance: regulation of membrane potential and cell turgor, enzymatic activity, protein synthesis and resistance to stress, such as presence of toxic lithium and sodium. Protein Trk1 is considered to be a key importer of potassium in yeast *Saccharomyces cerevisiae*. Trk1 is characterized by steady and low level of expression that is independent of external conditions and is therefore thought to be regulated on posttranslational level. The most distinctive feature of Trk1p is its alleged ability to switch affinity, from millimolar (low-affinity state) to micromolar (high-affinity state) ranges under potassium-limiting conditions or in the presence of toxic monovalent cations such as lithium and sodium. The precise nature of this mechanism remains unclear, however, phosphorylation has been observed to be involved in analogical process in plant transporter proteins. In Trk1, so far, only one amino acid (Leu949) has been suggested to play role in this process. In our work we selected 12 putative phosphorylation sites, based on prediction software and sequence conservation, to be examined in connection to regulation of activity and affinity of Trk1. We also aimed to unveil, in more detail, the role of Leu949 in Trk1-mediated potassium uptake and affinity switch. We used site directed mutagenesis to substitute selected residues (potential phosphorylation sites and Leu949) and to study the effects of these substitutions on growth under potassium limiting conditions, in presence of lithium and eventually on localization of Trk1.

COMPARISON OF THE MODELS OF DORSAL AND VENTRAL VISUAL STREAMS USING ELECTROPHYSIOLOGY IN HUMANS

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A large body of data suggests that visual information is processed in the brain in two separate streams originating from the visual cortex: the dorsal and ventral in parietal and temporal lobes respectively. Originally, the role of the dorsal stream was suggested in spatial perception ('Where'), while the role of the ventral stream – in object identity coding ('What'). Some studies, using electrophysiology in macaques and fMRI in humans, have shown that both streams are active in both identity and location processing. Nevertheless, other scalp EEG studies have demonstrated different time courses for the processing of this information. The aim of this project will be the clarification of the roles of both visual streams and the identification of their location in the brain. We will use intracranial EEG (iEEG) recordings as the major method. The iEEG data will be recorded in pharmacoresistant epilepsy patients during the experiment with a picture presentation. It will be directed either on the activation of the dorsal stream, by the location change of objects on the picture, or on the activation of the ventral stream, by the identity change of objects. The iEEG method has a more precise spatial resolution compared with the scalp EEG. It will enable us to localize the brain regions activated by position and identity changes and validate in this way the separation of both visual streams. Our results will contribute to a better specification of the visual processing models with more precise timing.

A NOVEL CLASS OF BIOACTIVE LIPIDS AS POSSIBLE PRECURSORS OF FAHFA LIPIDS MEDIATORS

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Fatty acid esters of hydroxy fatty acids (FAHFAs) are endogenous lipids derived from oxygenated fatty acids. They consist of a fatty acid (e.g. palmitic acid, PA) esterified to the hydroxyl group of a hydroxy fatty acid (e.g. hydroxy stearic acid, HSA), abbreviated as PAHSA. These compounds are being studied for their anti-inflammatory and antidiabetic properties, but their metabolism remains unclear. Recently, a novel class of FAHFA-containing TAG-estolides was discovered. Their structure consists of a fatty acid esterified to the hydroxyl group of a hydroxy fatty acid that is bound to a glycerol backbone alongside two other acyl chains. FAHFA-containing TAG-estolides were analyzed using LC-MS system consisting of Vanquish UHPLC System coupled to QExactive Plus mass spectrometer. Chromatographic separation of such a mixture of metabolites is a challenging task owing to the complexity of TAG-estolides structure. So far, we managed to separate different TAG-estolide classes but further method development is needed for better separation according to the position of acyl chains and the hydroxy fatty acid branching. The nature of FAHFA-containing TAG-estolide structure suggests that it can serve as a precursor of free FAHFA. Because FAHFAs were found to be upregulated during fasting, murine epididymal white adipose tissue (eWAT) at fed and fasted state was analyzed. We hypothesized that FAHFAs could be liberated by lipolysis during fasting resulting in increased levels of free FAHFAs compared to the fed state. Thus, we anticipated decreased levels of TAG-estolides in the fasted state. Levels of FAHFAs (9-PAHSA, 5-PAHSA, and 9-PAHPA) significantly increased in the fasted state indicating that FAHFAs are liberated from a specific cellular pool activated during lipolysis. Interestingly, the levels of TAG-estolides increased in the fasted state as well suggesting that metabolic regulation is more complicated and yet not fully elucidated.

