Center for Experimental
and Applied Physiology
Faculty of Pharmacy
University of Belgrade

Centar za eksperimentalnu
i primenjenu fiziologiju
Farmaceutskog fakulteta
Univerziteta u Beogradu

2nd Symposium in Biomedicine: Basic and Clinical Neuroscience

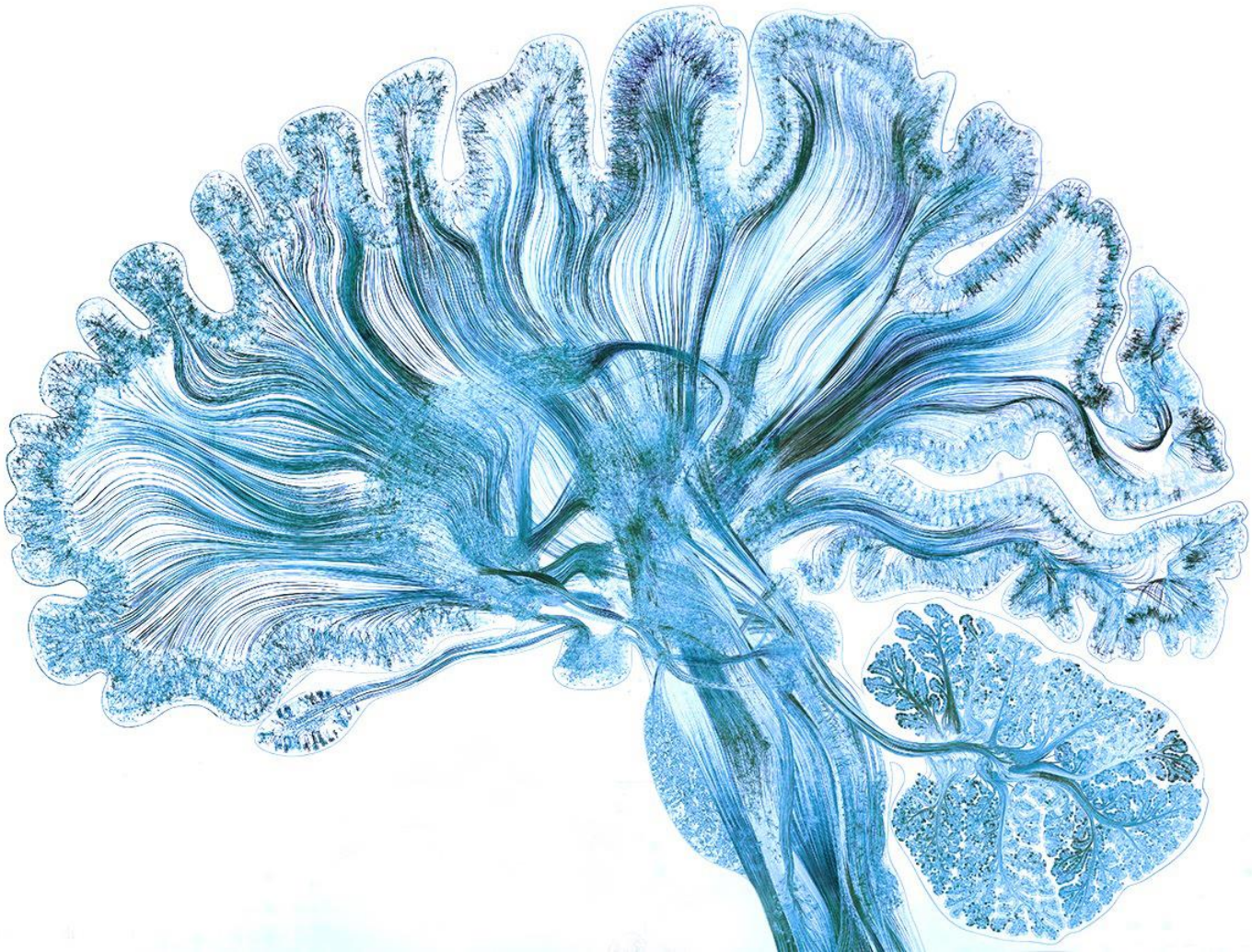
May 9, 2019

2. Simpozijum iz biomedicine: bazična i klinička Neuronauka

9. maj 2019.

ABSTRACT BOOK

KNJIGA SAŽETAKA



Programme / Program

8:45 – 9:00	**Opening / Otvaranje**
9:00 – 9:30	**Prof. Magnus Ingelman-Sundberg**, Karolinska Institutet, Sweden. *Spheroid in vitro 3D system as a tool for modeling interindividual variability in drug metabolism*
9:30 – 10:00	**Dr. Selma Kanazir**, Institute for Biological Research "Siniša Stanković„ Serbia. *Non-pharmacological interventions in ameliorating AD pathology*
10:00 – 10:30	**Prof. David Slattery**, Goethe University, Germany. *Novel mechanisms underlying the anxiolytic properties of oxytocin*
10:30 – 11:00	**Asist. dr Aleksandra Tomić**, Neurology Clinic KCS, Serbia, *Are there two different forms of functional dystonia? A multimodal brain structural MRI study*
11:00 – 12:00	**Coffee break & poster session / Kafe pauza & prezentacija postera**
12:00 – 12:30	**Prof. David Gurwitz**, Tel Aviv University, Israel. *Genomic biomarkers for personalized treatment of depression*
12:30 – 13:00	**Prof. Vesna Pešić**, Faculty of Pharmacy, University of Belgrade, Serbia. *Oxytocin affects expression of Itgb3 and Chl1, what are the consequences in depression?*
13:00 – 13:30	**Dr. Milica Jovičić**, Psychiatry Clinic, KCS, Serbia *Glucocorticoid receptor phosphorylation in stress-related disorders*
13:30 – 14:00	**Doc. Marin Jukić**, Faculty of Pharmacy, University of Belgrade, Serbia. *Clinical Impact of CYP2D6 Genotype on Exposure and Treatment Failure of Risperidone and Aripiprazole*
14:00 – 14:15	**Poster Awards / Dodela nagrade za najbolji poster**

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Arsen Pejin, BPSA (Belgrade Pharmacy Students' Association)

2nd Symposium in Biomedicine: Basic and Clinical Neuroscience

2. Simpozijum iz biomedicine: bazična i klinička Neuronauka

PLENARY LECTURES

PREDAVANJA

3D spheroid in vitro system as a tool for modeling interindividual variability in drug metabolism and toxicity.

Magnus Ingelman-Sundberg, Karolinska Institutet, Stockholm, Sweden

Hepatic *in vitro* systems should be able to provide a cellular phenotype similar to the situation in vivo in man. Using a model of 3D PHH spheroids we observed that drug metabolism was preserved for several weeks of cultivation (1-4) and that the metabolomics analyses revealed similar quantity of metabolites in freshly isolated hepatocytes as in 3D spheroids cultivated for 3 weeks (4). In addition using this 3D spheroid systems we have been able to mimic different kinds of liver disease like steatosis and fibrosis and found the system suitable for evaluation of drug candidates (5-8). The model system responds very well to gene silencing utilizing siRNA constructs and the importance of specific gene products can easily be evaluated. This also allows to study the importance of genetic polymorphism on drug metabolism and drug toxicity, where specific transporters or enzymes can be silenced. The system is especially valuable for examination of pharmacogenetics of low clearance drugs.

