PHD MEETING
TŘEŠŤ 2017

November 20 – 22, 2017

CONTENT

THE ORGANIZING COMMITTEE

Ph.D. students:
Pavel Adámek
Jaroslav Hrdlička
Radmila Kudláčková
Marek Ladislav
Zuzana Novosadová
Marian Rupert
Kristýna Skřenková
Romana Weissová
Jakub Žiak

Organizers:
Martin Horák
Diana Moosová

PROGRAM

20.11.2017 / MONDAY

9:15 Departure from Institute of Physiology CAS
11:15 Arrival to Třešť
12:00 – 13:00 Lunch
13:00 – 13:15 Opening of the Annual students meeting
13:15 – 14:25 Oral presentations 1
(Adámek, Skřenková, Hrdlička, Novosadová, Rupert, Žiak, Ladislav)
14:25 – 15:10 Invited speaker – doc. MUDr. Přemysl Jiruška, Ph.D.
15:10 – 16:50 Poster session 1 (2nd year of Ph.D.) ***+ Coffee break***
16:50 – 18:00 Oral presentations 2
(Trávníčková, Kovalčíková, Adamcová, Vodička, Kulhavá, Lichnerová, Brožka)
18:00 – 18:45 Invited speaker – Tereza Smejkalová, Ph.D.
19:00 – 20:00 Dinner
20:15 – 22:30 PHD Movie + discussion

21.11.2017 / TUESDAY

09:00 –09:40 Oral presentations 3
(Buchtová, Kleisnerová, Pošusta, Kuchaříková)
09:40 – 10:25 Invited speaker - Prof. RNDr. Jan Černý, PhD.
10:25 – 10:40 Coffee break
10:40 – 11:50 Oral presentations 4
(Ahuja, Vavřínová, Ivetič, Hubálková, Fábera, Eliášová, Vojtěchová)
12:00 – 12:45 Lunch
13:00 – 15:30 Trip
15:50 – 17:20 Poster session 2 (1st year of Ph.D.) ***+ Coffee break***
17:20 – 18:00 Oral presentations 5
(Hansíková, Olejníková, Chvojková, Kudláček)
18:00 – 18:15 Speech of the director – MUDr. Jan Kopecký, DrSc.
18:15 – 19:15 Workshop – Ing. Olga Zimmermannová, Ph.D.
19:15 Dinner, social program

22.11.2017 / WEDNESDAY

09:00 – 10:00 Oral presentations 6
(Vondráková, Radostová, Krausová, Svoboda, Czerneková)
10:00 – 10:15 Coffee break
10:15 – 11:00 Invited speaker – RNDr. Tomáš Mráček, Ph.D.
11:00 – 11:40 Announcement of winners, end of the conference
12:00 – 13:00 Lunch
13:30 Departure from Třešť
16:00 Arrival to Institute of Physiology CAS

USE STRAIN-SPECIFIC IMMUNE RESPONSE IN WHITE ADIPOSE TISSUE DURING COLD EXPOSURE

K.Adamcova[1], K.Bardova[1], P. Janovska[1], O. Horakova[1], P. Flachs[1], M. Svobodova[1], M. Rossmeisl[1], N. B. Danneskiold-Samsøe[2], J.M. Larsen[3], L. Madsen[2,4], S. Brix[3], K. Kristiansen[2], and J. Kopecky[1]

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Cold exposure (CE) was shown to activate lipid metabolism in epididymal white adipose tissue (eWAT), with a stronger effect in obesity-resistant A/J as compared with obesity-prone C57BL/6J (B6) mice. The ability of WAT to serve as a whole-body buffer for lipids depends in part on a sufficient vascular network, presence of adipocytes with high lipolytic/re-esterification capacity, and possibly on the extent of WAT remodelling under the conditions of changing energy demands. Since WAT metabolism is interconnected with tissue immune responses, we investigated whether the effect of CE on eWAT metabolism was mirrored by the content and polarization of eWAT macrophages. Two-month-old male A/J and B6 mice fed chow were maintained at thermoneutral temperature (30°C) or exposed to cold (6°C for 48 hours). eWAT was analyzed immunohistochemically and/or using quantitative PCR; macrophages were characterized using flow cytometry. CE decreased weight of eWAT with a more pronounced effect in A/J mice. The occurrence of UCP1-negative and ATGL- and DGAT1-positive paucilocular adipocytes was induced by CE in both strains, with a stronger induction in A/J mice. Our results document both strain-specific difference and influence of CE on eWAT abundance of macrophages. In A/J mice, lower macrophage population with decrease in M1 population by cold was observed in comparison with B6 mice. Overall the ratio of M2/M1 macrophages increased by CE only in A/J mice. These results suggest a causal link between the reduced content of M1 macrophages and relatively strong activation of lipid metabolism in response to CE in A/J mice.

MODULATION OF SYNAPTIC TRANSMISSION IN THE SPINAL CORD AS THE UNDERLYING MECHANISM OF PATHOLOGICAL PAIN

P. Adamek[1, 2], P. Mrozkova[1], V. Nerandzic[1], D. Spicarova[1] and J. Palecek[1]

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Dorsal horn of the spinal cord is the first point for modulation of nociceptive information from the periphery that is perceived as pain. Amplification or attenuation of spinal nociceptive signaling underlies pathological pain conditions in different diseases involving peripheral neuropathy and inflammation. Our projects were focused primarily on the role of presynaptic TRPV1 receptors in central branches of primary afferents in the spinal cord in a model of chemotherapy-induced neuropathic pain and after peripheral inflammation. Our results suggest that neuropathic pain related to therapeutic use of chemotherapeutic drug Paclitaxel is at least partially mediated by modulation of TRPV1 receptors function, by activation of Toll-like receptor 4 signaling pathway, including PI3-Kinase and other serine-threonine kinases. In another study TRPV1 receptors played an important role in synaptic modulation induced by anandamide precursor, endocanabinoid 20:4-NAPE in the model of peripheral inflammation. These results further supported possible interaction between the cannabinoid (CB1) and TRPV1 receptors in pain modulation. Burn injuries represent a clinical condition where pain control is crucial and existing analgesic drugs are insufficient. Our study suggested involvement of $Na_v1.7$ sodium channels in nociceptive processing in the spinal cord, as their inhibition by Protoxin-II significantly attenuated enhanced excitatory synaptic activity in a model of burn injury. Our results advance understanding of these nociceptive modulatory processes and may help to improve analgesic therapy in pathological pain states.

PROCESSING SPATIAL INFORMATION RELATIVE TO A MOVING OBJECT IN AN "ENEMY-AVOIDANCE TASK" IN RATS

N.Ahuja[1,2], V. Lobellova[1], E. Kelemen[1,2,3], A.Stuchlik[1,2,3]

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In real world environments, it is crucial for an animal to assess its own position (orientation) relative to moving objects such as predators or conspecifics while organizing its own movement. Our group has previously shown that rats are able to avoid a moving object by keeping safe distance from it and that this ability depends on intact hippocampus[1] and anterior cingulate cortex[2]. In the current study, we examined specifically the rat's ability to assess not only distance from a moving object, but also position relative to a moving object in a modified version of the enemy-avoidance task[1]. The task required the animals to assess whether they are on the left, on the right or in front of a moving robot in order to avoid a foot shock. We showed that the rats were able to avoid a circular zone (25cm in diameter) whether it is in front, or on the side of the robot. This ability was not based on recognizing prominent visual patterns on the robot; because the avoidance was not impaired when the robot was painted all-white and front differed from the back only by geometry of the surface. We conclude that the rats are capable of assessing their position in a reference frame of a moving object.

[1] Telensky P, Svoboda J, Blahna K, Bures J, Kubik S, Stuchlik A. Proc Natl Acad Sci USA. 108(13):5414-8, 2011
[2] Svoboda J, Lobellova V, Popelikova A, Ahuja N, Kelemen E, Stuchlik A. Neurobiol of Learn Mem. 139:144-148, 2017

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A NOVEL ONE TRIAL LEARNING PARADIGM AS A TOOL TO STUDY A FUNCTION OF ADULT NEUROGENESIS IN DENTATE GYRUS.

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

In a past two years we developed a novel one trial learning task, with aim to test a function of new neurons generated in dentate gyrus of hippocampus. It was to test a hypothesis that new neurons are THE substrate which links temporally discrete events together. To develop a fitting test we fused two paradigms – a light-dark place preference test and one-trial trace conditioning. Light-dark test was implemented first, to achieve uniform behavior of animals (rats) –spending almost whole 15minute session in the dark compartment. This was necessary for elimination of any other movement during the following test except for movement in response to conditioned stimulus. Next, trace conditioning test is implemented. First, animal is played 80dB sound for 3 seconds. Then, following 2 second pause, 1mV pulsating foot-shock is delivered. Shock is terminated only after animal escapes into a light compartment. During this one session, portion of animals form an association between the sound and electric shock. In fact, almost 30% of animals escape directly to the light compartment when response after sound presentation is recorded 24h-72h later. Main advantages of this task are that it is one trial and that the behavioral response is discrete (escaped/ not-escaped). The simplicity of the paradigm also indicates high future replicability. Our future plan is to compare identities of the active cells in DG with the regard to their age in escaping vs. not-escaping animals. We predict that escaping animals will utilize more new neurons during recollection compared to not-escaping animals.

THE EFFECT OF EARLY MATERNAL SEPARATION ON PLACE AVOIDANCE AS AN ANIMAL MODEL OF SCHIZOPHRENIA

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

As multifactorial disease, schizophrenia remains challenging both in human medicine and experimental research. Our objective is to establish animal model of psychosis based on early life stress, combining neurodevelopmental and environmental factors using valid behavioral test of cognitive coordination - the rotating arena (Carousel). The aim of our study is to establish novel animal model relevant to cognitive dysfunction in schizophrenia, based on the role of early life stress in neurodevelopment. Newborn rat pups (both genders) were individually separated for 3 hours daily from postnatal day 1 (PD1) to PD21 during light period of day. Control pups underwent *early handling* - the litter remains without mother for 15 min daily up to PD21 in order to decrease the stress sensitivity of pups. After weaning on PD30 rats were housed in pairs or alone to elicit social deficit. In PD120 rats were handled and tested in place avoidance task on rotating arena (Carousel) for 5 days followed by days of reversal learning (the location of avoided place was opposite).

PIN1 REGULATES STABILITY AND SOLUBILITY OF CDK5-PHOSPHORYLATED CRMP2A IN NEURONS.

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

Collapsin Response Mediator Protein 2 (CRMP2) is a microtubule associated protein promoting microtubule assembly and axon growth. Its activity is negatively regulated by CDK5 and GSK3β phosphorylation inducing growth cone collapse. In Alzheimer's disease (AD), CRMP2 is present in neurofibrillary tangles and its hyperphosphorylation is an early event in AD mouse model pathology. CRMP2 has two isoforms CMRP2A and B. Recently, we have shown that CRMP2A has a novel CDK5 target site - Ser27 (S27) phosphorylation of which leads to CRMP2A degradation. At the same time S27-phosphorylated CRMP2A is specifically bound and stabilized by prolyl isomerase Pin1. Physiological function of S27 phosphorylation or its role in AD pathology has not yet been shown. In the present work, we demonstrate that Pin1 not only stabilizes S27-phosphorylated CRMP2A but also regulates its solubility. We show that, in young mice expressing p25, CDK5 activator, phospho-S27 level is elevated, while in p25Tg Pin1KO double mutant mice S27 phosphorylation, as expected, decreases in soluble fraction. Nevertheless, the level of insoluble phospho-S27 rises in p25Tg Pin1KO mice already in the young age. Similar increase in the insoluble fraction is seen only in older control animals, when Pin1 level drops. Importantly, in 3xTgAD mouse model, levels of both soluble and insoluble phospho-S27 are significantly elevated and S27 hyperphosphorylation is present also in AD patient brains. These data indicate that Pin1 is important for maintenance of the soluble phosphoS27-CRMP2A and that Pin1 deficiency together with CDK5 hyperactivity could contribute to AD related CRMP2 pathology.

