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ABSTRACT BOOK

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CONTENTS

PLENARY LECTURES

PL. 1 (Inaugural lecture)
Personalized therapies for CNS diseases: needs and approaches

Kalpana Merchant, PhD, *TransThera Consulting Co., Portland, OR, USA*

CNS diseases, both psychiatric and neurological disorders, represent significant unmet medical needs and associated societal burden. The age-associated chronic, progressive, neurodegenerative disorders are of particular concern in light of the expected exponential increase in the worldwide aging population. From a drug discovery and development perspective, CNS disorders have been one of the most challenging areas, with perhaps the highest rate of clinical failure. The primary factor underlying the failures has been the lack of meaningful efficacy in patients. This is despite the fact that advances in genomic and drug discovery technologies have identified many novel molecular targets as well as potent and safe drug-like clinical candidates.

This talk will highlight major reasons associated with the attrition rate of CNS drugs and approaches to address these issues. Specifically, the focus will be on translational approaches that could result in dissecting syndromic CNS disorders into molecularly-defined disease states in individual patient populations. An important first step if to reform the current practice of diagnosing CNS diseases, which relies largely on blunt clinical instruments that are frequently based on patient-reported symptoms and clinical outcomes. These classifications fail to take into account the tremendous heterogeneity in the etiology and pathophysiology underlying a disorder as well as allostatic molecular changes that are known to occur within a patient over time. On the bright side, advances in genomic, imaging and other analytical "omics" technology platforms offer an unprecedented opportunity to derive insights into molecular mechanisms associated with CNS disorders. These insights could enable, on the one hand, re-classification of CNS diseases on the basis of objective biomarkers, and on the other hand, provide targets for therapeutic interventions that target pathophysiologic mechanisms in individual patient populations; i.e., personalized therapies.

The power of this approach is evident in the recent emergence of transformative therapies in the oncology area. Although CNS diseases have their unique set of challenges, specific examples from published literature will be discussed to demonstrate that it is possible to develop personalized therapies for CNS diseases.

PL. 2
Hereditary breast and ovarian cancers

Jan Lubinski, *International Hereditary Cancer Center, Pomeranian Medical University, Szczecin, Poland*

Hereditary breast and ovarian cancers are a group of syndromes that involve high predisposition to above cancers and an autosomal dominant with high penetrance pattern of inheritance.

<u>Genetic factors and risk assessment</u>
Breast and ovarian cancer among carriers of BRCA1 / BRCA2 mutations
Among BRCA1 carrier the average cumulative risk of breast cancer by 80 yrs of age is 70% and of ovarian cancer 45%. Among BRCA2 carriers these average cumulative risks are 70% and 10% respectively.
After a first breast cancer, BRCA1 and BRCA2 carriers have also a substantial risk of contralateral breast cancer. 70% of breast cancers that develop in BRCA1 carriers are "triple negative". BRCA2 carriers are "triple negative" in 15% only. Serous ovarian cancers predominate in both types of carriers.
Risk of breast or ovarian cancer is higher among carriers with a positive family history, however for ~40% of carriers breast/ovarian cancer family history is negative although the risk of these cancers is substantially increased.
By 70 yrs of age, the cumulative risk of breast cancer is ~7% among men with BRCA2 mutations.
Hereditary breast and ovarian cancer due to genes other than BRCA1 and BRCA2
High (>20-25%) lifetime risk of breast cancers has been reported also in carriers of mutations in such genes like PALB2, CHEK2, PTEN, TP53, STK11, CDH1 and, recently, RECQL.
Hereditary breast and ovarian cancer without an identified genetic cause
At present, majority of persons with a suspected hereditary predisposition to breast and ovarian cancer have not been found to have a mutation that is pathogenic. For these patients, integrating estimates of cancer risks according to family history with other clinical factors, are recommended.
The most popular tools include BOADICEA (ccge.medschl.cam.ac.uk/boadicea/), BRCAPro and Tyrer-Cuzick model (www.cancertechnology.co.uk/ibis-software-tyrer-cuzick-model).

<u>Screening for mutations</u>
Next generation sequencing (NGS)
NGS analyses are now available which allow BRCA1 and BRCA2 sequencing for 200 USD. With such cost it seems that **NGS of BRCA1/BRCA2 should be considered by all women**, because, actually, it is life-saving procedure.
In addition to BRCA testing NGS panels of other cancer risk genes are also available now. This cost is only slightly higher than NGS of BRCA1/BRCA2
Founder mutations
For several countries / ethnic groups genetic testing is much more cost-effective because of high prevelance of founder mutations. It is the well known effect in Israel, Slavic countries, French Canadians, Iceland, Cyprus and others.

Management in hereditary breast and ovarian cancer syndromes
BRCA1/BRCA2
Surveillance

Screening for breast cancer should include an annual MRI examination because of its high sensitivity (85-90%) in detection of early cancers. Mammography and USG of the breast performed together are showing sensitivity 2 times lower. USG and CA125 are detecting no more than 10% of early ovarian / fallopian tube cancers.

Prevention

Two the strongest procedures to reduce the risk of cancers among BRCA1/2 carriers are mastectomy – protection approaches 100% and adnexectomy - 5-fold protection. Preventive adnexectomy is associated also with ~80% reduction of all-cause mortality. Other procedures include: long breast feeding, delay of menarche, tubal ligation and tamoxifen. A very important is to do not use oral contraceptives (OC) at age under 25 yrs (increased risk of breast cancer diagnosed at age under 40 yrs) but to apply OC at women at age above 35 yrs (not increased risk of breast but lower of ovarian cancer).

Treatment

The 10-year survival rate among women with breast cancer and a BRCA1 mutation is much improved after adnexectomy – the risk of death is decreased by up to 70%. Tamoxifen in breast cancers in carriers is reducing by 50% the risk of contralateral breast cancer even in patients with ER(-), PR(-) tumours.

Breast cancers in carriers are poorly responding to taxanes, but are highly sensitive to cis-platin. Ovarian cancers in carriers which are sensitive to platin, have longer progression free survival (PFS) after applying PARP inhibitor – olaparib.

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PL. 3

Personalizing treatment of the schizophrenia spectrum through endophenotypes, rational pharmacology, and pharmacogenomics

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The atypical antipsychotic drugs, of which clozapine is the prototype, are now widely used to treat not only schizophrenia and schizoaffective disorder, but also bipolar disorder, treatment resistant depression (regardless of the presence of psychosis) behavioral disturbances of dementia, aggression, and suicide. All of the atypical antipsychotic drugs were introduced to treat schizophrenia but became more widely prescribed, leading to registration for some other indications for some of the drugs. However, they are not effective in all patients in these diagnostic categories, nor are they equally effective in all patients, for pharmacodynamic as well as pharmacokinetic reasons. In addition to treating positive, negative and mood symptoms, there is now extensive evidence that these drugs enhance cognitive function in a small but significant proportion of schizophrenia patients. This broad spectrum of action contrasts with the first generation of antipsychotic drugs which mainly target positive symptoms. A variety of receptor mediated effects underlie this broad spectrum of action. This talk will discuss the shared and unique pharmacology of the atypical antipsychotic drugs, and their utility in treating specific domains of psychopathology and cognitive domains. Recent candidate gene and genome wide association studies have shown promise in identifying biomarkers which predict efficacy and tolerability of these agents in specific patients, holding out the promise of precision medicine replacing trial error. The importance of prolonged duration of treatment for patients with treatment resistant schizophrenia will be highlighted.

PL. 4

Structural insights into G protein coupled receptor activation

Brian Kobilka, *Department of Molecular and Cellular Physiology, Stanford University*

G protein coupled receptors (GPCRs) conduct the majority of transmembrane responses to hormones and neurotransmitters, and mediate the senses of sight, smell and taste. Many metabolic processes are regulated by GPCRs, and several GPCRs are targets of therapeutics for metabolic disorders including diabetes and obesity. While GPCRs represent the largest class of pharmaceutical targets for a broad spectrum of diseases, the number of new approved drugs for GPCR targets over the past two decades has fallen short of expectations. We have applied protein crystallography and other biophysical approaches to provide structural insights into GPCR activation. I will discuss what these studies have taught us about GPCR signaling, and the potential impact of structural biology on drug discovery efforts.

PL. 5

How novel should the new drug targets be? Industry perspective

Anton Bespalov, *Partnership for Assessment and Accreditation of Scientific Practice*

There are multiple reasons why the discovery and development of new medicines for neuropsychiatric disorders continues to be a challenge and it may take a while until we understand all of these reasons. However, the declining productivity of drug R&D and the alarmingly high rate of clinical trial failures lead to rapid adjustments of drug R&D investment strategies. This is illustrated by many drug companies reducing their presence in certain therapeutic areas or leaving them completely. Years of research, compounds, targets, models are lost as the result of such strategic changes. There are three key factors, which can be addressed immediately (i.e., as opposed to the need to wait for a deeper understanding of disease biology or for novel clinical trial methodology) and retrospectively (i.e., to evaluate the validity of older targets and models). These factors are the robustness of preclinical data, the generalizability of preclinical efficacy claims, and the translational strategy to confirm target engagement. First, the rate of false positive preclinical data is estimated to be as high as 50-90% (Freedman, L.P.; Cockburn, I.M.; Simcoe, T.S. PLoS Biol 2015, 13, e1002165). Besides the obvious financial implications of this phenomenon, there are also difficulties with interpreting negative results of clinical trials that were initiated based on such false positive preclinical efficacy claims. Second, preclinical efficacy is often observed only under specific laboratory conditions. If efficacy in one mouse strain studied in one lab does not generalize to another mouse strain or another lab, can we expect that it would generalize from mice to humans? Third, for some therapeutic areas such as neuroscience, target engagement is an absolute must-have when planning translation of preclinical data into clinical efficacy (Morgan, P. et al. Drug Discovery Today 2012, 17, 419-424). Similar to discussions in other areas (e.g. Alzheimer's disease), we found, using an example of schizophrenia Phase II studies (1994-2014), that 80% of tested drugs most likely were not tested at doses engaging their CNS targets. Such evidence argues that the perception of clinical trial failures in neuroscience is, in many cases, inaccurate.

All together, these factors heavily contribute to many of the apparent failures in translation, and, when addressed, could lead to a dramatic improvement in the predictive validity of preclinical research, with only modest additional effort required by all stakeholders (academic, government, and industry). Analysis of the impact of these factors challenges the widely held view that the high rate of clinical failures invalidates both the drug targets that have been pursued as well as the preclinical models used to support efficacy claims. Further, there is a need for re-examination of well characterized candidate drugs and pharmacologically defined targets that were previously declared as having failed in clinical proof of concept studies.

PL. 6

Heteroreceptor complexes and their allosteric receptor-receptor interactions as a novel biological principle for integration of communication in the CNS: Targets for drug development in mental and neurological disorders

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The discovery of the central DA, NA and 5-HT neurons and their architecture opened up a new understanding of neuronal communication in the CNS. It led to the introduction of volume transmission taking place in extracellular fluid and CSF with diffusion and flow of soluble biological molecules and extracellular vesicles containing inter alia proteins e.g. receptors and homeoproteins and diverse types of RNA. Integration of synaptic and volume transmission strongly involves receptor-receptor interactions in heteroreceptor complexes in the plasma membrane. They are major modulators of the dynamics of the brain circuits.

The heteroreceptor complexes are novel targets for drug development through increased diversity, bias and specificity of the receptor protomers leading to a novel pharmacology[1]. Adaptor/scaffolding proteins participate in the heterocomplexes which can be of a higher order containing trimers, tetramers etc. The reciprocal allosteric receptor-receptor interaction produces a marked increase in the repertoire of recognition, pharmacology, trafficking and signalling of the participating GPCR protomers. GPCR protomers can change their function through the allosteric receptor-receptor interactions modifying single or several strands of amino acids in the receptors. This is e.g. achieved through changes in G protein selectivity, and in switching from G proteins to beta-arrestin or calmodulin mediated signaling. Their function may also change by forming heteroreceptor complexes with RTK and ion channel receptors. The balance between the different complexes in the plasma membrane is highly dynamic.

Electrostatic interactions may represent important hot spots in the receptor heteromer interface. The triplet puzzle theory describes that protriplet amino acid homologies participate in the receptor interface and was based on results of a mathematical approach when studying 48 pairs of receptors that form or do not form heterodimers. They help construct a kind of code that may determine which receptors should or should not form heterodimers.

Antagonistic A2AR-D2R interactions in heteroreceptor complexes, demonstrated with proximity ligation assay, in dorsal striato-pallidal GABA pathways lead to reduced D2R protomer recognition, Gi/o coupling and signaling. A2A antagonists significantly target these A2A protomers besides A2A homoreceptor complexes[2]. They increase locomotion and contralateral turning behavior in animal models of PD after subthreshold doses of L-DOPA and D2like agonists without worsening dyskinesias. A2A antagonists are therefore tested as antiparkinsonian drugs in clinical trials and heterobivalent drugs with A2A antagonist and D2 agonist pharmacophors are being developed.

Antagonistic A2A-D2 receptor interactions in heterocomplexes may inhibit cocaine-induced reward through increasing activity in the ventral striato-pallidal anti-reward GABA pathway.

Antagonistic A2AR-D2R interactions in this pathway also control cocaine-seeking behaviors in rats[3]. One possible new strategy for treatment of cocaine addiction is to activate the A2A protomer of A2A-D2-(Sigma 1) heteroreceptor complexes in the ventral striatum to restore the brake on D2 signaling in this anti-reward system. The accumbens D2 heteroreceptor complexes also give hopes of a promised land for drug development in schizophrenia, since inter alia salience dysregulation likely contributes to psychosis and is associated with increased meso-limbic DA activity.

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INVITED SESSIONS

S. 01-1

G Protein-coupled receptors (GPCRs) for nucleosides and nucleotides: structure-based ligand design and therapeutic potential

Jacobson KA, Ciancetta A, Balasubramanian R, Junker A, Toti K, Tosh DK, Gao ZG.

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Extracellular nucleosides and nucleotides act at G protein-coupled receptors (GPCRs): 4 adenosine receptors (ARs) and 8 P2Y receptors (P2YRs), respectively, in many organs and tissues. These GPCRs have promise as new therapeutic targets in cardiovascular, pulmonary, central nervous system, endocrine, ophthalmic conditions, inflammation, cancer and pain. However, additional selective agonist and antagonist ligands are needed, especially for the P2YR family. Among P2Y ligands, only well-validated antithrombotic $P2Y_{12}R$ antagonists are used clinically. However, the $P2Y_6R$ (UDP-activated) and $P2Y_{14}R$ (UDP-glucose-activated) have roles in immune and endocrine function. Also, $P2Y_6R$ activation promotes insulin release in pancreatic islets and glucose uptake in adipocytes and skeletal myocytes. A $P2Y_{14}R$ antagonist reduces sterile inflammation in the kidney and activates mast cells, suggesting its application to asthma.

 A_3AR agonists from our lab are efficacious in models of inflammation (entering Phase III trials in psoriasis and rheumatoid arthritis), cancer and chronic pain. A_3AR agonists are active in reversing and preventing the development of neuropathic pain resulting from chemotherapy-induced peripheral neuropathy or nerve injury in mouse and rat, without the development of addiction or tolerance. The protection is lost in A_3AR KO mice. The beneficial effects of A_3AR agonists are mediated in the peripheral, spinal cord and brain.We establish structure-activity relationships at ARs and P2YRs, to design and synthesize novel ligands as pharmacological probes and potential therapeutic agents. Detailed structural information from the X-ray crystallographic structures within these families enables rational design of novel ligands and guides modification of known agonists and antagonists. We recently reported high-resolution X-ray structures for $P2Y_1$ (Gq-coupled) and $P2Y_{12}$ (Gi-coupled) receptors for ADP. These GPCR structures facilitate the discovery of potent and selective ligands and their use in the identification of novel pharmaceutical targets.

Conformational constraint of normally flexible ribose with a bicyclic ring system increases the selectivity of nucleosides and nucleotides at GPCR subtypes. For example, in A_3AR agonists and $P2Y_1R$ ligands, a methanocarba ([3.1.0]bicyclohexane) ring system increases affinity and selectivity. The North (N) conformation is preferred at both receptors, and an X-ray structure of the $P2Y_1R$ complex with (N)-methanocarba nucleotide antagonist MRS2500 details its recognition. Alternatively, the $P2Y_1R$ complex with allosteric inhibitor BPTU was the first example of a completely extra-helical mode of binding of a high affinity GPCR ligand. $P2Y_{14}R$ models based on the closely related $P2Y_{12}R$ structure and molecular dynamics simulations of ligand biding enable an understanding of recognition of known ligands and the prediction of novel antagonist candidate molecules. This approach resulted in new $P2Y_{14}R$ antagonists.

S. 01-2
A$_{2A}$ and A$_{2B}$: closely related adenosine receptor subtypes with striking differences

Christa E. Müller, *PharmaCenter Bonn, Pharmaceutical Institute, Pharmaceutical Chemistry I, University of Bonn, Bonn, Germany*

Adenosine receptors (ARs) belong to the superfamily of rhodopsin-like (class A) G protein-coupled receptors (GPCRs).[1] They are subdivided into four different subtypes designated A$_1$, A$_{2A}$, A$_{2B}$ and A$_3$. While A$_1$ and A$_3$ ARs are G$_i$-coupled leading to a decrease in intracellular levels of the second messenger cAMP, A$_{2A}$ and A$_{2B}$ ARs are G$_s$-coupled receptors, and their stimulation leads to an activation of adenylate cyclase resulting in increased intracellular cAMP concentrations. A$_{2A}$ and A$_{2B}$ are the most closely related AR subtypes. Despite their high sequence homology and almost identical orthosteric agonist binding sites, they show crucial differences: while the A$_{2A}$ AR is activated by low, nanomolar concentrations of adenosine, the A$_{2B}$ AR requires high, micromolar adenosine levels of adenosine to be activated. Such high, micromolar adenosine concentrations are only observed under pathological, i.e. hypoxic or inflammatory conditions. Both, A$_{2A}$ and A$_{2B}$ ARs have recently been proposed as novel targets for the immunotherapy of cancer due to their expression on cells of the immune system. Their expression is often altered, in many cases upregulated, under pathological conditions. Both, A$_{2A}$ and A$_{2B}$ ARs, display a particularly high number of cysteine residues in the extracellular loop regions. Several X-ray structures of the A$_{2A}$ receptor in complex with agonists and antagonists have been determined, including high resolution structures. We have determined a homology model of the human A$_{2B}$ AR based on an X-ray structure of the human A$_{2A}$ AR and used it for docking studies.[2] Our goal has been to elucidate structural and functional differences of the A$_{2A}$ and A$_{2B}$ AR subtypes. To this end we explored the role of the extracellular disulfide bonds in both receptor subtypes.[3] Moreover we investigated the importance of the extracellular loop 2 for ligand binding and receptor activation by studying loop exchange mutants.[4] Finally we discovered that A$_{2A}$ and A$_{2B}$ ARs form heteromers that show a pharmacological behaviour distinct from that of the homomeric receptors (manuscript in preparation).

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S. 01-3
Design of specific chemical tools for investigation of proteolytic enzymes using unnatural amino acids

Marcin Drąg, *Wroclaw University of Technology, Wroclaw, Poland*
Unsubmitted

S. 02-1
Sphingolipids and the transition from depression to alcoholism

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Alcohol is a major psychoactive drug in western societies involved in many cultural activities. It was shown that alcohol can be instrumentalized, i.e. used to achieve goals that would be impossible to achieve or require more work load without alcohol use. Alcohol use can serve numerous instrumentalization goals, one of the most important goals being the self-medication for innate or induced psychiatric problems, like for depression and/or anxiety disorders (Müller & Schumann, 2011). There is a high comorbidity of depression and alcohol use disorder with bi-directional trajectories. While the neuropharmacology of alcohol is well known, neurobiological mechanisms for alcohol instrumentalization are poorly understood. Together with cholesterol and glycerophospholipids, sphingolipids are the most common lipids in brain membranes. Sphingolipids form lipid rafts and signaling platforms, which are membrane compartments enriched in G-protein-coupled receptors. Acid sphingomyelinase (ASM) hydrolyses sphingomyelin to ceramide and phosphorylcholine and, thus, represents a major regulator of sphingolipid metabolism, which was shown to be involved in emotional behaviour (Kornhuber et al., 2014). We found that overexpression of ASM in mice (tgASM) not only induces depression-like behaviour (Gulbins et al., 2013), but also enhanced consumption of alcohol and alcohol-deprivation-effects after repeated withdrawal in a free-choice drinking paradigm. ASM hyperactivity facilitates the establishment of the conditioned behavioural effects of alcohol, and thus, drug-memories. Furthermore, we found that free-choice alcohol drinking, but not forced alcohol exposure, reduces depression-like behaviour selectively in depressed animals by normalization of ASM activity. Using SolariX MALDI-MS slice imaging, we found that ASM hyperactivity induces sphingolipid and subsequent monoamine transmitter allostasis in the nucleus accumbens. Alcohol drinking restores sphingolipid and monoamine homeostasis selectively in depressed mice. Here we provide first mechanistic evidence for alcohol instrumentalization with the goal to self-medicate and ameliorate behavioural symptoms of a genetically-induced innate depression. We show that alcohol drinking normalises ASM function and re-establishes sphingolipid- and monoamine homeostasis in the nucleus accumbens of depressed mice. Thus, sphingolipid homeostasis emerges as a new mechanism to control depression-alcohol addiction comorbidity. This work was supported by funding from DFG grants KO 947/15-1, KO 947/13-1, GU 335/29-1 and MU 2789/8-1, and by funding from the Interdisciplinary Center for Clinical Research (IZKF) Erlangen, Project E13.