References

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Genomic biomarkers for personalized treatment of depression

David Gurwitz, Tel Aviv University, Israel

Major depressive disorder (MDD) is a major cause of morbidity in developed countries with lifetime prevalence estimated at up to 20% of the population. Over 30% of MDD patients fail to improve on SSRIs, the first line MDD therapeutics, while other classes of antidepressants are available since many decades (SNRIs, TCAs, serotonin ligands). In March 2019, S-ketamine nasal spray was approved by the FDA for patients diagnosed with treatment-resistant depression (TRD) – those who do not respond to antidepressants from at least two drug families. However, no genomic biomarkers are in place for diagnosing MDD patients who are likely to be later diagnosed as having TRD.
Our team, along with clinicians from Shalvata Mental Health Center (Israel) and a team from BIOCEV (Czech Republic) are collaborating on identifying genomic and metabolomic TRD biomarkers in blood samples from MDD patients that would allow prognosis of TRD and choice of the antidepressant therapeutics most likely to benefit individual patients. We collect longitudinal blood samples from MDD patients along with psychological score assessment (HAM-D and HAM-A) prior and following treatment with electroconvulsive therapy (ECT) or ketamine (14 or two weeks, respectively). Bloods are separated to peripheral blood mononuclear cells (PBMC) for RNA-seq and plasma for LC-MS spectrometry. I will present our preliminary findings on novel genomic and metabolomic biomarkers for prognosis and precision medicine of MDD.

Non-pharmacological interventions in ameliorating AD pathology

Dr. Selma Kanazir, Nataša Vasiljković-Lonačarević, Milena Jović, Desanka Milanović, Sanja Ivković, Milka Perović, Divna Lazić
Institute for Biological Research "Siniša Stanković", Serbia

Alzheimer's disease (AD) is a progressive, age-dependent, neurodegenerative disorder and the most
common cause of dementia. So far, the majority of clinical trials aimed at suppressing AD pathology have failed. Alternative approaches that are focused on life style interventions are widely used aiming to intervene at prodromal phase of disease when the symptoms are not yet apparent. Among them, the use of dietary restriction regimes and dietary supplementations are widespread due to proposed beneficial effects on neurodegenerative diseases. The mechanisms and therapeutic potential of these interventions still remain elusive.
We use transgenic animal model of AD, 5xFAD mouse, to investigate the potential of various dietary interventions to diminish and/or postpone the AD pathology. We will present the results showing the effects of dietary restriction (every- other-day, EOD, feeding regime) and the fish oil (FO) supplementation implemented at 3 months of age, when the disease pathology is in an early phase of progression. We have demonstrated that EOD feeding regimen exacerbates Alzheimer's disease-like neurodegenerative and neuroinflammatory changes irrespective of Aβ pathology. In regard to FO treatment we have revealed the novel mechanism underlying the FO effects by which microglia/macrophages create a physical barrier around amyloid plaques, thereby protecting surrounding neurons from Aβ cytotoxicity, and consequently preventing the neuritic dystrophy.

These finding suggest that FO consumption may play an important role in modulating microglial response and ameliorating AD pathology, while the caution should be made when using the food restriction approach in prodromal phase of this neurodegenerative disease.

Novel mechanisms underlying the anxiolytic properties of oxytocin

David Slattery, Goethe University, Frankfurt, Germany

The neuropeptide oxytocin (OXT) is currently discussed as a treatment option for a series of psychiatric disorders owing to its prosocial, stress-attenuating, and anxiolytic properties. OXT, when infused into the paraventricular nucleus of the hypothalamus (PVN), reduces anxiety- and fear-related behaviours in both rats and mice. The neuronal connections underlying its anxiolytic and fear-attenuating actions within various brain regions have progressively been revealed. In parallel, knowledge of intraneuronal signalling pathways coupled to the OXT receptor (OXTR) that underlie anxiolysis is growing, but gaps remain in our understanding concerning the molecular mechanisms that causally link OXTR recruitment and anxiolysis.
To address the effectors underlying the anxiolytic properties of intra-PVN OXT, we have performed a series of studies in the past years that have shed light on the intracellular signalling pathways that are coupled to OXTR activation. Here, I will describe these studies and reveal how the these results show that OXT recruits several intracellular signalling cascades to induce protein synthesis, possibly via recruitment of eukaryotic elongation factor 2 (eEF2), which mediates the anxiolytic effects of OXT within the PVN.

Oxytocin affects expression of Itgb3 and Chl1, what are the consequences in depression?

Vesna Pešić, Faculty of Pharmacy, University of Belgrade

Selective serotonin reuptake inhibitors (SSRIs) are the first-choice drugs in pharmacotherapy of major depressive disorder (MDD), but remission is only reached in a small percentage of patients, and introducing new strategies for efficient MDD therapy became a necessity. Intranasal treatment with the neuropeptide oxytocin showed some beneficial effects in post-traumatic stress disorder and autism spectrum disorders, however it was not much explored in MDD patients. Also, our previous *in vitro* genome-wide transcriptomic study on human lymphoblastoid cell lines exposed to the paroxetine resulted in increase of integrin β3 (*ITGB3*) gene expression, and pointed to the increased SSRI sensitivity. Our research team for some time investigates the effect of oxytocin on behavior in the chronic stress and oxidative stress surroundings. Also we are exploring whether there is a synergy between anxiolytic- and antidepressant-like effects of citalopram and oxytocin. Finally, we have analyzed brain alterations on molecular level as expression of ITGB3, CHL1, ITGAV and SIRT1. Our long-term research showed that in depression-like behavioral model, oxytocin exhibited anxiolytic- and antidepressant-like effects, and ameliorated molecular changes in the hippocampus and PFC; enhanced the effects of citalopram and this synergy persisted in reversing the reduction of the *Itgb3* gene expression in the PFC. These findings support hypothesis of possible association of elevated *ITGB3* gene expression and SSRI treatment successfulness and suggest that co-administering oxytocin along with an SSRI drug may have beneficial effect for the treatment of MDD patients, and should thus be considered in future clinical trials for enhancing the antidepressant effects of SSRI drugs in these patients.