EFFECT OF A1 ADENOSINE RECEPTOR AGONIST CCPA IN HIPPOCAMPAL EXCITABILITY DURING BRAIN DEVELOPMENT IN RATS

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The adenosinergic system may influence excitability in the brain. Endogenous and exogenous adenosine has anticonvulsant activity, presumably by activating A1 receptors. Administration of adenosine A1 receptor agonist 2-chloro-N6-cyclopentyladenosine (CCPA) may thus bolster anticonvulsant effect, but may be age-specific. Developmental changes in A1 receptors may thus have important role in hippocampal excitability. Hippocampal epileptic afterdischarges (ADs) were elicited in 12-, 15-, 18-, 25-, 45-, and 60-day-old rats. Stimulation and recording electrodes were implanted into the dorsal hippocampus. The A1 receptor agonist CCPA (0.5 or 1 mg/kg) was administered intraperitoneally 10 min before the first stimulation. Control animals were administered saline. All rats were stimulated with electrical pulses delivered at 60 Hz with increasing stepwise intensity (0.05 - 0.6 mA). Each age and dose group comprised 9-14 animals. AD thresholds and durations were evaluated. A1 receptors were detected in the hippocampus in 12-, 15-, 25-, 32-, and 45-day-old rats. Both CCPA doses significantly increased hippocampal AD thresholds in 12-, 15-, 18-, and 60-day-old rats compared to controls. The higher dose significantly decreased AD thresholds in the 25-day-old rats. AD durations exhibited corresponding changes to the high dose - significantly diminished in all age groups except for 25-day-old rats and significantly prolonged in 25-day-old rats. A1 receptor expression in the hippocampus was lowest in 12-day-old rats and increased thereafter. The adenosine A1 receptor agonist CCPA exhibited anticonvulsant activity at all developmental stages studied, except in 25-day-old rats in which it had a proconvulsant effect. Age-related differences might be due to developmental differences in presynaptic A1 receptors on axons of glutamatergic and GABAergic neurons in the hippocampus.

METABOLIC CHANGES IN MUSCLE DURING EARLY POSTNATAL DEVELOPMENT IN TWO DIFFERENCE MICE STRAINS

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Postnatal period is very important period due to programing to metabolic diseases. Changes in nutrition (switching from high-fat breast milk to high-carbohydrate solid diet) suggest changes in utilization of lipids or glucose and involvement of pyruvate dehydrogenase (PDH). *Gastrocnemius* muscle was obtained from obesity-prone C57BL/6J (B/6) and obesity-resistant A/J mice at 10 days (D), 15D and 28D after birth. Levels of Acylcarnitines (AC) and Amino Acids (AA) were quantified by FIA-ESI MS/MS (n=8). Gene expression was assessed by qPCR (n=6). Activity of pyruvate dehydrogenase (PDH) was determined by measurement of release $^{14}CO_2$ by decarboxylation of ^{14}C-pyruvic acid by PDH (n=7). Data evaluation was performed using t-test ($p < 0.05$). Levels of AC and AA significantly decreased during development in both strains. Strain-specific differences were observed in C0, C4-OH, C10, C12:1 and C12 levels. Expression level of *Acot2* decreased during development in both strains. *Acadl*, *Crat* and *Tfam* showed higher expression levels in A/J mice during development. At 10D, higher level of Pdk4 was observed in A/J mice in comparison with B/6 mice. PDH activity was lower in A/J mice (~1.7-fold) at 10D in comparison with B/6 mice. During early postnatal development, levels of AC and AA decreased probably due to change of nutrient composition. Strain-specific differences in gene expression and lower activity of PDH at 10D in AJ mice could indicate a preference to oxidize fatty acids than to utilize glucose. These results suggest greater ability to utilize fatty acid in A/J mice in early postnatal development of skeletal muscle.

EFFECT OF EPOXYEICOSATRIENOIC ACID ANALOGUE ON MYOCARDIAL TOLERANCE TO ACUTE ISCHEMIA/REPERFUSION INJURY AND POSTISCHEMIC HEART FAILURE DEVELOPMENT

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Protective role of epoxyeicosatrienoic acids (EETs) have been demonstrated in hypertension, ischemia/reperfusion (I/R) injury, inflammation or diabetes. But these effects are limited by low bioavailability of endogenous EETs. Levels of EETs in tissue can be elevated by inhibition of EETs degrading enzyme soluble epoxid hydrolase (sEH) or administration of agonistic EETs analogues. Aim of this study was to determine effect of EET analogue EET-A and sEH inhibitor cAUCB to acute I/R injury and heart failure (HF) development in hypertensive Ren-2 transgenic rat. In acute I/R study rats were treated with EET-A and/or cAUCB (in drinking water; 13 and/or 1 mg/kg/day respectively) for 14 days prior 20 min left anterior descending (LAD) coronary artery occlusion followed by 180 min reperfusion. Blood pressure was measured by telemetry, infarct size was assessed by tetrazolium staining and ECG was measured during I/R. For HF development experiments rats were treated with the same doses of EET-A and/or cAUCB for 5 weeks since 24 hours after 60 min LAD occlusion. LV geometry and function were assessed by echocardiography and at the end of the study also by P-V loop measurement. EET-A and cAUCB administered before I/R attenuated hypertension in Ren-2 transgenic rat and reduced the incidence of ventricle fibrillations in Ren-2 transgenic rat. Chronic treatment of EET-A and cAUCB tended to have positive effect on some functional parameters of HF progression but did not affect LV dilatation in rats after MI. EET-based therapy has the potential to be considered beneficial for patients with cardiovascular diseases.

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NEUROSTEROIDS ARE MORE POTENT INHIBITORS OF NMDA RECEPTORS AFTER INCREASED INTRACELLULAR CA^{2+} CONCENTRATION

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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 effect of Ca^{2+} on functional and pharmacological properties of NMDAR, and on NMDAR modulation by neurosteroids. Using electrophysiology, we show that the amplitude of agonist-induced responses of recombinant GluN1/GluN2B receptors or native NMDAR in primary hippocampal neurons were diminished by ~60% following receptor activation in the presence of 2 mM Ca^{2+}. The same Ca^{2+} stimulation resulted in increased receptor sensitivity to the inhibitory effect of neurosteroids. We tested the naturally occurring neurosteroid pregnanolone sulfate (IC_{50} changes 85→51 μM on GluN1/GluN2B and 105→43 μM on neurons) as well as synthetic analogues, which show an even higher increase in inhibitory effect after Ca^{2+} stimulation (e.g. pregnanolone hemipimelate, IC_{50} changes 65→15 μM on GluN1/GluN2B and 151→25 μM on neurons). This phenomenon is dependent on the intracellular C-terminal domain (CTD) of NMDAR. Deeper understanding of Ca^{2+} regulation of NMDAR function may help in finding new compounds with neuroprotective activity.

EFFECTS OF NEUROPROTECTANTS IN TRIMETHYLTIN MODEL OF NEURODEGENERATION

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Trimethyltin (TMT)-induced pharmacological model of neurodegeneration in rat shares important features with human neurodegenerative disorders (ND): cognitive impairment, neuroinflammation, oxidative stress, excitotoxicity, limbic system involvement and progressive pattern of action. Therefore, it is considered as an useful model of human ND which can be also used for testing the effects of neuroprotectants. The goal of our project was to test the effectivity of selected neuroprotectants – NMDA R antagonists (MK-801) and GABA potentiators (midazolam) and their combination in this model. Wistar rats (10-11 weeks) were used in the experiment. TMT (8 mg/kg) was applied IP. Neuroprotectants MK-801 (0,1 mg/kg) or midazolam (5 mg/kg) were applied IP 30 min before TMT and then daily for the next 10 days. Cognitive functions of the rats were tested in the Morris water maze 14 days after application of TMT. Twenty eight days after TMT, the rats were perfused and their brains were Nissl stained. A defined part of dorsal hippocampus was analysed. Trimethyltin caused a cognitive deficit in Morris water maze and hippocampus damage (decreased hippocampus area and CA1,2,3 length, increased cell loss score). A protective effect of MK-801 was not found. In midazolam treated rats we found a slight protective effect in both cognitive (Morris water maze, day 2) and histological (hippocampus area) parameters. We found that GABA potentiator midazolam, but not NMDA R antagonist MK-801, was therapeutically effective (at behavioral and histological level) in TMT-induced model of neurodegeneration in rat.

AUGMENTATION OF PURINERGIC MODULATION OF GABAERGIC TRANSMISSION IN THE SUPRAOPTIC NUCLEUS DURING INTENSE HORMONE SECRETION

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The release of hormones from the supraoptic nuclei (SON) of the hypothalamus is dependent on the rate and pattern of neuronal electrical activity that is regulated by excitatory, glutamatergic, and inhibitory, gamma-aminobutyric acid (GABA-ergic), synaptic inputs. ATP has been previously shown to be the major neuromodulator of SON neurons that potentiates glutamate and GABA release by activating presynaptic and extrasynaptic P2X receptors. Although presynaptic P2X responses have been described in many parts of the brain, the precise physiological function of this form of presynaptic facilitation is unknown. Therefore, in the current study we investigated purinergic facilitation under conditions of physiologically-induced increases in the activity of magnocellular neurons. Secretion of vasopressin and oxytocin is potentiated after short term fasting and subsequent refeeding. We examined changes in the P2X receptor-mediated modulation of GABAergic transmission in the SON during food state-related changes in hormone secretion. Experiments were performed on hypothalamic slices prepared from 30-day-old rats under provision of food *ad libidum* and rats after 48h of fasting and subsequent refeeding with standard chow. We observed a significant increase in the effect of ATP within the SON of refed vs normal rats, suggesting that rapid alteration of purinergic signaling may occur in association with potentiated hormone release.

PROLYL ISOMERASE PIN1 IS ESSENTIAL FOR SPINAL CORD REGENERATION IN MOUSE CONTUSION MODEL.

M. Kleisnerova[1], A. Alastrue-Agudo[2], R. Weissova[1], B. Eliasova[1], J. Ziak[1], P. Zjablovskaja[3], M. Alberich-Jorda[3], V. Moreno-Manzano[2], and M. Balastik[1]

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Microtubule-associated protein Collapsin Response Mediator Protein 2 (CRMP2) is an important regulator of axon growth and guidance. CRMP2 has been shown to bind heterodimers of tubulin and promote axon growth and branching. Recently, we have shown that one of its isoforms, CRMP2A, is stabilized by prolyl isomerase Pin1 specifically in distal part of axons and improves axon growth. Here we analyse the role of Pin1 and CRMP2A in regeneration of the central nervous system in mouse model of spinal cord injury (SCI). We overexpressed Pin1 or CRMP2A, or silenced Pin1 in mice directly upon SCI and for one month analysed their recovery using behavioural and histological tests. We found significantly improved locomotion in mice with overexpressed CRMP2A. In contrast, there was a significant reduction of the hind limb mobility in Pin1 knockdown group. Furthermore, we detected bigger lesion cavity, higher infiltration of inflammatory cells, less oligodendrocytes in the lesion interface and worse ingrowth of neurons into lesion area upon Pin1 knockdown. In addition, we found that upon SCI Pin1 regulates the level of IFN-β in microglia/macrophages which has been previously shown to affect SCI regeneration. Our findings demonstrate that isomerase Pin1 promotes SCI regeneration by regulating both neuron growth and the immune response.

MITOCHONDRIAL PROTEIN TMEM70: KEY ROLE IN THE BIOGENESIS OF ATP SYNTHASE VERIFIED IN A MOUSE KNOCKOUT MODEL.

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TMEM70 is a transmembrane protein localized in the inner mitochondrial membrane and involved in the biogenesis of the eukaryotic ATP synthase, but its detailed molecular role is still unknown. *TMEM70* mutations cause isolated deficiency of ATP synthase often resulting in a fatal neonatal mitochondrial encephalocardiomyopathy in patients.

To understand molecular mechanism of TMEM70 action, we generated tamoxifen (TAM) inducible knockout. Upon induction of Cre-mediated excision, the weight of mice decreased and they died by the week 8 post-induction. Despite the similar knockout efficiency in liver and heart, BN-PAGE showed more pronounced decrease of the fully assembled F_1F_o ATP synthase with F_1 subcomplex accumulation in liver than in heart. Furthermore, using high resolution CN-PAGE we demonstrated the differences in ATP synthase subcomplexes profile in liver of TAM treated mice than in other models of ATP synthase deficiency. By SDS-PAGE, we quantified OXPHOS proteins showing decreased level of F_1-alpha and changed levels of other complexes in liver. We also observed higher membrane potential measured by TPP^+-selective electrode and poor inhibition of state 3 (ADP) respiration by oligomycin measured by oxygraph in the liver mitochondria of TAM treated mice in comparison to controls. Liver damage was confirmed by hyperammonemia, increased blood levels of its indicators alanine aminotransferase and aspartate aminotransferase, increased oxidative stress and apoptosis.

In conclusion, induction of *Tmem70* knockout in adult mice impairs primarily liver function, and it resembles symptoms present during metabolic crises in patients. This contrasts with the primarily cardiologic presentation at disease onset in humans.