MOLECULAR MECHANISM OF 14-3-3 PROTEIN DEPENDENT REGULATION OF UBIQUITIN LIGASE NEDD4-2

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14-3-3 proteins belong to evolutionarily highly conserved family of regulatory proteins. By binding to specific phosphorylated motif of its binding partners regulate a variety of biological processes. One of several hundreds of binding partners of 14-3-3 proteins is ubiquitin ligase Nedd4-2 (NEDD4L), whose role is ubiquitination of various ion channels and membrane transporters. The best-described example is the regulation of the epithelial sodium channel (ENaC), which with its activity in the distal renal tubule, contributes to maintaining Na^+ homeostasis of the whole organism. Nedd4-2 is also involved in the regulation of several voltage-gated sodium channels (Na_vs) in cortical brain and dorsal root ganglion (DRG) neurons. Mutations of Nedd4-2 gene are associated with developmental disorders, hypertension and epilepsy. Dysregulation of Nedd4-2 in mice also leads to respiratory, renal, cardiac, and neural disorders and negatively affects the immune system. Phosphorylation of specific serine and/or threonine residues allows the binding of 14-3-3 proteins, which results in the inhibition of interaction between Nedd4-2 and its substrate. However, the structural nature of the mechanism of regulation by 14-3-3 protein has not been elucidated yet. The aim of our project is to reveal the structural basis of Nedd4-2 enzyme inhibition by 14-3-3 protein, identify the amino acids involved in the interaction between these molecules and describe the stoichiometry of the complex, its dynamics, size, shape and stability. We have prepared and purified the stable forms of Nedd4-2 protein and performed initial structural experiments.

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MODULATION OF NOCICEPTIVE SIGNALLING ON A SPINAL CORD LEVEL UNDER PATHOLOGICAL CONDITION

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Currently available analgesics do not often help patients to relieve their pain satisfactorily or possess serious side effects. Many chronic pain states are still difficult to treat. Several pathological pain states are underlain by modulation of nociceptive signalling on a spinal cord level. This project will concentrate to some crucial receptors in nociceptive transmission at spinal cord level and their interactions. The role of transient receptor potential vanilloid 1 (TRPV1) a ligand gated, non-selective cation channel and interactions with opioid receptors or cannabinoid receptors will be investigated. Spinal nociceptive signalling will be studied preferentially by recording of synaptic transmission in dorsal horn in laminae I and $II_{(outer)}$. Patch-clamp technic in whole-cell configuration will be used to record miniature (m), spontaneous (s) or electrically evoked (e) excitatory postsynaptic currents (EPSCs) from superficial dorsal horn neurons, which constitute with presynaptic endings of primary sensory neurons the first synapse on nociceptive pathways. Acute rodent spinal cord slices will be used for currents measurement. In addition Hargreaves test to quantify heat thresholds in the hind paws of mice and rats upon application of a radiant heat stimulus and mechanical or electrical aesthesiometry to quantify mechanical thresholds in the hind paws allow us to measure nociception in behavioural testing. Established animal models replicate aspects of the human pain pathway. In this project neuropathic pain model chronic constriction injury (CCI) to the sciatic nerve consisted of several ties ligatures around the nerve and inflammatory model induced by subcutaneous injection of carrageenan in the hindpaw will be used. Studying spinal nociceptive signalling under normal conditions with the emphases on modulatory mechanisms during development and maintenance of pathological pain states is critical to find new approaches in pain therapy.