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S. 02-2

Integrating serotonergic strategies into therapeutics for cocaine use disorder

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Cocaine use disorder continues to extract considerable personal, health and societal tolls across the globe. Effective and safe pharmacotherapeutic approaches are urgently needed to promote abstinence. The cycling course of cocaine intake, abstinence and relapse is tied to a multitude of behavioral and cognitive processes with impulsivity (rapid unplanned reactions to stimuli without regard for the consequences) and cue reactivity (attentional bias toward cocaine-associated cues) cited as two key phenotypes that set up vulnerability to relapse even years into recovery. Medications that suppress impulsivity and cue reactivity may provide value in enhancing abstinence following termination of cocaine use. The serotonin (**5-HT**) system provides modulatory control over impulsivity and cue reactivity, particularly through the G protein-coupled receptor (**GPCR**) 5-HT$_{2C}$ receptor (**5-HT$_{2C}$R**). A selective 5-HT$_{2C}$R agonist (e.g., Ro 60-0175, WAY163909) consistently reduces, and the 5-HT$_{2C}$R antagonist SB242084 increases, motor impulsivity (1- or 5-choice serial reaction time task). In the same dose range, these selective 5-HT$_{2C}$R agonists suppress cue- and cocaine-primed drug-seeking as well as the reinforcing and motivational effects of cocaine. We have reported that rats expressing the highest level of impulsivity or cocaine cue reactivity display the lowest levels of 5-HT$_{2C}$ R protein expression in the medial prefrontal cortex (**mPFC**). Employing virally-mediated 5-HT$_{2C}$R genetic deletion methods, we confirmed that the engineered loss of 5-HT$_{2C}$R in the mPFC resulted in aggregate elevation in impulsivity and cocaine cue reactivity. These data suggest that dampened 5-HT$_{2C}$R signaling capacity may contribute to phenotypic vulnerability to relapse and that normalization of 5-HT$_{2C}$R function may be useful to suppress relapse to cocaine use disorder promoted by impulsivity and cocaine cue reactivity. In addition to the therapeutic potential for selective 5-HT$_{2C}$R agonists (e.g., lorcaserin), a novel drug design strategy is to ameliorate 5-HT$_{2C}$R hypofunctionality through small molecule 5-HT$_{2C}$R positive allosteric modulators (**PAMs**) that augment the response to endogenous 5-HT. This presentation will highlight these serotonergic ligands as innovative pro-abstinence, anti-relapse therapeutics for cocaine use disorder.

S. 02-3

Impact of serotonin$_2$ receptors on cocaine-induced dopamine release in the medial prefrontal cortex: implications in the control of cocaine behavioral responses

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Background: Over the past decades, substantive work led to the proposal that serotonin$_{2A}$ (5-HT$_{2A}$R) antagonists, 5-HT$_{2C}$R agonists and 5-HT$_{2B}$R antagonists could be promising pharmacological tools for treating cocaine addiction. Microdialysis studies, showing that 5-HT$_2$R-dependent control of cocaine behavioral responses occurs independently of DA release in the nucleus accumbens (NAc) and the striatum, suggested that these modulatory effects could result from a post-synaptic interaction in these brain regions, possibly involving changes of DA transmission. However, a possible role of the medial prefrontal cortex (mPFC), known to regulate subcortical DA pathway activity and cocaine-induced behaviors, has not been investigated to date.

Materials and methods: Using *in vivo* microdialysis in freely moving rats, we assessed the effects of various 5-HT$_{2A}$R (MDL 100907, 0.5 mg/kg, s.c), 5-HT$_{2B}$R (RS 127445, 0.16 mg/kg, i.p.; LY 266097, 0.63 mg/kg, i.p) and 5-HT$_{2C}$R (SB 242084, 1 mg/kg, i.p.) antagonists, and of the 5-HT$_{2C}$R agonist Ro 60-0175 (1 mg/kg, i.p.), on cocaine (10 mg/kg, i.p.)-induced DA release in the mPFC.

Results: We found that both RS 127445 and LY 266084 potentiated cocaine-induced DA outflow, whereas MDL 100907 and SB 242084 had no effect. Also, the effect of cocaine on DA release was potentiated by Ro 60-0175.

Conclusions: These findings demonstrate that 5-HT$_2$Rs exert differential controls on cocaine-induced DA outflow in the mPFC. In keeping with the anatomo-functional relationships between the mPFC, the NAc and the striatum, it is tempting to suggest that 5-HT$_{2B}$R antagonists and 5-HT$_{2C}$R agonists, by potentiating cocaine-induced DA release in the mPFC, could trigger changes of subcortical DA transmission, resulting in the suppression of cocaine-induced behavioral responses.

S. 02-4

Defining the mechanism regulating the role of serotonin in the development of ethanol addiction in animals

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Serotonin (5-hydroxytryptamine, 5-HT) is one of the most important neurotransmitters in the brain that is synthesized in 5-HT neurons from tryptophan by tryptophan hydroxylase 2 (TPH2). Dysregulation of 5-HT homeostasis in the brain has been associated with many pathological states, including depression or drug addiction. Indirect evidence supports a link between disturbed 5-HT function in the brain and addictive behaviors, however, in the case of ethanol the effects of decreased 5-HT content are contradictory.

Recently generated mice deficient in the brain 5-HT by knocking out the *Tph2* gene (but leaving 5-HT neurons untouched) enabled to assess the role of central 5-HT in the developmen t of

alcohol addiction. Interestingly, *Tph2* null mutant mice consumed more ethanol in a two-bottle choice test compared to wild-type mice; a trend toward increased preference for alcohol was seen in the knockouts. Baseline phenotyping of *Tph2* knockout mice revealed that these animals exhibited the depression-like behavior in the forced swim test, but had no signs of anhedonia in the sucrose preference test compared with normal mice. Surprisingly, 5-HT-deficient mice consumed more food and drank more water, and also showed a tendency to increased locomotor activity. Together with high level of aggression and low anxiety phenotype, described previously in these animals, these data suggest that such behaviors may be the result of compensatory changes in the reward system rather than the lack of 5-HT *per se*.

We further studied two possible mechanisms that could contribute to the regulation of 5-HT levels, and thereby to the development of ethanol addiction in the wild-type animals. The first mechanism could be editing of *Tph2* mRNA, a form of post-transcriptional modification of the genetic code based on the conversion of adenosine-to-inosine (A-to-I) or cytidine-to-uridine (C-to-U) by adenosine deaminases acting on RNA (ADARs)). This process was recently discovered in humans and was shown to decrease enzymatic activity of TPH2 in the human brain. Surprisingly, deep sequencing analysis revealed that *Tph2* mRNA editing process does not exist in mice. The other conceivable mechanism that may also affect the activity of TPH2 enzyme involves changes in the transcript level of *Tph2*. Indeed, there have been no observable changes in the transcript level of *Tph2* in the brain of mice that had undergone the procedure of spontaneous ethanol drinking. However, when mice were divided on high- and low-ethanol preferring animals, subsequent statistical analysis demonstrated that low ethanol preference is associated with decreased level of *Tph2* transcript in the raphe nuclei.

The results emphasize the importance of *Tph2* gene in alcohol dependence. Further studies are needed in order to clearly answer the question whether the alterations in the expression level of *Tph2* correlate with changes in TPH2 enzymatic activity/5-HT level.

S. 03-1

Advances in nanosystems for drug delivery

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Various pharmaceutical nanocarriers, including liposomes and polymeric micelles, are our days frequently used for the delivery of a broad variety of both soluble and poorly soluble pharmaceuticals. Using nanoparticulate pharmaceutical carriers to enhance the *in vivo* efficiency of many drugs is now well established. Now, within the frame of this concept, it is important to develop multifunctional stimuli-responsive nanocarriers, i.e. nanocarriers that, depending on the particular requirements, can circulate long; target the site of the disease via both non-specific and/or specific mechanisms, such as enhanced permeability and retention effect (EPR) and ligand-mediated recognition; respond local stimuli characteristic of the pathological site by, for example, releasing an entrapped drug or deleting a protective coating under the slightly acidic conditions inside tumors facilitating thus the contact between drug-loaded nanocarriers and cancer cells; and even provide an enhanced intracellular delivery of an entrapped drug with its subsequent delivery to specific intracellular organelles, such as nuclei,

lysosomes or mitochondria. Additionally, these carriers can be supplied with contrast moieties to follow their real-time biodistribution and target accumulation. Among new developments to be considered in the area of multifunctional pharmaceutical nanocarriers are: drug- or DNA-loaded delivery systems additionally decorated with cell-penetrating peptides for the enhanced intracellular delivery; "smart" multifunctional drug delivery systems, which can reveal/expose temporarily hidden functions under the action of certain local stimuli characteristic for the pathological zone; new means for controlled delivery and release of siRNA; approaches for intracellular drug delivery and organelle targeting; application of nanocarriers co-loaded with siRNA and drugs to treat multidrug resistant tumors; and nanocarrier-based new targeted contrast agents for diagnostic imaging.

S. 03-2

Synthesis and properties of polyelectrolyte-coated nanocapsules

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Background: Traditional pharmaceuticals rarely demonstrate specific affinity towards the site of their action and as a rule, they distribute throughout the body upon administration. To reach the action site, a pharmaceutical agent has to overcome the inactivating action of the aggressive biological medium and cross a variety of biological barriers, which frequently results in at least partial drug inactivation and unfavorable pharmacokinetics. In addition, many pharmaceutical agents could provoke undesirable side effects in normal organs, tissues and cells. The solution of these complicating problems are targeted drug delivery systems.

Materials and methods: The nanocapsules were prepared via direct encapsulation of emulsion droplets in a polyelectrolyte multilayer shell. The emulsion drops stabilized by docusate sodium salt/poly-L-lysine interfasial complex (AOT/PLL) were encapsulated in multilayered shells formed by the LbL adsorption of polyelectrolytes, poly-L-glutamic acid (PGA) and PLL. The nanocapsules were modified by pegylation for biomedical application.

Results: Procedure for encapsulation of hydrophobic drugs in polyelectrolyte multilayer nanocapsules was proposed. Using AOT as emulsifier we obtained emulsion drops, stabilized by AOT/PLL interfacial complex. These drops were encapsulated by layer-by-layer adsorption of biocompatible polyelectrolytes. We used the saturation method for formation of consecutive layers and we determined the optimal conditions concerning concentration of surfactant and polyelectrolytes to form stable shells. The average size of the obtained capsules was 100nm. Pegylated external layer were prepared using PGA-g-PEG. The capsules were stable for at least a the period of 3 months. Model drugs were encapsulated in prepared nanocarriers.

Conclusions: Obtained nanocapsules loaded with model drugs, are good candidates for further biological experiments.

S. 03-3

Biocompatible polymeric nanoparticles as promising candidates for drug delivery

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Injectable nanoparticle drug carriers have been the object of extensive studies over the past thirty years. In spite of scalability and cost-effective manufacturing process, the ideal drug carrier should be small, nontoxic, biodegradable and biocompatible. It should be stable in blood and resistant to opsonization - process leading to recognition by macrophages of the mononuclear phagocytic system which triggers removal of drugs or particles from circulation, preventing them from reaching the target localization. Due to existence of the blood brain barrier, the best gatekeeper in the body toward exogenous substances, the brain is particularly challenging organ for drug delivery.

We focused on investigations of the interactions between different polymeric nanoparticles built from the polyelectrolytes pair PLL/PGA on emulsion droplets, using the layer-by-layer technique with the saturation method with external layers prepared using PGA grafted by polyethylene glycol and a murine macrophage cell line RAW 264.7 and a human monocytic leukemia cell line (THP-1). The physicochemical properties of nanoparticles: their charge, size and surface modifications, have influenced viability, phagocytosis potential and endocytosis in these cell lines. The conjugation of polyethylene glycol seemed to be an effective method for overcoming the problem of rapid elimination of the nanocarrier by macrophages. It significantly lowered toxic effects of nanoparticles as well as their internalization by phagocytic cells, what we showed by performing cell viability and cytotoxicity tests, vybrant phagocytosis assay, flow cytometry experiments and confocal microscopy imaging.

In aging population the incidence of neurological disorders will increase, thus development of drug delivery vehicles that mediate transendothelial transport of medicines across the blood brain barrier is worthwhile. The cell viability and cytotoxicity studies showed that polymeric PLL/PGA and PGA-PEG particles have no toxic effect on human blood brain barrier endothelial hCMEC/D3 cell line. Our studies reveal that nanoparticle surface modification influences its entry pathway and processing in hCMEC/D3 cells. All studied nanoparticles are internalized in energy-dependent way, but only PEG-modified ones undergo both active and passive internalization. Conclusions coming from our experiments strongly support the hypothesis that the PLL/PGA nanoparticles are promising candidates for drug delivery.

S. 03-4

Neuroprotective activity of (bio)polyelectrolyte-coated nanocapsules containing curcumin

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Background: Experimental data demonstrated the neuroprotective potential of curcumin (CUR), however its clinical usage is limited due to its low bioavailability (poor water solubility and photostability) and potential peripheral and central toxicity. To overcome these limitations the nanoparticulate CUR delivery technology has been proposed.

Materials and methods: Two types of (bio)polyelectrolyte-coated nanocapsules containing CUR were synthesized using nanoemulsification technique and layer-by-layer (LbL) saturation method. The nanocapsules were formed with usage of FDA-approved surfactant AOT (docusate sodium salt) and biocompatible polyelectrolytes: poly-L-lysine (PLL) and poly-L-glutamic acid (PGA). The average size of nanocapsules (NC1-CUR: AOT/PLL-CUR and NC2-CUR: AOT/PLL/PGA-CUR) was around 100 nm. Nanocapsules biocompatibility and neuroprotective potential were studied in human neuroblastoma SH-SY5Y cells by biochemical cell viability (MTT reduction) and toxicity (LDH release) assays.

Results: CUR when given alone at concentration above 10 uM was toxic for cells, but at concentration 5 uM attenuated the hydrogen peroxide (H_2O_2)-induced cell damage. Further studies showed the toxic effect of positively charged nanocapsules (NC1-CUR), whereas negatively charged one (NC2-CUR) were safe to SH-SY5Y cells. Moreover, only the NC2-CUR, but not NC1-CUR protected the cells against the H_2O_2-induced damage with the similar efficiency as did CUR (5 uM) when given alone. The neuroprotective effect of NC2-CUR was not connected with its influence on intracellular ROS production but with prevention of H_2O_2-induced decrease in mitochondrial membrane potential. As measured by UPLC and mass spectrometry methods, the intracellular concentration of CUR in cells after administration in encapsulated form was about 20 times lower than when CUR (5 uM) was added directly to cell culture.

Conclusions: The obtained data constitutes a strong basis for future targeted delivery of curcumin with improved efficiency and potentially reduced toxicity.

S. 03-5

Evaluation of protective action of polydatin, free form and in nanocapsules, in the hippocampal organotypic cultures treated with lipopolisaccharide

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Background: Polydatin is a natural stilbene compound extracted from the dried roots of the herb *Polygonum cuspidatum*. Several reports have shown that polydatin exerts various beneficial effects not only in the periphery but also in the central nervous system. Recent data underline its anti-apoptotic and anti-inflammatory properties in the brain. To verify this hypothesis we measured the protective effects of polydatin, itself and in nanocapsules on changes induced by lipopolisaccharide (LPS) in hippocampal organotypic cultures (OHC).
Materials and methods: OHC were prepared from hippocampi of 7-8-day-old rats. On the 7th day *in vitro* slices were pre-treated for 30min with various concentration of free (0.1-50μM) or nano-encapsulated form (1:1;1:2) of polydatin and then stimulated with LPS (1μg/ml). After 24h and 48h the cell death/viability (MTT, LDH tests) parameters were estimated. Additionally we evaluated the pro-inflammatory cytokine and nitric oxide (NO) release. Furthermore to study the putative mechanism underlying the effects of polydatin treatment, we examined the intracellular pathways, which are considered to mediate the expression of pro-inflammatory factors.
Results: We demonstrated that free polydatin (5 and 10μM) and encapsulated (1:1;1:2) showed beneficial action in MTT and LDH assays. Moreover, both forms of polydatin inhibited NO and cytokine release, enhanced by LPS. Interestingly, the effect of fee as well as encapsulated polydatin were stronger after 48h. Furthermore, our preliminary data suggested the involvement of NRF2-KEAP1/NF-KappaB pathways in the observed, beneficial action of polydatin.
Conclusions: Taken together, polydatin in both forms, shows the beneficial properties in OHC stimulated by LPS. We postulate that encapsulation of protective agents may be considered as novel delivery systems in modern therapies based on the inflammatory modulation.

S. 03-6

Synthesis of water-solubleintrinsically fluorescent amino functionalized polyhedral oligomeric silsesquioxanes based nanoparticles for biomedical applications

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unsubmitted

S. 04-1

The effect of co-treatment with risperidone and escitalopram on dopamine and serotonin level in the rat frontal cortex

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Background: Schizophrenia is a devastating psychiatric disorder that impairs mental and social functioning. Although schizophrenia has been long known as a serious disease, its etiology and pathophysiology are still unknown. Positive symptoms of schizophrenia (e.g. delusions, hallucinations) are due to an excess of DA signaling and recently are treated with a second generation of antipsychotic drugs. The negative symptoms of schizophrenia (e.g. social withdrawal, cognitive deficits) are alleviated by novel antidepressant drugs as an adjunct to antipsychotic treatment. It was demonstrated that co-administration of risperidone and selective serotonin reuptake inhibitors improved depressive symptoms and cognitive dysfunction in animal models of schizophrenia. Our early results showed that combination of fluoxetine or mirtazapine with risperidone increased DA and 5-HT level in the rat frontal cortex more efficiently than the drugs given separately.

Material and methods: The present study was aimed to find up whether combination of various doses of risperidone and a novel SSRI, escitalopram is effective in increasing cortical DA and 5-HT release. The extracellular level of DA and 5-HT in the rat frontal cortex was examined using brain microdialysis in freely moving rats. The dialysate concentration of DA and 5-HT was assayed by high performance liquid chromatography (HPLC).

Results: It was found that risperidone (0.2 and 1 mg/kg) and escitalopram (5 and 10 mg/kg) significantly increased cortical DA and 5-HT. Combination of different doses of risperidone and escitalopram was more efficient in enhancing extracellular concentration of DA and 5-HT than drugs given separately.

Conclusions: Thus, co-administration of both drugs in sub-maximal doses may be used to treat positive and negative symptoms of schizophrenia and allows to minimise the drug's side effects.

S. 04-2

Modeling co-existence of depression and cocaine addiction in rats: the effects of N-Acetylcysteine-amide (AD4) on cocaine reward, extinction and seeking behaviorin bulbectomized rats

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Background: Depression is a serious problem today. Several clinical reports indicate a high comorbidity between depression and cocaine addiction. One of the leadinghypotheses

explaining the correlation between depression and addiction is called 'self-medication'. Depressed patients may use addictive substances to feel better and toprovide a temporary escape from anhedonia.Recently, small molecules like N-acetylcysteine or AD4were postulated to be useful in drug addiction.

Material and methods: Male Wistar rats with implanted catheters intravenously and with olfactory bulbs removal (OBX) or SHAM-operated controls were trained to self-administer cocaine (0.5 mg/kg/infusion)paired with the conditional stimuli (tone+light). Other groups of animalsunderwent cocaine self-administration followed by 10-days extinction procedures during which the animals received saline instead of cocaine; there was nopresentation of the conditioned stimuli. Later on, reinstatement was induced byinjection of cocaine or contextual stimuli previously paired with cocaineself-administration. AD4was given to rats (i) during the maintenance phase, (ii) during extinction (iii) beforeinduction of cocaine-seeking behavior.

Results: AD4 (25-75 mg/kg) given acutely did not alter the rewarding effects of cocainein OBX rats and SHAM-operated.However, AD4 (6.25-50 mg/kg) decreased cocaine seeking and relapse triggered by cocaine priming or the drug-associated conditioned cues in both phenotypes. Furthermore, repeated treatment with AD4 (25 mg/kg) during extinction, reduced reinstatement of drug seeking behavior in SHAM-operated and OBX rats.

Conclusions: These results suggest that AD4 may be effective in patients suffering from cocaine use disorder as well as in patients with comorbid depression and cocaine addiction.

S. 04-3

The role of AMP-activated kinase (AMPK) in epithelial-mesenchymal transition (EMT)

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Background: AMP-activated kinase (AMPK) is a metabolic sensor that maintain cellular energy homeostasis. It is also involved in tumorigenesis and metastatic progression processes, however its role remains unclear. There is lack of sufficient evidence that AMPK cellular level is adjusted in response to influence of cytokines such as HGF, however it seems that this process occurs among other changes related to epithelial - mesenchymal transition (EMT) – a process necessary to develop metastasis ability of cancer cells.

Material and methods: Three cervical carcinoma cell lines according to different clinical stage of cancer have been used. C-4I, represents an early stage of cancer. HTB-35 cells indicate the implementation of EMT and HTB-34 cells regained epithelial phenotype. Thus, these lines are model of tumorigenesis and metastatic progression of cervical carcinoma. qRT-PCR analysis were performed to examine the level of AMPK catalytic subunits ($\alpha 1$ and $\alpha 2$) for cell lines cultured under standard conditions, as well as stimulation of HGF. Simultaneously, Western Blotting was used to investigate AMPK total and phosphorylated protein amount.