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POTENTIATION OF DISEASE-ASSOCIATED DE NOVO MUTATIONS OF HUMAN NMDA RECEPTORS BY NEUROSTEROIDS

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N-methyl-D-aspartate receptors (NMDARs) are glutamate gated ion channels that play a crucial role in synaptic plasticity and memory formation processes. Their hypofunction is thought to be implicated in several neurodevelopmental disorders. Recently have been identified de novo missense mutations in genes encoding the GluN2B subunit of the NMDAR that were found in patients suffering from an autism spectrum disorders and/or intellectual disability. We performed a functional analysis of these mutant forms of human NMDARs and identify mutations that lead to decreased current responses due to the lower open probability (Po) of these receptors. Pregn-5-en-20-on-3β-yl sulfate (PE-S) is an endogenous neurosteroid that potentiates the action of NMDARs by increasing the Po. Using the patch-clamp technique on HEK293 cells we tested the possibility to rescue the currents by using neurosteroids that potentiate the activity of NMDARs. We tested a two different structural motives of steroids that leads to potentiation of NMDARs – PE-S and Pregn-5β-an-20-on-3β-yl-2´-butyric acid (PA-But) at 7 mutations located nearby or within the membrane regions of GluN2B subunit (hGluN2B-V558I; W607C; V618G; D668N; E807K; G820A). We found out that potentiation by these steroids was dependent both on type of steroid and type of the mutation. Further, in case of mutation (hGluN2B-E807K) was potentiation by PE-S and PA-But significantly increased compared to non- mutated form of the receptor. Our results provide an opportunity for designing new drug candidates for treatment of diseases associated with the hypofunction of the NMDARs.

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THE ROLE OF SMALL INTESTINE IN DIFFERENTIAL METABOLIC EFFECTS OF VARIOUS LIPID FORMS OF DIETARY OMEGA-3 FATTY ACIDS

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Omega-3 fatty acids are known for their hypolipidemic and anti-inflammatory effects. Metabolic responses to Omega-3 might depend on their lipid form, with phospholipids showing better efficacy than triacylglycerols. Aim of this study was to investigate changes in cellular and metabolic processes in small intestine after feeding with various forms of Omega-3. C57BL/6N mice were fed for 8 weeks with high-fat diet (cHF), cHF diet supplemented with fish oil (Omega-3 as triacylglycerols; TG) or Krill oil (Omega-3 as phospholipids; PL-H) at a dose matching the omega-3 content in cHF-F. cHF diet containing Krill oil at a dose matching the amount of fish oil in the TG diet (PL-L), was also used. At the end, mice underwent glucose tolerance test. TAG content was assesed in liver and samples of small intestine were used for expression profilling. Mice in both PL groups gained less body weight and improved glucose tolerance compared to cHF. Liver steatosis was decreased in PL-H group. The most regulated pathways in small intestine compared to cHF included: lipid metabolism (all groups), cytoskeleton remodelling (TG and PL-L), and peroxisomal fatty acid oxidation, retinol and glutathione metabolism and ketone bodies biosynthesis (PL-H). Omega-3 efficiently regulated intestinal lipid metabolism and cellular processes in form dependent manner. Moreover, prevention of body weight gain and improvement of glucose tolerance were more pronounced after feeding with Omega-3 in form of phospholipids. Our findings support the idea that a differential regulation of intestinal lipid metabolism contributes to whole-body metabolic effects of various lipid forms of Omega-3.

LONG-TERM FLUCTUATIONS OF SEIZURE PROBABILITY IN TETANUS TOXIN MODEL OF EPILEPSY

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The long-term fluctuations in seizure probability are well documented in both patients and various models of epilepsy. The most frequent type of the fluctuation is clustering. The aim of our study was to explore the mechanisms which govern seizure clustering. Epilepsy was induced in adult rats (n=7) by injection of 10 ng of tetanus toxin into the right dorsal hippocampus. Electrodes were implanted bilaterally into dorsal hippocampus and motor cortex. The animals were subjected to >2 weeks of continuous video-EEG monitoring. EEG was analyzed semi-automatically using routines written in Matlab. Signal power during seizures and rate of inter-ictal discharges were calculated for hippocampal and cortical channels separately. According to behavioral correlates, seizures were manually classified as convulsive or non-convulsive. We have shown that during the course of a single cluster, the brain undergoes complex changes which manifest by progressive increase of inter-seizure interval which is paralleled by increase in behavioral severity of seizures and increased signal power and IED rate in the motor cortex. We hypothesize that early focal non-convulsive seizures cause an increase in brain propensity to seize and facilitate seizure spread. In contrast, later generalized convulsive seizures act as a negative feedback mechanism reducing the seizure frequency and their cumulative effect promotes cluster termination.

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COMPARISON OF HUMAN SALIVA COMPOSITION ACCORDING TO TOOTH DECAY AND GENDER

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Most people, worldwide, suffer from dental caries. Only a minor part of the global population (about 10%) is caries-resistant and the reason for their resistance is unknown. Only few studies have compared the composition of salivary proteins between groups of people with carious teeth and people with no caries. In this study, we compared the differences in the proteomic composition of saliva between caries-resistant and caries-susceptible females and males, aged between 20 and 45 years, by gel-free method nano-liquid chromatography tandem mass spectrometry (Label-Free Quantitative Proteomics). Our results demonstrate that the observed differences in the protein levels might have influence on the caries resistance. A total of 19 potential markers of tooth caries were found (7 proteins with significantly higher expression in caries-resistant groups and 12 in caries-susceptible groups). The present study is the first complex proteomic and gender project where salivary proteins of caries-free and caries susceptible people were compared by label-free MS. These results could be beneficial to oral health research and medical care in dentistry.

MOLECULAR INSIGHT INTO THE N-METHYL-D-ASPARTATE RECEPTOR CHANNEL GATING: THE ROLE OF LILI MOTIF OF M3-S2 LINKERS

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N-methyl-D-aspartate receptors (NMDARs) mediate excitatory synaptic transmission in the CNS and it has been shown that dysregulation of NMDARs is involved in neurological and psychiatric disorders. Channel opening is the key step in the NMDAR gating that allows the flux of ions. Several lines of evidence indicate that the rearrangement of M3 helices in activated receptor makes the central cavity of the channel accessible therefore implying a crucial role of the M3S2 linkers in channel opening. To answer the fundamental question, what are the initial steps in NMDAR channel opening, we embarked on functional, molecular biology and molecular dynamics studies of GluN1/GluN2B receptors and focused on the M3-S2 linkers.

The results of our experiments show that mutations (deletion/glycine substitution) in the M3-S2 linker of GluN1 and GluN2B subunits profoundly affect the NMDAR channel function: i. Mutated NMDARs open spontaneously and as a consequence of receptor activation by a single agonist; ii. The effect of deletions is stratified – spontaneous activity and singleligand induced responses are more pronounced at deletions closer to the M3 helix; iii. The degree of spontaneous activity and single-agonist responses differ for GluN1 and GluN2B subunits. Combining functional data and computational biology we show that the extracellular channel gate is formed by GluN1(L657) and GluN2B(I655) (LILI motif).

Our data provide new insight into the mechanism of NMDAR ion channel gating and describes LILI motif crucial for the transduction of the energetics of agonist binding to the ligand binding domain to pore opening.

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ALEURIA AURANTIA LECTIN AFFECTS THE FUNCTIONAL PROPERTIES OF THE GLUN3A-CONTAINING N-METHYL-D-ASPARTATE RECEPTORS

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N-methyl-D-.aspartate receptors (NMDARs) are a subclass of glutamate receptors that play an essential role in mediating excitatory neurotransmission and synaptic plasticity in the mammalian central nervous system. NMDARs form a heterotetrameric complex composed of GluN1, GluN2(A-D) and GluN3(A, B) subunits. The activation of NMDARs plays a key role in brain development and memory formation. Abnormal regulation of NMDARs plays a critical role in the etiology of many neuropsychiatric disorders. The GluN3A is nonconventional subunit of the NMDARs, and GluN3A-containing NMDARs have distinct functional properties compared with NMDARs composed of the major GluN1 and GluN2 subunits. Many studies using heterologous expression systems and mammalian neurons have found that both GluN1 and GluN2 subunits are extensively *N*-glycosylated. Recently, our electrophysiological analysis with respective lectins revealed that the pre-incubation of cerebellar granule cells and also HEK cells expressing the GluN2B-containing NMDARs with these lectins alters their functional properties. However, little is known regarding the role of *N*-glycosylation on the functional properties of the GluN3A-containing NMDARs. We found that various plant lectins as well as human galectins profoundly regulate the function of GluN3A-containing NMDARs, mostly by reducing their desensitization properties. Interestingly, our data also showed that the effect of one of the most potent plant lectin, Aleuria Aurantia Lectin (AAL), is mediated by single glycosylation site within the GluN3A subunit. Our findings suggest that the *N*-glycosylation of GluN3A-containing NMDARs may be critically involved in many basic processes associated with glutamatergic neurotransmission.

AGING OF THE CIRCADIAN CLOCK IN PANCREAS

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In the pancreas, the circadian clock temporally controls its physiological functions, including insulin release. Age-dependent impairment of the pancreatic and other metabolic functions has been well documented; however, it is not known whether the impairment was due to worsening in the circadian clock function. The aim of our study was to find out whether aging affects basic properties of the circadian clock in pancreas in comparison with clock in lungs.
The properties of the circadian clocks were studied *in vivo* in Wistar rat pancreas and also *in vitro*, using organotypic explants of pancreas and lungs from *mPer2*^{Luciferase} young (9 months) and aged (25 months) mice. The results revealed no difference in clock gene expression *in vivo*; however, we observed tissue-specific effects of aging on the mPer2^{Luc} rhythmic protein production. Whereas aging did not affect the amplitude dampening in the pancreas, it significantly speeded up the dampening rate in the lungs. The circadian clocks in the lungs, but not in the pancreas, were entrained by the in vitro treatment. Our results demonstrate that aging does not impair *in vivo* rhythms of clock genes expression. Aging also does not compromise *in vitro* rhythms in mPer2^{Luc} protein in the pancreas but significantly affects formal properties of the circadian clock in the lungs. These findings indicate vital significance of the pancreatic clock along the course of life.

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STRESS AFFECTS EXPRESSION OF THE CLOCK GENE *BMAL1* IN THE SUPRACHIASMATIC NUCLEUS OF NEONATAL RATS VIA GLUCOCORTICOID-DEPENDENT MECHANISM

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The reactivity of the circadian clock in the suprachiasmatic nuclei (SCN) to stressful stimuli has been controversial but most studies have confirmed the resilience of the SCN to stress. We tested the hypothesis that during a critical period shortly after birth, the developing SCN clock is affected by stress. Mothers of two rat strains with different sensitivities to stress, i.e., Wistar rats and spontaneously hypertensive rats (SHR), and their pups were exposed to stressful stimuli every day from delivery, and clock gene expression profiles were detected in the 4-day-old pups' SCN. The glucocorticoid receptors antagonist mifepristone was administered to pups to block the effect of the glucocorticoids. The glucocorticoid receptors were detected at the mRNA and protein levels in the SCN of 4-day-old pups. The mothers responded to stressful stimuli with an elevation of plasma glucocorticoid levels; the response was stronger in SHR mothers and it caused the phase shift of *Bmal1* expression rhythm in the SCN of their pups. In the Wistar rat pups, not the maternal stress itself but its combination with daily manipulation of the pups increased plasma glucocorticoid levels and shifted the *Bmal1* rhythm in their SCN which was completely blocked by mifepristone. *Per1* and *Per2* expression profiles remained phase-locked to the light/dark cycle. The results demonstrate that the SCN is sensitive to stressful stimuli early after birth in pups maintained under light/dark conditions and the effect is mediated via glucocorticoid-dependent pathways.

HUMAN COMPUTER INTERACTION USING SURFACE ELECTROMYOGRAPHIC SIGNALS IN VIRTUAL REALITY ENVIRONMENT

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Biosensors for analysis of electrical signals in muscles (electromyography; EMG) are used in medical applications as well as in the human–computer interaction. This work concerns with development of fast online analysis methods and signal transformations to control actions. Algorithms are optimized and embedded into small integrated portable stand-alone USB device, which is capable to process and analyze the signal in realtime onboard. It is connected via USB and a custom developed driver. Device provides continuous quantitative estimate of muscle activation and can emulate various types of virtual switches. The recognized control signals are used for general control of a computer and can be transformed to various types of control signals (switches and virtual sliders, text input, motor control and virtual reality controller). Device was successfully tested as assistive technology with healthy and also disabled participants. Most recent part of work is a game engine plugin, which can be used in Unity (3D engine) on Windows platform. It was successfully applied in realtime virtual reality custom developed environment. Such a system can be utilized for rehabilitation tasks, muscle activation training or movement tracking. Finally it can extend experience for disabled users.