Clinical Impact of CYP2D6 Genotype on Exposure and Treatment Failure of Risperidone and Aripiprazole

Marin Jukić, Faculty of Pharmacy, University of Belgrade

Background: The polymorphic CYP2D6 enzyme metabolizes the antipsychotics risperidone and aripiprazole to their active metabolites 9OH-risperidone and dehydroaripiprazole. The aim of this study was to quantify the impact of the *CYP2D6* genetic variability on risperidone and aripiprazole exposure and treatment success.
Methods: The study included 1,288 risperidone- and 1,334 aripiprazole-treated patients from the Diakonhjemmet Hospital, Oslo, Norway, out of which 725 risperidone- and 926 aripiprazole-treated patients were eligible for the analyses of pharmacokinetic parameters. Subgroups were defined by the CYP2D6 genotype-determined metabolizer status: poor metabolizers, intermediate metabolizers, normal metabolizers, and ultrarapid metabolizers. Drug exposure was quantified by the dose-normalized sum of parent drug and active metabolite serum levels. Treatment failure rate was measured as the incidence of switches from risperidone or aripiprazole to another antipsychotic drug.
Results: The CYP2D6 genotype significantly influenced risperidone and aripiprazole metabolism resulting in an approximately 1.6- and 1.4-fold increase in risperidone and aripiprazole active moiety exposure in poor and intermediate metabolizers in comparison to normal metabolizers, respectively (p<0.001). Compared with normal metabolizers, i) the clinicians reduced daily doses of risperidone and aripiprazole administered to poor metabolizers by 19% and 14%, respectively and ii) risperidone treatment failure rate was increased 2.3- and 1.6-fold in ultrarapid (p=0.003) and poor metabolizers (p=0.015), respectively.

Interpretation: The *CYP2D6* genotype had a substantial clinical impact on risperidone and aripiprazole exposure and on therapeutic failure rate of risperidone. Preemptive CYP2D6 genotyping would be of value for the individualization of risperidone and aripiprazole dosing and treatment optimization.

Are there two different forms of functional dystonia? A multimodal brain structural MRI study

Aleksandra Tomić, Neurology Clinic, KCS, Serbia

Introduction: This study assessed brain structural alterations in two diverse clinical forms of functional (psychogenic) dystonia (FD) - the typical fixed dystonia (FixFD) phenotype and the "mobile" dystonia (MobFD) phenotype, which has been recently described.
Methods: Forty-four FD patients (13 FixFD and 31 MobFD) and 43 healthy controls were recruited. All subjects underwent 3D T1-weighted and diffusion tensor (DT) magnetic resonance imaging (MRI). Cortical thickness, volumes of gray matter (GM) structures, and white matter (WM) tract integrity were assessed.
Results: Normal cortical thickness in both FD patient groups compared with age-matched healthy controls were found. When compared with FixFD, MobFD patients showed cortical thinning of the left orbitofrontal cortex, and medial and lateral parietal and cingulate regions bilaterally. Additionally, compared with controls, MobFD patients showed reduced volumes of the left nucleus accumbens, putamen, thalamus, and bilateral caudate nuclei, whereas MobFD patients compared with FixFD demonstrated atrophy of the right hippocampus and globus pallidus. Compared with both controls and MobFD cases, FixFD patients showed a severe disruption of WM architecture along the corpus callous, corticospinal tract, anterior thalamic radiations, and major long-range tracts bilaterally.
Conclusion: This study showed different MRI patterns in two variants of FD. MobFD had alterations in GM structures crucial for sensorimotor processing, emotional, and cognitive control. On the other hand, FixFD patients were characterized by a global WM disconnection affecting main sensorimotor and emotional control circuits. These findings may have important implications in understanding the neural substrates underlying different phenotypic FD expression levels.

Glucocorticoid receptor phosphorylation in stress-related disorders

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Dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis has long been associated with stress-related psychiatric disorders. For instance, a significant percentage of depressed patients exhibit increased blood cortisol levels, and this effect is higher in those with comorbid anxiety. Effects of cortisol are mediated through the glucocorticoid receptor (GR), including the feedback regulation of the HPA axis. The activity of the GR can be modulated by numerous cellular factors, which act either to stimulate or inhibit the GR-mediated transcription of specific genes. Specific kinases which phosphorylate the GR alter the receptor conformation and thereby change its transcriptional activity. There are several amino-acid residues on the human GR that can be phosphorylated, with serines 211 and 226 receiving the most attention. Phosphorylation of human GR at serine 226 was found to blunt hormone signaling by enhancing nuclear export of the GR, while GR phosphorylation at serine 211 enhances GR transcriptional activity. Here, we present current findings concerning the status of GR phosphorylation in persons with stress-related disorders and we discuss the possibility of targeting the GR signaling pathway in these patients.

2nd Symposium in Biomedicine: Basic and Clinical Neuroscience

2. Simpozijum iz biomedicine: bazična i klinička Neuronauka

POSTER SESSION

POSTER SESIJA

P 01
Tianeptine: the proteomic insight in molecular effects of its prolonged use as a nootropic

Ivana Perić, Andrijana Stanisavljević, Dragana Filipović
Vinča Institute of Nuclear Sciences, University of Belgrade, Mike Petrovića Alasa 12-14, 11001 Belgrade, Serbia

Tianeptine (Tian), an atypical antidepressant and anxiolytic, has recently found use as a nootropic too, given to its acute effect of improving cognitive performances, such as alertness, concentration mental clarity and informational processing. However, there are evidence of addiction and abuse of Tian, whereby data about the molecular effects of its prolonged use are scarce. Because the hippocampus acts as a cognitive function center, while cognitive performances are enhanced by Tian treatment, we sought to identify ongoing molecular changes in the hippocampus. Hence, we performed a comparative proteomic study to investigate the effects of Tian (10 mg/kg/day) treatment of adult male Wistar rats during three weeks on cytosol and non-synaptic mitochondria (NSM) sub-proteomes of the hippocampus. The purity of cell fractions was confirmed with Western blot. For comparative proteomic study we used HPLC-LTQ Orbitrap XL mass spectrometer and Sieve 2.0 software for relative quantification using STRING 11.5, for the identification of modified biological pathway. Bioinformatic analysis of proteomic data showed that Tian, compared to vehicle-treated controls, predominantly acted by increasing the expression of proteins in both examined fractions. According to STRING, Tian affected the proteasome and redox system, vesicle-mediated process and protein transport in cytosolic fraction. In NSM, Tian increased the expression of energy-related proteins, possibly indicating on enhanced energy metabolism. Also, Tian increased the expression of glyceraldehyde-3-phosphate dehydrogenase expression bound to NSM. Our data suggest that proteins from both cytosol as well as NSM fractions are targets of prolonged effect of Tian.

P 02
Layer/subregion-protective effect of olanzapine on parvalbumin and GAD67 cells number of dorsal hippocampus of chronically socially isolated rats

Stanisavljević Andrijana, Ivana Perić, Dragana Filipović
Vinča Institute of Nuclear Sciences, University of Belgrade, Mike Petrovića Alasa 12-14, 11001 Belgrade, Serbia