Results: The aim of this study was to investigate changes in expression of AMPK as a result of HGF impact. Our analysis showed immense discrepancies in AMPK transcript and protein amount between examined cell lines. C-4I line presented the highest AMPK mRNA level, as well as protein. HGF stimulation resulted in decreased expression of AMPK$\alpha 1$ catalytic

subunit. HGF influence was also observed for HTB-35 which showed upregulation of AMPK α2 subunit.

Conclusions: Performed analysis allow to trace the process of metabolic deregulation of cancer cell in metastatic progression. Decrease of AMPK activity corresponds with increasing capabilities for invasiveness and metastasis of cancer cells.

S. 04-4

Characterization of bone marrow-derived mesenchymal stem cells obtained from children diagnosed with hypoxic-ischemic encephalopathy

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Background: Mesenchymal stem cells (MSC) are gaining increased interest of regenerative medicine as a tool in cellular therapies of pathological processes in nervous system, driven by excessive inflammation and neurodegeneration. MSC, which originally populate adult and neonatal tissues, have anti-inflammatory and immunomodulatory properties. Moreover, MSC are known to release many trophic factors, that can mediate extensive tissue repair. The results of our recent clinical studies demonstrated that autologous MSC transplantation in children with hypoxic-ischemic encephalopathy (HIE) led to an improvement of the clinical status of patients. The aim of this study was to evaluate properties of patients' autologous MSC.

Materials and methods: CD271⁺ cells from mononuclear fraction of autologous bone marrow were isolated. Flasks were incubated in standard culture conditions. The MSC phenotype was analyzed with antibodies for CD3, CD45, CD73, CD90 and CD105. In order to evaluate changes in immunomodulatory and tissue remodeling properties, MSC were additionally seeded on 6-well plate and after reaching 80% confluence, incubated with INF-γ, TNF-α or IL-1β. RNA were isolated from MSC in various conditions and reverse transcription was performed. Gene expression was determined by qPCR analysis.

Results: Expanded MSCs were positive for CD73, CD90, CD105 and negative for CD3 and CD45. Gene expression analysis have shown, that MSC express genes for neurotrophic, proangiogenic factors and also ones related to tissue remodeling. We have also evaluated the changes in immunomodulatory and tissue remodeling properties in control conditions and after incubation with proinflammatory cytokines.

Conclusions: Obtained results confirmed unique properties of MSC and gave us presumptive evidence to explain their neuroregenerative potential in HIE treatment.

S. 04-5

Serum miR-29c as a potential biomarker for abdominal aortic aneurysm

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Background: MicroRNAs, which are a class of small, non-coding RNA molecules, offer great potential as biomarkers because of their stability in bloodstream and characteristic expression in different diseases. Up to date little is known about their role in abdominal aortic aneurysms. Thus, the aim of this study was to perform unbiased molecular screening of microRNAs in serum collected from patients with AAA in comparison to non-aneurysmal controls in order to select potential biomarker of this disease.

Materials and methods: Study population consisted of two groups: 50 patients with AAA, who were admitted for elective surgery and 50 controls matched by age and gender to the patients. Total RNA was isolated from serum after addition of a spike-in microRNA using standard method based on mini-columns optimized for purification of small RNAs. Screening phase of the study was performed on Open Array Platform for a complete panel of human miRs. MicroRNA, which showed significant differential expression were validated using real-time PCR with TaqMan probes and analyzed for a potential biomarker efficacy in diagnosis of the disease.

Results: At least twofold differences in serum expression of 19 microRNAs (14 upregulated, 5 down-regulated) were noted between AAA patients and controls. Man-Whitney rank test results limited candidates to 10 miRs, and hsa-miR-29c was selected for further analysis. Serum hsa-miR-29c was significantly upregulated in AAA patients compared to the controls (RQ=8.73, p=0.0263) and correlated with the diameter of the aneurysm. Logistic regression model did not reveal statistically significant association between well-known risk factors (hypertension, hypercholesterolemia) and has-miR-29c level in serum samples.

Conclusions: miR-29c in AAA patients' serum has a robust predictive and diagnostic potential biomarker (area under the ROC curve = 0.929 [95% CI 0.901-0.956, p<0.05]). However, it remains to be established its prognostic value for aortic rupture.

S. 04-6

Hepatotoxicity of posaconazole oral formulation in relation to ABCB1 polymorphism among children with haematological malignances

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Background: Posaconazole (PCZ) as a broad-spectrum triazole agent is approved for the prophylaxis and treatment of invasive fungal infections. It is both a substrate, and an inhibitor of the P-glycoprotein. This suggests an impact of the ABCB1 polymorphism on its pharmacokinetics and toxicity. PCZ treatment appears to be well-tolerated but in some patients it can lead to abnormal liver function tests. Limited data are available for pediatric patients, especially with malignant diseases and current guidelines are mainly based on the extrapolation of adult data. The aim of the study was to determine the influence of ABCB1 polymorphism on the hepatotoxicity of PCZ oral formulation in a pediatric population.

Materials and methods: ABCB1 C3435T SNP was determined in a total number of 40 children (7 months - 18 years), receiving PCZ oral formulation for prevention of fungal infections. The PCR-RFLP method using the Sau3AI enzyme was performed. Biometrical and biochemical data were analyzed. ALT, AST, GGT and bilirubin serum levels, were checked before, 7, and 20 days from the beginning of treatment. ABCB1 genotype was correlated with the liver function parameters. The study was approved by the Ethics Committee of Wrocław Medical University.

Results: The observed genotype frequencies of ABCB1 were 20% for 3435CC, 40% for 3435CT and 40% for 3435TT. Elevated ALT, AST and GGT serum levels were found in more (45, 30, 25%) patients at 20 compared with day 7 (20, 10, 5%) of PCZ treatment, while bilirubin values were increased only at day 7. The frequency of the abnormal liver tests was higher among subjects carrying 3435CT and 3435TT genotype.

Conclusions: No significant relationship between ABCB1 polymorphisms and bilirubin serum levels was observed in pediatric patients. Liver enzyme concentrations seem to be influenced by the ABCB1 C3435T SNP during PCZ treatment. The ABCB1 polymorphism may have an impact on the hepatotoxicity of posaconazole oral formulation among children.

S. 04-7

The impact of prenatal tobacco exposure on the cerebral mass of the neonate

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Background Maternal tobacco smoking is one of the main risk factors for many neurological disorders and it is changing the neurological development of neonate. The main aim of this study was to assess cerebral mass, based on head circumference measurements in neonates exposed to tobacco smoke in utero. The relative proportions of the cerebral and body mass was also determined.

Materials and methods: The study included 147 neonates admitted to the Neonatal and Intensive Care Department of the Medical University in Warsaw. They were divided into three treatment groups based upon the maternal status as active, second-hand smoke (SHS) or non-smokers determined by maternal urinary cotinine concentration and a questionnaire. Cotinine concentration (major metabolite of nicotine) was measured by high-performance liquid chromatography with spectrophotometric detection and norephedrine as an internal marker, following earlier liquid–liquid extraction.

Results: Neonates whose mothers were active smokers (maternal urinary cotinine concentration >200 ng/mg of creatinine) during pregnancy had a lower head circumference and in consequence a lower cerebral mass significantly more frequently when compared with those whose mothers were nonsmokers ($P = 0.002$). The risk of lower cerebral mass was 3.9 (1.4–10.8, CI 95%). It was also seen among neonates whose mothers were SHS exposed with urinary cotinine concentration 5–200 ng/mg of creatinine: 1.9 (0.7–5.2, CI 95%); however, this difference was not statistically significant. A negative correlation was seen between cerebral mass and maternal urinary cotinine concentration (correlation coefficient $r = -23$, $P = 0.006$). In all neonates groups the ratio of cerebral to body mass was similar.

Conclusions: Active smoking by mothers during pregnancy inhibits the neonate brain growth (as well as body mass) which perhaps could be associate with the negative behavioral and cognitive outcomes of children in the future.

S. 05-1

The role of glial cells in the experience of pain: how much is important the sex factor?

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Although the understanding of pain mechanisms has significantly improved in the recent years, much more is yet to be discovered. Broadening of our knowledge is needed in order to find innovative treatments for fighting pain, mainly neuropathic pain. As a matter of fact, all current therapeutic options for neuropathic pain are unsatisfactory and commonly associated with severe side effects. We studied the complex network of interactions along the nociceptive pathways through the study of behavioral responses and immunohistochemical alterations in the expression of specific protein markers at peripheral and central level in the Chronic Constriction Injury (CCI) model of neuropathic pain in CD1 adult male and female mice. In particular, we focused our attention on the link between pain and glial function. As a matter of fact, a growing relevance of glial cells has highlighted their role in physiological and pathological processes related to pain: generation and maintenance of neuropathic pain are associated to glia activation in central and peripheral nervous system. In the spinal cord, the activation of microglia and astrocytes is accompained by morphological changes (such as hypertrophy and proliferation) as well as changes in glial markers and in signaling pathways (such as phosphorylation of mitogen-activated protein kinases). At the periphery, Schwann cells have a fundamental role in degenerative/regenerative processes after injury, removing myelin debris and promoting axonal regeneration. We observed time-dependent differences in the time-course of neuropathic pain and in the activation of glia and astrocytes, as well a fundamental role played by sex. CCI induced mechanical allodynia that gradually decreased in male mice with a complete recovery occurring 81 days post surgery. Moreover, CCI induced an activation of microglia and astrocytes that completely disappeared in male 121 days after CCI. On the contrary, in female mice both allodynia and gliosis were still present 121 days after CCI. At the periphery a different expression of important proteins associated with nerve injury and repair supported faster nerve regeneration in males than in females. In both male and female mice 17beta-estradiol resulted in analgesic effects, facilitated recovery and counteracted gliosis.

Our findings provide new insights in the comprehension of neurobiological mechanisms involved in pain modulation and demonstrate the importance of the sex factor for the development of more specific and targeted therapy against neuropathic pain.

S. 05-2

Molecular basis of OA to guide the identification of new therapeutic targets

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Pain in OA has been classically attributed to joint structural damage and is generally classified as nociceptive pain. However, disparity between the degree of radiographic structural damage and the severity of symptoms, such as pain and functional limitations in OA patients, implies that factors other than the joint pathology itself, also contribute to the pain. Continuous and intense nociceptive input from the OA knee joint may drive peripheral and/or central sensitization, which may arise from chronic nociceptor stimulation and subsequent modification of pain-transmitting neurons.

This presentation will provide an overview of studies examining the impact of OA (chemical model) on both structural (alterations in suchondral bone architecture and bone density visualized by X-ray computed microtomography) and molecular changes (dysregulation of extracellular matrix metalloproteinases; gene-expression profiling in knee cartilage; signalling events in endocannabinoid systems in the knee joint, DRG and spinal cord) during disease progression. The role of significant axonal injury to DRG cells including those innervating targets outside of the knee joint such as hindpaw skin will also be discussed. More recent studies highlight the possible role of cannabinoids in bone remodelling processes during OA. Consequently the putative role of endocannabinoid system on osteoblast migration will be addressed. New attractive targets not only to control pain symptoms (modulation of pain transduction and transmission) but also to regulate bone cells' metabolism, which can benefit in management of human OA, will be indicated.

S. 05-3

Interleukin-6 and signal transducer gp130 as important regulators of neuroimmmune interactions in the pain pathway

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Cytokines of the IL-6 family are not only key regulators of innate immunity and inflammation, but are also essential factors for the differentiation and development of the somatosensory system. In addition, they regulate nociceptive processing in the peripheral as well as the central nervous system. In particular, IL-6 and Oncostatin M induce hypersensitivity to mechanical and heat stimuli by modification of transducer ion channel function such as TRPV1 phosphorylation and membrane insertion. Long-lasting changes in ion channel machinery of nociceptive primary afferents result from the induction of IL-6 like cytokines which signal to their target cells via receptor homo- or heteromers containing the IL-6 signal transducer glycoprotein 130 (gp130). Since global ablation of gp130 results in a lethal phenotype, transgenic models with conditional gp130 deletion in primary nociceptive afferents expressing the nociceptor specific sodium channel Nav1.8 are available to explore the role of gp130 in nociceptors. These SNS-gp130$^{-/-}$ mice were largely protected from developing long-lasting signatures of hypersensitivity in preclinical models of pathological pain. SNS-gp130$^{-/-}$ mice showed reduced mechanosensitivity to strong mechanical forces in the von Frey assay *in vivo* and this was associated with a reduced sensitivity of nociceptive primary afferents *in vitro*. In the spared nerve injury (SNI) model for

neuropathic pain, SNS-gp130$^{-/-}$ mice exhibited a significantly reduced mechanical hypersensitivity. In line with these findings, mRNA expression of the mechanotransducer ion channel Transient Receptor Potential Ankyrin 1 (TRPA1) was significantly reduced in dorsal root ganglia (DRG) from SNS-gp130$^{-/-}$ mice [2]. This was reflected by a reduced number of neurons responding with calcium transients to TRPA1 agonists in primary DRG cultures. Upregulation of TRPA1 function was associated with the expression of gp130 as it was not found in SNS-gp130$^{-/-}$ mice. In addition, depletion of gp130 significantly impaired neuroregenerative properties at the crossroad of cytokine and neurotrophin signaling via STAT3 pathways, and the release of neuropeptides [3]. Such alterations of key signaling pathways may be the reason for a significant role of gp130 expressed in nociceptors in a preclinical model of autoimmune arthritis [1]. Our results closely link gp130 to lesion induced alterations of neuroimmune functions, both of which have been shown to contribute to hypersensitive pain states. We suggest that gp130 has an essential role in mutual communication between the immune and nervous systems and is critically important for the generation in particular of neuropathic pain disorders.

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S. 05-4

Pharmacological modulation of intracellular signaling pathways in microglia can attenuate neuropathic pain symptoms and enhance opioid effectiveness

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The neuropathy may now be considered as neuro-immune disorder, since it is known that the activation of spinal glia results in the release of both pro- and anti-nociceptive cytokines. Neuropathic pain treatment remains a challenge because it is resistant to alleviation by morphine. The spinal microglial production of immune factors (IL-1alfa, IL-1beta, IL-6, IL-10, IL-18, CCL2) is believed to play an important role not only in nociceptive transmission, but also in opioids effectiveness. We believe that modulation of the mitogen-activated protein kinase (MAPK) and nuclear factor kappaB (NF-κB) pathways can influence the neuropathic pain development. Our results have shown that minocycline (p38MAPK inhibitor) diminished microglia activation and spinal M1 factors (IL-1beta, IL-18 and CCL2) that were previously elevated following sciatic nerve injury. Moreover the minocycline enhanced spinal M2 factor (IL-10). Besides, the minocycline prevented the development of flaccid paralysis following high-dose of dynorphin A, suggesting a neuroprotective effect. Interestingly, primary microglial cell culture studies confirmed the presence of mu- and kappa-opioid receptors and provide evidence for the lack of delta-opioid receptors. Interestingly, the analgesic effect of delta-opioid receptor ligands (DPDPE, deltorphine II) under neuropathy is not diminished in contrast to mu- and/or kappa-opioid ligands (morphine, DAMGO, U50.488H, SNC80). Therefore, delta-opioid receptor agonists appear to be the best candidates for new drugs to treat neuropathic pain. Interestingly, also the parthenolide (NF-κB inhibitor) attenuated the allodynia/hyperalgesia and enhanced opioid effectiveness, however increased microglia activation after sciatic nerve injury. Nevertheless, parthenolide reduced the protein level of M1

(IL-1β, IL-18, and iNOS) and enhanced M2 (IL-10, TIMP1) factors. In addition, it downregulated the phosphorylated form of NF-κB, p38MAPK, and ERK1/2 protein level and upregulated STAT3. In primary microglial cell culture we have confirmed that IL-1β, IL-18, iNOS, IL-6, IL-10, CCL2 and TIMP1 are of microglial origin. Summing up, minocycline and parthenolide directly or indirectly attenuates neuropathy symptoms and promotes M2 microglia/macrophages polarization. We suggest that neuropathic pain therapies should be shifted from blanketed microglia/macrophage suppression toward maintenance of the balance between neuroprotective and neurotoxic microglia/macrophage phenotypes. Our study provide evidence for some new important mechanisms underlying the development of neuropathy which led to identify some possible diverse approaches to the pain treatment.
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S. 06-1
Molecular pathophysiology of aspirin exacerbated respiratory disease
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unsubmitted

S. 06-2
Eicosanoid imbalance in aspirin exacerbated respiratory disease
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unsubmitted

S. 06-3
Leukotriene overproduction and platelet activation in AERD

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Aspirin-exacerbated respiratory disease (AERD) refers to the development of bronchoconstriction and rhinitis with/without urticaria if exposed to ASA/NSIADs in patients with chronic asthma. Key features of AERD are overproduction of cys-leukotriens(LT) and intense eosinophilic infiltration in upper and lower airways, which are associated with higher prevalence of severe asthma and chronic rhinosinusitis(CRS). Since the role of platelet aggregated eosinophils has been reported in upper airway inflammation of AERD patients, recent findings demonstrated that platelet activation markers were elevated in association with eosinophil activation in AERD compared to ATA patients.
A two-step cluster analysis analyzing 302 AERD patients enrolled at Ajou University Hospital cohort demonstrated 4 different subtypes, in which atopic status, female ratio, peripheral eosinophil counts, and the prevalences of CRS/nasal polyp, urticaria and severe asthma were significantly different among 4 subtypes. Moreover, frequency of asthma exacerbation and anti-asthmatic medication requirements including medium/high dose ICS/LABA and systemic steroid were significantly different among 4 subtypes.
Metabolomic analysis demonstrated that urinary LT E4 metabolite levels were significantly higher in subtype 1&3, while 15-LO metabolites were significantly higher in subtype 3. Mast cell-derived metabolite was significantly higher in subtype 2. Urinary LTE4 metabolite levels

were significantly higher in AERD patients carrying HLA DPB1*0301 and CysLTR1-634 T allele, both of which were reported as strong gene markers for AERD.

These findings suggest that stratified medicine with applying biomarkers of each subtype from an AERD cohort will be able to achieve better outcome toward precision medicine in the management of AERD.

S. 06-4

Concentration of selected eicosanoids in exhaled breath condensate during allergen-induced early asthmatic response

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In allergic asthma patients exposure to relevant allergens leads to immediate airway bronchoconstriction referred to as early asthmatic response (EAR). It has already been demonstrated that EAR depends on IgE-mediated mast cell activation, however the exact role of individual mediators is not fully understood. Allergy to house dust mites (HDM) is a risk factor for asthma. It was therefore of interest to evaluate concentration of exhaled eicosanoids in HDM-allergic patients (HDM-APs) during an EAR induced by exposure to Dermatophagoides pteronyssinus (Dp) allergen extract.

The studied population included HDM-APs who responded to bronchial challenge with EAR (responders, Rs) and those who did not produce significant bronchoconstriction (NRs) after allergen challenge. In order to evaluate local production of selected eicosanoids exhaled breath condensate (EBC) samples were obtained before (T0) and after the last Dp extract inhalation (TEAR), which in Rs resulted in significant bronchoconstriction. High-sensitivity methods based on mass spectrometry were used to evaluate concentration of selected 5-LOX- and COX-derived eicosanoids, including leukotriene -B4 (LTB4), -C4 (LTC4), -D4 (LTD4) and -E4 (LTE4), 5-oxo-eicosatetraenoic acid (5-Oxo-ETE), prostaglandin – D2 (PGD2), -E2 (PGE2) and 8-Iso-PGE2 in EBC samples.

At baseline, among the studied eicosanoids the greatest concentration in EBC was demonstrated for LTB4 and 5-Oxo-ETE. The mean concentration of COX-derived eicosanoids was several fold lower than that of 5-LOX-derived mediators.

The baseline lung function tests were comparable in Rs and NRs. However, Rs were characterized by greater serum Dp-specific IgE concentration, greater bronchial hyperreactivity expressed as histamine PC20 and greater peripheral blood eosinophilia.

Analysis of exhaled eicosanoids in relation to the pattern of response to allergen challenge demonstrated significantly lower baseline concentration of 5-Oxo-ETE and PGD2 in Rs in comparison to NRs. The concentration of other eicosanoids in EBC did not differ significantly between the two groups. In Rs significant increase in concentration of 5-Oxo-ETE, 8-Iso-PGE2 and LTD4 in EBC was seen at TEAR. No change in concentration of any of the studied eicosanoids could be demonstrated at TEAR in NRs. Finally we searched for a possible associations between 5-Oxo-ETE and clinical/immunological parameters of HDM-APs. The increase in EBC 5-Oxo-ETE concentration inversely correlated with the change in the number of circulating eosinophils at TEAR. Interestingly, exhaled 5-Oxo-ETE concentration correlated at T0 with LTD4, while during an EAR with 8-Iso-PGE2.

Allergen-induced EAR is associated with altered local metabolism of arachidonic acid derived lipid mediators. 5-Oxo-ETE may represent an interesting target for therapeutic intervention in allergic asthma patients.

S. 07-1

Personalized or precision medicine?