ANALYSIS OF NEUROTRANSMITTER LEVELS IN THE ANTERIOR CINGULATE CORTEX IN AN ANIMAL MODEL OF OCD-LIKE CHECKING BEHAVIOR INDUCED BY QUINPIROLE SENSITIZATION

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Obsessive-compulsive disorder (OCD) is one of the most prevalent psychiatric disorders and it has been studied for a long time. We still do not know neural and neuronal changes underlying its pathogenesis. This study analyzes neurochemical basis of OCD-like checking behavior in rats in the structure proposed to play a pivotal role in OCD, the anterior cingulate cortex (ACC). The chronic pharmacological animal model administrating D2-like receptor agonist quinpirole has been used to model OCD checking behavior in rats in a square open-field arena with several objects. In different phase of receptor sensitization, a microdialysis probe has been implanted to the ACC and after a recovery of brain tissue 5 samples were collected and frozen. Chromatographic separation of neurotransmitters was performed by LC/MS on UPLC Ultimate 3000 RSLC using gradient elution in positive ionization mode for 10 minutes. At this point we are in the phase of optimizing the analytical procedure. We predict increased activity of the monoamine neurotransmitters and glutamate during checking behavior manifestation in comparison with control group of animals and concurrently continued growth of their levels in the course of dopaminergic sensitization.

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CHARACTERIZATION OF MUTANTS IN THE FIRST TRANSMEMBRANE DOMAIN OF PURINERGIC P2X7 RECEPTOR THAT CONTROL CHANNEL CONDUCTIVITY.

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Purinergic P2X receptors (P2X1-7) are ATP-activated cation channels that are composed of two transmembrane domains (TM1 and TM2), a 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 pore dilatation and receptor sensitization, which causes an increase in its permeability to larger organic cations. To explore the molecular mechanisms by which the large pore is generated, we analyzed the electrophysiological properties and Ethidium bromide (EtBr) uptake of alanine mutants in TM1 of the rat P2X7R. We substituted one by one all (22) residues in TM1 (from G27 to D48) of the rat P2X7 receptor with alanine and expressed wild type (WT) and alanine mutants in HEK293 cells. After stimulation with receptor-specific agonist, we measured BzATP-induced membrane current from single cells using patch clamp recordings in a whole-cell configuration. Cellular accumulation of fluorescent dye and BzATP-stimulated changes in fluorescence EtBr (20 μM) was measured using an epifluorescent microscope. Eight substitutions (G27A, K30A, H34A, F43A, Y40A, L45A, M46A and D48A) forms receptors, that exhibited significantly decreased initial current amplitude and dye uptake ability. Molecular modeling revealed that most of these residues (G27, K30, H34, L45 and M46) line one wall of TM1 helix oriented outside from the pore. These results indicate that TM1 might be involved in interaction with another transmembrane molecule possibly important for P2X7 receptor pore formation.

N-GLYCOSYLATION OF THE GLUN SUBUNITS REGULATES MULTIPLE STEPS IN THE TRAFFICKING OF NMDA RECEPTORS

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N-methyl-D-aspartate receptors (NMDARs) play critical roles in excitatory neurotransmission, synaptic plasticity, and excitotoxicity. It is generally believed that both the number and type of NMDARs present at the neuron's cell surface are regulated at multiple levels, including early processing (synthesis, subunit assembly, processing within the endoplasmic reticulum, and intracellular trafficking to the cell surface), lateral diffusion, internalization, recycling, and degradation. Previous studies have shown that posttranslational modifications such as phosphorylation and palmitoylation regulates trafficking of NMDARs, however, little is known regarding the *N*-glycosylation of the NMDARs. Using a combination of electrophysiology, microscopy and biochemistry in two experimental model systems – lines of human fibroblasts derived from patients with various forms of congenital disorders of glycosylation (CDG) and cultured hippocampal neurons incubated with specific inhibitors of *N*-glycosylation, we studied the effects of impaired N-glycosylation machinery on the trafficking of different types of the NMDARs. Our research findings show that the *N*-glycosylation pathway regulates multiple steps in the trafficking of the NMDARs into the excitatory synapses, both on the endoplasmic reticulum level and on the neuronal cell surface. Together, our data show that the *N*-glycosylation is essential for proper functioning of glutamatergic excitatory transmission in the mammalian brain.

BLOOD BRAIN BARRIER PERMEABILITY CHANGES IN CEREBRAL ISCHEMIA

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Stroke is according to the World Health Organization the world's second most common cause of mortality or long lasting disability. Stroke or focal cerebral ischemia is a focal disruption of cerebral blood flow typically caused either by embolic (thrombus) vessel occlusion or hemorrhagic which cause local loss of perfusion pressure. Ischemic origin accounts for 80-85% of all cerebrovascular accidents. Stroke similarly to other diseases, such as brain trauma, infections or tumors, can induce epileptogenesis (the process by which a normal brain becomes epileptic) is accompanied by serious blood-brain barrier (BBB) impairment. The common consequence of a BBB dysfunction is increased permeability leading to extravasation of plasma constituents and vasogenic brain edema. The BBB impairment can persist for long periods, being involved in secondary inflammation and neuronal dysfunction, thus contributing to disease pathogenesis. Our goal is to measure BBB impairment after cerebral ischemia in laboratory animals and search for possible biomarkers of postischemic epilepsy. For this purpose we use confocal (CellVizio) and widefield microscopy or computed tomography (Albira) with specified markers as Ultravist, AuroVist, Fluorescein sodium or Evans Blue. We are able to monitor the opening of BBB and detect extravasation of blood elements into brain extracellular tissue.

HUMAN ADIPOSE TISSUE-DERIVED STEM CELLS - CHARACTERISTICS DEPENDING ON PATIENT FACTORS

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Adipose tissue-derived stem cells (ASCs) are adult stem cells of mesenchymal origin. ASCs are very suitable for tissue engineering as they are abundant in many patients and can be easily harvested by minimally invasive surgery such as liposuction. There are many patient factors that can influence yields, proliferation, and differentiation of harvested ASCs. In our study, we focused on the lipoaspirates obtained by liposuction performed under low (-200 mmHg) or high (-700 mmHg) negative pressure from each individual patient. The region of liposuction was the abdomen, inner thighs or outer thighs. The proliferation activity and phenotypic characteristics of the isolated cells were studied in order to find possible differences between the cells obtained under low and high pressure or from different regions. The proliferation activity of the cells was evaluated by cell counting and cell proliferation assay kit (MTS), the phenotype of cells was characterised using their CD surface markers by flow cytometry. Our current results show higher yields of isolated cells from the thigh region. The cells from low pressure liposuction from thighs generally tend to have a higher proliferation activity than the cells from high pressure liposuction. However, there are considerable differences among the patients. We have not observed distinctive differences in CD markers between cells obtained under low or high pressure liposuction. Our primary results also showed the ability of ASCs to differentiate into vascular smooth muscle cells (VSMCs) depending on the medium composition. The differentiation potential of different ASCs into VSMCs will be further studied.

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BLOOD PRESSURE RESPONSE TO NOREPINEPHRINE AND EPINEPHRINE AFTER SYMPATHECTOMY OF SPONTANEOUSLY HYPERTENSIVE RAT

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Sympathetic innervation of resistance arteries and sympathoadrenal system play an important role in development of high blood pressure in spontaneously hypertensive rat (SHR). We performed chemical sympathectomy (SYMPX) on 20-week-old SHR and normotensive Wistar-Kyoto (WKY) rats by daily intraperitoneal administration of guanethidine (30 mg/kg per day) for two weeks. SYMPX was confirmed by visualization of catecholamines in femoral artery by sucrose-potassium phosphate-glyoxylic acid (SPG) solution. Blood pressure was measured in conscious animals 24 hours after cannulation of carotid artery. Plasma levels of norepinephrine and epinephrine were measured in intact control and sympathectomised rats. Control SHR had higher basal mean arterial pressure (MAP) and heart rate (HR) than WKY rats. SYMPX decreased both parameters, the strain difference being preserved. The effect of acute intravenous administration of norepinephrine or epinephrine on MAP and HR did not differ between control SHR and WKY rats. After SYMPX, MAP response to norepinephrine or epinephrine was shifted to the left and increased in both strains, the effects being augmented in SHR. Plasma level of catecholamines did not differ between control SHR and WKY rats. Plasma norepinephrine decreased after SYMPX in both strains, while plasma epinephrine increased after SYMPX, the effect being more pronounced in WKY. MAP decrease after sympathectomy of SHR and WKY rats seems to be counteracted by increased sensitivity of arteries to catecholamines and by increased plasma level of epinephrine. It would be interesting to study molecular basis of the compensatory changes in arteries and in adrenal medulla of sympathectomised rats.

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THE ROLE OF MICROBIOTA IN THE REGULATION OF PERIPHERAL COMPONENTS OF THE HPA AXIS AND LOCAL METABOLISM OF GLUCOCORTICOIDS

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Recent evidence suggests, that gut microbiota are involved in regulation of the hypothalamo-pituitary-adrenal axis. One of the approaches to determine the role of gut microbiota in stress is the use of germ free animals. In present study we investigated the role of repeated psychosocial stress in germ free (GF) and specific pathogen free (SPF) mice. Male BALB/c mice were exposed to repeated psychosocial stress, where naïve male was placed into the cage of an older male. Mice were allowed to interact freely for 10 min., subsequently they were divided by steel partition to avoid injuries for another 50 min. This procedure was repeated for 5 consecutive days and social interactions were recorded. After last stress session mice were taken out of isolator and sacrificed. The changes in behavior during social interactions and mRNA levels of neurohormones in adrenal gland and pituitary were investigated. Stress increased the expression of adrenal tyrosine hydroxylase and phenylethanolamine N-methyltransferase and this effect was significantly more pronounced in GF than CV mice. No effect of gut microbiota was found in expression of proopiomelanocortin in pituitary; however mRNA expression of Fkbp5 was higher in GF mice both in basal and stress conditions. GF mice also spent less time manifesting defensive behavior during interaction with residents, at the same time there was no difference in offensive behavior of residents. We demonstrated up-regulation of key enzymes of catecholamine synthesis in adrenals of GF mice after repeated social stress and showed that microbiota modulates local glucocorticoid metabolism in Adrenal Gland.

BEHAVIOURAL CHARACTERIZATION OF RATS PRENATALLY EXPOSED TO LIPOPOLYSACCHARIDE

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Environmental factors including infection during pregnancy could play an important role in development of mental diseases. According to the hypothesis, an activation of maternal immune system disrupts maturation of foetal brain. As a consequence, some behavioural changes could appear in the offspring. In our study, pregnant rat females (Wistar) received subcutaneous injection of bacterial lipopolysacharide (LPS; 1 mg/kg) or saline (control) from gestational day 7, and every other day to a delivery. The offspring of both sexes, at the age of 1.5 month (development) and 3-4.5 months (adulthood), was exposed to tasks testing various aspects of behaviour: beam walking (motor coordination), elevated plus maze (anxiety), open field (locomotion, exploration, anxiety), Morris water maze (spatial navigation, working memory) and Carousel maze (cognitive coordination, long-term memory). We found that rat males and also females prenatally exposed to LPS (but not to saline) showed hyperlocomotion and lower anxiety in several tasks in adulthood with some signs of these changes also during development, and that the effect was task-specific for males and females. On the other hand, rats of LPS-treated dams were not impaired in motor coordination or the water maze, however, cognitive performance of all groups was poor in the Carousel maze. We conclude that prenatal exposure to maternal immune activation led to some behavioural changes which were probably caused by disruption of brain development.

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THE ROLE OF ENDOTHELIN RECEPTORS IN NEUROCHEMICAL CHANGES DURING FOCAL CEREBRAL ISCHEMIA IN IMMATURE RAT BRAIN

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Perinatal and neonatal period of life is time of high risk for occurrence of the focal cerebral ischemia (FCI). Perinatal ischemia is followed by acute seizures and mechanisms involved in the development of seizures remain to be unclear. The aim was to investigate the role of endothelin receptors in the development of seizures following activation of endothelin receptors in immature brain. To induce FCI intrahippocampal infusion of endothelin-1 (ET-1; 40pmol/µl) was used in 12 days old rats. To investigate the role of ETB endothelin receptors we infuse selective agonist of ETB receptors (4-Ala-ET-1; 40pmol/µl). Microdialysis study on anesthetized animals and video-EEG monitoring were performed to identify neurochemical and electrophysiological correlates during two hours after the drug infusion. Our results showed that the activation either ETA or ETB receptors led to development of seizures and particular metabolic changes. Neurochemical correlates mostly copied EEG findings and showed specific time-locked changes. For instance, ET-1 infusion changed Glu/GABA ratio during the first hour. In contrast, Ala-ET-1 increased inflammatory response (leukotrienes, prostaglandines) and oxidative stress. We suggest that in ischemic condition early appearance of seizures caused by disbalance Glutamate/GABA; whereas ETB receptors modulated hyperexcitability via inflammatory mechanisms in immature brain.