Chronic social isolation (CSIS) induces anxiety- and depressive-like behaviours in rats, and affects the number of parvalbumin-positive (PV^+) cells in the subregions of dorsal hippocampus. We determined whether an atypical antipsychotic olanzapine (Olz) applied during the last three-weeks of six-weeks of CSIS (7.5 mg/kg/day) may reverse CSIS-induced behavioural changes in the adult, male Wistar rats. Also, we investigated the effects of Olz and/or CSIS on the number of PV^+ cells, as well as, on the GABA-producing enzyme $GAD67^+$ cells in Stratum oriens (SO), Stratum pyramidale (SP), Stratum radiatum (SR) and Lacunosum moleculare (LM) of CA1, CA2, CA3 subregions, as well as, in molecular layer-granular cell layer (ML-GCL) and Hillus (H) DG of dorsal hippocampus. Olz reversed CSIS-induced behavioural changes and antagonized the reduction of PV^+ cells numbers in SP CA1 and $GAD67^+$ in SP/SR/SLM of CA1 but failed to antagonize CSIS-induced decrease of PV^+ cells number in SO CA1, SO/SP CA2, SP CA3, layers of DG, and $GAD67^+$ in SLM CA2/3 and ML-GCL DG induced by CSIS. In controls, Olz decreased the number of PV^+ cells in SO CA2, SO/SP/SR CA3, and $GAD67^+$ in SO CA2/3 and layers in DG. Furthermore, Olz in CSIS rats decreased the number of PV^+ cells in SR of CA3 and $GAD67^+$ in SO of CA3, as comapred to CSIS alone. Our data show that antidepressant- and anxiolytic-like effects of Olz coincided with layer/surbegional modulation of GABAergic system, preventing CSIS-induced decrease of PV^+ $GAD67^+$ cells number in certain layers of CA1 subregion of dorsal hippocampus.

P 03
Novel toxicological approach in irritable bowel syndrome pathophysiology

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Irritable bowel syndrome (IBS) is a well-known functional gastrointestinal disease characterized by frequent or consistent changes in intestinal transit and permeability, pain, bloating, and extreme discomfort, in the absence of clearly distinguishable intestinal tissue or molecular impairments. Previous studies of our group showed that IBS is also characterized by a strong behavioural component including cognitive performance, affective spectrum symptoms, and social impairments, in some IBS rodent animal models. Moreover, it was shown that these aspects could be correlated with gut microbiota changes, inflammation and oxidative stress changes. In this way, the ethiology and pathophysiological mechanisms of IBS are currently not fully understood making IBS a multifactorial, multigenetic, and environmental-correlated disease. However recent studies suggested that IBS could be due to a severe allergic reaction to ingested food which leads to the previously described

inflammatory changes occurring in IBS intestinal linning. Thus, increased mast cells levels in colonic epithelium alongside increased passage of several pathological *Enterobacteriaceae* species, such as *Escherichia coli* and *Salmonella sp.* through mucosa linning has been hypothesized to be a part of the immunologic hypersensibility which triggers the inflammatory response in IBS. Therefore, based on the recent studies, in this poster we aim to present further evidence of this proposed mechanism of action in IBS pathophysiological development.

B.I.M. and C.A. are currently supported by a research grant for Young Teams offered by UEFISCDI Romania, no. PN-III-P1-1.1-TE-2016-1210, contract no. 58 from 02/05/2018, called "Complex study regarding the interactions between oxidative stress, inflammation and neurological manifestations in the pathophysiology of irritable bowel syndrome (animal models and human patients)".

P 04

Expression of ectonucleotidases in rat hippocampus after ovarian hormones deprivation and estradiol replacement

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Purinergic signaling is the main signaling system in brain. ATP acts as a fast excitatory transmitter, while adenosine sets a global inhibitory tone within neuronal networks. ATP and adenosine are interconnected by ectonucleotidase enzymes, which convert ATP to adenosine. Existing data point to the converging roles of ovarian steroids and purinergic signaling in synapse formation, refinement and activity in the hippocampus. Therefore, we examined spatial distribution and expression of ecto-enzymes NTPDase1, NTPDase2, and ecto-5'-nucleotidase (eN) after ovariectomy (OVX) and estradiol replacement (E2) in female rat hippocampus. The target ectonucleotidases are predominantly localized in synapse-rich hippocampal layers. The most represented NTPDase in the hippocampal tissue is NTPDase2, being at the same time the mostly affected ectonucleotidase by OVX and E2. Specifically, OVX decreases the expression of NTPDase2 and eN, whereas E2 restores their expression to control level. Impact of OVX and E2 on ectonucleotidase expression was also examined in purified synaptosome (SYN) and gliosome (GLIO) fractions. SYN expresses NTPDase1 and NTPDase2, both reduced following OVX and restored with E2. GLIO exhibits NTPDase2-mediated ATP hydrolysis, which falls in OVX, and recovers by E2. These changes in the activity occur without parallel changes in NTPDase2 protein abundance. The same holds for eN. The lack of correlation between NTPDase2 and eN activities and their protein abundances suggest a non-genomic mode of E2 action, which is studied further in primary astrocyte culture. Since ovarian steroids shape hippocampal synaptic networks and regulate ectonucleotidase activities, cognitive deficits seen after ovariectomy may arise from the loss of E2 modulatory actions on ectonucleotidases in the hippocampus.
Supported by the Ministry of Education, Science and Technological Development, Republic of Serbia, grants 41014 and 173044.

P 05

Early administration of valproic acid disturbs the sociability of zebrafish (*Danio rerio*)

Madalina-Andreea ROBEA[1], Alin CIOBICA[2,3,4], Gabriel PLAVAN[2], Stefan-Adrian STRUNGARU[2], Mircea NICOARA[1], Karl Ægir KARLSSON[5]
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Aims. Decreased sociability is one of the main symptoms of autism spectrum disorder (ASD). The high impact of drugs on embryonic development leads to an abnormal morphology and functioning of central nervous system (CNS). Valproic acid (VPA) is an antiepileptic drug used to treat epilepsy, and has been linked with ASD in new born children. We used zebrafish as an animal model to observe the impact of VPA on sociability status after administration of the compound in the early hours of life.
Methods. In this study was conducted an experiment using the zebrafish to understand and demonstrate the damaging effects of this drug on social interaction. Valproic acid was administrated during the first 24, 48 and 72 hours using a dose of 48 µM. A control group simulated the administration of VPA. Each fish was tested for social behavior in an experimental tank. The video images were recorded and analysed using EthoVision XT 7 (NOLDUS, Netherlands). The experimental tank was divided in three sub-areas: one for the group, one for the experimental fish and the last one was empty. The velocity and time spent in the sub-areas was measured. Each trial had 20 minutes duration.

Results. After the administration of VPA, the experimental fish showed impairements in locomotor activity and in social interaction. In comparison to the fish from control group, the fish from the group with the longest exposure had the most impaired social behavior (VPA administration for 72 hours). In fact, the sociability started to decrease as the administration period increased.
Conclusions. Our findings validate and extend prior work. Valproic acid can be a potential inductor for ASD symptoms in a zebrafish animal model.