MITOCHONDRIAL PHOSPHOLIPASE A2Γ PARTICIPATES IN CELLULAR ANTIOXIDANT AND ANTI-INFLAMMATORY PROTECTION IN VIVO

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Redox-dependent regulations play an essential role in a wide range of biological activities. Mitochondrial calcium-independent phospholipase A2γ (iPLA2γ) belongs to a family of enzymes that participate in cellular signaling by simultaneously producing free fatty acids and lysophospholipids. Using mitochondria isolated from wild-type (WT) and iPLA2-KO mice, we have found previously that the activity of iPLA2γ increases following the intrinsic gain in oxidant production by mitochondria or following the addition of extrinsic oxidants, such as *tert*-butyl hydroperoxide or H_2O_2. Our results also show that iPLA2γ-liberated fatty acids induce H^+ transport mediated by mitochondrial uncoupling protein UCP2, and by mitochondrial adenine nucleotide translocase in heart and brain mitochondria, leading to a decrease in the mitochondrial protonmotive force and subsequent decrease in mitochondrial superoxide production. Using wt, iPLA2-KO and UCP2-KO mice, we further tested the hypothesis that iPLA2γ participates in cellular antioxidant and anti-inflammatory protection *in vivo*. We found that steady-state serum levels of the pro-inflammatory cytokine interleukin-6 (IL-6) and also of protein carbonylation, which is a marker of oxidative stress, were higher in iPLA2γ-KO as well as UCP2-KO mice compared to wt controls. The wt IL-6 serum levels increased to the levels detected in both types of KO mice following the administration of iPLA2γ-selective inhibitor R-BEL. The injection of the endotoxin lipopolysaccharide also resulted in a marked IL-6 increase in iPLA2γ-KO mice compared to the wt controls. These results show that redox upregulation of cytokine expression is prevented by the antioxidant and anti-inflammatory action of iPLA2γ in synergy with UCP2, which ceases in the respective KO mice or upon pharmacological inhibition of iPLA2γ.

METABOLIC ADAPTATIONS TO MITOCHONDRIAL DYSFUNCTION IN CELLULAR MODELS

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Mitochondria are essential for the maintenance of cellular homeostasis. They play a critical role in metabolism, covering most of the cellular ATP demands and providing biosynthetic intermediates for anabolic pathways. The redox reactions held in the mitochondrial matrix (mainly Krebs cycle) provide substrates for oxidative phosphorylation (OXPHOS) consisting of complexes I-V located in the inner mitochondrial membrane. The first four enzymes form the electron transport chain (ETC) and generate a proton gradient, which is coupled to ATP production by complex V (ATPase). Defects in OXPHOS cause mitochondrial dysfunction and lead to human pathologies. Mitochondrial dysfunction triggers adaptive rewiring of metabolic pathways aimed at compensation of the deficiency.

To describe the rewiring under various situations we established cellular models in HEK293 cells affecting either complex IV (COX), or ATPase, which we characterized by structural (native electrophoreses) and functional (mitochondrial respiration) methods. COX defect (COX6b-KO) was associated with complete loss of assembled enzyme and impaired respiration, which was compensated by increased glycolysis. Full loss of ATPase was achieved by knock-out of catalytic subunit β (Beta-KO) resulting in impairment of coupled respiration compensated by upregulated glycolytic activity. Similar but less severe phenotype was observed in MLQ-KO causing intermediate ATPase defect associated with decreased functional capacity of the enzyme due to instability of proton channel domain. Ultimately, DAPIT-KD represented the mildest defect of ATPase. It prevented ATPase oligomerization and led to slight increase in glycolysis, yet without associated decrease in respiration. Using proteomic, steady-state metabolomics and metabolic tracing approaches, we aim to decipher the adaptive mechanisms that restore metabolic homeostasis and whether those responses show selectivity towards the primary nature of OXPHOS defect.

IDENTIFICATION OF TRANSMEMBRANE AND EXTRACELLULAR VESTIBULE RESIDUES CONTRIBUTING TO P2X7 RECEPTOR CHANNEL GATING AND CONDUCTIVITY