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TUNING THE BRAIN CIRCUITS: AN INTRODUCTION INTO AXONAL PRUNING

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The pattern of neuronal connectivity is established during embryonic and early postnatal period. Axon from a single neuron usually send branches to multiple targets. However, in later development, considerable amount of initial axonal tracts are pruned into the adult pattern of projections. Precise regulation of both axon growth and selective pruning of inappropriate connections are key for development of functional neural circuits, but molecular cascades regulating these proceses are still porely understood. Semaphorin 3A and its downstream factor collapsin response mediator protein 2 (CRMP2) have been shown to regulate axon growth and guidance in vitro, while Semaphorin 3F (Sema3F) is esential for pruning of already established connections. Now, by a combination of *in vitro* and *in vivo* experiments, we demonstrate that CRMP2 mediates not only axon guidance in Sema3A gradients but also axon and dendritic spine pruning through Sema3F. We demonstrate that CRMP2 deficiency leads to increased spine density and defective pruning of infrapyramidal bundle of mossy fibers and corticospinal axons from visual cortex in *crmp2*$^{-/-}$ mice, similar as shown in *Sema3F*$^{-/}$ mice. We further demonstrate that Sema3F is inable to induce axon retraction in dissociated *crmp2*$^{-/-}$ hippocampal neurons *in vitro*. Moreover, analysis of axon guidance in knockouts revealed defects in corpus callosum and peripheral nerves. In conclusion, we identified CRMP2 as a novel mediator of pruning. Our results further indicate that CRMP2 participate in both axon guidance and elimination.

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POSTERS

EXISTENCE OF CIRCADIAN RHYTHMS IN EXPRESSION OF CELL CYCLE GENES AND THEIR CHANGES IN AGING AND COLORECTAL TUMOURIGENESIS

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Mammalian endogenous timekeeping mechanism called circadian clock allows the organism to adapt and anticipate natural periodic changes. Circadian clock generates circadian rhythms which participate in the regulation of a number signalling pathways. Disruption of the circadian regulatory mechanisms seems to be associated with the development and the progression of tumours including colorectal cancer. The progression of tumourigenesis is influenced by age and is linked to disruption of the cell cycle machinery, which is also controlled by circadian clock. Cell cycle is a dynamical process where accurate transitions of cell cycle phases depends on the dynamic expression of the cyclin (CCN)/cyclin-dependent kinase (CDK) complexes. Expression and degradation of these complexes are regulated by CDC25 phosphatases and WEE1/MYT kinases. Therefore, we compared the 24-hr expression profiles of genes encoding the key CCNs (*Ccnd2, Ccnd3, Ccne2, Ccna2, Ccnb2),* CDKs (*Cdk6, Cdk4, Cdk1, Cdk2*), CDC25s *(Cdc25a, Cdc25b, Cdc25c),* WEE1 and MYT1 in healthy colon of young (14 weeks) and old mice (52 weeks) and in chemically induces primary colorectal tumours of 52 week-old mice. Using RT-qPCR we proved circadian rhythmicitys in *Ccnd3, Ccne2, Ccna2, Ccnb2, Cdk4, Cdk1, Cdk2, Wee1, Myt1, Cdc25b and Cdc25c* in normal colon of young mice. In contrast, this rhythmicity disappeared during aging, except for *Cdc25b* and was absent also in tumours. In summary, our results indicate circadian regulation of cell cycle machinery and larger effect of aging than neoplastic transformation on these diurnal changes.

THE ROLE OF DEMETHYLASE FTO AND ADIPOKINES IN THE HEART: EFFECT OF CHRONIC HYPOXIA

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Adaptation to chronic hypoxia renders the heart more tolerant to ischemia/reperfusion injury. This adaptation is enabled by physiological changes on the cellular level. One of these changes is a shift in the energy metabolism. This process can be regulated by fat mass and obesity associated (FTO) protein, demethylase epigenetically regulating cell protein synthesis. Heart metabolism can be also modulated by adipokines leptin and adiponectin. This project aimed to study the role of FTO and adipokines in the chronically hypoxic heart. Adult male Sprague-Dawley rats were adapted to continuous normobaric hypoxia (CNH; 12% O2; 3 weeks). The FTO protein level was measured in the left (LV) and right (RV) ventricles of both hypoxic and normoxic animals. Under the normoxic conditions, the level of FTO in the RV was by 50% higher than in the LV. CNH led to a significant increase of FTO protein level in the heart by 21% in the LV and by 27% in the RV. We observed lower levels of adiponectin (52% decrease) in the plasma of hypoxic rats. All in all, we showed that CNH increases level of FTO in the heart, which may potentially participate in the ischemia-resistant phenotype.

NOVEL LIPID MEDIATORS – FAHFA BRANCHED-CHAIN FATTY ACID ESTERS OF HYDROXY FATTY ACIDS

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The study is focused on lipid extraction techniques optimization, using liquid liquid extraction, solid phase extraction as pre-separation methods and ultra performance liquid chromatography coupled with mass spectrometry for extraction and subsequent identification of branched-chain fatty acid esters (FAHFA). This newly discovered class of lipid molecules is associated with insulin secretion, which could improve whole body and local glucose metabolism, providing potential for diabetes 2 type treatment. Liquid liquid extraction was optimized using citrate buffer and ethylacetate. Solid phase extraction of biological samples was optimized on columns using reversed phase columns. SPE column HyperSep SI Silica 500 mg/ 10 ml was the most effective for FAHFA isomers separation from biological samples. Chromatographic separation of FAHFA was performed on UPLC Ultimate 3000 RSLC equipped with Accucore C30 2.6 µm 2.1 x 250 mm column using isocratic elution in negative ionization mode for 30 minutes. UPLC was coupled to QTRAP 5500 a hybrid, triple quadrupole, linear ion trap mass spectrometer. We characterized the regioisomers of 6 FAHFAs – POHSA, OAHPA, PAHPA, OAHSA, PAHSA and SAHSA. We identified 9 regioisomers 5-, 6-, 7-, 8-, 9-,10-, 11-, 12-, 13-PAHSA in serum and white adipose tissue of human and mice.

CHANGES IN μ-OPIOID RECEPTORS EXPRESSION IN RAT LYMPHOCYTES AND FOREBRAIN CORTEX AFTER MORPHINE TREATMENT - *IN VIVO* AND *IN VITRO* STUDIES

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Morphine is a widely used opioid analgesic drug and a substance of abuse. The effects of morphine are mediated by binding to opioid receptors (ORs), predominantly to μ-ORs mainly expressed in CNS. Despite the fact, that the primary role of ORs is in brain cells, they are expressed in other mammalian cell types including the immune cells. The mechanisms how morphine influences the immunity are still unclear. The immunomodulation may include direct interaction with immune cells and indirect actions – by a neural-immune network. ORs expression in immune cells is normally very low, but it can be enhanced by stimuli like mitogens or opioids. This study is focused on possible morphine-induced changes of μ-ORs expression in rat lymphocytes and forebrain cortex. Western blot analysis of forebrain cortex showed no change in μ-OR protein level after prolonged morphine exposure, compared to control rats. Interestingly, in splenic lymphocytes of these animals, μ-ORs protein expression was up-regulated by morphine treatment. 48-hour cultivation of splenic lymphocytes with morphine resulted in the similar outcome. In immunoblots, bands of μ-ORs were not detected in control, while in morphine-treated cells´ samples, signals for μ-ORs were obtained. Flow cytometry analysis showed significantly more of morphine-treated lymphocytes expressed μ-ORs compared to control. Mitogen (Concanavalin A) stimulation of lymphocytes results in dramatic up-regulation of μ-ORs protein expression and in a number of cells expressing μ-ORs compared to non-stimulated cells. This is the first study documenting changes in protein level of μ-ORs in rat splenic lymphocytes developed by morphine or Concanavalin A.

CARDIAC ISCHEMIC TOLERANCE IN SPONTANEOUSLY HYPERTENSIVE RATS WITH MITOCHONDRIAL GENOME FROM LEWIS STRAIN

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Spontaneously hypertensive rat (SHR) with mitochondrial genome from Lewis strain (SHRmtLEW) is one of the conplastic rat models with essential hypertension. SHRmtLEW was made on SHR genetic background, but the mitochondrial genome was replaced with Lewis rats. Chronic normobaric hypoxia (CNH) represents a cardioprotective phenomenon which improves myocardial ischemic tolerance in normotensive rats. It has been shown that cardioprotective action of CNH is related to activation of mitochondrial function. Therefore, the aim of our study was analyzed the effect of CNH on cardiac ischemic tolerance in SHR and SHRmtLEW, respectively, and normoxic controls. Adult male rats were adapted to CNH for 3 wks. After that, normoxic and chronically hypoxic rats were subjected 20 min coronary artery occlusion and 3 hrs reperfusion. The incidence and severity of ischemic and reperfusion arrhythmias were analyzed. Myocardial infarction was determined by tetrazolium at the end of reperfusion. In the separated groups of rats, protein levels of MnSOD and OXPHOS were assessed. There were found no differences in cardiac ischemic tolerance between normoxic SHR and SHRmtLEW, respectively. CNH significantly decreased myocardial infarct size and increased expression of complex III in both SHR strains. The lower protein levels of MnSOD in normoxic and hypoxic SHRmtLEW was observed. It seems that mitochondrial genome from Lewis strain do not play an important role in cardiac ischemic tolerance of SHRmtLEW.

IN VITRO APPROACH TO STUDY THE ROLE OF GLUCOCORTICOIDS IN THE DEVELOPMENT OF CIRCADIAN CLOCK

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Synchronization of the circadian clock with external environment is crucial for proper function of all metabolic and behavioral processes in organism. The circadian system consists of the principal clock in suprachiasmatic nuclei (SCN) of the hypothalamus and peripheral clocks in various organs and tissues. SCN are developed gradually during ontogenesis, when neurogenesis is completed already during embryonic stage, but synaptogenesis is finished postnatally. Notably, rhythmical expression of clock genes, which are responsible for the molecular mechanism of the circadian clock, is detected already at the late embryonic stage. The pups are born with SCN clocks synchronized with the maternal circadian system but the mechanism of entrainment is unknown. Mother affects the circadian rhythms of fetuses via either behavioral or hormonal signals. Possible candidates for hormonal signaling are glucocorticoid hormones because their receptors are present in the embryonic brain, they are released in a circadian manner and they pass through the placenta. To ascertain the role of glucocorticoid signaling, we have established the *in vitro* model of long-term cultivated organotypic explants of embryonic SCN from Per2::LUC mouse. *In vitro* results demonstrate that 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 impact of the glucocorticoids on circadian rhythmicity of PER2 protein in the SCN *in vitro*, as well as restoration and synchronization of rhythms in explants after glucocorticoid treatment. These findings support the role of glucocorticoids as entraining cues for the fetal clock.

ADHESION, GROWTH AND DIFFERENTIATION OF OSTEOBLAST-LIKE CELLS ON MATERIALS FOR BONE IMPLANTS

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Electrochemical anodization, along with various mechanical and chemical methods, is currently the most common type of surface modification of titanium and its alloys used in biomedicine. It has been applied to achieve alteration of the surface topography, chemistry, roughness, and to enhance its adhesive properties. High biocompatibility and suitable physical properties make Ti alloys a frequent choice for designing and fabricating implants for medical applications predominantly in orthopaedics and dentistry. We focused on testing and improving Ti-6Al-4V ELI biomaterials in cooperation with research institutions and private companies developing and producing such materials. The metallic samples used in this study were modified by plasma electrolytic oxidation (PEO) with use of electrolytes of a different composition to induce development of a homogeneous TiO_2 layer on its surface. *In vitro* interactions of human osteoblast-like cell line Saos-2 with the sample surface were investigated. Initial cell attachment, spreading, morphology, cell population density, viability, calcium deposition and expression of selected osteogenic markers, e.g. collagen type I, alkaline phosphatase and osteocalcin, were evaluated on cultured cells. The cells' behavior was then correlated with physicochemical properties of the material surface, such as its topography, roughness, wettability, surface layer chemical composition *etc.* The results are also compared with those obtained in cells cultured on control samples of untreated alloys as well as microscopic glass coverslips and bottom of standard polystyrene cell culture wells. Based on these results the modified material with the most promising combination of properties for the use in temporary bone implants was selected.