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unsubmitted

S. 07-2

Genome studies and the risk of cancer

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unsubmitted

S. 07-3

One treatment for all? A case of cardiovascular patient

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Personalized medicine became a key concept in modern medicine. However, it is often understood differently. Doctors have been using personalized approach for centuries, when they analyzed holistic pictures of their patients. However, in recent decades we started to emphasise the usage of data from clinical trials, that tried to unify patients' populations and hence treated patients in unified manner. This resulted in great improvement in patient's care, but it seems that we are coming to the end of such approach. Increasing costs of huge clinical trials and the amount of data we gathered on individual factors that affect the response pushes towards more directed, individualized trials and hence more personalized approach. But we should not discard evidence based medicine (EBM). It always had personalization as its major goal. One often forgets that in EBM the evidence has to be used in particular situation based on the clinical expertise of the doctor and personal values and preferences of the patient[1]. In other fields personalized medicine is often perceived as genomic medicine. In cardiovascular medicine the most common diseases are caused by multiple genetic alterations, and the effect of a single polymorphism is relatively small, so the situations when a sole genetic investigation will result in particular treatment will be relatively rare, however as our knowledge of genomics and our comprehension of the interaction between particular genetic and epigenetic factors increase, we will see more input from genetics to risk stratification and hence treatment strategies. Advent of the era of genomic medicine creates also an opportunity for future genetic interventions. One of the recent problems was tissue and cell specificity of gene transfer interventions. Usually genes that improve myocardial viability and resistance to ischemia may simultaneously promote neoplastic growth. New genetic methods, including CRISPR allow long lasting and safer interventions in somatic cells. We can expect also tissue or cell specific interventions.

Maybe in near future we will be able to fix changes precisely in sites that are required in cells that we want. How all these new methods will affect everyday cardiology practice? Doubtlessly this will be a great change, it will require great effort from all doctors and healthcare systems. However, there always will be a place for an individualized interpersonal relation with a vulnerable human – the patient.

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S. 08-1

Role of antibiotics on neuroprotection and neuroinflammation

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unsubmitted

S. 08-2

Matrix metalloproteinases and neurodegenerative progression in Parkinsonism

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Matrix metalloproteinases (MMPs), matrixins, are extracellular proteases part of the metzincin family. MMPs are calcium-dependent zinc-containing endopeptidases which are not only responsible for degradation of the extracellular matrix (ECM) but as well MMPs can play a pivotal role in neuroinflammation. In the naive brain it have been detected quite low levels of the mRNA, protein expression or enzymatic activity of MMPs (in the hippocampus, the cerebral cortex or the cerebellum). In normal conditions, MMPs are mainly present in the cell bodies and dendrites of neurons (specially on dendritic spines in excitatory synapses) having very low MMPs' expression in glial cells (microglia, astrocytes or oligodendrocytes).
Among MMPs, neuronal MMP-9 can control synaptic plasticity in learning and memory as can regulate the shape of dendritic spines and the function of excitatory synapses. As extracellular protease, MMP-9 has the ability to digest gelatin, a denatured collagen but, additionally, has recognized targets as different growth factors (and their precursors), cell surface receptors, and cell adhesion molecules. Moreover, MMP-9 activates proinflammatory cytokines (tumor necrosis factor α, TNF-α or interleukin 1a, IL-1a) and, in turn, up-regulated cytokines increase secretion of MMP-9 by glial cells.
In order to maintain homeostasis, the expression of MMPs in normal physiological conditions is low but there is a significant increase activity related with either acute or chronic neuroinflammation in some neurodegenerative disorders, in stroke, in psychiatry disorders or in traumatic brain injury. In particular, it has been demonstrated a possible association between a risk of PD and the 1562 polymorphism of the MMP-9 gene. Furthermore, in the substantia nigra pars compacta (SNpc) and in the striatum of post-mortem brains of PD patients and Parkinsonian animals there are increased levels of both tissue inhibitors of MMPs (TIMPS) and active different MMPs (up-regulation of MMP-9) in the neurons and in the microglia. Additionnally, MMP-9 knockout increases the number of remaining dopaminergic neuron after MPTP intoxication with a correlation of decrease of microglia and astrocytes activation. In Parkinsonism, MMP-9 is activated not only inside the brain cells but as well in the ECM, as

this activation is maintained chronically during the neurodegenerative process. Then, even if the initial steps of neuroinflammation are considered as favorable reactions to harmful stimuli, the primed microglia can release excessive amounts of proinflammatory cytokines driving to chronic neurodegeneration. Then, when the inflammation overactivity is maintained a common play of MMPs could be crucial for its progression: i) intracellular active MMPs (driving lipid peroxidation and apoptosis) can increase/maintain dopaminergic cell death in the SNpc; and ii) MMPs in the ECM keep on a *vicious circle* between oxidative stress and inflammation which promotes chronic microglia activation with increased release of different pro-inflammatory cytokines. Then, the protective and salubrious physiological function of MMPs would turn into a noxious effect boosting the neurodegenerative progression in Parkinsonism.

S. 08-3

L-DOPA effect on hydroxyl radical in a Parkinsonian rat

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There is ongoing debate as to whether L-DOPA accelerates progression of Parkinson's Disease (PD) by increasing oxidative stress in dopaminergic neurons. To address this hypothesis, a model of severe PD was produced by bilateral 6-OHDA (134 µg icv) treatment of rats at P3. As adults these rats were acutely treated ip with L-DOPA (60 mg/kg; carbidopa pretreatment, 12 mg/kg), then terminated at 1h for analysis of striatal dopamine (DA) and 3,4-dihydroxybonzoic acid (3,4-DHBA), an indirect measure of hydroxyl radical ($HO^\bullet$). In these rats striatal $HO^\bullet$ levels (i.e., 3,4- DHBA) were overtly reduced by L-DOPA, indicating that L-DOPA is neuroprotective and unlikely to enhance progression of PD. In a companion study half the control and 6-OHDA rats were additionally treated at P3 with 5,7-dihydroxytryptamine (5,7-DHT) to largely destroy striatal serotonin (5-HT) nerves. In adulthood, in vivo microdialysates were assessed for $HO^\bullet$ before and after PCA (1mM in microdialysis perfusate). While prominent DA release was evoked in 6-OHDA lesioned rats, the additional 5,7-DHT lesioning resulted in a 3-fold greater DA release; and this DA exocytosis occurred without an increase in $HO^\bullet$. This finding indicates that 5-HT nerves normally suppress DA release in parkinsonian rats. Moreover, under the above experimental conditions, the series of studies indicate that enhanced DA formation (i.e., L-DOPA treatment) and enhanced DA exocytosis (i.e., PCA) are not accompanied by increased $HO^\bullet$ formation and therefore do not predispose to dopaminergic neurodegeneration. Supported by NINDS.

S. 08-4

NO and glutamate interaction in treatment of L-DOPA-induced dyskinesia

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Background Amantadine is the noncompetitive antagonist of N-methyl-D-aspartate (NMDA), receptor activated by the excitatory neurotransmitter glutamate. It is the only effective medication used to alleviate dyskinesia induced by L-3,4-dihydroxyphenylalanine (L-DOPA) in Parkinson's disease patients. Unfortunately, adverse effects as abnormal involuntary movements (AIMs) known as L-DOPA-induced dyskinesia limit its clinical utility. Combined effective symptomatic treatment modalities may lessen the liability to undesirable events.
Likewise drugs known to interfere with nitrergic system reduce AIMs in animal models of Parkinson's disease. **Material and methods** We aimed to analyze an interaction between amantadine, neuronal nitric oxide synthase inhibitor (7-nitroindazole, 7NI) and nitric oxide donor (sodium nitroprusside, SNP) in 6-hydroxydopamine-(6-OHDA)-lesioned rats (microinjection in the medial forebrain bundle) presenting L-DOPA-induced dyskinesia (20mg/kg, gavage, during 21 days). **Results** We confirm that 7NI-30mg/kg, SNP-2/4mg/kg, and amantadine-40mg/kg, individually reduced AIMs. Our results revealed that co-administration of sub-effective dose of amantadine (10mg/kg) plus sub-effective dose of 7NI (20mg/kg) potentiate the effect of reducing AIMs scores when compared to the effect of the drugs individually**.** No superior benefit on L-DOPA-induced AIMs was observed with the combination of amantadine and SNP. **Conclusions** The results revealed that combination of ineffective doses of amantadine and 7NI represents a new strategy to increase antidyskinetic effect in L-DOPA-induced AIMs. It may provide additional therapeutic benefits to Parkinson's disease patients from these disabling complications at lower and thus safer and more tolerable doses than required when either drug is used alone. To close, we discuss the paradox of both nitric oxide synthase inhibitor and/or donor produce AIMs reduction through targeting nitric oxide synthase.

S.09

Workshop for Polish young scientist - Tips for writing a successful grant proposal
Liana M, Kowalczyk M.
National Science Centre, Poland

S.10-1
Non-invasive detection of cell death in diabetes and other pathologies

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Minimally-invasive detection of cell death could prove to be an invaluable biomarker in many physiologic and pathologic situations, ranging from normal human development to diabetes, cancer and neurodegenerative disease. Cell-free circulating DNA (cfDNA), released from dying cells, is emerging as a diagnostic tool for monitoring cancer dynamics and graft failure. However existing methods rely on DNA sequence differences in source tissues, so that cell death in tissues with a normal genome cannot be identified. We developed a method of detecting cell type-specific cell death in humans, based on tissue-specific methylation patterns in cfDNA that are independent of variations in nucleotide sequence[1]. We interrogated tissue-specific methylome databases to identify cell type-specific DNA methylation signatures for several different cell types, and developed a method to detect these in mixed DNA samples. We isolated cfDNA from plasma of donors, treated it with bisulfite, PCR-amplified and, using massive parallel sequence technology, sequenced pre-determined regions to quantify cfDNA carrying the methylation markers of the cell-type of interest. Pancreatic beta-cell DNA was identified in the circulation of recently diagnosed type-1 diabetes patients and islet graft recipients, oligodendrocyte DNA in patients with relapsing multiple sclerosis, neuronal/glial DNA in patients after traumatic brain injury or cardiac arrest, and exocrine pancreas DNA in patients with pancreatic cancer or pancreatitis. Since each cell type contains its own, unique methylation signature, this approach can be further developed to trace cell death of any of the hundreds of different cell types in the human body, offering a minimally-invasive window for monitoring and diagnosis of a broad spectrum of human pathologies, as well as better understanding of normal tissue dynamics. In the setting of diabetes, future work is aimed at tracing beta-cell death throughout the course of both Type 1 and Type 2 diabetes. Additional studies are planned to monitor cell death in tissues associated with short- and long-term complications of this devastating disease.

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S.10-2
The deadly duo of obesity and type 2 diabetes - can new drugs treat both?
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unsubmitted

S.10-3

Vascular complications of diabetes – completely avoidable at last?

Leszek Czupryniak, *Medical University of Warsaw, Poland*
unsubmitted

S.10-4

Insulin resistance - does it play a role in pathogenesis of chronic complications and treatment of diabetes?

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Insulin resistance (IR) is defined as a state of impaired biologic response to endogenous and/or exogenous insulin which affects carbohydrate, lipid and protein metabolism and also mitogenic activity. Diabetes mellitus is a chronic disease which leads to the development of diabetic complications. Macroangiopathy is still a major problem in type 2 diabetes mellitus and impaired insulin action is involved in pathogenesis of this complication. Recent data provides evidence that mild cognitive impairment and other neurodegenerative disorders are also more common in type 2 diabetic patients and are related to IR. Insulin receptors are widely expressed in the central nervous system and insulin action in the brain is related not only to the regulation of energy homeostasis but also cognitive function. "Insulin Resistant Brain State" is considered as an important step in the development of type 2 diabetes. Impaired action of insulin in brain of patients with Alzheimer disease has been described recently. Additionally, several epidemiological studies have shown increased cancer risk in patients with type 2 diabetes mellitus, which is attributed to long term exposure to hyperinsulinemia secondary to IR. The question arises if improvement of insulin sensitivity play an important role in the prevention and treatment of type 2 diabetes mellitus and its complications. Firstly, behavioral intervention (diet, physical activity) are the simplest way to improve insulin sensitivity, however the beneficial effect of lifestyle modification has been proven only in prediabetic state. In large, randomized, controlled Look AHEAD Study, in patients with established type 2 diabetes mellitus, intensive lifestyle modification for 10 years, despite differential weight loss, did not reduce the cardiovascular events in adults with type 2 diabetes. However, marked improvement of HbA1c has been observed. Current pharmacologic approaches to improve insulin sensitivity include two antidiabetic drugs - metfromin and pioglitazone. Metformin in the treatment of type 2 diabetes is recommended as a first-line therapy and during the progression of the disease, in combination with other oral antidiabetic drugs and insulin. In the UKPDS and UKPDS-follow up Study metformin has been proven to reduce macrovascular complications of diabetes. Pioglitazone is a powerful insulin sensitizer in muscle and liver. According to the recommendations, this drug can be used as a second-line therapy, also in combination with other drugs and insulin. Additionally, bariatric surgery in morbidly obese patients with type 2 diabetes improves insulin sensitivity. Currently, new molecules are under investigation to target insulin resistance and in consequence prevent and treat type 2 diabetes mellitus.

S.11-1

Analysis and recovery of neuronal microcircuit activity in Huntington's disease using two-photon microscopy and optogenetics

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Huntington's disease (HD) is an autosomal dominantly inherited neurodegenerative disorder characterized by motor, cognitive, and psychiatric disturbances caused by expression of a polyglutamine stretch in the protein huntingtin (Htt). Here, we asked whether cortical circuit activity is affected at early stage of the HD prior to neurodegeneration and the establishment of symptoms in the cortex of HD transgenic animals with an insertion of 150 Q into the mouse Htt gene (HD150). To examine this hypothesis, we studied the network activity of a neuronal population in the layer 2/3 of the visual cortex using 2-photon calcium imaging. We chose the visual cortex as a model of study because the functional activity of this structure is altered in HD (Rub et al., 2014). An imaging chamber was implanted above the targeted cortical region in the lightly anesthetized mouse; using a sharp patch pipette, we injected the Ca2+ indicator Oregon-Green BAPTA1-AM using the bolus-loading technique (Stroh et al., 2013). We characterized the OGB-1 AM staining in the layer 2/3 of the V1 in WT and HD150 mice and found no difference in the density of stained cells. The analysis of spontaneous activity indicated that both WT and HD150 mice exhibit silent and active cells. Notably, the suprathreshold activity of the cortical microcircuit as mirrored by neuronal Ca2+ transients of individual neurons was significantly higher in HD150 mice compared to WT mice. Indeed, the frequency of calcium transients was significantly higher in HD150 mice suggesting an increased cortical activity. Next, we classified the neurons based on their rate of spontaneous activity: 1) low activity (<0.3 trans/min), 2) normal activity (0.3-3 trans/min) and 3) hyperactivity (3< trans/min). This analysis revealed that highly active cells were only recorded in HD150 mice whereas no highly active cells were observed in control mice (p<0.01 Mann-Whitney test). In addition, the analysis of the frequency distribution showed a significant shift towards hyperactivity in HD150 mice compared to WT mice.

Our data underscore the importance of the assessment of cortical microcircuit activity in HD mice, as we could reveal the emergence of a hyperactive phenotype in vivo for the first time. This population might be instrumental for the dysregulation of cortical network in this mouse model of HD, and represents a target for therapeutic interventions.

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S.11-2
Biomarkers in Parkinson's disease

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Parkinson's disease is the second most common neurodegenerative disease. Symptoms like movement disorders and non-motor symptoms may cause patient's serious disability. Etiology of this disease is still unknown, which makes the diagnosis and treatment difficult. Levodopa is commonly considered as the most effective symptomatic therapy. Motor complications: response fluctuations and dyskinesias following the levodopa treatment are recognized as the most common therapeutic problem.

Establishing of markers of the Parkinson's disease constitutes one of the contemporary research aims. As a consequence, current research is focusing on finding clinical, imaging, and biochemical biomarkers for prodromal phase, diagnosis, as well as disease progression of Parkinson's disease. The research includes the following parameters: clinical (non-motor symptoms), biochemical biomarkers (metabolic, neurotrophic factors, neuroinflammation, oxidative stress, iron and other metals, α-synuclein, CSF biomarkers), imaging biomarkers (nuclear imaging, transcranial sonography, magnetic resonance imaging).

Serum/plasma metabolomics is considered an appropriate tool for understanding metabolic pathways in Parkinson's disease.

A number of studies have been performed worldwide to assess the genetic, biological and pharmacological factors influence on levodopa dose response (including cathecol-O-methyltransferase, dopa-decarboxylase and dopamine transporter gene polymorphisms). It is commonly accepted that the development of biomarkers could ultimately deliver personalized therapeutic strategies.

An appropriate refinement of novel technology may result in the development of biomarkers that better diagnose Parkinson's disease and improve the efficiency of disease-modyfying therapies.

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S.11-3
CSF biomarkers of Alzheimer's disease
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S.11-4
Blood biomarkers of Alzheimer's disease

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Alzheimer's disease (AD) is the most common age-related dementia affecting over 45 million people worldwide. The effectiveness of AD therapies depends on the early diagnosis of AD but currently clinical diagnostic criteria allow only for the diagnosis of probable or possible AD in patients with clinical dementia and with mild cognitive impairment (MCI), which later leads to AD. The diagnosis of AD is based on neuropsychological tests and cerebrospinal fluid (CSF) biochemical assay of tau protein and the Aβ peptide, which requires an invasive lumbar puncture and can be supported by expensive brain imaging techniques. In light of the preferable biomarker criteria defined by FDA, progress in AD diagnostics relies, to a great extent, on identification of novel biomarkers of early AD stages, preferably in easily available blood samples. Accordingly, all biological paradigms have been utilized in search for blood AD molecular signatures, including proteomics, lipidomics, transcriptomics, metabolomics, and epigenomics. One of the most promising approaches to the identification of blood AD biomarkers concentrates on circulating microRNAs (miRNAs), small noncoding RNA molecules, which showed altered expression in biofluids in many disease states including cancer.

Our recent research using the quantitative real time polymerase chain reaction (qRT-PCR) led to identification of 9 novel miRNAs, which show a consistently changed expression pattern in blood plasma during early AD, in patients with MCI,, and in more advanced stages of AD, as compared to blood samples from non-demented age-matched subjects (patent application P.416956, PCT/IB2016/052440). The reliability of our methodology for assessment of miRNA levels in blood is confirmed by simultaneous identification of 6 miRNAs, which were reported in 4 different studies as miRNAs whose levels were changed in AD patients. We found that these 6 miRNAs were indeed dysregulated in blood samples from AD patients comparing to non-demented age-matched controls. The present data indicate that miRNA profiling may provide a novel method of determining whether a person is afflicted with AD. This method is less invasive, less expensive, faster, and potential test would be more available in a clinical setting than any other approved method for supporting AD diagnostics, such as conventional CSF biochemical assays or brain imaging techniques. Such a methodology may revolutionize future diagnostics, shifting its focus from symptoms to molecular signatures.

S.12-1
Molecular mechanism of activity of non-basic ligands of 5-HT$_6$ receptors

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Background: The activity of serotonin receptors ligands has been linked to formation of charge-assisted hydrogen bond with Asp$^{3.32}$, supported by countless compounds containing protonated nitrogen in physiological conditions presumably interacting with this residue of the 5-HTRs. There is, however, a group of ligands that lack this structural feature, yet remain active towards 5-HT$_6$R, thus denying the established mechanism of interactions with serotonin receptors. Research described herein aims to explain the molecular mechanism of interactions between atypical ligands with 5-HT$_6$ receptor.

Materials and methods: The basis for the study is a series of non-basic compounds derived from indolyl-sulphonyl moiety with their affinity towards 5-HT$_6$R evaluated in radioligand binding assay. The interactions with the receptor were investigated by docking to a series of homology models of 5-HT$_6$R developed on available crystal structures of serotonin receptors 1B and 2B (PDB codes: 4IAR and 4IB4, respectively) and molecular dynamic simulations.

Results: The results prove that the mode of interactions between non-basic ligands and 5-HT$_6$R does not involve Asp$^{3.32}$ and the specific and non-specific contacts are formed with TMH5,7 and ECL2, where number of residues unique for serotonin receptor 6 were identified.

Conclusions: The designed series of tool compounds along with computational studies allowed to explain the mechanism of action of atypical ligands of 5-HT$_6$R. It emerged that interaction with Asp$^{3.32}$ is not necessary for high affinity binding and several residues from ECL2 and TMs 5 and 7 can compensate for that.

S.12-2
Monoamine oxidase B activity of novel xanthine derivatives

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Background: Monoamine oxidase B (MAO-B) is known for its crucial role in neurodegenerative diseases. MAO-B inhibitors, such as selegiline and rasagiline are drugs registered in the treatment of Parkinson's disease. We investigated new compounds from the group of tricyclic xanthine derivatives as potential MAO-B inhibitors.

Material and methods: Compounds were investigated for inhibition of human recombinant MAO-B. The Amplex Red® Monoamine Oxidase kit was used. Inhibition activity was measured in presence of the reference substrate, p-tyramine (200µM). Data were calculated in GraphPad Prism 7 free trial. Instant JChem was used for structure database management, search and prediction.

Results: We investigated 90 new compounds for their activity towards MAO-B in one concentration (1µM). Compounds, which exhibited more than 50% of the maximum inhibition

activity (presented by pargyline in 10µM conc.) were chosen for further investigation. For 13 compounds IC_{50} and K_i values were experimentally calculated. IC_{50} values ranged between 45nM and 8300nM.

Conclusions: From the large group of investigated compounds we managed to find xanthine derivatives that exhibit inhibition activity towards MAO-B. The structure-activity relationship can be helpful in drug development in this group of compounds.

S.12-3

Pharmacophore modeling of UDP-N-acetylmuramoylalanine glutamate ligase inhibitors – methodology and application for virtual screening procedure

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Background: UDP-N-acetylmuramoylalanine glutamate ligase (MurD) is one of the most promising biological targets in development of next-generation of anti-bacterial compounds. Along with other members of the amide ligases (MurC-F), MurD inhibits the synthesis of peptidoglycan (PG) – key bacterial metabolite, crucial for bacterial growth. Anti-bacterial action of MurC-F is indeed very promiscuous, given that this metabolic pathway is common for bacteria and inhibitors of a single enzyme may be multipotent antibacterial agents.