P 06
Anatomical and cellular distribution of ecto-nucleoside triphosphate diphosphohydrolase 2 in rat brain

Milorad Dragić[1,2], Marija Adžić[1], Nataša Mitrović[2], Ivana Grković[2], Nadežda Nedeljković[1]
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Extracellular ATP and adenosine modulate various processes in the central nervous system, including neurotransmission, gliotransmission, synapse activity and blood flow. Their extracellular levels are tightly controlled by ectonucleotidase enzymes, which sequentially hydrolyze ATP to adenosine. ATP and ADP are principally degraded by ectonucleoside triphosphate diphosphohydrolases 1 and 2 (NTPDase1 and 2), which exhibit different preferences and affinity towards the nucleotides. Namely, NTPDase1 degrades ATP and ADP equally well to AMP, while NTPDase2 preferentially catalyzes ATP, with accumulation of ADP. NTPDase1 has wide distribution in the brain and dominant microglial localization, while regional and cellular distribution of NTPDase2 in CNS is largely unknown. Present immunohistochemical study shows somatic localization of NTPDase2 at $MAP2^+$ and $calbindin^+$ neurons in the thalamus, Purkinje cell layer, deep cerebellar nuclei, in the brainstem nuclei, in the caudoputamen and sensorimotor cortex and tectum of midbrain. Synaptic localization of NTPDase2 is observed in the hippocampus and caudoputamen. Besides, NTPDase2 is localized at $GFAP^+$ and VIM^+ fibrous astrocytes in the white matter throughout of brain. The regional and cellular pattern of NTPDase 2 expression suggests its dominant localization in the motor areas, underpinning its possible role motor functions.

P 07
The effect of short-term fasting on the insulin signaling in the rat hypothalamus

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The aim: Our previous study[1] showed that short-term fasting increased insulin expression in hypothalamus. The aim of the follow up study was to investigate the effect of short-term food deprivation on the insulin signaling pathways in the same brain region. Therefore, we analyzed expression of IRS1, IRS2, PI3K, AKT, ERK and theirs phosphorylated forms in the rat hypothalamus.
Methods: Two months old male Wistar rats were exposed to six-hour fasting beginning at 6 pm. Controls (C) had free access to food and were sacrificed simultaneously with the fasting counterparts. Western blot was used to determine the levels of the examined proteins in both the cytosolic fraction and total protein isolate.
Results: The amounts of IRS1, IRS2, and their phosphorylated forms (pIRS1-Tyr612, pIRS2-Ser731) were not altered after six-hour fasting, nor was that of PI3K-p85. Fasting did not change the levels of AKT1/2/3 and phospho-AKT1/2/3-Ser473 either. ERK1/2 and phospho-ERK1/2-Thr202/Tyr204 levels were also not significantly different between control and experimental rats when measured in the total protein isolate. However, fasting upregulated the levels of phosho-ERK1/2 in the cytosolic protein fraction.
Conclusion: Although hypothalamic insulin content was increased after short-term fasting, no differences in PI3K/AKT signaling pathway activation were observed. However, results indicate that locally produced insulin may potentially be involved in the activation of MAPK signaling pathway.

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P 08

Short term fish oil treatment alters fatty acid composition and cholesterol-related gene expression affecting the visual cycle in mouse retina and RPE

Irena Jovanovic Macura[1], Ivana Djuricic[2], Marjana Brkic[3], Desanka Milanovic[1], Sladjana Sobajic[2], Selma Kanazir[1] and Sanja Ivkovic[1]
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Age-related macular degeneration (AMD) is a progressive and degenerative disease of the retina and major cause of blindness among the elderly population, whose etiology is not known. Changes in cholesterol metabolism and in the uptake of unsaturated fatty acids (DHA) in the retina and retinal pigmented epithelium (RPE) have been implicated in the pathogenesis of AMD. Because the continuous renewal of retinal membranes requires a constant supply of omega-3 fatty acids by RPE cells, diets rich in DHA may improve retinal function and may delay the development of exudative AMD. Thus, we hypothesized that short-term DHA supplementation (3 weeks), may serve as a prophylaxis in AMD prevention. We used real-time PCR to quantify the expression levels of genes involved in retinal cholesterol metabolism and fatty acid uptake in retina and RPE in control and FO treated animals (4 months-old). We analyzed the retinal expression pattern of genes regulating biosynthesis (*hmgcr*, *lxrβ*, *srebp-2*), transport (*abca1*, *apoE*) and elimination (*cyp27*, *cyp46*) of cholesterol and its metabolites and of two different DHA transporters - *adipoR1* and *mfsd2A*, necessary for the photoreceptor proper function. As a functional outcome we analyzed the expression profile of visual cycle genes in RPE. Our results showed that FO supplementation elicited significant changes in the phospholipid composition and transcriptional networks of the cholesterol-mediated and DHA transporter genes. Finally, the FO supplementation decreased the expression of the key regulatory genes of the visual cycle in RPE.

P 09

Fructose-rich diet and walnuts supplementation differentially regulates hypothalamic and hippocampal glucose transporters expression

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Introduction: Metabolic syndrome (MS) is a major health risk challenge. Previous studies have shown that fructose-rich diet can induce MS whereas a nut-rich diet may reduce the risk of developing MS. The aim of this study was to examine the effects of fructose-rich diet and walnuts supplementation on the glucose transporters (GLUT) expression in rat hypothalamus and hippocampus.
Methods: Male Wistar rats were divided into four groups: (C) - standard chow diet and drinking water; (F) - standard chow and drinking water supplemented with fructose (10%) during 15 weeks; (CW) - standard diet supplemented with 2,4 g of walnuts during the last 6 weeks of the experiment; (FW) - standard chow and drinking water supplemented with fructose (10%) during 15 weeks whereas in the last 6 weeks standard diet was supplemented with walnuts. Hypothalamic and hippocampal membrane protein fraction was isolated using the subcellular protein fractionation kit. The amount of GLUT1, 2 and 3 was measured by Western blot method.
Results: In the rat hypothalamus, all treatments increased GLUT1 and GLUT2 protein levels, whereas GLUT3 was increased only in CW and F groups. In the rat hippocampus, GLUT1 content was increased only in FW group, whereas GLUT3 level was decreased in the same group. Decreased amount of membrane GLUT2 was detected in hippocampal tissue of all experimental groups.
Conclusion: Fructose-rich diet and walnuts supplementation increased membrane GLUT1 content in rat hypothalamus and hippocampus. In contrast, GLUT2 and GLUT3 membrane content in examined brain regions was differently regulated under applied dietary regimes.

P 10

Sex differences in the regulatory changes of HPG axis during experimental autoimmune encephalomyelitis in rats

2ND PRIZE

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Multiple sclerosis (MS) is a chronic inflammatory neurodegenerative disease, two to three times more common in women than in men. Because the effects of neuroinflammation on the reproductive status have not been fully explored, our aim was to investigate the impact of experimental autoimmune encephalomyelitis (EAE), the rat model of MS, on hypothalamo-pituitary-gonadal axis.

EAE was actively induced in Dark-Agouti rats of both sexes. The animals were examined daily for disease symptoms, weight changes, and estrous cycle phase. The animals were sacrificed at the onset, peak, and end of the disease. Hypothalamic and pituitary tissues were dissected for qRT-PCR analyses. Blood was collected for LH measurements. In separate experiments, groups of male and female animals at the peak of EAE and naïve controls received a subcutaneous injection of buserelin acetate, a potent synthetic GnRH analogue.
The obtained data implied that hypothalamic neuroinflammation occurs during onset and/or peak of the disease in both sexes (upregulation of *Gfap*, *Il1b*, *Il6*, *Ccl2* and *Spp1* mRNA expression). However, hypothalamic *Kiss1* and *Gnrh* mRNA expression was affected differently in males and females, as well as mRNA expression of pituitary signature genes - *Lhb*, *Fshb* and *Gnrhr*. LH levels drop transiently following the course of the disease; in females, this drop coincided with the arrest in diestrus. Nevertheless, the pituitary remained responsive to buserelin treatment.
Our results indicate that EAE affects the regulation of hypothalamo-pituitary-gonadal axis in both sexes. Further analyses are needed to elucidate the causes and details of differences in hypothalamic response to neuroinflammation.