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P2X receptors (P2X1-7) are ATP-activated cation channels that are composed of two transmembrane domains (TM1 and TM2), ligand-binding ectodomain, and intracellular N- and C-termini. The P2X7 receptor (P2X7R) is involved in neurodegeneration, neuropathic pain, release of inflammatory cytokines and is strongly deregulated in many tumors. In the prolonged presence of agonist, the opening of P2X7R channel is followed by receptor sensitization, which increase its permeability to larger organic cations. To explore the molecular mechanism(s) by which large pore formation is regulated, we substituted one by one all residues in TM1 (from G27 to D48) and TM2 (from G326 to I355), and selected (conserved or unique) residues in the extracellular vestibule (K49, Y51, Q52, F322, G323, G326, K327, F328, Q332) with alanine, and measured BzATP-induced current and Ethidium bromide (EtBr) uptake by HEK cells expressing wild type (WT) and alanine P2X7R mutants. Our results revealed that the TM1 mutants G27A, H34A, Y40A, F43A, L45A, and M46A showed significantly reduced dye uptake, current amplitude and expression in the membrane, indicating that they are important for P2X7R trafficking. TM1 mutants K30A and D48A showed reduced dye uptake and membrane current, but without significant changes in the plasma membrane expression. Clusters of conserved residues in the extracellular vestibule (Y51, Q52, F328) are also important for the P2X7R trafficking, and conserved residue G323 is important for P2X7 receptor trafficking and vestibule structure. Alanine substitution of non-conserved F322 dramatically increased P2X7R sensitivity to agonist and enhanced the rate of dye uptake, indicating that aromatic residue at position 322 is important for pore opening. Overall, our data suggest that charged residues in the TM1 domain are critical for the P2X7R function, and transition between extracellular and central vestibule, near position F322, represents a promising new target for the allosteric control of P2X7R sensitivity and channel gating.

GALECTIN-3 IN CARDIOVASCULAR TISSUE ENGINEERING

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There is an effort to optimize the inner surface of vascular grafts. One of the major disadvantages of vascular grafts used nowadays is a high risk of thrombus formation. To avoid this complication, it is essential to create a confluent layer of endothelium on the inner surface of vascular graft. Aim of our research is to invent a novel bioactive surface which would enhance self-endothelialization of vascular graft. Galectin-3 is a 28 kDa protein belonging to galectin protein family. Characteristic feature of galectin proteins is high afinity to β-galactosides. Cytoplasmatic membrane-localised galectin-3 is able to bind carbohydrate groups of glycoproteins present in extracellular matrix or on the surface of cells and to promote cell adhesion.

To elucidate the adhesive properties of galectin-3, we compared the effect of immobilized galectin-3 on initial adhesion of two different cell types using xCELLigence RTCA SP real-time sensing device. Our results show that the optimal galectin-3 concentration for enhancement of cell adhesion is the same for both studied cell types – adipose derived mesenchymal stem cells and human umbilical vein endothelial cells. Our preliminary results also suggest that the ability of immobilized galectin-3 to enhance cell adhesion is not mediated by interaction of galectin-3 with carbohydrates.

These findings will be applied in our further research which will be focused on construction of artifical vascular grafts. We plan to modify the inner surface of vascular graft with covalently binded β-galactosides or with galectin-3. We presume that these modifications will lead to faster endothelialization of vascular graft and to decreased risk of thrombus formation.

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POSTNATAL DEVELOPMENT OF EPIGENETIC MODIFIERS AND MTOR PATHWAY PROTEINS IN THE HEART

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Epigenetic modifications play an essential role in postnatal development. Fat mass and obesity-associated (FTO) protein is an important demethylase of m6A in mRNA and its activity is associated with the mammalian target of rapamycin (mTOR) pathway. The aim was to investigate the expression of proteins involved in the growth and development of cells (mTOR pathway) together with proteins responsible for the epigenetic modification in rat heart during postnatal development. Left ventricles of Fisher rat`s offspring were collected on postnatal days (d) 1, 4, 7, 10, 12, 14, 18, 21, 25, 28 and 90. The expression profiles of target proteins were examined utilizing SDS-PAGE/Western blotting. The protein level of FTO displayed a steady decrease from d1 to d90 in both male and female hearts. Both demethylase AlkB Homolog 5 (AlkBH5) and methyltransferase like 3 (METTL3) exhibited a dramatic decline of their levels from d1 to d4 with a subsequent steady decrease from d4 to d90. Levels of mTOR and its downstream target p70 S6 kinase revealed a similar pattern as FTO, a steady decrease from d1 to d90. Interestingly, the level of the phosphorylated form of p70 S6 kinase (Thr389) exhibited two peaks at d1 and d12 together with a dramatic decrease in its levels in the periods of d1-d10 and d12-d25. These results showed that the decrease of both writers and erasers of methyl modification together with a reduction of mTOR pathway activation occurs in rat hearts during the early postnatal development, which may attenuate ribosome biogenesis and protein synthesis.