Material and methods: Due to the increasing numbers of published MurD inhibitors (87 structures in February 2016) some standard *in silico* approaches may be utilized for the discovery of new ligands. Here we have used pharmacophore modeling pipeline, as described before. All known MurD inhibitors were hierarchically clustered using Canvas with manual refinements to ensure proper chemotypes classification. Multiple hypotheses were developed for each cluster, employing the previously utilized approach. After application of DUD-like test set, one model per cluster was selected (according to Yourden's statistics value) to form the linear combination of pharmacophore models, i.e. the first, general pharmacophore hypothesis of MurD inhibitors.

Results and conclusions: This combination was applied as one of the steps in the virtual screening protocol for reducing space of ca. 8M of compounds from seven commercial databases and 100K compounds from virtual library of easily synthetically accessible compounds.

S.12-4

Genomic and transcriptomic determinants of resistance in anthracycline therapy in pediatric acute leukemias

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Background: The aim of the study was to identify genomic and transcriptomic changes in the context of *ex vivo* resistance to anthracyclines (DNR-daunorubicin, DOX-doxorubicin, IDA-idarubicin, MIT-mitoxantrone) in pediatric acute leukemias (AL).

Materials and methods: The *in vitro* drug resistance profile was determined in MTT assay performed on mononuclear cells taken from 155 patients with AL. Gene expression and CGH array profiles were prepared on the basis of cRNA or gDNA hybridization to human genome microarrays, respectively. Validation of array results was performed by RT-qPCR with the use of UPL probes for 20 genes (*ABCG1, ANXA1, CDKN1A, ARAP1, FGR, HK3, SERP1, ITGAM, PCDH9, DUSP2, RETN, IFIT3, RUNX1, TCF7, WDR26, WNK1, CASP1, ITGB2, TBL1XR1, TTC28*) comparing to 4 reference genes (*ACTB, GAPDH, SDHA1, TUBA1B2*).

Results: Ontological analysis of transcriptomic resistance profiles revealed that overexpression of hydrolases is characteristic for DNR, DOX and MIT. Wnt signaling pathway genes were downregulated for DNR, IDA and MIT. Chemokines and cytokines showed upregulation for DNR and MIT. According to aCGH results, we identified frequent genomic changes, such as amp8p12-p11.21 in blasts resistant to DNR and DOX, amp14q32.33 in blasts sensitive to DNR, IDA and MIT. Gene expression profiles indicated several potential target genes for each drug, but 2 of them were common for all (*DUSP2, HK3*).

Conclusions: Basing on the microarray analysis validated in qPCR, we selected overexpressed target genes *DUSP2* and *HK3,* correlated with resistance to anthracyclines. *DUSP2* inactivates kinases and negatively regulates MAPK pathway, which controls proliferation and drug-induced apoptosis of hematopoietic cells. *HK3* is activated by transcription factor PU.1, which activates transcription of the antiapoptotic *BCL2A1* and inhibits transcription of the *TP53* tumor suppressor.

S.12-5
Methylation of CD146 (MCAM) gene promoter as a cause of CD146 silencing level in breast cancer cell lines

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Background: The cell adhesion molecule CD146 (MCAM) was first described in melanoma cancer. Recently, the aberrant expression of CD146 has been implicated in tumor progression of several cancers. Studies demonstrate that CD146 plays a critical role in promoting breast cancer, by increasing cancer cells motility and in consequence their metastatic potential. Despite the significant correlation between CD146 expression and high tumor grade, the molecular basis for the high level of this protein in tumors, has not been demonstrated.

Materials and methods: We analyzed the CD146 expression at the mRNA and the protein level in human breast cancer cell lines (MCF7, T47D and MDA-MB-231) and in the normal breast cell line MCF10A. We compared results with the CD146 expression in cells after 5-aza-deoxycytidine treatment. Next we study the CD146 promoter by methylation-specyfic PCR(MSP) to reveal CpG methylation in all tested lines.

Results: The use of 5-aza-deoxycytidine, as demethylation agent, resulted in an increase in the CD146 expression at the mRNA and the protein level in all tested cell lines. Interestingly, in parallel to the increase of CD146 expression, we observed morphological changes in the treated cells. Analysis of the CD146 promoter by MSP indicated correlation between demethylation and re-expression CD146 in all, cancer and normal breast cells.

Conclusions: Hypermethylation occurs at CpG islands in the CD146 gene promoter region and is associated with gene inactivation. After 5-aza-deoxycytidine treatment, we observed demethylation of gene promoter with re-expression at the mRNA and protein level in all analyzed cells.

S.12-6
TRAIL-producing *Lactococcus lactis* bacteria as a vehicle for induction of colorectal cancer cell apoptosis

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Background: Despite the progress of medicine, the need for therapeutic strategies, which more selectively eliminate cancer cells, is still urgent. TRAIL (Tumor Necrosis Factor-Related Apoptosis-Inducing Ligand) has been shown to induce apoptosis of tumor cells without cytotoxic effect on normal cells, however, its therapeutic activity depends on the way of administration. *Lactococcus lactis* is a non-pathogenic bacteria, which may serve a vehicle for

TRAIL expression and *in vivo* administration. The aim of the study was to determine the tumoricidial effect of hsTRAIL expressed by *L. lactis* bacteria, harbouring recombinant plasmid pUSP45TRAIL3.

Material and methods: hsTRAIL-cDNA sequence was synthesized, inserted into the pTrc99A plasmid vector and introduced into the *E.coli* DH5α host strain in order to perform initial screening of hsTRAIL antitumor activity. Afterwards, hsTRAIL-cDNA was cut out from the *E.coli* plasmid and together with cDNA of *L.lactis* usp45 gene coding signal peptide, was inserted into the pNZ8148 plasmid vector. This final hsTRAIL-secretable vector (pUSP45TRAIL3) was introduced into the *L.lactis* NZ9000 host strain. The presence of usp45-hsTRAIL-cDNA insert was verified by plasmid digestion with restriction enzymes. Production of TRAIL was assessed by Western blot analysis of cell lysates. Preliminary tests for antitumor activity of hsTRAIL produced by *L.lactis* were performed *in vitro* with human colorectal HCT116 cell line.

Results: Supernatants from *L. lactis* cultures expressing hsTRAIL showed antitumour activity against colorectal cancer cells *in vitro*. The presence of hsTRAIL protein in the lysates proved its production by transformed strain.

Conclusions: hsTRAIL produced by *L. lactis* maintains its antitumor activity *in vitro*. More studies are required to confirm advantages of *L.lactis* as the applicable vehicle for TRAIL administration *in vivo*.

S.12-7

The impact of magnetic field on ion influx of the neuronal cells

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Background: Transcranial magnetic stimulation (TMS) is a noninvasive approach used to stimulate small regions of the brain. Recent studies show that TMS improves motor symptoms of Parkinson's disease. However, the relationship between the clinical efficacy of TMS and its mechanism is still obscure. Calcium ions generate versatile intracellular signals that control key functions in all types of neurons. In this study we aim to understand whether the application of a magnetic field can affect the ion influx in neuronal cells.

Materials and methods: SH-SY5Y human neuroblastoma cells were differentiated with retinoic acid, usually used as a cell model for Parkinson's disease. Culture medium has been previously magnetized by flowing between two coils in a magnetic circuit and medium was changed before each assay. Calcium imaging was carried out to measure changes in intracellular calcium concentration upon stimulation of ionotropic and metabotropic receptors. Behavior of voltage-gated ionic currents in magnetic condition was studied by Patch-Clamp.

Results: The solvation shell of the ions present in the culture medium increased when the medium was subjected to an external magnetic field. The calcium imaging showed that the variation in intracellular calcium concentration was affected when the ions in the extracellular

medium was magnetized. The variation of this response depended on the receptor that was stimulated, however a decrease was observed for most of the receptors studied. Ionic currents were also affected in the medium magnetization conditions.

Conclusions: Our results suggest that the magnetization of ions of the extracellular medium alone is enough to cause a disturbance in cellular signaling mediated by these ions. Further studies are necessary to clarify the specificity and physiological significance of the effects induced by the external magnetic stimulus in neuronal cells.

S.12-8

The influence of BoNT/A administration on IL-1 family members in rat neuropathic pain model – *in vivo* and *in vitro* evidences

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Background: Participation of cytokines in neuropathic pain development is established, however their role in botulinum neurotoxin serotype A (BoNT/A) analgesic effects is unclear. The aim of study was to verified the influence of BoNT/A injection on neuropathic pain symptoms and protein level of IL-1 family members.

Material and methods: Experiments were performed on male Wistar rats. The BoNT/A was single intraplantar injected (*i.pl.;* 300 pg) at day 5 following chronic construction injury CCI, than at day 7 after CCI the allodynia (von Frey test) and hyperalgesia (cold plate test) were evaluated. Western blot analysis was used to indicate the influence of BoNT/A administration on protein level of IL-1 family members in the primary microglial cell cultures and in neuropathic pain model at the spinal cord and DRG level. Experiments were carried out according to IASP rules. The results were assessed by ANOVA analysis.

Results: The CCI-induced neuropathic pain symptoms parallel with increase of pronociceptive IL-1beta and IL-18 in spinal cord and DRG. The single *i.pl* BoNT/A injection diminished allodynia and hyperalgesia as well as decreased IL-1beta and IL-18 protein level in the spinal cord and/or DRG. Additionally BoNT/A injection increased level of IL-1RA in the DRG. Lipopolysaccharide stimulation of microglial cells induce up-regulation of IL-1beta and IL-18 parallel with decrease of IL-1RA and IL-18BP protein level. The BoNT/A treatment down-regulate the IL-1beta and IL-18, but had no influence on IL-1RA and IL-18BP protein level.**Conclusions:** Our studies shows that BoNT/A possess analgesic properties which can be correlate with its diminishing influence on the level of pronociceptive IL-1 family members.

S.13-1
MMP-9, extracellular protease – a culprit of aberrant synaptic plasticity

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Matrix metalloproteinase 9, MMP-9 is an extracellularly operating enzyme that has been demonstrated as important regulatory molecule in control of synaptic plasticity, learning and memory. Either genetic or pharmacological inhibition of MMP-9 impairs late phase of long-term potentiation at various pathways, as well as appetitive and spatial memory formation, although aversive learning remains apparently intact in MMP-9 KO mice. Strikingly, blocking of MMP-9 activity in the central amygdala impairs appetitive, but not aversive learning and memory. MMP-9 is locally translated and released from the excitatory synapses in response to neuronal activity. Extrasynaptic MMP-9 is required for growth and maturation of the dendritic spines to accumulate and immobilize AMPA receptors, making the excitatory synapses more efficacious. Animal studies have implicated MMP-9 in such neuropsychiatric conditions, as e.g., epileptogenesis, autism spectrum disorders, development of addiction, and depression. In humans, MMP-9 appears to contribute to epilepsy, alcohol addiction, Fragile X Syndrome, schizophrenia and bipolar disorder. In aggregate, all those conditions may be considered as relying on alterations of dendritic spines/excitatory synapses and thus understanding the role played by MMP-9 in the synaptic plasticity may allow to elucidate the underpinnings of major neuropsychiatric disorders.

S.13-2
Functional organization of perisomatic inhibition in the basolateral amygdala

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The basolateral amygdala (BLA), a cortical structure is one of the main output stations of the amygdala region. This area plays an essential role in several higher order cognitive functions. In spite of its fundamental role in key physiological and pathological processes, our knowledge of its microcircuit structure is surprisingly limited. This statement is particularly true for the organization of amygdalar GABAergic circuits, which are critically involved in neuronal operation. By combining high resolution confocal microscopy, electron microscopy and *in vitro* electrophysiology extended with optogenetics, we identified three GABAergic interneuron types giving rise to the vast majority of the perisomatic inputs of the principal cells in the BLA. Basket cells expressing a Ca^{2+} binding protein parvalbumin (PV) or type 1 cannabinoid receptor (CB1) targeted both the soma and dendrite of principal cells. PV-expressing axo-axonic cells, however, specifically innervated the axon initial segments of principal cells, a restricted region where the action potential generation had the highest likelihood. In spite of the different membrane surface innervated by the interneurons, all the three GABAergic cell types controlled the spiking of amygdalar principal cells with similar efficacy evaluated in paired recordings. Our analysis uncovered that there was a strong correlation between the inhibitory efficacy and the number of boutons targeted the perisomatic region. In contrast, perisomatic inhibitory cells received excitatory inputs from amygdalar principal cells with different properties. PV-containing interneurons were excited by neighbouring principal cells with high probability, while CB1-expressing basket cells received intra-amygdalar excitatory inputs from principal

cells located evenly within the amygdala. The unitary events originated from principal cells had distinct properties, too. PV-containing cells received large and fast unitary events, while unitary events in CB1-expressing basket cells were small and slow. Finally, we studied the connectivity among the three interneuron types. Basket cells were found to be innervated each other within, but not between their own categories, forming two parallel basket cell networks. Our data together show that the principal cells in the basolateral amygdala prefer to excite PV-containing basket cells, while activation of CB1-expressing basket cells needs substantially higher activity levels. These observations support a hypothesis that the two basket cell types can fulfil specific functions in network operation during various brain states.

S.13-3
Neuronal circuits in the central amygdala underlying socially transferred fear

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In its simplest form empathy can be characterized as the capacity to share the emotional state of another being (emotional contagion). Tuning one's emotional state to that of another increases the probability of similar behavior, which thereby allows for a rapid adaptation to environmental challenges. Emotional contagion, commonly observed in animals, including rodents, is well described at the behavioral level, but the neural circuits necessary for sharing emotions are not well understood. In the simple model of socially transferred fear that we have developed we showed that a brief social interaction with a fearful cage mate (demonstrator) activates the amygdala of the observers, especially its central part (CeA). The CeA is critical in fear learning and controls defensive responses. To directly test the hypothesis that CeA neural circuits are involved in socially transferred fear, we optogenetically activated 'social fear' neurons in CeA. We used *c-fos*-driven targeting of channelrhodopsin into neurons involved in social interaction to specifically manipulate their activity during subsequent tests. We showed that activation of 'social fear' neurons in CeA of the observers enhances exploratory behavior of the familiar environment but inhibits social interaction and ultrasonic communication. Their activation in the novel environment resulted in increased anxiety reflected by shortened exploration of anxiogenic stimuli. Thus, by activation of CeA neurons involved in social interaction with a fearful partner we were able to reproduce behavioral changes observed as a consequence of the real interaction. We also showed a population of the CeA neurons that was activated by socially transferred fear but not by non-social fear. To further characterize this subpopulation we identified their anterograde and retrograde projections by functional mapping methods. Especially dense anterograde projections have been found in the periaqueductal gray (PAG) and dorsal raphe nuclei (DRN); the structures implicated in fear and anxiety. Taken together, out results show that the neural circuits within the CeA control socially transmitted fear and that these circuits involve groups of neurons that are at least partially different than the ones involved in non-social fear.

S.13-4
Neuronal circuits and mechanisms of fear behaviour
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unsubmitted

S.14-1

Circulating miRNAs as future biomarkers for a cerebrovascular immune-inflammatory atherosclerotic diseases

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unsubmitted

S.14-2

Contemporary approach to acute stroke treatment

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unsubmitted

S.14-3

Patients stratification in stroke management

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unsubmitted

S.14-4

Central and systemic inflammatory mechanisms in cerebral ischemia

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The contribution of central and peripheral inflammatory processes to brain injury is widely recognised. However, inflammatory changes after stroke occur at different temporal and spatial scales, therefore complex imaging technologies, transgenic models and both *in vivo* and *in vitro* approaches are required to understand the mechanisms involved. Of these, imaging early inflammatory events after acute brain injury and correlating these with functional outcome remain technically challenging. We have recently developed novel approaches to visualize early inflammatory changes after brain injury with *in vivo* two-photon and SPECT imaging. These include the assessment of microglia-neuron interactions, calcium responses, blood brain barrier (BBB) injury, oxidative stress and perfusion changes in real time, during and after cerebral ischemia, induced by middle cerebral artery occlusion using a common intraluminal filament- or a remote filament approach. Our results suggest that changes in BBB injury after the onset of ischemia can be detected with either two-photon imaging or SPECT much earlier (within 2 h) than by using histology. Changes at the capillary / small vessel level as assessed by two-photon imaging show a good correlation with BBB injury seen in full brain hemispheres based on SPECT imaging studies. However, successful reperfusion after cerebral ischemia is followed by spontaneously occurring perfusion deficits later, which is further impaired by preceding systemic inflammation. Systemic inflammation also leads to larger BBB injury, which is apparent as early as 2 h after the onset of ischemia and is associated with impaired functional outcome. SPECT imaging in mice is also capable of detecting very early (within 2 h) inflammatory changes in the lung and the gut, which are known target organs for post-stroke inflammation and infection leading to poor clinical outcome in patients. In line with this, changes in autonomic innervation and the gut microbiota are seen in response to cerebral

ischemia and genetic deletion of inflammasomes sensing diverse host- and pathogen-derived danger molecules improves outcome after stroke. Understanding central and systemic inflammatory mechanisms in brain diseases will be essential for the development of novel diagnostic and therapeutic tools for the clinic.

S.15-1

Interneuronal plasticity and psychiatric disorders

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Several lines of evidence have pointed to alterations in the development, structure and physiology of interneurons as crucial factors involved in the etiopathology of mental disorders, including major depression and schizophrenia. However, the cellular and molecular bases of these alterations are still unclear. Research in our laboratory during the last years has been focused in the analysis of inhibitory networks in key regions involved in these psychiatric disorders in animal models and patient's brains, specially in the prefrontal cortex and the limbic system. We have also studied the effects of antidepressant and antipsychotic drugs on these inhibitory circuits. Our research, together with that in other labs, has shown that the structure, connectivity and neurotransmission of interneurons are altered. It has also demonstrated that the polysialylated form of the neural cell adhesion molecule (PSA-NCAM) is a key player in this interneuronal plasticity. PSA-NCAM is expressed by a substantial proportion of cortical interneurons and influences their structure and connectivity. The analysis of the expression of this plasticity-related has revealed alterations in its expression in the brain of psychiatric patients. These alterations were also observed in inhibitory networks of novel animal models of schizophrenia and depression. These models showed changes in the expression of PSA-NCAM and other plasticity-related molecules and structures, as well as alterations in dendritic arborization and spine density, reorganization of their connectivity and alterations in the expression of molecules related to inhibitory neurotransmission.

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S.15-2

Early-life stress and dysfunction of the medial prefrontal cortex as risk factors for mental disorders

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Clinical data clearly show that early-life stress (ELS) increases the risk for several mental disorders like, e.g., mood disorders, anxiety disorders, behavioral disorders and addiction. Moreover, ELS is associated both with early-onset (in childhood and adolescence) and late-onset of these psychopathologies. Therefore, it has been hypothesized that ELS interferes with

brain development and maturation. From the brain regions highly implicated in the above-mentioned mental disorders, the medial prefrontal cortex (mPFC) focuses the special attention, because it displays prolonged developmental trajectory and it accomplishes its final maturation during adulthood. During preadolescence and adolescence periods essential remodeling of the mPFC occurs, including, apoptosis and pruning of overproduced cells and synapses and extensive myelination. Thus, during adolescence, the malfunctions which have originated earlier in life, e.g., as a result of ELS, may easily manifest themselves. However, the knowledge about the impact of ELS on adolescent brain is scarce. All these facts prompted to apply the animal model of ELS, i.e., maternal separation (MS) procedure in rats (3 h/day, from postnatal day 1 to 14) and investigate its effects on mPFC function and rat behavior during adolescence. Our study showed that MS male rats displayed an increase in anxiety-like behavior and prolonged impairment in fear memory. The behavioral phenotype of adolescent MS rats strongly indicated the dysfunction of the mPFC. Histological and immunohistochemical studies revealed that MS increased the numbers of neurons and astrocytes in the mPFC of adolescents. Oppositely, the number of microglia cells was decreased. Moreover, we found that activation of several proteins engaged in cell cycle regulation and apoptosis, e.g., caspase-3 and PARP was affected by MS. Additionally, it was showed that MS reduced gene expression of proapoptotic Caspase-9 and BAK1 and elevated mRNA levels of Bcl-2, known for its antiapoptotic activity. These results indicated that MS interfered with cell survival/apoptosis processes in the mPFC. We also studied the effects of MS of structural and functional plasticity in the mPFC. MS caused the atrophy of basal dendritic tree and reduced spine density in pyramidal neurons. It was also showed that MS decreased gene expression of several proteins involved in synaptic plasticity processes and neurotransmitter release, like e.g., sialyltransferase ST8SIA2, neuroligin-3, synapsin-2 and vesicle-associated membrane protein. What is more, MS impaired long-term potentiation processes in the mPFC. Finally, we found that MS reduced gene expression of some proteins involved in metabolic processes, e.g., malate dehydrogenases and subunits of cytochrome C oxidase. Summing up, all these results suggest that ELS may cause a dysfunction of the mPFC on both structural and functional levels manifested in adolescence and lead to development of early-onset psychopathology.

S.15-3
Neuroinflammation in alcohol addiction

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Neuroinflammation is thought to play an important role in neuropathological problems caused by chronic alcohol (ethanol) use. Enhanced immune signaling due to alcohol use may also promote development of alcohol addiction. Recent preclinical findings indicate that, 1) binge and chronic alcohol exposure increase brain immune signaling in rodent models through Toll-like receptor 4-dependent mechanisms, 2) modulation of neuroimmune signaling by transgenic techniques affects mouse alcohol drinking, and 3) pharmacological modulation of neuroimmune signaling affects alcohol drinking. However, little is known what role alcohol-induced neuroinflammation plays in the development of addiction.