P 11
Effects of a high-fat diet on expression of the hypothalamic NADPH oxidases

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Background and Aims - Obesity is an increasing epidemic worldwide; therefore, numerous clinical and basic studies focus on the potential mechanisms underlying this complex disorder. High-fat (HF) diet-induced obesity is associated with mild inflammation in peripheral tissues. NADPH oxidases (NOX) are recognized as a physiological source of reactive oxygen species (ROS) contributing to the inflammation. Previous studies proposed that similar events may also occur in the hypothalamus, a cerebral area responsible for the regulation of energy homeostasis.
Purpose of this study was to examine the effects of a HF diet on expression of the hypothalamic NADPH oxidases. We also examined expression of transcription factor HIF-1alpha (HIF-1α) owing to its potential involvement in the upregulation of the NOX system.
Methods - Male rats were placed on HF diet starting at 9 weeks of age and lasting for 12 weeks. Expression of NOX 2/gp91phox, NOX 4, p22phox subunit, and HIF-1α in the hypothalamus was evaluated by Western blot analysis.
Results - The HF diet significantly increased the expression of NOX 4 and its p22phox subunit in the hypothalamus. No significant changes in the levels of NOX 2 and HIF-1α were observed.
Conclusions – The present study suggests that NOX 4 but not NOX 2, might be involved in the onset of the hypothalamic inflammation underlying the HF diet-induced obesity. The involvement of HIF-1α needs to be further investigated.

P 12
Can sneezing leave you paralized? A case report of spinal fracture and flaccid paraplegia after sneezing

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Introduction: Sneezing is a universal protective reflex that can be caused by a number of stimuli, irritans or chronic imphlamatory deseases. The closed - airway sneeze results from a voluntary effort to repress the sneeze and restrict the audible rush of air through the nasal cavities. Active occlusion of oral cavity and nasopharynx results in airway pressures up to 20 times the pressure in a normal sneeze. The transference of this high pressure to other parts of the respiratory tract may result potential variety of injuries.
Methods: Retrospective analysis of medical history documentation.
Case report: We reported a case of 67-year old male patient with past medical history of prostate cancer, lower back pain and lower extremities paraparesis, with a suden onsent of flaccide paraplegia, as well as impairment of previous lower back pain. Three days before this event, the patient had violent episode of sneezing while simultaneously obstruction both nostrils. After performing clinical and neurological examination, magnetic resonace and electromyoneurography we found that onset of flaccid paraplegia was most likely caused with pathological fracture of thoracic and lumbal spine which was prevopously weakened by metastatic implantation.
Conclusion: Sneeze injuries are rare clinical entity but can be life treating, especially when a closed – airway sneeze is attempted. To our knowlage, this is the first reported case of spine fracture and flaccid paraplegia triggered by sneezing.
Key words: sneezing, paraplegia, pathologic fractures

P 13

Acute restraint stress promotes anxiety-like behaviour and changes excitability of the central nervous system

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Introduction: Response to stress exposure has been identified as one of the risk factors for developing anxiety-like and depressive behaviour. While majority of studies indicate anxiogenic effect of acute stress, others imply its vital role in evolutionary adaptation. Possible mechanisms underlying the susceptibility to stress-provoked anxiety are inflammation, hippocampal neurogenesis, changes in gene and protein expression. Moreover, some researches imply protective effect of acute stress on animal seizure models by activating endogenous opioids and NO. Therefore, the aim of our study was to determine the influence of acute restraint stress (ARS) on anxiety-like behaviour and behavioural characteristics of lindane-induced seizures.
Methods: Adult male Wistar rats have been divided into control (C) and ARS group. Rats were stressed in restraining device for 1h prior to behavioural testing. After 20 min of rest, locomotor and exploratory activity have been registered in automated Open Field chamber. Independent measures included total ambulation distance and time, centre ambulation distance and time, time in the centre, and number of rearings. Afterwards, convulsions were induced by i.p. administration of lindane and seizure behaviour was evaluated. Seizure behaviour was assessed by incidence, seizure latency and the seizure severity.
Results: ARS significantly decreased total ambulation distance and time ($p<0.05$), and time in the centre ($p<0.05$) while the number of rearings was not significantly different between the groups. Furthermore, latency to seizures and duration of ictal periods was significantly different in ARS compared to the C group.
Conclusion: Our results strongly indicate that ARS protocol induces anxiety-like behaviour and changes the excitability of the CNS in male rats.
Key words: Stress, Anxiety, Epilepsy, Lindane, Seizure,

P 14

Effects of lithium on noradrenergic turnover in the prefrontal cortex of chronically stressed rats

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Chronic stress can provoke depressive-like behaviors in rats. In pathophysiology of mood disorders lithium is known as an effective drug in the long-term stabilization of moods. It is known that depressive disorder is caused by insufficient signaling by monoamines, particularly noradrenaline (NA). Because of the significant role of NA in regulation of numerous brain functions in stress conditions, monitoring the changes of noradrenergic turnover in the prefrontal cortex (PFC) in chronically stressed rats treated with lithium may be very important in research of lithium role in reduction of functional deficiency of NA in pathological conditions. Therefore, in this study we examined: levels of enzymes involved in NA reuptake (noradrenaline transporter-NET), storage (vesicular monoamine transporters-VMAT2) and degradation (monoamine oxidase-MAO and catechol-O-methyltransferase-COMT), as well as concentrations of NA in the PFC of chronically stressed rats treated with lithium. An additional aim of the study was to test the behavior of chronically stressed rats treated with lithium. The investigated parameters were quantified by Western blot analyses and assay of enzyme activitie. We found that lithium treatment decreased high protein levels of NET and VMAT2, as well as the enzyme activity of MAO A in chronically stressed rats to the level found in unstressed animals. In addition, lithium treatment decreased concentration of NA and immobility of animals with depressive-like behavior. In conclusion, modulation of noradrenergic turnover in the prefrontal cortex of chronically stressed rats by lithium reduced functional deficiency of NA and stabilized behavior in animals with depressive-like behavior.
Acknowledgments: This work was supported by the Ministry of Education and Science of the Republic of Serbia, Contract No.III 41027.