IMPACT OF THE CIRCADIAN RHYTHMS DISTURBANCES DURING THE DIFFERENT PERIODS OF LIFE ON THE FEMALE REPRODUCTIVE SYSTEM AND FERTILITY

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Infertility is an extremely significant problem nowadays. According to the statistic, from 12% until 15% of couples are unable to conceive after 1 year of having unprotected intercourses. Approximately 40% of women with infertility faces the challenge of disturbed ovulation. It may be a consequence of primary ovarian insufficiency (POI), age-related diminished ovarian reserve or concomitant endocrine disorders. Nevertheless, the most widespread cause is polycystic ovary syndrome (PCOS) that affects 1 in 10 women of childbearing age.

PCOS is a relatively complicated, heterogeneous endocrine disorder with multiple metabolic aberrations. The most important clinical signs of this syndrome include hyperandrogenemia, insulin resistance and chronic anovulation with characteristic species of ovaries on USG. The underlying PCOS mechanism has not been fully understood. However, the role of oxidative stress seems a most plausible cause of PCOS pathogenesis.

Recently, pieces of evidence have been accumulated that circadian clocks are closely coupled with the antioxidant defense ability and especially with mitochondrial functioning. Moreover, circadian system affects the synthesis of hormones and neuropeptides related with the hypothalamic-pituitary-gonadal axis (kisspeptins, leptin, GnRH, etc.), which disrupted regulation may subsequently lead to the development of the vast malfunctions of fertility.

Based on this knowledge, the main goal of my future research is to investigate the impact of circadian system malfunctions via light pollution (continuous light exposure) and shifts in daily rhythms induced at different life periods (from the intrauterine development till the adulthood) on the hypothalamic-pituitary-gonadal axis, oxidative stress in reproductive organs, and fertility.

INHIBITION OF GLIAL CELLS ACTIVATION BY MINOCYCLINE ATTENUATES ACUTE EFFECT OF PACLITAXEL ON PRESYNAPTIC TRPV1 RECEPTORS IN THE SPINAL NOCICEPTIVE PATHWAY.

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Paclitaxel treatment of cancer patients leads to development of neuropathic pain difficult to treat with available analgesics. We have shown previously that modulation of nociceptive transmission at the first sensory synapse in the spinal cord dorsal horn plays an important role in this process. TRPV1 receptors at the presynaptic endings show a significant attenuation of tachyphylaxis to repeatedly applied capsaicin after the paclitaxel treatment. This process is mediated by TLR4 receptors and is dependent on PI3K activation [1,2]. In the current experiments, we have used an inhibitor of microglia activation to assess possible involvement of glial cells in the process. Whole-cell patch clamp recordings of miniature excitatory postsynaptic currents (mEPSC) from spinal cord dorsal horn neurons (lamina I, II) were made to measure tachyphylaxis after repeated capsaicin (200 nM) application. Paclitaxel (50 nM) was used for acute application between capsaicin applications. Minocycline (100 µM) was used for incubation of slices and for acute application during the recordings in corresponding groups. In the control experiments, second capsaicin evoked mEPSC frequency reduced to 42 % of the first one. Paclitaxel application reduced the tachyphylaxis to 66 %. Acute minocycline application did not prevent paclitaxel induced attenuation of the tachyphylaxis. Incubation in minocycline for 90 minutes before the measurement led to significant recovery of the tachyphylaxis and the second capsaicin response was reduced to 48 %. Our results show that *in vitro* incubation of spinal cord slices in minocycline reduced the effect of paclitaxel on TRPV1 mediated response to capsaicin. This indicates a significant involvement of microglial activity in the paclitaxel induced enhancement of capsaicin evoked response. Further experiments will be needed to determine the exact pathways between microglial activation and modulation of presynaptic TRPV1 function.