In experimental animals, one way to induce neuroimmune signaling is through systemic administration of lipopolysaccharide (LPS) which produces acute sickness reaction lasting for

one to two days. Our own studies focus on testing whether LPS-induced neuroinflammation could be a useful model for analyzing how enhanced immune signaling affects consumption of alcohol, and modulates the effects of alcohol, such as rewarding effects, anxiolysis and sedation. We have analyzed how pretreatment with LPS affects long-term alcohol drinking of mice. In a free-choice 24-h model, pretreatment with 1 mg/kg LPS did not affect alcohol intake of male C57Bl/6J mice, while the mice pretreated with the 1.5 mg/kg dose of LPS consumed less high concentrations of alcohol than controls. Similar results were obtained from the drinking-in-the-dark binge drinking model, where LPS pretreatment did not alter 15% alcohol drinking monitored for several weeks after LPS insult. Thus, these results after a single LPS treatment did not support the hypothesis that neuroinflammation *per se* enhance alcohol drinking. In search for a model to study interactions of immune signaling and alcohol consumption we are now analyzing whether repeated LPS treatments between binge drinking periods modulate alcohol intake.

In conclusion, although it is clear that chronic high-dose consumption of alcohol induces various immune signaling molecules in the brain, it is not known, what is the impact of these changes on motivation to consume alcohol? Also, further research is needed for better understanding how enhanced immune signaling modulates positive and negative reinforcing effects of alcohol and brain reward circuitries.

S.15-4

Enriched environment and development of schizophrenia-like abnormalities

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Several findings have indicated that schizophrenia is a neurodevelopmental disorder with symptoms appearing at the end of abnormal brain development starting years before illness onset. The epidemiological and genetic studies have also suggested that not only genetic but also environmental factors, especially early live experience during critical period of brain development might contribute to the onset or progression of schizophrenia. The perinatal period of life has been shown to be critical for proper brain development and it has been implicated in the pathophysiology of the disease. In addition, adolescence has been pointed to be crucial for exacerbation of symptoms in the predisposed brain. During this period, a synaptic pruning process with excessive elimination of synapses as well as myelination process occur, mainly in the prefrontal cortex.

The limited epidemiological data have shown that environmental interventions during early childhood might protect against the onset of schizophrenia. In addition, the positive effect of exposure to enriched environment (EE) during childhood /adolescence has been observed in the prevention of development of schizophrenia symptoms in the animal neurodevelopmental model of schizophrenia based on postnatal pharmacological interventions or genetic modifications. However, whether the exposure to EE during adolescence might prevent the abnormalities in brain development and impairments in behavioural response in adulthood after prenatal exposition to environmental factors, is still under investigation. Therefore, in the

present study we determined the effect of exposure to EE during early adolescence (23rd-29th day of life) on the development schizophrenia-like abnormalities in a neurodevelopmental model of schizophrenia based on prenatal administration of the antimitotic agent methylazoxymethanol (MAM) at embryonic day 17 (E17). MAM administration at E17 induced behavioural dysfunction observed as sensorimotor gating deficits, impairment in social interaction and recognition memory disruption in adult rats. Exposure to EE in defined adolescent period prevented the development of the above behavioural impairments in adult prenatally MAM treated rats.

A decrease in glutamic acid decarboxylase (GAD) 67 expression in prefrontal cortex is commonly observed in neurobiological features of schizophrenia. Prenatal MAM administration induced a decrease in GAD67 mRNA and protein level in adult medial prefrontal cortex (mPFC), and EE inhibited the decrease in GAD 67 expression induced by prenatal MAM administration in adult mPFC. Moreover, exposure to EE normalized the GAD67 mRNA level in prenatally MAM treated adult rats by influencing impaired epigenetic regulation of Gad1 gene promotor. Thus, the obtained results might suggest that exposure to EE during early adolescence might be able to prevent the abnormalities in brain development and behavioural dysfunction in adulthood.

S.16-1
Identification of endogenous and surrogate ligands for orphan GPCRs

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We previously reported putative endogenous ligands, l-α-amino acids, L-tryptophan and l-phenylalanine, for the orphan receptor GPR139[1]. However, it is still an open question what constitutes the endogenous agonist of GPR139, as we have recently discovered an endogenous peptide that can activate this receptor with higher potency. We have also identified published and new small-molecule allosteric antagonists for the orphan receptor GPRC6A[2], and agonists for GPR32 (in preparation) and GPR132[3], by computational methods. Funded by the ERC and the Lundbeck Foundation, two new 5- and 7-year programmes aim to identify endogenous peptide ligands for orphan receptors using bioinformatics, pharmacological tool compounds by focused screening libraries, and G protein inhibitors by structure-based drug design. Tailored tools for GPCR analysis and ligand design have been made available through the GPCR database, GPCRdb (gpcrdb.org).

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S.16-2
Differences of agonist and antagonist binding to GPCRs

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G-protein-coupled receptors (GPCRs) mediate most of our physiological responses to external stimuli including neurotransmitters, hormones, and diverse environmental signals. As GPCRs are involved in many diseases and neurological disorders, they are targets for many presently used medicines and are of utmost interest for the development of novel therapeutic compounds. There is a rich diversity of available ligands for even single GPCR subtype. These ligands are often characterized as inverse agonists that suppress basal activity, full agonists that maximally activate the receptor, partial agonists that produce submaximal activity even at saturating concentrations, and neutral antagonists that occupy the orthosteric binding site but do not affect basal activity. To complicate matters further, the efficacy of a ligand may depend on the downstream signaling pathway used to quantify activity. GPCRs are not simple ON/OFF machines but can exist in thousands of final states thereby leading the cell response in required direction [1]. Although this functional versatility is important for normal physiological signaling, it makes identifying effective therapeutics very challenging.

GPCR ligands are highly diverse and include peptides, nucleotides, lipids, amino acids, and glycoproteins. Although there is little sequence homology among these receptors, the core structure of GPCRs is conserved and consists of seven transmembrane helices that are connected by intracellular and extracellular loops. The recent advances in GPCR crystallography provided about 30 structures of unique GPCRs, which led to discovery and design of novel lead-like compounds through structure-based strategies. The second key step in drug development for GPCRs is identification of compounds with desired selectivity and functional profiles. One of the methods to distinguish agonists and antagonists of opioid receptors is solvent accessible surface area (SASA) [2] of optimized ligand-receptors structures. For other receptors, having more structures crystallized with antagonists but also agonists, more direct methods could be applied to discover GPCR ligands with tailored pharmacological properties.

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S.16-3
Exploiting secondary binding pockets in aminergic GPCRs

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Fragment based drug discovery (FBDD) employs growing and linking strategies for optimization. Structural information on G-protein coupled receptors (GPCRs) made FBDD available on this class of targets, however, most reported programs applied a growing strategy starting from orthosteric fragment binders. We developed a sequential docking methodology to

support the identification of primary (orthosteric) and secondary site binders and linking of these fragment hits. Predicting the binding mode of multiple fragments bound to a single target we assessed the sampling and scoring accuracy for the first and second site binders in self- and cross-docking situations. The prospective validation of this approach was performed on dopamine receptors using the human dopamine D3 receptor crystal structure and a human dopamine D2 receptor homology model. Two focused fragment libraries were docked in the primary and secondary binding sites, and best fragment combinations were enumerated. Similar top scoring fragments were found for the primary site, while secondary site fragments were predicted to convey selectivity. A set of linked compounds created from the best scored primary and secondary site binders were synthesized from which we identified a number of D3 favoring compounds including one with 200-fold D3 selectivity. The structural assessment of the subtype selectivity of the compounds allowed us to identify further compounds with high affinity and improved selectivity.

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S.16-4
In silico exploration of chemical and GPCR ligand space

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G protein-coupled receptors (GPCRs) are the protein class most frequently targeted by present-day drugs, owing to their ubiquitous usage as signal transducers in nature. The past nine years saw the determination of a multitude of X-ray structures of diverse GPCRs. Thanks to these structures, we can now apply structure-based chemoinformatic methods. For instance, we identified binders with predefined binding profiles for chemokine receptors. Moreover, we can investigate the mechanistic behavior of GPCRs through MD simulations.
 I will highlight key lessons learned from docking multi-million compound libraries to different GPCRs. The most prominent example is the first unbiased screen we did with the β 2 – adrenergic receptor, which produced six novel binders – some of them with chemotypes previously undescribed for this target – and a most potent compound with an affinity of 9 nM [1]. I will also show how we identified derivatives of these compounds, further elaborating on the unprecedented chemotypes. Further examples include the chemokine receptors CXCR3 and CXCR4, where we identified potent ligands with tailored selectivity profiles with hit rates exceeding 50 % [2]. The malleability of GPCRs seems to make multi-conformation screenings a good strategy, as we have shown for the A 1 subtype of the adenosine receptors. Different receptor conformations produced different ligand sets, and all in all we identified 20 new ligands for this receptor system. At the low-throughput end, I will talk about the docking-based in-depth analysis of four ligands of the orexin receptor subtype 2 [3]. The challenge in this system were the unusual binding mode of the crystallographic ligand as well as the comparative scarcity of binding site features.
I will also present a database of virtual compounds optimized towards high likelihood of synthetic tractability [4]. It currently holds 21 M compounds, based on approx. 8000 building blocks, thus opening up large areas of chemical space. Two dozen compounds have already

been synthesized and tested in biochemical assays, demonstrating the validity and potential of the approach. I will show compounds that have been designed for the β_2 -adrenergic receptor and the kinase Pim-1.

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S.17-1
Competitive GlyT1 Inhibitors

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Background: Schizophrenia is prevalent in about 1% of the population worldwide. Approved antipsychotic drugs primarily affect positive symptoms such as delusions and hallucinations by modulation of dopaminergic function. In contrast, cognitive and negative symptoms, such as anhedonia, are poorly medicated and represent a large unmet medical need. A potential treatment option for these symptoms could be the modulation of the N-methyl-D-aspartate (NMDA) receptor, whose hypofunction has been linked to pathophysiological processes. Several therapeutic concepts to address this NMDA receptor hypofunction are under investigation such as GlyT1 inhibition, D-serine addition, DAAO inhibition, and KATII inhibition.[1] GlyT1 inhibition is seen as one of the most intriguing options. Indeed, Phase II data from Roche on their non-competitive allosteric GlyT1 inhibitor RG1678 demonstrated efficacy on the negative symptoms of schizophrenia with limited side effect.[2,3] A subsequent phase II/III trial revealed a bell-shaped dose-response curve and changes in hemoglobin.[2,3] AbbVie has focused on competitive GlyT1 inhibitors, offering potential advantages with respect to efficacy and liability for peripheral side effects. Unlike non-competitive inhibitors, AbbVie's inhibitors at efficacious concentrations would be displaced by high glycine, limiting the potential detrimental effects of enhanced glycine levels by GlyT1 inhibition.

Results: Lead optimization led to potent, selective and competitive GlyT1 inhibitors with favorable ADME and PK profiles. In vivo characterization of GlyT1 inhibitors showed good correlation between ex vivo target occupancy and in vivo efficacy in the L687,414-induced hyperlocomotion model. 14d rodent tox revealed no peripheral side effects with regards to hemoglobin levels.

Conclusions: AbbVie has identified potent and selective, competitive GlyT1 inhibitors with good pharmacokinetic properties and in vivo efficacy. In addition these compounds show a linear dose-response curve and no peripheral side effects. As such, selective, competitive GlyT1 inhibitors might proof to be a potential treatment option for negative and cognitive symptoms of schizophrenia while avoiding some of the limitations seen with non-competitive inhibitors.

Disclosures: All authors are employees of AbbVie. The design, study conduct, and financial support for this research were provided by AbbVie. AbbVie participated in the interpretation of data, review, and approval of the publication.

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S.17-2

Analysis of Selvita's libraries: generation of high quality hit compounds for early discovery programs

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The importance of the optimization of physicochemical and ADME properties at early stage of a drug discovery program is now well established. Back in the early 1990's, poor pharmacokinetics accounted for approximately 40% of late stage attrition. The integration of early ADME testing into the drug discovery process helped reduce attrition rates due to poor pharmacokinetics and bioavailability to less than 10% in the year 2000. Finding the right balance between efficacy, ADME and toxicity still remains a challenge for project teams and can require a lot of time and optimization effort. The emergence of *in silico* drug discovery methods (virtual screening, bio- and chemoinformatics, data mining of complex HTS data sets, extensive use of chemogenomics databases) and focused libraries containing high quality novel and diverse compounds have increased the chances to discover hit compounds with good efficacy and optimal properties from the beginning of a project. This has translated into a higher success of obtaining preclinical or clinical candidates. Firstly, this presentation will describe an overview of Selvita and its Drug Discovery Research capabilities. Subsequently, an analysis of Selvita's internal libraries of compounds regarding physicochemical and ADME properties will be discussed. Finally, two case studies will be shown. The first case study describes the discovery of inhibitors of a protein kinase which prevents Tau hyperphosphorylation and results in a reduction of neurofibrillary tangles (NFTs). The screening of our library identified highly selective hits with optimal properties to penetrate the Blood Brain Barrier. Several of them have been selected for further *in vivo* pharmacokinetics studies and have shown good brain exposure. The second case study shows the development of first-in-class small molecule modulators of inflammasome complex which is effective in therapeutic indications like autoimmune disorders. In this study, hit compounds from our library inhibit the production of IL1β in human PBMC stimulated with LPS and nigericin with low micromolar IC50. The compounds have good ADME properties what translates into a significant reduction of IL1β level in animals pretreated with two of them after LPS administration.

S.17-3

Knowledge-based design of a novel selective pan-FGFR kinase inhibitor - CELONKO project
Aleksandra Stańczak, *CelonPharma, Poland*
unsubmitted

S.18-1

Oscillations of intracellular calcium concentration and neural transcription factor expression coding for neural fate determination of embryonic stem cells

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Oscillations of intracellular calcium concentrations participate in many cellular processes. Spatial and temporal patterns of calcium spike and wave activity are important for fate determination in the development of the Xenopus laevis nervous system and participate in neurotransmitter specification of differentiating neurons. As a suggested underlying mechanism for stem cell differentiation into defined phenotypes, calcium spike patterns in neural precursor cells code for the expression and activation of neural transcription factors, which then induce the expression of proteins of an advanced stage of differentiation. Any interference with this calcium transient activity affects the fate of differentiation. Voltage-gated calcium channels and purinergic ATP-activated receptors are already expressed at early stages of development, and in vitro and in vivo data provided evidence for their participation in intracellular calcium transient signaling and control of neural differentiation. Using mouse embryonic stem (ES) cells as in vitro model for neuroectodermal differentiation into neuronal and glial phenotypes, we tracked calcium transient activity together with rhythmic neural transcription factor expression. Time-lapse imaging with calcium-sensitive fluorescent probes was combined with luminescence imaging of stable transfected cells with Mash-1 or Neurogenin-2 promoter-protein fusioned to a luciferase reporter construct. Spontaneous calcium transients observed as spikes or waves, based on their frequency and duration, depended on calcium mobilization from extracellular and intracellular pools, respectively. Further, addition of ATP inducing increases in cytosolic calcium concentration by activation of ionotropic and metabotropic purinergic receptors augmented frequencies and amplitudes of intracellular calcium oscillations together with rhythmic changes in Mash-1 and Neurogenin-2 expression levels. The results of Pharmacological assays let us to conclude that ES cells pre-differentiated into neural stem cells augmented Mash-1 expression as a result of P2Y2 purinergic receptor activity, while expression rates of this neural transcription factor were reduced as consequence of P2X7 receptor activity. Mash-1 and Neurogenin-2 oscillatory expression patterns depended on voltage-gated calcium channel activation, as probed by the time-lapse fluorescence and luminescence imaging technique. Overall, our studies show that temporal oscillations of Mash-1 and Neurogenin-2 expression patterns code for neural phenotypes (differentiation into neurons or astrocytes) as well as for GABAergic neuron specification, providing novel insights into mechanisms of neurogenesis.

S.18-2

Synthetic matrix-assisted hMSC delivery: Defining recovery neurobiology of injured spinal cord

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Mesenchymal stromal stem cells (MSCs) isolated from adult tissues such as bone marrow or lipoaspirate offer tangible potential for regenerative medicine considering their availability and capacity for autologous transplantation. MSCs have been studied in the settings of stroke, amyotrophic lateral sclerosis, and spinal cord injury (SCI), showing promising effects for neurological disorders. However, MSC translational progress has been hampered by poor graft survival, which also prevents a precise understanding of molecular and cellular bases for MSC-mediated neural repair *in vivo*. We have now devised a human MSC (hMSC) delivery formula through investigating the interactive molecular events of hMSCs incorporated in a uniquely designed poly(lactic-co-glycolic) acid scaffold via inflammatory exposures or an original dorsal root ganglia organotypic co-culture system. In a rat model of spinal cord injury, we demonstrated that tailored scaffolding augments hMSC stemness, engraftment, and function, resulting in robust motosensory improvement, neuropathic pain mitigation, tissue damage amelioration, and myelin preservation. The scaffolded hMSCs, without committing neural transdifferentiation, exerted multimodal effects of neurotrophism, anti-autoimmunity, anti-inflammation, angiogenesis, and neurogenesis. We determined that hindlimb locomotor capability was restored by scaffolded hMSC-enhanced integrity and function of sub-midbrain circuits of serotonergic reticulospinal pathway, propriospinal network, neuromuscular junction, and central pattern generator. Therefore, the combinatorial regimens described have provided an adult stem cell platform for investigating molecular events underlying the neural repair impact of non-transdifferentiated hMSCs. Our approach enabled investigation of mechanistic essentials of recovery neurobiology for injured mammalian spinal cord. The uncovered neural circuits as well as molecular and cellular targets offer biological underpins for designing clinical rehabilitation protocols and developing therapeutics to treat disabilities and complications of traumatic SCI.

S.18-3

Neuroprotective activity of hematopoietic stem/progenitor cells

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Advance of research on pathophysiology of stem cells (SCs) gives a chance on elucidation of the mechanisms regulating both normal human development as well as pathologic processes. Recently, a growing body of evidence suggests that SCs might be used in the adjuvant therapy of severe neurodegenerative disorders. Although there is currently a lack of compelling evidence for the effectiveness of cell-replacement therapies, the main benefit that is accessible from transplanted cells would be paracrine secretory activity, which is theoretically more convincing and fits nicely into the concept of adjuvant/supportive roles for SC-based therapy. Neurotrophic factors regulate survival, development, and function of nervous tissue. Illumination of their physiological role in the maintenance of central nervous system

homeostasis as well as regeneration of damaged tissue have ignited expectations to heal neurodegenerative diseases, including amyotrophic lateral sclerosis. It has been demonstrated that SCs that are genetically modified with viral vectors (e.g., MSCs transduced to express NT-4) are capable of long-term survival after transplantation when NTs (e.g., NT-4 or BDNF) are continuously delivered and that this survival results in significant improvements in functional parameters that are observed with objective methods. On the other hand, there are evidences for the presence of neurotrophins and their receptors in distinct hematopoietc cell populations derived from umbilical cord blood and bone marrow, showing that these cells express NTs and NT receptors at both the mRNA and protein levels. Of note, NT expression is greater under stress-related conditions. Interestingly, autohemotransfusion of cord blood in premature newborns may lead to improvement of peripheral blood parameters as well as influence the probability of development of some typical complications. Furthermore, bone marrow-derived hematopoietic Lin- SCs *administered* via a lumbar puncture noticeable modulated expression of both NFs as well as angiopoetic and proinflammatory factors in the cerebrospinal fluid. Overall, the advances in experimental studies suggest that hematopoietic SC-based therapy might represent a novel treatment modality for the repair and regeneration of injured neural tissue. However, further extensive studies are definitely required to understand the mechanisms of SC actions, particularly their paracrine activities, and to present SCs as a new treatment option for clinical approaches.

S.18-4

Progenitor cell imaging in cardiovascular regeneration: A need or a fancy?

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Background: Our team, focused since 2002 on the interface between basic and clinical science in cardiovascular regeneration medicine, early identified several fundamental, unresolved issues of progenitor cell therapy in acute myocardial infarction in the context of both basic mechanisms and clinical implementation.
Material and methods: In 2003-2016 we embarked on pre-clinical and clinical investigator-initiated myocardial regeneration research projects aimed to elucidate some fundamental issues that we have identified. Our work centered on (i) verification and optimization of cell delivery using the transcoronary route, (ii) visualization of progenitor cells myocardial uptake area in relation to the infarcted zone, and (iii) identification of the determinants of myocardial cell uptake in recent infarction, (iv) development and implementation of novel cellular therapy approaches, leading to first-in-man investigations.
Results: Our principal achievements include (1) design, bench-testing and evaluation in a randomized study in man of a novel system for physiological transcoronary administration of cellular therapies, (2) clinical implementation of the cell-delivery system that we developed, (3) identification of the infarct border-zone, rather than the central zone, as the area of preferential uptake of progenitor cells (using, for the first time, hybrid imaging combining the most sensitive technique for progenitor cell visualization in vivo in humans with state-of-the

art myocardial infarct imaging in vivo), (4) identification of the infarct size, rather than the degree of global left ventricular contractility impairment as a key factor of the magnitude of progenitor cell homing, and (5) novel therapeutic approaches involving the use of allogenic but largely non-immunogenic cell types available 'off-the-shelf' and on large scale (first-in-man studies).

By today, over 200 patients have been studied by our team in investigator-run and industry-supported clinical projects of myocardial regeneration in acute myocardial infarction and heart failure.