P 15

In silico reconstruction of human dopamine transporter and design of novel neuroprotective drugs for Parkinson's disease

1ST PRIZE

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Abnormally folded alpha-synuclein protein, dysfunctional mitochondria, increased oxidative stress and reduced dopamine neurotransmitter synthesis are a
ll extremely well characterized phenomena in Parkinson's disease (PD) and are thought to be interconnected. While direct targeting of these areas has demonstrated neuroprotection *in vitro* and *in vivo*, there has been a major lack of success in clinical trials. A critical component in the failure of these clinical trials is the inability to specifically target drugs to dopamine producing neurons in the brain.
New drugs targeting the dopaminergic neurons by specific uptake through the human dopamine transporter (hDAT) could represent a viable strategy for establishing selective neuroprotection. Molecules able to increase the bioactive amount of extracellular dopamine, thereby enhancing and compensating a loss of dopaminergic neurotransmission, and to exert neuroprotective response because of their accumulation in the cytoplasm, are required.
By means of homology modeling, molecular docking and molecular dynamics simulations, we have generated 3D structure models of hDAT in complex with substrate and inhibitors. Our results clearly reveal differences in binding kinetics of these compounds to the hDAT in the open and closed conformations, critical for future drug design. The established *in silico* approach allowed the identification of three promising substrate compounds that were subsequently analyzed for their efficiency in inhibiting hDAT-dependent fluorescent substrate uptake, through *in vitro* live cell imaging experiments. Taken together, our work presents the first implementation of a combined *in silico*/*in vitro*-approach enabling the selection of promising dopaminergic neuron specific substrates.

P 16

Identification of potential dual histamine H_3 receptor antagonist and serotonin reuptake inhibitors through ligand-based and structure-based approaches

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The major depressive disorder (MDD), routinely treated with selective serotonin reuptake inhibitors (SSRIs), is the second leading cause of disability worldwide. However, the treatment of MDD is complicated by high prevalence of residual symptoms connected to increased risk of relapse. Some of the most common residual symptoms are cognitive dysfunction and fatigue. Histamine H_3 receptor (H_3R) antagonists are both, pro-cognitive and wake-promoting agents. In pre-clinical study it was suggested that dual histamine H_3R antagonist and SSRI may have utility as a more efficient antidepressant therapy. The aim of this *in silico* study was identification of novel dual SSRI/H_3R antagonist using ligand-based and structure-based drug design techniques. Starting from structures and activities of known dual ligands, two GRIND-based 3D-QSAR models have been developed, SERT model (R^2 = 0.97; Q^2 = 0.79; SDEP= 0.124) and H_3R model (R^2 = 0.86; Q^2 = 0.75; SDEP= 0.184), and 3D-pharmacophores were constructed. Further, homology model of H_3R was built and refined with molecular dynamics. The hypotheses of binding modes for dual ligands were generated with molecular docking on H_3R model and X-ray structure of SERT. In the second part of this study, ligand-based and structure-based virtual screening models were generated and validated. Prospective screening of ZINC database was performed in order to extract novel chemotypes of dual ligands. Final selection of ligands was performed based on generated pharmacophore and docking models as well as predicted pharmacokinetic properties. Few novel compounds were emphasized as promising starting point for development of new classes of dual antidepressants.

P 17

Structure and ligand based drug design strategies in the development of novel serotonin 5-HTt$_{2a}$ receptor antagonists

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The serotonin 5-HT_{2A} receptors are widely distributed throughout the central and the peripheral nervous system where they play a key role in many physiological functions. Abnormal activity of 5-HT_{2A} receptors is associated with various neurological disorders, such as depression, schizophrenia, anxiety, and Parkinson disease. In order to analyze 3D-structure of the

pharmacophore as well as binding kinetics of 5-HT_{2A}-R antagonists, we have combined ligand and structure based approaches. Three-dimensional quantitative structure-activity relationship (3D-QSAR) study in combination with molecular docking and molecular dynamic (MD) simulation was used to identify key substituents responsible for high binding affinity and selectivity of 5-HT_{2A} antagonists. The study was performed on wide range of structurally diverse antagonists that were divided into three different clusters: clozapine, ziprasidone, and CHEMBL240876 derivates. We have obtained three different inactive, antagonist-bound, conformations of this receptor by using the 50ns long MD simulations with each cluster representative. Subsequently, these conformations were used as templates for docking studies in order to find virtually bioactive conformations of ligands. Selected virtually bioactive conformations were used for calculation of specific molecular descriptors (Grid Independent Descriptors- GRIND) and 3D-QSAR model building. The 3D-QSAR approach was used to select the most influential variables which were used for clarifying the structural features required for 5-HT_{2A} antagonists. The reliability and predictive power of the model was assessed using an external test set compounds and showed reasonable external predictability. The study provides valuable information about the key structural features that are required in the rational drug design of novel 5-HT_{2A} antagonists.

P 18
Treatment of pediatric multiple sclerosis

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Multiple sclerosis is a chronic, autoimmune, inflammatory, demyelinating disease of the central nervous system. The exact etiology of MS is still not clarified, although it is known that autoimmune, genetic and environmental factors play an important role in the development of MS, making it multifactorial disease. The disease most often begins between second and fourth decade of life, but it can also begin in the childhood. Multifocal inflammatory changes and damage to myelin are the key pathological features of MS. Clinical events that characterize MS are relapse and progression. Therapy of pediatric MS involves treatment of relapses, immunomodulatory therapy and symptomatic treatment. The disease-modifying drugs (DMDs), which are base for MS therapy, are able to modify the course of the disease i.e. immunomodulatory drugs. They are divided it to two categories: First - line and second – line immunomodulatory therapy. First line immunomodulatory therapy (Interferon beta-1a, Interferon beta-1b and Glatiramer acetate) have shown significant therapeutic effectiveness by reducing the frequency of clinical relapses and further progression of the disease. The disadvantage is that they are only allowed in children older than 12 years. Second-line immunomodulatory therapy (Natalizumab, Mitoxantrone, Fingolimod, Teriflunomide, Azathioprine, Cyclophosphamide, Rituximab, Dimethyl fumarate) are allowed in pediatric population only as a part of ongoing clinical studies. Although those medications reduce the number of relapses by 68% (First-line IMT only by 30%), they have many serious side-effects. Treatment of relapses/exacerbations also involves the use of intravenous doses of corticosteroids which are still gold standard for relapsing- remitting MS.

P 19
Quantification of antidepressants and antipsychotics exposure increase in CYP 2C19/CYP 2D6 slow metabolizers: Systematic review and meta-analysis

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In a recent period, development of new antidepressants and antipsychotics entered a quiescent phase, so clinicians can rely only on handful of effective drugs in the treatment of psychiatric disorders. At very best, half of the psychiatric patients respond to the first administered psychotropic drug and treatment-resistant patients are also numerous. Therefore, it is of paramount importance to utilize current therapeutic options as effectively as possible. Administering appropriate dose is very important since underdosed patients will not respond to treatment, while overdose can cause serious adverse drug reactions. In order to determine the most appropriate dose, the clinicians need an accurate estimate of drug metabolism and exposure. Most of the currently used antidepressants and antipsychotics are metabolized via polymorphic CYP 2C19 and CYP 2D6 enzymes. Metabolic capacity of both enzymes is genotype-determined. Poor metabolizer status (PM) defined as complete absence of enzymatic activity and intermediate metabolizer status (IM) defined as greatly reduced enzymatic activity, can both cause a significant increase in drug exposure. When making genotype-governed dose adjustments, clinicians need to know the magnitude of exposure increase in PM and IM. However, due to small sample size of the previous studies, the exposure increase magnitude still cannot be estimated with sufficient precision. Therefore, the aim of this meta-analysis is to pool all these studies and estimate to the best possible degree the magnitude of drug exposure increase caused by the CYP 2C19 and CYP 2D6 PM and IM status compared with normal metabolizers (NM).