INTRODUCTION OF APEX TAG INTO ANAEROBIC PROTIST

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The intermembrane space (IMS) of aerobic mitochondria contains proteins of the respiratory chain and machineries for the protein import and assembly. However, we do not know anything about the IMS of hydrogenosomes and mitosomes. The reason is that it is impossible to isolate IMS with classical methods like cell fractionation. Recently, the new ascorbate peroxidase (APEX) tag has been developed. It is suitable for EM as well as fluorescence visualization and behaves like biotin ligase when exposed to biotin-phenol. Altogether, it allows compartment specific protein labeling and their subsequent isolation. We have managed to establish this technique for visualization of mitosomal matrix in *Giardia intestinalis*. Our further aim is to use APEX tag for characterization of IMSs of hydrogenosomes and mitosomes of *Trichomonas vaginalis* and *Giardia intestinalis*, respectively.

Y CHROMOSOME SHORT TANDEM REPEATS VARIABILITY IN SLAVIC-AVAR POPULATION FROM 8.-9. CENTURY

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In archeogenetics, analysis from historical samples provides glimpses into variability of biological individuals and populations, such as reconstruction of human migrations and microevolution. However exhumed historical biological material, such as bones, are confronted with handling and molecular analysis complications. First of all disadvantages is low quantity and damaged DNA biomolecules. Second, there is a high risk of modern DNA contamination which is prererably amplified by the main tool of the field - polymerase chain reaction, instead of authentic historical molecules. In this work, we present the survey of 17 male subjects from slavic-avar burying ground, dated on 8.-9. century A.D., archeological site Cífer-Pác, localized on Avar Khaganate territory (curently Slovakia). Aim of this work was analysis of Y chromosome short tandem repeats (STR) variability in exhumed male human petrous bone, for the purpose of determination range and origin of gene flow from asian populations. Overall, with hypersensitive amplification we reliably detect endogenous nuclear DNA. We identify haplotypes of the Y chromosome STR markers in 17 individuals and determine Y chromosome haplogroups in 11 individuals. In conclusion, with fylogenetic networks we determine gene flow by two male individuls, who have haplotypes connected with migration of population from Asia.

EFFECTS OF FAT SPECIFIC PGC-1β DELETION ON METABOLIC FUNCTIONS OF ADIPOSE TISSUE IN MICE DURING COLD EXPOSURE

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Peroxisome proliferator-activated receptor gamma (PPARγ) is engaged in the regulation of energy homeostasis in metabolically active tissues including white and brown adipose tissues (WAT/BAT). One of the processes under control of PPARγ in adipose tissue is futile cycling based on lipolysis and fatty acid reesterification (TAG/FA cycling). Several effects of PPARγ are mediated by interactions with PPARγ coactivators 1 alpha/beta (PGC-1α/β), which are responsible for mitochondrial biogenesis and regulation of mitochondrial oxidative functions. The aims of this study were to find a link between PGC-1β and regulation of TAG/FA cycling in WAT and to characterize the role of PGC-1β in metabolic and thermogenic functions of BAT during cold exposure. Male C57BL/6J mice with adipose tissue specific PGC-1β deletion and their wild-type littermates were either exposed to cold for 2 or 7 days, or kept at thermoneutral temperature. The levels of gene expression and proteins were quantified using qPCR and Western blotting analysis. The results show that inactivation of PGC-1β probably does not affect TAG/FA cycling in WAT. In BAT of mice with PGC-1β deletion, increased weight of tissue and upregulation of the expression of genes connected with regulation of mitochondrial oxidative functions were observed. Levels of PGC-1α gene expression in BAT of cold exposed animals positively correlated with UCP1 gene and the expression of both genes was elevated in mice with PGC-1β deletion. In conclusion, PGC-1β plays nonredundant role in BAT and compensation of its ablation by increased expression of several genes is not sufficient to maintan fully functional tissue.

THE ROLE OF NEUROKININ RECEPTORS IN MODULATION OF NOCICEPTIVE SYNAPTIC TRANSMISSION, CHARACTERIZATION OF NOVEL TRANSGENIC MICE STRAIN WITH FLUORESCENTLY LABELLED NK1 RECEPTORS

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Physiological pain is an essential defense mechanism of organism, but as much as 20% of individuals at some point of their lives suffer from chronic pain. Mechanisms of chronic pain are still not well understood and effective treatment of chronic pain is in many cases not available. Recent discoveries suggested that activated neurokinin 1 receptor (NK1R) after internalization is still affecting synaptic modulation through endosomal signaling. These findings may explain why NK1R antagonists failed in pain related clinical trials and may still represent a suitable target for effective analgesic treatment. NK1R is a heterotrimeric GTP-binding protein coupled receptor (GPCR) activated by endogenous agonist substance P (SP) that plays a role in central sensitization of nociceptive pathways. At spinal cord level NK1Rs are localized in postsynaptic membranes of dorsal horn neurons. In collaboration with the Czech Center for Phenogenomics we have developed transgenic mice strain with NK1Rs labeled with mCherry fluorescent protein. In this project we will first characterize the expression and functional properties of NK1Rs in these animals. We will use histological techniques for visualization of NK1R and electrophysiological techniques in spinal cord slices to evaluate their properties and role in synaptic transmission. Combined optical and electrophysiological techniques will be used at confocal facility in BIOCEV. The goal will be to further evaluate the role of NK1Rs in nociceptive signaling. Parallel to this project will be a study examining the role of cytokines in modulation of opioid induced analgesia and TRPV1 receptors signaling in a model of neuropathic pain.

PATHOGENESIS OF ACUTE KIDNEY INJURY AND EXTRACELLULAR DNA

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Acute kidney injury is an abrupt impairment or loss of renal functions and its causes are numerous. Acute kidney injury may lead to a number of health complications, including death. Nowadays, disorders of renal functions are diagnosed on the basis of laboratory findings, such as elevated levels of blood urea nitrogen or plasma creatinine. Current knowledge of extracellular DNA (ecDNA) indicates that it can be used as sensitive, fast and noninvasive diagnostic method. The aim of this thesis was monitor changes in levels of extracellular DNA in acute kidney injury. Partial aims were to compare levels of ecDNA between different animal models with various pathological mechanisms of kidney injury, to compare levels of ecDNA with biochemical markers of renal functions routinely used in clinical practice, to compare levels of ecDNA measured by qPCR and QUBIT and to prove therapeutic effect of DNase I in model of acute kidney injury. Based on the results of measuring markers of renal functions - levels of plasma creatinine and blood urea nitrogen we confirmed significantly higher in individuals with acute kidney injury compared to control samples. Levels of ecDNA measured by qPCR and QUBIT method were also higher in animals with acute kidney injury compared to control individuals. We found a positive correlation between levels of ecDNA and levels of plasma creatinine. After administration of DNase I to animals with glycerol induced acute kidney injury we measured lower levels of ecDNA compared to animals without DNase I therapy, which indicates that DNase I may have therapeutic effects.

INTERACTION BETWEEN CIRCADIAN CLOCK AND MACROPHAGES IN THE ADIPOSE TISSUE

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Well-functioning circadian system is crucial component of healthy organism and its disruption can result in impairment of metabolic functions with possible consequential development of obesity and type 2 diabetes mellitus. Obesity is in general accompanied by enhanced migration of pro-inflammatory polarized macrophages (M1) into adipose tissue. We have shown that interaction of this type of macrophages with adipose tissue had a significant effect on rhythmic expression of clock genes in adipocytes. We further investigated effects of high fat diet and diet, which was enriched by omega-3 fatty acids (namely EPA and DHA), on circadian oscillations in white adipose tissue and differently polarized macrophages. This diet significantly affected oscillations in adipose tissue and in non-polarized (M0) and so-called alternatively polarized macrophages (M2), which are characterized by their anti-inflammatory properties. These results support previous findings of effect of omega-3 fatty acids on metabolism and suggest their effect on circadian system as well.

DUAL ROLE OF INTERICTAL DISCHARGES IN HIPPOCAMPAL ICTOGENESIS

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It has been demonstrated that interictal epileptiform discharges (IEDs) can have either pro-ictogenic or anti-ictogenic effects, while certain studies claim that IEDs do not interfere with the transition to seizure. In this study, we have explored the multifaceted role of IEDs in ictogenesis from a dynamical perspective. Using high potassium (>8 mM) hippocampal seizure model in vitro, we found that in isolated CA1 slices with the absence of IED activity, the duration of the inter-seizure period was 50.3±2.4 s. In intact slices which generate IEDs, the presence of IEDs was associated with a prolonged inter-seizure period (69,8±2,1 s). In this preparation, seizure initiated hypersynchronous heralding spike across the entire CA1 area due to an incoming IED. To evaluate the mechanisms beyond the dual effect IEDs, we have determined the resilience of the CA1 network to pertubations using active probing. Results showed delayed recovery from the pertubation with approaching seizure suggesting progressive loss of resilience of CA1. Active probing approach also demonstrated that pro-ictogenic effect of IEDs occurred during the state of low resilience while seizure delaying effect of IEDs dominated during the state with high resilience. This study suggests that the effect of IEDs can be defined by the dynamical state of the seizure-generating network at the moment of the discharge occurrence. If the CA1 network displays low resilience to perturbations, then the originally anti-ictogenic IEDs can shift the dynamics of the CA1 network into the ictal regime.

SEARCH FOR BIOMARKER OF POSTISCHEMIC EPILEPSY

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Cerebral ischemia (stroke) is the most common cause of epileptogenesis in adult patients. Patients after stroke are monitored in the long term with the aim to find biomarker for postischemic epileptogenesis. Several MRI sequences and EEG monitoring are included in our measuring protocol. Our approach to MRI data and methodology used for MRI processing in our study is summarized in this poster. We have developed several semiautomatic algorithms for detection of regions effected by ischemia and a software for assessment of blood-brain barrier (BBB) impairment in the brain. Detected ischemic regions are parametrised and together with parameters of BBB impairment are monitored in the time. All parameters are then compared across two groups of patients: patients which stay healthy and patients which has developed epilepsy. Collected statistic will be used for indentification of biomarker of postischemic epileptogenesis. Currently 60 patients were analysed and others are being recruited. Our preliminary results show that BBB impairment caused by stroke significantly exceeds ischemic region, thus it might plays an important role in postischemic processes in surrounding brain tissue.

CHARACTERIZATION OF METABOLIC EFFECTS OF DIETARY *n*-3 FATTY ACIDS IN TRANSGENIC PPARα-HUMANIZED MICE

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Obesity is tightly connected with metabolic diseases including insulin resistance, type 2 diabetes or dyslipidemia. Peroxisome proliferator-activated receptor (PPAR)-α is a key transcription factor involved in the regulation of lipid metabolism. Polyunsaturated fatty acids of *n*-3 series, including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), are endogenous ligands of PPARα, and they are used as dietary supplements in order to lower triacylglycerol levels in circulation and to prevent cardiovascular disease. Wax esters represent a relatively novel lipid form of EPA and DHA, which could be isolated from marine zooplankton *Calanus finmarchicus*. Mice of the 129S1/SvImJ inbred strain, including wild-type (WT) mice as well as transgenic mice either expressing the human form of PPARα (hPPARα) or lacking PPARα completely (PPARα-KO) were used. Animals were fed for 8 weeks the following diets: (i) low-fat chow diet, (ii) obesogenic high-fat diet (cHF; lipids ~32 wt%), and (iii) the cHF diet supplemented with EPA/DHA as wax esters (ω3Cal; replacing 15% of lipids). Administration of ω3Cal affected metabolic parameters only in PPARα-KO mice, in which body weight gain and adiposity were reduced. The ω3Cal intervention also reduced lipid accumulation in the liver, total as well as non-HDL cholesterol levels in plasma, and fasting blood glucose levels. In conclusion, the inability of ω3Cal to induce metabolic changes in hPPARα mice could be explained by relative resistance of these animals to obesogenic effects of cHF diet.On the contrary, yet unidentified PPARα-independent alternative mechanism(s) are likely involved in some of the effects of ω3Cal observed in PPARα-KO mice.