Hybrid imaging, combining single-photon emission tomography (cells) and magnetic resonance techniques (tissue to be regenerated) that we introduced for the first time, has played a key role in our investigations.

Conclusions: In conclusion, our work has played an important role in advancing the concept of regeneration therapy in patients with the thus-far 'irreversible' ischaemic loss of myocardial contractile tissue. Our fundamental contributions to the progress of the field have received wide international recognition (cf. http://www.nature.com/nmat/journal/v13/n2/full/nmat3868.html).

We continue to actively contribute to further independent academic research and, in parallel, large-scale research projects in collaboration with industry to progress the field to the stage of routine clinical applications.

S.19-1

Aversive memory and its modulation in an animal model of stressor-related disorder

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A number of brain diseases, from Alzheimer's disease to anxiety disorders, are characterized by alterations in memory functioning, which make research findings on memory processes particularly appealing for their potential clinical implications. Memory consolidation can be schematically described as the time-dependent stabilization of a memory trace, through the involvement of neural systems and cell processes, which leads to long-term memory formation. As concerns memory for aversive events, in different studies we have contributed to unveil the crucial role of different neural mechanisms underlying the consolidation (and reconsolidation) of fear memories. Fear allows organisms to cope with dangerous situations and remembering these situations has an adaptive role. However, recalling memories for traumatic events can induce re-experiencing the trauma, thus resulting in a maladaptive fear and psychopathological disorders in humans, such as Post-Traumatic Stress Disorder (PTSD). In this context, we have found that fear sensitization, a non-associative component of fear memory, is mainly involved in the onset of PTSD-like symptoms in an animal model, suggesting the importance of targeting fear sensitization to improve behavioural strategies aimed at mitigating long-lasting outcomes of traumatic experience.

Indeed, we have found that the reduction of fear sensitization after a traumatic experience, through an unconditioned stimulus (US) re-evaluation procedure, was able to restore an

adaptive expression of fear and to persistently attenuate anxiety levels. Since fear sensitization takes on an important role in the onset of PTSD, we decided to further investigate the nature of this memory component. In particular, we decided to study its relationship with generalization, the response not just to the original conditioned stimulus, but also to new stimuli that resemble that stimulus. Our results argue in favour of a substantial independence between the two processes. Results show that (i) footshock intensity differentially affected generalization and sensitization, (ii) generalization increased over time while sensitization was time-independent, (iii) context similarity and contextual memory retrieval affected generalization but not sensitization, (iv) the lack of ERK1 protein in knockout mice increased sensitization but not generalization.

Overall, these results indicate that sensitization has to be taken carefully into account when therapeutic approaches for traumatic disorders are designed.

Acknowledgements

S.19-2

Molecular adaptations of signaling pathways in animal models of addiction

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Disturbances in the glutamatergic projections arising from the dorsomedial prefrontal cortex (dmPFC) to the nucleus accumbens (NAc) underlie addictive diseases induced PFC hypofunction and contribute to drug seeking behavior. Studies in rat model of cocaine self-administration (SA) demonstrated that early withdrawal from cocaine is a critical time point for development of ERK dependent intracellular signaling alterations within dmPFC responsible for deficits in cortico-acumbal glutamatergic transmission and compulsive behavior[1]. Two hours after last cocaine SA session significant decrease in tyrosine phosphorylation of ERK/MAP kinase was observed. Simultaneously, in presynaptic terminals of NAc occurred the increase in phosphorylation of synapsins, the proteins regulating neurotransmitter release. Infusion of BDNF into dmPFC immediately after last cocaine SA normalized changed by cocaine phosphorylation level of ERK in dmPFC and synapsins in NAc. Moreover, BDNF infusion restored normal glutamate release in NAc three weeks later during the cocaine-primed reinstatement test and attenuated compulsive behavior for so long. Further results revealed significant decrease in tyrosine phosphorylation of NMDA receptor subunits GluN2B, GluN1 in PFC of rats two hours after last cocaine SA session. These changes were accompanied by increased activation of striatal-enriched protein tyrosine phosphatase (STEP), indicating STEP as a mediator of the ERK dependent deficits in corticostriatal neurotransmission[1].

Whereas the ERK dependent neuroadaptation was proven in addiction, the up-regulation of cAMP signaling seems to be engaged during more prolonged abstinence. Our previous studies of the expression of G alpha subunits (G□) in rat models of addiction revealed that morphine place preference reduced mRNA levels of G□□i□□□ in PFC and NAc and increased G□(s) suggesting tendency to up-regulation of cAMP pathways in corticostriatal projection in morphine addiction [2]. In cocaine SA model one week of abstinence increased phosphorylation of PKA targets: CREB, GluA1 in dmPFC and synapsinI in the NAc. Intra dmPFC infusion of

PKA inhibitor (Rp-cAMPs) on day 7 of abstinence normalized these phosphorylation levels. Moreover, the inhibition of PKA reduced cocaine seeking in cue-induced relapse test.
Understanding of mechanisms of the addictive drugs-induced deficits of signaling transmission in corticostriatal projection and their progress over time may help to create pharmacological strategies to reduce the compulsive behavior and cease progression of addiction disease.
The lecture originated in the frame of cooperation between the Italian National Research Council (CNR) and the Polish Academy of Sciences PAS. The work was supported by statutory funds of the Institute of Pharmacology PAS.

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S.19-3

Compensatory mechanisms as possible targets of pharmacotherapy in neurodegenerative diseases - study on transgenic models

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Neurodegenerative diseases represent one of the main causes of mental and physical disability, especially among highly developed societies. The etiology of the majority of late-onset neurodegenerative diseases remains undefined and even for those caused by identified genetic mutations, the direct pathway leading from the gene alteration to the final cell death has not yet been clearly elaborated. Currently available pharmacotherapy relies on disease symptomatology, and – apart from alleviating typical features – do not restores neuronal functioning neither prevent cell loss.
Most of the classic animal models of neurodegeneration are based on applying neurotoxins – an effective strategy for studying the phenotype but generating immediate neuronal death, thus giving poor opportunity to observe molecular changes associated with real, slow neurodegenerative process. Advancements in genetic engineering over the last two decades have brought many transgenic lines exploited as an alternative, genetic models of various neurodegenerative diseases. These were created either by targeting precisely the same causative genes as involved in human disorders (e.g. Huntington's disease, some rare familiar forms of Parkinson's and Alzheimer's disease), either the genes controlling subcellular changes and processes affected in diverse neuropathological conditions: oxidative stress, rRNA synthesis, inflammation, mitochondrial and proteasomal dysfunction, accumulation of protein aggregates, apoptotic and autophagic responses. Surprisingly, many of these models did not fully recapitulate the inevitable neuronal loss or at least not to this extend as expected, supporting the concept that different genetic, cellular and environmental factors may contribute to the final cell death. Some failed even to show phenotypic alterations associated with modelled diseases, providing further evidences that humans and primates are more vulnerable than mice to the same triggers inducing neurodegeneration. These observations provided clues into the possible compensatory mechanisms that may protect neurons from death in evaluated models. In our own study we focus attention mainly on the role of noradrenaline in Parkinson's disease (PD) as this neurotransmitter has been recently proposed to be involved in compensatory effects in PD dopaminergic neurodegeneration. We have shown that stimulation of noradrenergic system

may indeed enhance the resistance of dopaminergic neurons in new genetic mouse model of PD based on ribosomal stress.

Elucidating these mechanisms may further our understanding of the preclinical deficits observed in neurodegenerative diseases and provide insight into the pathogenic triggers underlying the initial, symptomless phase of their onset. These could lead to opportunities for more successful, neuroprotective and neurorestorative therapies, not only those restricted to the compounds that simply delay the onset of degeneration.

S.19-4

MicroRNAs biogenesis in Parkinson's disease - targeting DICER activity in dopamine neurons

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Background: MicroRNAs are small non-coding RNAs that regulate mRNA stability and translation efficiency providing additional dimension to the landscape of gene regulation. MicroRNAs are expressed as pri-microRNAs which are processed in the nucleus to pre-microRNAs and then in the cytoplasm by endonuclease Dicer to mature microRNAs. Recently it has been proposed that disturbances in microRNAs biogenesis contribute to cancer, depression and degeneration of motoneurons in ALS. Moreover, rapid upregulation of Dicer activity is required for specific response to neurotrophins, while decreased Dicer expression has been observed in aging tissues, including the brain. We investigate here the importance of Dicer and microRNAs biogenesis pathway for the survival and functions of dopamine (DA) neurons.
Materials and methods: We studied the role of Dicer in DA neurons by: 1) selective tamoxifen-inducible ablation of *Dicer1* gene in adult DA neurons of Dicer/DATCreERT2 mice; 2) pharmacological upregulation of Dicer activity by enoxacin in cultured embryonic midbrain DA neurons; 3) assessment of *Dicer1* and microRNAs abundance in aged midbrain DA neurons.
Results: Deletion of Dicer in adult DA neurons causes their progressive degeneration accompanied with the loss of striatal dopamine and motor deficits. Conversely, upregulation of Dicer activity with enoxacin improves DA neurons survival in culture. We also observe downregulation of Dicer mRNA and decreased microRNAs levels in aged DA neurons.
Conclusions: The age-related decrease of Dicer levels and reduced microRNAs biosynthesis can contribute to DA neurons vulnerability. Increasing microRNAs abundance is a potentially viable neuroprotective strategy for DA neurons in Parkinson's disease.

S.19-5
Structure, biology and therapeutic potential of CDNF/MANF family of neurotrophic factors

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Current drug treatments for neurodegenerative diseases are symptomatic and there is no treatment to stop or slow down the progression of the disease. Cerebral dopamine neurotrophic factor (CDNF) and mesencephalic astrocyte-derived neurotrophic factor (MANF) compose a novel CDNF/MANF-family of neurotrophic factors with unique structure and mode of action. We have shown that CDNF and MANF protects dopamine neurons, but more importantly, restores dopamine circuitry when given after 6-hydroxydopamine or 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine in rat and mouse Parkinson's disease animal models, respectively. We have also shown that MANF protects neurons also against cortical ischemia-reperfusion injury when given as recombinant proteins or expressed via adeno-associated viral vector. Invertebrates have a single protein homologous to vertebrate CDNF and MANF. MANF and CDNF are expressed in the brain and particularly in secretory tissues. Drosophila MANF knockdown leads to severe axon degeneration in dopaminergic neurons and in elevated levels of endoplasmic reticulum (ER) stress markers. MANF knockout mice develop type 1 diabetes and have also elevated ER-stress. CDNF and MANF structures consist of two domains: an amino-terminal saposin-like domain that may interact with lipids and a carboxy-terminal domain that may protect cells against endoplasmic reticulum stress. Carboxy-terminal domain of MANF has two important motifs: the CKGC sequence (a CXXC motif) that could be involved in redox reactions and the C-terminal RTDL sequence, an ER retention signal. We have recently shown that CKGC sequence is important for neuroprotective effect both in vitro and in vivo, and that RTDL deletion abolished the neuroprotective effect in vitro, but not in vivo when applied as recombinant protein to cerebral cortex before middle cerebral artery occlusion in rats. Recent data indicate that their mode of action differs from other neurotrophic factors and in addition to conventional support for neuronal survival their protective effects are mediated by mechanisms involved in ER stress.

S.20-1
(*R,R'*)-4'-methoxy-1-naphthylfenoterol acts as bitopic, GPR55 + β2-adrenergic receptor, ligand in various tumor cell lines

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Background: (*R,R′*)-4′-methoxy-1-naphthylfenoterol (MNF) was designed as an agonists of β2-adrenergic receptor (β2AR), but it is also a potent competitive inhibitor of the oncogenic GPR55 receptor.

Materials and methods: The antitumorigenic effects of MNF effect were investigated using [³H]-thymidine incorporation, cell motility assays, western blotting, and internalization of the fluorescent GPR55 ligand – T1117.

Results: MNF inhibited the proliferation of 1321N1 and U118 astrocytoma cell lines and UACC-647, M93-047 and UACC-903 melanoma cell lines in a β2AR-dependent manner. These effects of MNF were mediated by the activation of adenylyl cyclase, accumulation of cAMP and PKA phosphorylation. Furthermore, MNF elicited rapid drop in the ERK phosphorylation and inducted the activation of eEF2, thus blocking *proproliferatory* signaling and inhibiting the protein synthesis, respectively.

MNF inhibited protumorigenic signaling in GRP55-positive human HepG2 hepatocarcinoma and PANC-1 pancreatic carcinoma cells. MNF attenuated the internalization of fluorescent GPR55 agonists T1117 and inhibited cell motility in *in vitro* hound healing assay. In the mouse xenograft model, daily MNF treatment for 21 days led to a ~2-fold reduction in the expression of EGFR, PKM2, β-catenin in PANC-1 tumor tissue relative to vehicle-treated controls. These data suggest that MNF exerts its antitumor effects by inhibiting GPR55-mediated activation of selected cancer biomarkers.

Conclusions: In conclusion, MNF is a bitopic ligand that acts as an agonist of the β2-adrenergic receptor and an antagonist of GPR55 and, therefore, may have therapeutic potential for the management of cancer.

S.20-2

The alterations in blood plasma chitotriosidase immunoblotting pattern and O-glycans expression are associated with type 2 diabetes?

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Background: Chitotriosidase (CHIT1) is the first active chitinase discovered in human plasma. The exact functions of this enzyme are unexplained, but its involvement in the immunological and inflammatory processes has been suggested. Increased level of CHIT1 is also observed in type 2 diabetes (T2D). Since immunoreactivity and glycosylation profile of this protein have not been studied yet, we analyzed CHIT1 immunoblotting pattern and O-glycans expression in healthy subjects and T2D patients.

Materials and methods: CHIT1 concentration and activity were measured using immunoenzymatic and fluorometric techniques, respectively. Plasma samples from healthy and diabetic individuals, were pooled and electrophoresed in SDS-polyacrylamide gel. In Western

blotting, specific antibodies anti-human CHIT1 were applied and chemiluminescent substrate was used for detection of CHIT1 bands. The glycosylation was studied with lectin-ELISA with biotinylated lectins: Jacalin, *Maclura pomifera* and *Vicia villosa*, detecting complete and truncated O-glycans, respectively.

Results: We observed significantly higher CHIT1 concentration and activity in diabetic patients than in controls (p<0.001). In the examined groups, bands corresponding to CHIT1 molecular mass had slightly different locations: 49 kDa and 45 kDa for healthy and T2D subjects, respectively. The expression of glycotopes reacting with applied lectins was lower in T2D and significantly different in analyzed groups (*p*<0.01).

Conclusions: The differences in molecular masses of CHIT1 in examined groups may derived from the alterations in CHIT1 glycosylation observed in our study. In diabetes the higher CHIT1 concentration and activity was accompanied by significantly decreased CHIT1 reactivity with lectins, what may affect CHIT1 properties and have association with disease progression. Further analysis of CHIT1 glycome may be helpful in better understanding of chitotriosidase biological function in T2D pathology.

S.20-3

The impact of incretin-based drugs on some non-traditional risk factors for cardiovascular disease (CVD) in rats with fructose-induced metabolic syndrome

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Background: Incretin-based drugs, currently used as antihyperglycemic agents for treatment patients with type 2 diabetes, apart from the ability to reduce blood glucose can also exert several cardioprotective effects. The present study was undertaken in order to answer the question whether the four-week treatment with sitagliptin (a dipeptidyl peptidase-4 inhibitor [DPP-4i]) or exenetide (a longer-acting glucagon-like peptide 1 receptor agonist [GLP-1a]) can influence some nontraditional risk factors for CVD, such as plasma level of oxidized low density lipoprotein (oxLDL), endothelin 1 (ET1), monocyte chemoattractant protein 1 (MCP-1), platelet activating factor (PAF), apolipoprotein A-I (ApoA-I) as well as brain natriuretic peptide (BNP).

Materials and methods: Normal (CON) and fructose-received (FRU) rats were treated with sitagliptin (S) (5.0 and 10 mg/kg p.o) or with exenatide (E) (5 and 10 μg/kg, s.c.). Additionally, NaCl injected (NaCl) and caloric restricted(RC) groups were created. Plasma levels of oxLDL, PAF, ET1, MCP-1, ApoA-I and BNP were measured by enzyme immunoassay technique.

Results: No significant differences were found in plasma oxLDL, PAF, MCP-1, ET1, ApoA-I and BNP concentrations between groups CON *vs* FRU. The treatment with exenatide resulted in oxLDL concentration reduction in CON+E10 *vs* CON+NaCl (-33,54%, P<0,05) and in FRU+E5 and FRU+E10 *vs* FRU+RC (30,06% and -25,08%, respectively; P<0,05). Neither exenatide nor sitagliptin administration affects the plasma levels of PAF and MCP-1. Sitagliptin

had no impact on ET1, ApoA-I and BNP concentrations but exenatide increased ET1 in CON+E5 *vs* CON (+137%; P<0,001), ApoA-I in FRU+E5 *vs* FRU (+221,15%; P<0,001) and decreased BNP in FRU+E5 and FRU+E10 by 36,3% (P<0,05) and 36,6% (P<0,05), respectively.

Conclusions: Incretin-based drugs have heterogenous impact on the CVD risk. Sitagliptin (DPP-4i) seems to be neutral, but exenatide (GLP-1a) can improve some nontraditional risk factors for CVD.

S.20-4

Brain serotonin regulates the expression of cytochrome liver P450 via neuroendocrine pathways involving the paraventricular and arcuate nuclei of the hypothalamus

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Background: Changes in functioning of the brain serotonin system may occur during pathological states (e.g. depression) or pharmacotherapy. The paraventricular (PVN) and arcuate nuclei (ARC) of the hypothalamus produce hormones regulating the expression of cytochrome P450 (CYP), such as CRH, TRH and somatostatin (PVN) or GHRH (ARC).

Materials and methods: Experiments were performed on male Wistar rats. A set of experiments consisting of serotonergic system damage or activation was performed to prove that changes in the brain serotonin level (especially in the PVN or ARC) can affect the expression and activity of CYP isoforms in the liver.

The concentrations of serotonin, dopamine and noradrenaline were measured in different brain structures. The activity and expression level of CYP isoforms in the liver microsomes were estimated (using HPLC, Western Blotting and real-time PCR respectively). The hormone and cytokine levels were measured in the serum using ELISA kits.

Results: Serotonergic system of the brain exerts a negative effect on the expression of CYP isoforms: 1A1/2, 2C11 and 3A1/2 in the liver. Changes in the expression of cytochrome P450, caused by damage or by activation of serotonergic system are opposite and hormones involved in this regulation are growth hormone, testosterone, corticosterone and thyroid hormones. The serotonergic innervation of the paraventricular nuclei (PVN) regulates CYP2C11 expression negatively, while that of the arcuate nucleus (ARC) regulates this isoform positively *via* GH secretion.

Conclusions: The obtained results indicate the involvement of the brain serotonergic system in the neuroendocrine regulation of liver cytochrome P450 expression, which may be of physiological and pharmacological significance.

S.20-5

Proteomic analysis of mitochondria isolated from the frontal cortex and hippocampus of apolipoprotein E knockout mice treated with Alda-1, an activator of mitochondrial aldehyde dehydrogenase (ALDH2)

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Background: Recently, it has been shown that mitochondrial dysfunction and oxidative stress play important roles in the pathogenesis of neurodegeneration. Mitochondrial aldehyde dehydrogenase (ALDH2), an enzyme responsible for the detoxification of reactive aldehydes, such as 4-hydroxy-2-nonenal (4-HNE) - the end products of lipid peroxidation, is considered to exert protective function in mitochondria.

Material and methods: The aim of our study was to use the methods of differential proteomics in concert with molecular and morphological techniques to elucidate the changes in the frontal cortex and hippocampus of apolipoprotein E knockout (apoE$^{-/-}$) mice (an animal model of the early stages of Alzheimer's disease) upon prolonged treatment with Alda-1 – a small molecular weight activator of ALDH2.

Results: Despite the lack of significant morphological changes in the brain of apoE$^{-/-}$ mice as compared to age-matched wild type animals, proteomic and molecular approach revealed many changes in genes and proteins expression indicating the impairment of energy metabolism, neuroplasticity and neurogenesis in brains of apoE$^{-/-}$ mice. Importantly, prolonged treatment of apoE$^{-/-}$ mice with Alda-1 led to the beneficial changes in genes and proteins expression related to neuroplasticity and mitochondrial function.

Conclusions: The pattern of changes suggested mitoprotective effect of Alda-1, however the exact functional consequences of the revealed alterations require further investigation.

S.20-6

Influence of dehydroepiandrosterone on pain threshold and monoamines concentration in STZ-induced neuropathic pain model

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Background It is well known that dehydroepiandrosterone (DHEA) has an ambiguous influence on nociception. It shows pronociceptive effects after acute administration, whereas under chronic treatment it induces an opposite effect. The aim of the study was to investigate the impact of DHEA on pain thresholds in a rat model of diabetic neuropathy and examine its effects on monoamine neurotransmission.

Material and methods. Studies were performed on male Wistar rats. Changes in nociceptive thresholds were determined using mechanical and thermal stimuli. Diabetes was induced by a single administration of STZ at 40 mg/kg. DHEA was administered orally at 10 mg/kg for 14 consecutive days. After a sub-chronic treatment with DHEA, rats were given 6-hydroxydopamine (6-OHDA, 20μg) intrathecally 3 days before testing. Another group received 4-Chloro-L-phenylalanine (p-CPA, 200 mg/kg) intraperitoneally. Monoamine levels (NA, 5-HT) were analyzed in homogenates from the L4-L6 segment of the spinal cord and from the periaqueductal grey (PAG) with the HPLC method.