P 20

Hyperdopaminergism-induced excitotoxicity in transgenic mice, carriers of the human CYP2C19 gene

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Introduction: Dopaminergic neurotransmission is responsible for many physiological functions of the brain. The degeneration of dopaminergic neurons in the nigrostriral pathway leads to the characteristic hypokinetic condition called parkinsonism. Transgenic mice, carriers of the human CYP2C19 gene, exhibit motor deficits and elevated dopamine concentrations in the brain.
The Aim: To examine the consequences of the increased dopaminergic neurotransmission of CYP2C19Tg mice on the number of dopaminergic neurons.
Materials and Methods: After immunohistochemical processing of the tissues of five CYP2C19Tg and five control mice, selectively stained dopaminergic neurons were examined by Olympus BX50 microscope and their total numbers were counted in the substantia nigra (SN) and ventral tegemental area (VTA).
Results: The number of dopaminergic neurons in SN was decreased by 13.5% and in VTA by 12% in the transgenic mice compared to control mice.
Conclusion: There are indications that an increase of dopaminergic neurotransmission of CYP2C19Tg mice causes a reduction in the number of dopaminergic neurons, likely by overactivation induced excitotoxicity.

P 21

D1 dopamine receptor antagonist reverses motor phenotype in transgenic mice, carriers of the human CYP2C19 gene

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Inroduction: The motor system dysfunction in the brain may lead to motor disorders. A transgenic mouse, carrier of the human CYP2C19 gene (CYP2C19Tg), exhibits an altered motor function with signs of hyperkinesia. Furthermore, CYP2C19Tg mice exhibit increased dopamine brain concentration, as compared to controls.
The Aim: The aim of this study was to investigate whether hyperkinesia in CYP2C19Tg mice has an impact on their motor coordination and motor task performance. It was also investigated whether modulation of dopaminergic neurotransmission affects the motor phenotype and motoric skills of these mice.
Material and Methods: In this experiment, 11 CYP2C19Tg and 12 control mice were used. Motor skills were assessed by using: (1) Rotarod test, by measuring time spent on the apparatus and (2) Beam walking test, by measuring time needed for crossing the narrow beam. Tests were performed in untreated mice, as well as after administering ecopipam (selective D1 antagonist) and raclopride (selective D2 antagonist).
Results: Decrease of the time CYP2C19Tg mice spent on the rotarod compared with control mice did not reach statistical significance. The CYP2C19Tg mice moved slower on the beam compared with control mice. Ecopipam reversed the time CYP2C19Tg mouse needed to cross the beam to the values of the control mice.
Conclusion: Hyperkinesia in CYP2C19Tg mice reduces their performance in the Beam walking test and ecopipam was able to reverse this phenotype.

P 22

Changes in the level of Brain-derived neurotrophic factor (BDNF) in the model of chronic stress induced by long term corticosterone treatment

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Introduction: It is well known that chronic stress has negative influence on adult neurogenesis, which consequently may lead to atrophy of hippocampus and prefrontal cortex. Influence of neurotrophic factors (neurotrophins), particularly of brain-derived neurotrophic factor (BDNF), is essential for neuronal function and growth. Corticosterone is the main glucocorticoid in rodents and it is released during stress exposure, due to increased hypothalamic-pituitary-adrenal axis activity.
Objective: Aim of this experiment was to determine the influence of long-term corticoserone treatment on BNDF protein amount in hippocampal and prefrontal cortex tissue of experimental animals.

Materials and methods: Male Wistar rats, 8 weeks old, were randomly divided into two experimental groups (6 animals in each group). First group (CORT group) was treated with corticosterone per os (100 mg/L, 21 day) . Second experimental group (control group) was treated with solvent (2% Tween® 80) per os, for 21 day. After isolation of brain tissue, amount of BDNF protein in hippocampus and prefrontal cortex is determined using Western blot method. The results were analyzed by Student t-test.
Results: Obtained results point out to negative effect of corticosterone treatment on BDNF protein amount in the brain tissue of experimental animals. Precisely, three-week corticosterone treatment significantly decreased amount of BDNF protein in hippocampus ($p= 0.002$) and prefrontal cortex ($p= 0.011$) of experimental animals, compared to control group.
Conclusion: In chronic stress model induced by three-week-long corticosterone treatment, reduction in the level of BDNF protein in both, hippocampal and prefrontal cortex tissue was noticed. This reduction could consequently underlay inadequate support of normal growth and development of neurons in these brain structures. Results of this study suggest that reduction of BDNF level could be one of the mechanisms underlying effects of chronic stress on atrophy of hippocampus and prefrontal cortex.

P 23

Changes in the level of Brain-derived neurotrophic factor (BDNF) in the model of chronic stress induced by long term adrenocorticotropic hormone treatment

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Introduction: The physiological stress response, mediated by increased activity of HPA axis and high concentration of glucocorticoids, has an essential role in the survival. Prolonged exposure to stress has negative impact on cell proliferation and differentiation in adult hippocampus, and reduces number of neurons in hippocampus as well as in prefrontal cortex. Brain-derived neurotrophic factor (BDNF) substantially affects neuronal survival and differentiation.
Aim: To examine BDNF expression in rat prefrontal cortex and hippocampus after chronic administration of ACTH.
Material and Methods: Adult male Wistar rats were 8 weeks old at the beginning of the experiment. Animals were divided into two groups; after 7 day-long habituation, rats were treated subcutaneously with saline solution (400µl/day) or ACTH (10mg/400µl/day), for 21 days. BDNF expression in rat hippocampus and prefrontal cortex was analyzed using Western blot. After homogenization, tissue samples were prepared for electrophoresis protein separation (SDS-PAGE). This was followed by protein transport from gel to membrane and incubation with primary anti-BDNF antibody and secondary antibody with HRP enzyme, while luminescence quantification was assessed using Fujifilm intelligent dark-box II.
Results: We showed that chronic ACTH treatment in rats resulted in lower BDNF expression in the hippocampus ($t(6) =3.008$; $p=0.024$), relative to control group, while difference in prefrontal cortex was not significant ($t(6) =1.727$; $p=0.135$).
Conclusion: The present results are in accordance with a number of studies focused on stress modeling, that underline significance of BDNF level change as a potential mechanism involved with atrophy of certain brain structures, but also underline the possibility of modulation of BDNF expression in therapy.

REGISTRATION

1000 RSD payment at Faculty account **840-1127666-05**; reference number **45441** (**500 RSD** for PhD students). On-site registration: **1500 RSD** Students and poster presenters: free admission

REGISTRACIJA

Uplatom na račun fakulteta **840-1127666-05** sa pozivom na broj **45441** u iznosu od **1000 RSD**, (**500 RSD** za doktorande). Uplatom na licu mesta: **1500 RSD.** Studenti i izlagači postera ne plaćaju registraciju.

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