Literature reference:

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DELETION OF MITOCHONDRIAL PROTEIN DAPIT DOES NOT AFFECT HEART ISCHEMIC TOLERANCE OF THE SPONTANEOUSLY HYPERTENSIVE RATS

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Mitochondria play an essential role in ATP production, which is needed for the proper function of the cells. Lack of oxygen and necessary nutrients during the ischemia lead to metabolic changes, and rapid decrease in ATP production. Thus changes in mitochondrial functions have a cruicial effect on the cell damage during acute myocardial infarction. Protein DAPIT (diabetes-associated protein in insuline-senzitive tissue) is one of the components of the F_0 subunit of mitochondrial ATP synthase, however its role in the heart is still not well known. Our study was performed on spontaneously hypertensive rats (SHR) and unique transgenic strain with deleted gene Usmg5 (upregulated during skeletal muscle growth 5), which encodes DAPIT protein (SHR-DAPIT). Their hearts were perfused according to Langendorff under constant flow, and after stabilization exposed to regional ischemia with subsequent reperfusion. We determinated the extent of ischemia-reperfusion injury using planimetric analysis, and evaluated the incidence of ischemic and reperfusion ventricular arrhythmias using electrocardiogram. The results show, that the absence of gene encoding DAPIT protein in transgenic strain SHR-DAPIT did not affect ischemic tolerance of the heart.

DIFFERENT METHODS OF FIRING RATE ESTIMATION

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Classical rate coding paradigm suggests that the information communicated through neurons is embedded in the electrical impulses (called action potential or spikes) sent per time window. Number of spikes per time window is traditionally defined as the neuronal firing rate. The concept of neuronal firing rate is essential for the rate coding hypothesis, which is still the most commonly investigated scenario in neuronal activity analysis along with temporal coding hypothesis. The estimation of dynamically changing firing rate from neural data can be challenging due to the variability of spike times, even under identical external conditions; hence a wide range of statistical measures have been employed to solve this particular problem over the years. We review the established methods of firing rate estimation, from classical to most recent ones.

ROLES OF ABERRANT DECISION MAKING, ERROR SIGNALING AND COORDINATION OF RESTING BRAIN NETWORKS IN CLINICAL SYMPTOMS OF OCD USING ANIMAL MODELS AS TRANSLATIONAL RESEARCH TOOLS

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OCD is a debilitating psychiatric condition featuring as the fourth most frequent mental health disorder. While a large number of the brain imaging studies have shown alterations to the structure or function of the front-striatal network, this framework rendered insufficient in being translated into effective treatment of OCD. The present project fills the knowledge gap standing between brain structure and functions and OCD symptoms by combining a novel DM model of OCD with relevant behavioral (Eriksen's Flanker Task, Monetary Incentive Delay Task, Stop Signal task etc.) and brain imaging experiments (BOLD-fMRI, tractography) to explain the severity and qualities of OCD symptoms. We will evaluate the dependencies between OCD symptoms, neurophysiological and psychological variables, and test their relations within the DM network model of OCD. Animal studies involved in this project will provide a deeper understanding of neuronal activity by combining decision making experiments (Carousel maze and reference-dependent choice behavior test) with animal models of OCD (QNP and 8-OH-DPAT models) and molecular imaging of immediate-early genes. In animal part of the project, we have confirmed impaired spatial choice on a Carousel maze in both models. We have successfully implemented the molecular imaging of IEGs as markers of neuronal activity in a QNP rat model of OCD. The results suggest over-activation of populations in OFC and a relative hippocampus deactivation, consistent with human findings. The project has the potential to design and test a predictive and mechanistic model of OCD which would allow linking individual patient's symptoms to a specific dysfunction of a particular neuro-cognitive process and its neural representation. Information about an altered stage of DM will be used in identifying novel targets of pharmacological intervention.