DIMENSIONS OF THE NARROW PORTION OF THE GLUN1/GLUN3A SUBTYPE OF NMDA RECEPTOR CHANNEL

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N-methyl-D-aspartate (NMDA) receptors are a subgroup of the ionotropic glutamate receptor family, which are found at excitatory synapses of the mammalian central nervous system. The NMDA receptors contain two GluN1 subunits and two GluN2A-D and/or GluN3A-B subunits. In contrast to all other ionotropic glutamate receptor subunits (iGluRs), GluN1/GluN3 are insensitive to glutamate; however, they bind glycine with high affinity. All GluN subunits contain the extracellular N-terminal domain (NTD), the ligand binding domain (LBD), the transmembrane domain (TMD) and the intracellular C-terminal domain (CTD). Each TMD is composed of three transmembrane (TM) helices (M1, M3 and M4) and a pore loop composed of the M2 helix and an extended region. The extended region forms the narrowest part of the ion channel called the selectivity filter, which plays a central role in ion permeation, ion selectivity, and channel block. A large number of studies investigate the functional properties of the GluN1/GluN2 subtypes, but few focus on the GluN1/GluN3 subtypes. The estimated pore size of the NR1-NR2A subtype is 5,5 A, however, there is lack information about the pore characteristics of the NR1-NR3A subtypes. The purpose of my experiments was to measure the GluN1/GluN3 pore size permeabilities to cations of different sizes to estimate the size of its ion channel pore. My data show that large organic cations such as arginine (Arg), histidine and tetramethylammonium (TMA), when added in millimolar concentrations to the extracellular solution, showed no measurable permeability at both GluN1/GluN2A and GluN1/GluN3A subtypes. However, tris-(hydroxymethyl)-aminomethane (Tris), which is not permeable at the GluN1/GluN2A subtypes, was permeant at the GluN1/GluN3A subtype. These findings suggest that the narrow portion of the pore of GluN1/GluN3A subtype has a higher mean diameter in than the pore of GluN1/GluN2A subtype.

PROTECTIVE EFFECT OF COGNITIVE TRAINING DURING ADOLESCENCE ON NEURONAL COORDINATION DEFICIT IN A PHARMACOLOGICAL MODEL OF SCHIZOPHRENIA

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Schizophrenia places an enormous burden on patients, caregivers and society. Cognitive deficit is an integral part of the disorder and the best predictor of functional outcome. Although there is no established treatment addressing cognitive deficits, early intervention seems crucial. Animal models of schizophrenia, such as NMDAR antagonist MK-801 administration, display impaired cognitive control and neuronal coordination. We tested if early cognitive training prevents detrimental effects of MK-801 on coordination of neuronal activity. During adolescence (PD 50-55), 48 male Long Evans rats were trained in active place avoidance on a rotating arena to engage cognitive control processes. In adulthood (PD 90+), subjects received MK-801 i.p. (0.15 mg/kg) followed by two exploration sessions in either the same or in two distinct environments. Hippocampal CA1 ensembles are activated by exploratory behavior. Increased similarity between ensembles representing two different environments indicates impaired neuronal coordination. We determined overlap between these ensembles by analyzing expression pattern of IEGs *Arc/Homer1a* using catFISH procedure. Exploration increased and MK-801 decreased IEG expression. In accord with our hypothesis, a trend toward decrease in similarity of neuronal ensembles representing two distinct environments in rats that received adolescent cognitive training was observed. However, the effect was not significant due to high variance. This variance probably resulted from uncontrolled disturbances during resting periods in the vivarium, which increased background IEG expression. Conclusiveness of this study might be enhanced by increasing the number of subjects. Our data demonstrate the critical importance of quiet baseline conditions during experiments utilizing IEG expression to map neuronal activity.

THE MODIFIED CELLULOSE MATERIAL AS A PROMISING WOUND DRESSING FOR ACCELERATED HEALING OF WOUNDS.

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Faster and better wound healing requires functionalized wound dressings and skin substitutes, which enhance cell proliferation and extracellular matrix production. We aimed to utilize a simple cellulose wound dressing as a scaffold for construction of dermo-epidermal substitute which are little presented on the market. The dermis was presented by primary human dermal fibroblasts cultured on the cellulose material. And the epidermis was presented by primary human keratinocytes cultured on a collagen gel which separated fibroblasts and keratinocytes. We monitored adhesion and vitality of the cells by fluorescent microscopy. Because the pristine material did not support the fibroblast adhesion, we used two strategies to improve the material properties. We either incorporated fibroblasts into the collagen gel prepared on the material or we coated the material with a fibrin mesh, seeded fibroblasts and thereafter covered it with the collagen gel. Fibroblasts migrated into the later prepared collagen gel which served after that for keratinocytes adhesion. Both strategies successfully improved fibroblast adhesion and proliferation. However, in the case of fibrin mesh fibroblasts proliferated faster, so the collagen gel contained higher amount of fibroblasts. It seems from our data that the higher amount of fibroblasts promoted better keratinocytes proliferation. But, this will be a subject of following investigation necessary for better clinical treatment of acute and chronical wounds.

IDENTIFICATION OF STRUCTURAL MOTIFS CRITICAL FOR 5β-PREGNAN-20-ON-3-α-YL2´-BUTYRIC ACID-INDUCED POTENTIATION OF N-METHYL-D-ASPARTATE (NMDR) RECEPTORS

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N-methyl-D-aspartate receptors (NMDARs) are glutamate-activated ion channels which play a critical role in excitatory synaptic transmission. Their function can be modulated by a wide range of compounds, including neurosteroids. Experimental data from several laboratories indicate that steroids with a "bent" structure at the A/B ring – like an endogenous neurosteroid 5β-pregnan-20-on-3α-yl sulfate (3α5βPA-S) – inhibit NMDAR responses, while steroids with a flat structure – like a naturally-occurring steroid pregnenolone sulfate (PE-S) – potentiate them. To evaluate structure-to-function relationship of the potentiating effect of pregnane steroids we prepared pregnane analogues, in which the ester bond was replaced 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.Our experiments show that analogs with short C3 residues (e.g. 3α5βPAcarboxylate and 3α5βPA-acetate) inhibit NMDAR responses. However, derivatives with elongated aliphatic chain (3α5βPAbutyrate (3α5βPABut) and 3α5βPApropionate) potentiate the responses, which is unexpected due to their "bent" structure. 3β5βPABut also demonstrated a strong potentiating effect on NMDAR and was chosen as a representative compound for further experiments. Subsequent analysis showed that 3β5βPABut has a disuse dependent effect at GluN1/GluN2B receptors and is similar to PE-S in terms of the on- and off- kinetics. Further experiments showed additive effects of both steroids, which suggest distinct binding sites or mechanisms of action. Alanine scanning mutagenesis indicates that amino acid residues in the membrane domain of the receptor affect the degree of 3β5βPA-But potentiation. Our results indicate new possibilities for the development of neurosteroid-like drugs to treat disorders associated with NMDAR hypofunction.

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BETULINS AS A NEW CLASS OF NMDA RECEPTORS MODULATORS

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N-methyl-D-aspartate receptors (NMDARs) are ionotropic glutamate receptors highly permeable to Ca^{2+} that mediate the vast majority of the excitatory synaptic transmission in the CNS and play an important role in synaptic plasticity, memory, and learning. NMDARs function can be influenced by a variety of endogenous and exogenous allosteric modulators, including neurosteroids. Betulin- derivatives (BD) are naturally occurring pentacyclic lupane triterpenoids with a similar structural formula to neurosteroids. BDs are known for their anti-cancerous and anti-inflammatory effects however their effects on the central nervous system are only poorly understood. The aim of our study was to investigate the effect of BD on NMDARs. Patch clamp technique was used to record responses induced by glutamate from recombinant GlutN1/GlutN2B receptors expressed in HEK293 cells. Our results show that 5 of the 9 BDs tested (1 positively charged, 5 negatively charged and 3 neutrals, at 30 µM BD) have potentiating effects that varied from 5% to 70%. One BD showed an inhibitory effect of 70% and one BD showed a mixture of both potentiating and inhibitory effects. The remaining two compounds were not dissolved at this concentration. Our next experiments will be aimed to: *i.* extend our knowledge on structural to functional correlated BDs at NMDAR and *ii.* to analyze the effect of BD on human NMDARs with single point mutations at the sites relevant to those found by genetic screening of autism, mental retardation, and epilepsy.

ANIMAL MODEL OF SCHIZOPHRENIA INDUCED BY DIZOCILPINE (MK-801): SPATIAL AND INTERVAL-TIMING STRATEGIES ON A ROTATING ARENA

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The objective of this study was to test a rat model of psychosis induced by dizocilpine (MK-801) in a variant of active place avoidance task aimed at both spatial-based and temporal-based avoidance strategies. We expected that we will be able to separate both types of strategies in this animal model and to influence them differently. We used a modified active place avoidance task on a rotating arena for rats. This task required the rats to localize a to-be-avoided sector defined in the stable room coordinate frame while the arena rotated. However, we also introduced periodic intervals during which the spatial strategy usage was blocked by darkness and rats had to rely on temporal updating of the sector position in relation to arena rotation. After pretraining in 20-min daily session during four weeks, we have applied MK-801 (0,12 mg/kg) and recorded changes in spatial and temporal performances in the final week. This specific dose of dizocilpine disrupted timing strategy but not performance based solely on spatial integration. Rats were not able to avoid the sector during intervals of temporal strategy demands and displayed a higher number of errors in these phases. The results corroborated the hypothesis that both types of strategies are separable.

MODELLING OF DEGRADATION AND A SOFT FAILURE MOMENT DURING THE OPERATION OF A SUPERCAPACITOR APPLYING SELECTED DIFFUSION PROCESSES

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An important requirement imposed on storage batteries nowadays is to have sufficient capacity. At the same time, a high level of availability, reliability and safety is required as well. Our intention is to determine a capacity degradation threshold and the moment the soft failure of a graphite supercapacitor (SC) occurs. If we do not take into account the idle state, the functioning of the supercapacitor might be expressed by charging and discharging processes under different operating conditions given by the allowed extent of SC design. When looking for the degradation threshold and the moment of soft failure occurrence we performed the experimental part of measuring in the climatic chamber. We performed and recorded the processes of SC charges and discharges at different temperatures: 40 °C, 25 °C and −42 °C and at different charging and discharging currents: 2A, 4A, 6A and 8A. The experimental results were used to model mathematically the SC discharge process. Appropriate tools used for SC discharge are diffusion processes. In this case, we apply a Wiener process with drift and an Itô process.

SORPTION OF METAL IONS BY CROSS-LINKED N-2-SULFOETHYLCHITOSAN WITH A 0.7 DEGREE OF MODIFICATION

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The purpose of the study was investigation of sorption properties of N-2-sulfoethylchitosan towards transition metal ions and study of influence of different conditions on selectivity of this sorbent. Crosslinked N-2-sulfoethylchitosan with 0.7 degree of substitution (SECH 0.7) was synthesized by the methods of chitosan transformation. Samples of SECH 0.7 with different degrees of crosslinking were examined by swelling test. Capacities of these samples towards silver (I) and copper (II) ions were estimated under different pHs. Breakthrough curves for adsorption copper (II) and silver (I) ions on SECH 0.7 in simultaneous presence in solution were plotted and described using mathematical models. It was found that selectivity of the SECH 0.7 it is a function of crosslinking degree, i.e. the higher the crosslinking degree the more selective the sorbent becomes. Therefore, sorption of transition metal ions from the single, binary and multi component systems for the most crosslinked SECH 0.7 sample in static mode was carried out. Sorption isotherms for these systems were obtained and fitted using Langmuir, Freundlich, and Redlich–Peterson models. Capacities of SECH 0.7 for metal ions were calculated. Thus, sorbent SECH 0.7 selectively collects silver and copper ions and other metal ions barely interfere.

3D dSTORM OF MITOCHONDRIAL NUCLEOIDS AT REDUCED MITOCHONDRIAL DNA REPLICATION IN PANCREATIC ISLET □-CELLS OF DIABETIC GOTO KAKIZAKI RATS

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Hypertrophic pancreatic islets (PI) of Goto Kakizaki (GK) diabetic rats contain lower number of β-cells than non-diabetic Wistar rat PI. Remaining β-cells contain highly reduced mitochondrial (mt) DNA per nucleus (copy number) within the fragmented mt network. Such a profound mtDNA decrease might originate from declining replication machinery or decreased transcription factor TFAM. Moreover, it might be reflected by an altered morphology of nucleoids, which are complexes of mitochondrial DNA with proteins responsible for DNA packaging (TFAM), transcription and replication (DNA polymerase polγ, Twinkle helicase, mtSSB, *etc.*). To elucidate these changes, we have immunostained nucleoids of insulin-positive cells by TFAM and Twinkle antibodies and visualized them using a 3D dSTORM imaging. Alternatively, we have employed incorporation of 5-bromo-2-deoxyuridine (BrdU) or 5-ethynyl deoxyuridine (EdU) for staining of newly replicating mitochondrial DNA (mtDNA) itself. The indirect 3D immunocytochemistry was employed with a secondary antibody conjugated to Alexa-647. 3D dSTORM measurement was performed on a Vutara SR-200 nanoscope (currently Bruker). For nucleoids segmentation and their 3D rendering, Delaunay tessellation and subsequent modeling by principal component analysis was used [1]. We have found that despite the profound mtDNA decline, the apparent nucleoid number (spatial density) was constant. In β-cells of GK rats (*vs*. Wistar) Eos-TFAM-visualized nucleoids were by 10% smaller (composed of 72% localized TFAM) while Eos-mtSSB-contoured “nucleoids” were by 25% smaller but 1.25 times denser. TFAM/mtDNA determined by qPCR of TFAM and ND5 subunit remained constant, when both were suppressed equally as the mtDNA.

TRANSMEMBRANE CYSTEINES DIFFERENTIALLY CONTRIBUTE TO TRPA1 ACTIVATION

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Mammalian transient receptor potential ankyrin 1 (TRPA1) channel is an excitatory, nonselective cation channel implicated in somatosensory transduction, neurogenic inflammation, itching and pain. The major activation mode of TRPA1 is a covalent modification of specific N-terminal cysteine and lysine residues by electrophilic compounds such as allyl isothiocyanate and cinnamaldehyde [1], but cysteines outside the N-terminal region may also contribute to channel gating [2]. Here, we investigated the role of the cysteine residues located within or near the transmembrane region of human TRPA1 using point mutagenesis and electrophysiology. We show that although the cysteines C703, C727, C773, C786, C834 and C856 are not likely to be involved in electrophilic binding, some of them substantially contribute to voltage- and calcium-dependent TRPA1 activation. Mutations at C703, C773, C834 and C856 primarily perturbed the voltage-dependent gating, whereas the C727A and C727S mutations significantly accelerated calcium-dependent inactivation. Significant outward rectification was observed for the C856A- and C856S-mediated currents, resulting from decreased inward currents at negative membrane potentials. This result suggests that mutations at C856 might cause conformational change of the S4-S5 linker leading either to changes in voltage-dependence of the channel gating kinetics or blockage of the channel pore by sodium ions under the ionic conditions used. Taken together, except for C786 in the first intracellular linker, cysteine residues within and near the transmembrane region of TRPA1 are required for normal activation gating induced by electrophiles and voltage and for modulation by external calcium.

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CHARACTERIZATION OF GLUTEN FREE FOOD

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Celiac disease is defined as a chronic immune-mediated intestinal enteropathy induced by gluten in genetically predisposed people. In the last few years, the rise of celiac cases has led to a marked increase in gluten free products on the Italian market. Food technologists have been looking for alternatives ingredients to wheat flour for the production of bread, pasta, biscuits, snacks and pizza. The most commons alternatives ingredients used are maize and rice flour, but also flour of sorghum, millet, quinoa and amaranth. In recent years, however, several studies have been suggesting that gluten free diets can lead to nutritional imbalances. The hypothesis is that the attempt to replace wheat flour has led to the production of food characterized by an increase in carbohydrates and lipids and by a decrease in micronutrients, such as vitamins and minerals. The purpose of this experiment is to evaluate the lipid composition of a representative group of gluten free wafer available on the italian market, compared with wafers formulated with gluten. In particular, it has been evaluated the fat content, the fatty acid profile and the sterol content and composition, using gas chromatography and mass spectrometry. The results does not show any significant difference in lipid content among the two groups, probably due to the fact that these two groups were quite inhomogeneous. However, some differences were found related to fatty acids profile, in fact gluten free wafers contain mostly long chains fatty acids, while wafers with gluten mostly short and medium chains fatty acids.

STRUCTURE-DEPENDENT POTENTIATION OF P2X RECEPTOR CHANNELS BY TESTOSTERONE ANALOGUES

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Purinergic P2X receptors (P2XRs) are ATP gated cation channels comprising of seven subunits (P2X1-7), which can assemble as homo- or hetero-trimers and are widely distributed in the peripheral and central nervous system. Endogenous molecules such as divalent cations and phospholipids can allosterically modulate P2XRss, however, little is known about modulation by neurosteroids that are known to modify neuronal activity by acting on ionotropic glutamate and GABA(A) receptors via a fast, non-genomic action. Here, we examined the effect of testosterone and several newly synthesized testosterone analogues on activity of P2XRs by using electrophysiological recordings in HEK293 cells expressing recombinant P2X2 and P2X4 receptors. Additionally, we tested the effect of testosterone analogues on endogenously expressed P2XRs in isolated anterior pituitary cells, particularly gonadotrophs which express P2X2R subtype. Our data show that several testosterone analogues potentiated the ATP-induced current in both P2X2R and P2X4R expressing cells in a concentration dependent manner but no potentiating effect on GABA(A) receptor was found. Potentiating effect was also observed in pituitary gonadotrophs. The comparison of chemical structure of the neurosteroids and their corresponding modulatory effect on P2XR responses showed that the modulation depends on both the lipophilicity, the length and the branching of ester moiety at position C-17 on the D-ring of testosterone. These results revealed structural requirements of putative steroid site(s) for proper P2XR-neurosteroid interactions which could serve as a guide for synthesis of new molecules. Detailed knowledge about modulatory sites on P2XRs is a prerequisite for development of new drugs against P2XR-based disorders.

THE FLUORESCENCE SPECTROSCOPY STUDY OF THE INTERACTION BETWEEN PROCASPASE-2 AND THE 14-3-3 PROTEIN

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Apoptosis is a process of programmed cell death that maintain number of cell in tissues. This set of biochemical pathways occurs for instance during aging or immune reactions. Caspases were identified to play a crucial role in apoptotic pathways and they are named due to their specific cystein protease activity. Our research is focused on human procaspase-2 interaction with the 14-3-3 (described in *Xenopus laevis*) [1]. The sufficient NADPH level induces phosphorylation of caspase-2 and 14-3-3 protein binding prevents procaspase-2 maturation. The nutrient depletion promotes the 14-3-3 protein release and caspase-2 activation. Procaspase-2 as an proenzyme consists of 3 domains: CARD, p18 (connected with linker containing $pSer^{139}$ and $pSer^{164}$) and p12 [2,3]. In current study we tested conformational behavior of procaspase-2 and its changes upon complex formation with 14-3-3 protein using set of fluorescence measurements. Four procaspase-2 tryptophan mutants, containing single tryphofan residue, were prepared at various positions. Values of mean fluorescence lifetimes (τ_{mean}) clearly show the different vicinity in individual mutants after 14-3-3 binding with exception of Trp^{188} which seems to be buried within the structure of procaspase-2. Data obtained from fluorescence anisotropy determined Trp^{151} and Trp^{426} with flexible vicinity with significant restriction after 14-3-3 binding. On the other hand Trp^{188} and Trp^{218} have been shown very rigid.

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MECHANISMS OF HIPPOCAMPAL CIRCADIAN CLOCK SYNCHRONIZATION

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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, the suprachiasmatic nuclei of the hypothalamus, and peripheral clocks. Recently, a circadian clock has also been found in the hippocampus, a key brain region for the process of memory formation. However, little is still known about its function and synchronization to exogenous cues. The role of the hippocampus in secretion of glucocorticoid hormones (GCs) and high presence of glucocorticoid receptors makes GCs likely candidates for hippocampal clock synchronization signal. The aim of our study was therefore to test the possible role of GCs in hippocampal clock synchronization. To achieve this, we investigated the impact of GC 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. For measuring the response to the GC stimulation in vitro, we used long-term cultivated organotypic hippocampal explants from Per2::LUC mice. Our results demonstrate that the absence of GCs abolishes rhythmical clock gene expression in the hippocampus, with exogenously administered GC analog dexamethasone partly reversing this abolition. Using the organotypic explants, we found that the GC signaling directly influences circadian rhythmicity of PER2 protein *in vitro*. These findings support the role of GCs as hippocampal clock synchronizers. Our results provide insight into plausible mechanisms of hippocampal circadian clock dysfunction in disorders accompanied by memory impairment and should also be taken into consideration when administering systemic GC therapy.

NOVEL CALMODULIN AND S100A1 BINDING SITE IN DISTAL TRPM4 N-TERMINUS

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Transient receptor potential (TRP) channels are calcium-permeable cation channels that are involved in a wide variety of physiological processes, e.g. thermosensation, mechanosensation, taste and vision. The membrane topology of TRP channels indicates six transmembrane domains flanked by long N- and C-termini that contain binding sites for ligands. TRPM4 is a member of TRP family, which plays a key role in calcium-activated signalling cascades involved in the cardiac conduction, immunity response or insulin secretion. Several conserved TRPM4 ligand binding domains modulating permeability of TRPs, which are typically present at the intracellular N- and C-termini, have been identified. The most common TRPM4 channel modulators include calcium binding proteins (CBP) calmodulin (CaM), S100A1; ATP and phosphatidylinositol phosphates. Here, we present two novel binding sites for CaM and S100A1 localized in distal part of TRPM4 N-terminus. Identification of ligand binding sites in TRPM4 and determination of the amino acid sequence contributing to protein-protein interactions were performed using fluorescence methods. Data obtained from fluorescence experiments led to the conclusion that the TRPM4 binding domains for calmodulin and S100A1 are overlapped. These results are also supported by De Novo molecular models of the complexes conforming that the interactions are formed by positively charged (K271, R273, R274) and hydrophobic (L263, V270, L276) residues of TRPM4. Taking together, our data provides a new potential mechanism for TRPM4 regulation. Further insights into TRPs modulation may allow the treatment of human diseases associated with TRP channel regulation disorders.

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DIFFERENTIAL REGULATION OF COLLAPSIN RESPONSE MEDIATOR PROTEIN 2A BY PROLYL ISOMERASES FKBP12 AND PIN1 IN NEURONS

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CRMP2 (collapsin response mediator protein 2) is an important regulator of neural development. It plays a significant role in neurons polarization and axon guidance. CRMP2 in its non-phosphorylated state binds tubulin dimers and promotes their polymerization, which leads to microtubule growth. After phosphorylation (by kinases CDK5 and GSK3beta) CRMP2 loses its affinity to tubulin which results in growth cone collapse. Hyperphosphorylated CRMP2 has been also linked to pathological states of aging neurons as it is present in Alzheimer's disease neurofibrillary tangles. CRMP2 exists in two alternatively spliced isoforms - CRMP2B and longer CRMP2A. Recently, we have shown that phosphorylated CRMP2A isoform is specifically bound by phospho-specific prolyl isomerase PIN1. Prolyl isomerases are enzymes that catalyze conformational changes of peptide bond between Proline and another amino acid. These proteins can play important roles not only as chaperons during protein maturation but also in signalling by changing conformation and function of its substrates. We have shown that PIN1 regulates CRMP2A stability and axonal growth. Using immunoprecipitation and pull down assays we now show that another Alzheimer's disease-linked prolyl isomerase - FKBP12 also binds CRMP2A isoform. Importantly, unlike PIN1, FKBP12 binds preferably non-phosphorylated CRMP2A. Thus, rather than being redundant in CRMP2A isomerisation, PIN1 and FKBP12 regulate CRMP2A in its distinct phosphorylation states and play unique roles in neural development and neurodegeneration.

INHIBITORS OF MITOCHONDRIAL GLYCEROPHOSPHATE DEHYDROGENASE – MODES OF ACTION AND UTILITY IN TARGETING CANCER PROLIFERATION

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With increasing incidence of various cancer types, there is a growing need for development of new strategies for targeted treatments. Rapid growth and fast proliferation of some tumors require specific adaptations of cellular metabolism comprising high rates of glucose utilization and subsequent NADH reoxidation. To sustain high glycolytic rate, many cancer types depend on functional mitochondrial respiration (aerobic glycolysis) and shuttle systems for NADH transfer between cytosol and mitochondria. Glycerophosphate shuttle represents one such system, which can be highly regulated through the expression of one of its components, mitochondrial FAD-dependent glycerol-3-phosphate dehydrogenase (mGPDH). An increased mGPDH expression appears in numerous cancer cell lines, among others in melanomas (A375) and in prostate cancer (DU-145, PC-3, LNCaP). In our work, we focussed on utility of established and proposed inhibitors of mGPDH (metformin, phemformin, alpha tocopheryl succinate and iGP-1) on enzyme itself and on proliferation of cancer cells. By measuring of the enzyme kinetics, alpha-tocopheryl succinate (alpha-TOS) and iGP-1 were shown to inhibit mGPDH activity. In the melanoma cell line A375 was demonstrated strong anti-proliferative effect of alpha-TOS. Taken together, our data demonstrate increased mGPDH expression in several cancer cell lines but it remains to be proved, whether targeting of mGPDH represents feasible approach for anti-tumour therapies.

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