SYMPATHECTOMY-INDUCED BLOOD PRESSURE REDUCTION IN ADULT NORMOTENSIVE AND HYPERTENSIVE RATS IS COUNTERACTED BY ENHANCED CARDIOVASCULAR SENSITIVITY TO VASOCONSTRICTORS

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The effect of chemical sympathectomy on cardiovascular parameters and compensatory role of adrenal hormones, renin-angiotensin system and cardiovascular sensitivity to vasoconstrictors were studied in spontaneously hypertensive (SHR) and normotensive Wistar-Kyoto (WKY) rats. Sympathectomy was induced in 20-week-old rats by daily intraperitoneal guanethidine administration (30 mg/kg b.w.) for two weeks. Basal blood pressure (BP), heart rate (HR) and restraint stress-induced cardiovascular changes were measured by radiotelemetry. BP response to catecholamines was determined in rats with implanted catheters. Sympathectomy decreased BP only transiently, after 14 day guanethidine treatment BP returned to basal values in both strains. Sympathectomy lowered HR, improved baroreflex sensitivity and decreased low-frequency domain of systolic blood pressure variability (marker of vascular sympathetic activity) permanently. Guanethidine also attenuated BP and HR response to restraint stress. On the other hand, BP response to catecholamines was augmented in sympathectomized rats, which was not due to de novo synthesis of vascular adrenergic receptors. Sympathectomy caused adrenal enlargement, enhanced expression of adrenal catecholamine biosynthetic enzymes and elevated plasma adrenaline level in both strains, especially in WKY rats. Guanethidine also increased plasma levels of aldosterone and corticosterone only in WKY rats. In conclusion, the sympathectomy produces a transient decrease in BP, chronic decrease in HR and improvement of baroreflex sensitivity. The effect of sympathectomy on BP was counteracted by increased vascular sensitivity to catecholamines in WKY and SHR and/or by enhanced secretion of adrenal hormones, which was more pronounced in WKY rats.

INVOLVEMENT OF MICROBIOTA ON mRNA EXPRESSION IN PITUITARY AND ADRENALS IN ACUTELY RESTRAINED MICE.

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Gut microbiota are capable of influencing brain functions and shaping behavioral and neuroendocrine response to stress. One approach to examine the effect of microbiome on stress reactivity is using animals in which the microbiome is absent. In the present study we investigated the effect of acute restraint stress (ARS) on corticosterone response and expression of genes involved in regulation of stress response in pituitary and adrenal glands of germ free (GF) and specific pathogen free (SPF) mice. Because transfer of mice from the isolator is a stressful procedure, three groups of mice were used: restrained group transferred from the isolator during ARS session; control group transferred from the isolator at the day of experiment and "IVC" group transferred to sterile individually ventilated cages (IVC) one week before the sacrifice. The results showed, that both ARS and transfer of animals from the isolator increased plasma corticosterone and this effect was more pronounced in GF than SPF mice. In pituitary, the genes encoding *Crhr1* and *Pomc* were more expressed in GF mice and ARS downregulated *Crhr1*. Expression of *Fkbp5* in pituitary was upregulated by ARS, but no difference was observed between GF and SPF mice. Expression of glucocorticoid receptor was similar in all groups. In adrenal gland, the expression of ACTH receptor gene *(Mc2r)* was similar in all groups. Steroidogenic acute regulatory protein (*Star)* was up-regulated by stress without any effect of microbiome. Cholesterol side chain cleavage enzyme (*Cyp11a1*) was slightly higher in stressed SPF mice. The results show that microbiota modulate the expression of *Pomc* and *Crhr1* in pituitary but have only limited effect on expression of steroidogenic genes in adrenals.

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Poster session 1

01 Alice Abbondanza
02 Tomáš Bárta
03 Peter Búran
04 Kristýna Čunátová
05 Šárka Danačíková
06 Klára Gotvaldová
07 Dragana Hajduković
08 Viktor Kuchtiak
09 Lucie Leňková
10 Aleksandra Marković
11 Jakub Masaryk
12 Veronika Palůchová
13 Pavel Pohl
14 Pavla Průchová
15 Guillermo Puertas
16 Dmytro Semenovykh
17 Šárka Škutová
18 Kateřina Adamcová
19 Nikhil Ahuja
20 Pavla Hubálková
21 Anežka Kahancová
22 Jan Kudláček
23 Julia Pajorová

Poster session 2

24 Daniela Alexová
25 Zina Ben Salem
26 Andrea Beňová
27 Milica Drapšin
28 Anna Gunia
29 Martina Hochmalová
30 Sofiia Moraresku
31 Monica Pontearso
32 Antonín Sedlář
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