Results: Pronociceptive effects surfaced after acute treatment with DHEA, whereas sub-chronic administration produced an antihyperalgesic effect in response to mechanical and thermal stimuli. DHEA alone increased NA and 5-HT concentrations in SC and PAG after sub-chronic treatment, whereas both toxins lowered the levels of these monoamines. Thus, depletion of NA and 5-HT was correlated with a decrease in the analgesic activity of DHEA.

Conclusions: Our results suggest that DHEA could be indicated as a drug to improve treatment of neuropathic pain disorders. Moreover, one of the possible mechanisms of its action involves modulation of monoamine concentration, previously shown to have a significant impact on neuropathic pain management

S.20-7
MMP-9 activity: friend or enemy of traumatic brain injury?

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Background: Epilepsy in 20% of cases, develops as an effect of traumatic brain injury (TBI). Recent evidences indicate important role of extracellular matrix metalloproteinase-9 (MMP-9) in neuronal circuitry remodeling and synaptic plasticity. The aim of the present study was to establish and characterize the experimental model of TBI and evaluate the MMP-9 activity changes and dendritic spines reshaping after brain injury.

Material and methods: As an animal model of TBI we used Controlled Cortical Impact CCI. After different time points we analyzed: lesion volume, MMP-9 activity and changes in dendritic spines parameters.

Results: Progressive degeneration of cortex (Cx) and structural changes in the hippocampus (Hp) were observed during 30d after brain injury. This effect was MMP-9 expression level dependent. In mice with deficiency of MMP-9 the degeneration volume degree was significantly lower, compared to wild type mice and mice with overexpression of MMP-9. The gel zymography analysis showed strong time-associated elevation of MMP-9 activity in ipsilateral Cx and Hp within 30d after CCI. Density of the spines labeled with fluorescent dye DiI, measured after 7 and 14d after CCI was significantly decreased in ipsilateral Cx and CA1 field of Hp. As levels of MMP-9 are altered in brain after trauma, we aimed also at characterizing variations in dendritic spines shape related with its function. Length to width ratio of spines as a main parameter describing spine shape, was decreased in ipsilateral Cx, CA1 and DG what indicates spine shortening. Moreover we observed increase of spine head width in ipsilateral CA1 and DG.

Conclusions: We described the correlation between TBI and MMP-9 activity & action and indicated that MMP-9 might be important for major dendritic spine reshaping, observed after brain injury, which in consequence may lead to altered sensibility of neuronal circuits to trigger seizures.

S.20-8

Imipramine attenuates mitochondrial disturbances in frontal cortex- study in animal model of depression

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Background: It is well known that mitochondria are intracellular organelles crucial in the production of energy. The CNS is highly dependent on oxidative metabolism hence mitochondrial dysfunction affects neuronal function and could form the basis of psychiatric disorders. Therefore, mitochondria may be an interesting target for therapeutic intervention. We investigated the impact of prenatal stress and chronic imipramine treatment on the mitoproteome of the frontal cortex of adult male rats after prenatal stress.

Materials and methods: Pregnant SD rats were subjected to stress sessions from 14th day of pregnancy until delivery. Adult control and prenatally stressed rats were injected with imipramine (10mg/kg i.p.) once a day for 21 days. Rats were decapitated 24 hours after the last injection. 2DE-LC-MS/MS was applied to investigate the changes of expression mitochondrial proteins.

Results: 2D electrophoresis coupled with MS identified 9 differentially expressed mitochondrial proteins, which belonged to the groups of metabolic enzymes, apoptosis regulators and oxidative stress factors in frontal cortex of prenatally stressed rats treated with imipramine compared to appropriate control. The most spectacular was up-regulation of 2',3'-cyclic-nucleotide 3'-phosphodiesterase (fold change +2.7). Recently, the involvement of this protein in the suppression of brain inflammatory processes is raised. Moreover, 2',3'-cyclic-nucleotide 3'-phosphodiesterase also exhibits anti-apoptotic action, provides Ca2+ buffering and maintains membrane homeostasis.

Conclusions: Our study demonstrated the impact of imipramine administration on the brain mitochondria proteins in frontal cortex of prenatally stressed rats. Based on our data the anti-inflammatory and anti-apoptotic imipramine action may be postulated. The exact consequences of the revealed alterations require further investigation.

POSTER SESSIONS

P.1 Preclinical research – Neurobiology

P.1-01
Behavioral sensitization to mephedrone in mice

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Background: Mephedrone is a cathinone derivative that possesses powerful psychostimulant and hallucinogenic effects. It has been speculated that mephedrone may act by increasing release and reuptake inhibition of serotonine and dopamine. Mephedrone has a high abuse and health risk liability, with increased tolerance, impaired control and a compulsion to use, the predominant reported dependence symptoms. However, the precise mechanisms underlying its psychoactive effects remain unclear. The present study was aimed to show the mephedrone sensitization to locomotor activity in mice.

Material and methods: Sensitization to locomotor activity was induced by chronic administration of mephedrone in (2.5 and 5 mg/kg/day ip, 5 days) in male albino Swiss mice. Locomotor activity was assessed for 10 and 30 min, following the first and fifth dose of drug,. After a 7- or 14-day interval, acute dose of mehedrone (2.5 mg/kg or 5 mg/kg respectively) was administered and locomotor activity was assessed in a similar manner.

Results: The present experiments demonstrated that administration of mephedrone in the doses of 2.5 and 5 mg/kg for 5 days, its 7- or 14-day cessation and followed injection of the challenge dose of mephedrone (2.5 and 5 mg/kg respectively) induces behavioral sensitization to locomotor activity in mice.

Conclusions: Repeated exposure to mephedrone induced behavioral sensitization in mice. Further research on the mechanism of mephedrone sensitization is warranted.

P.1-02
Interaction of mephedrone coadministration with diazepam or ethanol in a plus-maze in mice

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Background: Mephedrone is a substituted cathinone derivative that has gained popularity over recent years as a recreational drug due to its powerful psychostimulant and hallucinogenic effects. It acts as a non-selective substrate for monoamine transporters, facilitating neurotransmitters release. However, both precise mechanisms of its action and the effects of coadministration of mephedrone with other psychoactive drugs remain unknown. The present study investigated the effects of an acute mephedrone injections with ethanol or diazepam, which mechanisms of action are connected with GABAergic neurotransmission.

Material and methods: The experiments were carried out on male Swiss mice. Mephedrone (0.05, 0.125 and 0.25 mg/kg), diazepam (0.2 mg/kg) and ethanol (1.2 g/kg;10% solution) were administered intreperitoneally (ip). The anxiety-like behaviour was examined in the elevated plus-maze (EPM) in mice.

Results: The present experiments demonstrated that administration of mephedrone at the doses of 0.05 and 0.125 mg/kg with the threshold dose of diazepam resulted in significant anxiolytic effect in the EPM in mice. Interestingly, we have observed the intensification of anxiolytic effect after coadministration of the lower dose of mephedrone (0.05 mg/kg) with ethanol and anxiogenic effect after coadministration of the higher dose of mephedrone (0.25 mg/kg) with ethanol.

Conclusions: The obtained results indicated that mephedron may influence, at least partly, GABAergic neurotransmission. Additionally, these experiments are of special significance, since alcohol and diazepam are widely co-abused with amphetamine derivatives such as mephedrone.

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P.1-03
Brain development, schizophrenia and enriched environment

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Background: Schizophrenia is a neurodevelopmental disease with genetic and environmental background. The first symptoms of schizophrenia are seen in adulthood, but the factors cause the risk of this disease might be present in early stages of life. Our previous study showed that prenatal administration of methylazoxymetanol (MAM) modeling neurodevelopmental aspects of schizophrenia evoked the decrease in GAD67 mRNA and H3K4me3 protein expression in the medial prefrontal cortex (mPFC) of adult rats. In the present study we investigated, whether

the exposure to enriched environment (EE) during early adolescence is able to modulate MAM-induced alternations in GAD67 and H3K4me3 expression in adult animals.

Material and methods: Rat pregnant females were injected ip with 22 mg/kg MAM or saline at E17. Animals were housed in an EE or standard environment (SE) 24 hours for 7 days in early adolescence (P23-P29). The expression of GAD67 mRNA and protein level was measured in the mPFC of adult rats at P70 using RT PCR and Western blot analysis, respectively. We also used immunoprecipitation assay (ChIP) to revealed changes to histone modification (H3K4me3) at the Gad1 promotor after prenatal MAM injection.

Results: The present study indicates that EE exposure in early adolescence prevented the decrease in GAD67 mRNA and protein level in adult mPFC induced by prenatal MAM administration. We also observed that exposure to EE blocked the decrease in association of the Gad1 promotor with H3K4me3 in the mPFC induced by MAM administration.

Conclusions: The obtained results indicate that exposure to EE in early adolescence regulates the GAD67 expression in adult mPFC altered by prenatal MAM administration via epigenetic mechanisms and may attenuate the neurochemical changes which are responsible for appearance of schizophrenia-like symptoms.

P.1-04

The involvement of adenosinergic system in sensitization to morphine withdrawal signs in rats – changes in dopamine receptor expression

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Background: Experimental data inform that not only do the dose and time duration of dependent drugs affect the severity of withdrawal episodes. Previous withdrawal experiences may intensify this process, which is referred as sensitization to withdrawal signs. Adenosine and dopamine (DA) receptors may be involved in this sensitization.

Material and Methods: Rats were continuously and sporadically treated with increasing doses of morphine for 8 day. In rats, sporadically treated with morphine, morphine administration was modified by adding 3 morphine-free periods. The selective A1 or A2A adenosine agonists were given during each of the morphine-free periods (6 injections in total). On the 9ᵗʰ day, morphine was injected. One hour later, naloxone was administered to induce morphine withdrawal signs. The animals were placed into cylinders and the number of jumpings was recorded. Next, the rats were decapitated and brain and brain structures (striatum, hippocampus and prefrontal

cortex) were dissected for molecular experiments (qRT-PCR and western blotting analysis) within DAergic pathways.

Results: We demonstrated that previous experiences of opioid withdrawal intensified subsequent withdrawal signs. Adenosine ligands attenuated the sensitization to withdrawal signs. Significant differences in mRNA and protein expression of DA receptors were observed between continuously and sporadically treated with morphine rats. Adenosine agonists modified the expression of DA receptors.

Conclusions: Results demonstrated that intermittent treatment with morphine induced alterations in the DAergic system which may be responsible for sensitization to morphine withdrawal signs. Although adenosine ligands attenuate this sensitization, they are not able to fully restore the physiological brain status.

P.1-05

Plasma concentration of oxidative stress biomarkers in a group of patients with peri-implantitis

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Background: Peri-implantitis includes both soft tissue inflammation and a progressive loss of supporting bone in dental implants. The elevation of lipid peroxidation (LPO) and protein carbonylated (PC) levels occurs in chronic inflammatory processes. The purpose of this research was to determine whether the patients with peri-implantitis have a higher plasma concentration of LPO and PC products, which considered being two important of oxidative stress markers, and comparing these data with the plasma of blood from patients without peri-implantitis.

Methods: A survey carried out on 84 patients with dental implantation of both sexes. Among them 31 patients were without complications and 53 patients were with peri-implantitis. In the plasma was determined the content of LPO and PC products.

Results: In the patients after implant, therapy observed a moderate increase of LPO and PC levels. Development peri-implantitis resulted in a greater enhancement of free- radical oxidation in the blood. Schiff bases and PC concentration were slightly higher in the peri-implantitis group than in the healthy groups of patients. Moreover, Schiff bases concentration was higher in the peri-implantitis group than in the group of the patients without complication.

Conclusions: The plasma concentration of Schiff bases in patients after implant therapy can be consider as a marker of the peri-implantitis development.

P.1-06

Signaling pathways involved in the antidepressant-like effect of zinc

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Background: Recent studies indicated that antidepressant-like effect of zinc observed in forced swim test (FST) in rats is associated with the modulation of glutamate signaling through the NMDA or AMPA receptors and the serotonergic system through the 5-HT1A receptors. The aim of these studies was to investigate the hypothesis that antidepressant-like effect of zinc observed in FST might be also mediated through the modulation of PKA, CaMKII, MAPK/ERK, PI3K and PKC mediated signaling pathways that are implicated in the neuroplasticity, neurogenesis or cell survival.

Materials and methods: To test this hypothesis Spraque Dawley rats were pretreated with H-89 (an inhibitor of PKA); KN-62 (an inhibitor of CaMKII); U0126 (an inhibitor of MAPK/ERK); LY294002 (an inhibitor of PI3K) and GF1090203X (inhibitor of PKC). Zinc (5mg/kg) was administered intraperitoneally (*i.p*) 30min before the test but the signaling pathway inhibitors were administered 45 min before the test by intracerebroventricular (*i.c.v*) route.

Results: Zinc given alone at the dose of 5mg/kg significantly decreased the immobility time of rats in FST. Pretreatment with either LY294002 (10nmol/2µl); H-89 (1µg/2µl); GF109203X (5ng/2µl); KN-62 (1ug/2ul) or U0126 (5ug/2ul) blocked anti-immobility effect of zinc in this test but did not influence the behavior of rats when given alone. Zinc (5mg/kg) given alone decreased the locomotor activity of the rats. Pretreatment with inhibitors did not influence the locomotor activity of rats and did not affect the zinc induced decrease in this parameter.

Conclusions: The obtained results increase knowledge regarding the mechanisms underlying the antidepressant-like action of zinc by indicating that zinc anti-immobility effects observed in FST may depend on the activation of PKA, CAMKII, PKC, PI3K and ERK and modulation of intracellular signaling pathways mediated by these kinases.

P.1-07

Kissorphin (KSO) ameliorated spatial memory and cognitive flexibility impairment induced by chronic ethanol treatment in the Barnes maze task in rats

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Background: Ethanol exerts numerous pharmacological effects through its interaction with opioid system. Chronic ethanol consumption is often accompanied by numerous cognitive deficits and may lead to the long-lasting impairments in spatial learning and memory. Moreover, ethanol abuse leads to the functional and neuroanatomical changes in the prefrontal cortex and hippocampus, the regions involved in memory processes. Kisspeptins are peptides that may interact with opioid system and are produced in areas involved in the consolidation of memory and orientation. Furthermore, they may exhibit neuroprotective activity against neurodegenerative proteins (β-amyloid, prion protein, amylin).

Material and methods: The aim of our study was to reveal whether a new derivative of kisspeptin-10, an anti-opioid peptide - kissorphin (KSO) (Tyr-Asn-Trp-Asn-Ser-Phe-NH$_2$) counteracts spatial memory deficits induced by chronic ethanol administration in the Barnes maze test. KSO (1; 3 and 10 nmol/300 μl, *iv*) was given 15 min either before the probe trial or the reversal learning in the Barnes maze task.

Results: Our study demonstrated that this peptide was effective in reversing ethanol-induced deficits in the short-term spatial memory (probe trial) or cognitive flexibility (reversal learning). KSO reduced latency to find the target hole and decreased a number of errors in both sessions. The most pronounced effect was seen after administration of KSO at the dose of 10 nmol.

Conclusions: In conclusion, the ethanol-induced spatial memory impairment may be reversed by pharmacological manipulation of the endogenous opioid system.

P.1-08

MMP-9 contribution to synaptic plasticity of alcohol addiction

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Background: Matrix metalloprotease 9 (MMP-9) is an extracellularly operating protease shown to play the key role in the morphological reorganization of dendritic spines as well as specific forms of learning and memory. The aim of this study was to investigate the role of MMP-9 in functional and structural plasticity in physiological conditions and upon development of alcohol addiction.

Materials and methods: Mice, housed in the IntelliCage system, were constantly monitored for alcohol consumption and motivation to reward. Using confocal microscopy, we evaluated changes in spine morphology. We then performed patch clamp recordings to test the strength of synapses in central amygdala.

Results: MMP-9 KO mice display lower motivation towards ethanol compared to wild type mice (WT). Moreover, in central amygdala, chronic alcohol drinking produced alterations in dendritic spine shape of both WT and KO but interestingly, more pronounced changes were observed in WT high alcohol consumers affecting mostly mushroom spines which had enlarged spine head in high alcohol consumers. To test the functional relevance of altered structural plasticity we performed electrophysiological analysis of the strength of glutamatergic synapses in central amygdala. We investigated the formation and fate of silent synapses (immature synapses considered as substrates for increased plasticity) and discovered that alcohol consumption elevates the number of silent synapses, especially in the course of extended withdrawal. MMP-9 KOs, however, showed no synaptic adaptations neither after alcohol drinking nor withdrawal.

Conclusions: These data suggest that MMP-9 is involved in synaptic plasticity associated with alcohol addiction. The change of spine morphology together with elevated silent synapse number might represent ongoing circuitry reorganization that primes neurons for enhanced learning, which might lead to compulsive ethanol use.

P.1-09
Ceftriaxone as an anti-relapse drug for cocaine use disorder – preclinical evaluation

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Background: The number of people using psychoactive substances increases over the years and effective addiction therapy is lacking. Recent literature data demonstrate that repeated administration of drugs of abuse, including cocaine, leads to glutamate system homeostasis impairment. Glutamate homeostasis is provided among others by excitatory amino acid transporter EAAT2, which was shown to be diminished in brain of animals after cocaine self-administration. The EAAT2 activity and expression is increased following treatment with the brain-penetrant antibiotic ceftriaxone. Taking under consideration these findings, we aimed to establish if repeated administration of ceftriaxone could abolish the preference to drug-paired chamber induced earlier by cocaine administration.

Materials and methods: In the present study, we used male Wistar rats (250-280 g) and employed the unbiased conditioned place preference (CPP) with cocaine (15 mg/kg i.p) to establish animals showing cocaine-paired chamber preference. After completion of CPP animals received ceftriaxone (100 mg/kg i.p) or vehicle (2 ml/kg i.p.) for 7 days in their home cages. After ceftriaxone treatment rats were re-exposed on the CPP cages to establish whether ceftriaxone could abolish the reinstatement of the place-conditioned response.

Results: Our results show that ceftriaxone attenuated context-induced reinstatement of CPP ($p < 0.05$) seen as the diminished the difference (ca. 100 s) between time spent in cocaine- paired vs. saline-paired chamber.

Conclusions: Daily ceftriaxone treatment attenuated reinstatement of drug-seeking behavior but it did not stop it fully in CPP model of cocaine addiction. These behavioral outcomes will be discussed in context of molecular changes in the EAAT2 mRNA and protein expression in rats exposured to cocaine-saline and cocaine-ceftriaxone treatment schedules.

P.1-10

Effects of acute stress on the expression of galanin, galanin-like peptide and three galanin receptor subtypes in rat neuropophysis

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Background: From our experiments it became evident that oxytocin and galanin produced antianxious and antistress effects. Galanin (Gal)) and galanin-like peptide (GalLP) are acting via three G-protein coupled receptors (GalR). GalR1 and GalR3 activation of Gi/o inhibit adenylyl cyclase activity and GalR2 coupled with Gq/11 activates phospholipase C. Different distribution and density of GalR subtypes and activating different signaling systems could explain a wide range of Gal effects. Thus, the aim of our studies was estimating mRNA expression of galaninergic system in rat neurohypophysis (NH) after acute stress.

Materials and methods: Male Wistar rats were exposed to restraint stress for 60 min. Treatment of animals was in accordance with the Declaration of Helsinki Guiding Principles on Care and Use of Animals. Real time qPCR was done in the CFX96 Real-Time System and the quantification analysis by using the Optical System Software.

Results: We demonstrated the expression of mRNA Gal and GalLP in NH under basal condition and after stress application. Acute stress revealed decreasing of Gal mRNA expression by 50% while expression of mRNA GalLP has been increased by 50%. These data suggest the different involvement of these neuropeptides in NH in the response to stress. We have demonstrated the presence of all three GalR subtypes with the highest expression of GalR2. All GalRs respond to stress by reducing the expression of mRNA, the strongest response elicited GalR1.

Conclusions: After stress application, we observed decreased mRNA expression of Gal, increased GalLP and slightly decreased expression of mRNA of GalR subtypes. The expression of GalRs could be involved in NH function with dependence on distribution, pharmacology and signal transduction. Our data support the idea that galaninergic system is involved in the modulation of neurohypophyseal function under physiological state and after stress.

P.1-11

The expression of galaninergic and oxytocinergic systems in rat neurohypophysis

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Background: Our research is focused on oxytocinergic and galaninergic system in neurohypopysis (NH). Thus, the aim of our studies was detection of expression of galanin (Gal),

galanin-like peptide (GalLP) and their receptors (GalRs), localization and detection of possible colocalization with oxytocin in rat NH.

Materials and methods: We used male Wistar rats. Treatment of animals was in accordance with the Declaration of Helsinki Guiding Principles on Care and Use of Animals. 5µm sections of NH were prepared on Leica CM1850 Cryostat and immunohistochemical detection of peptides was done with primary antibodies and antibodies with coloid gold markers. The specificity of reaction was confirmed by Western Blot. Samples were observed with fluorescent microscope Leica DM5000 B and electron microscope EM JEM 100B. Obtained data were processed and analyzed with NIS Elements software and/or ImageJ.

Results: We determined the immunofluorescence labeling of Gal, GalLP, GalRs and oxytocin in NH. In order to determine the site of Gal and GalLP expression, we studied their colocalization with neurons and pituicytes using neuronal and glial markers. Our results indicate that both peptides are expressed in neurons, in pituicytes with close colocalization with oxytocin, also close to Herring bodies. In the same way we estimated localization of GalRs. We found the most pronounced expression of Gal R2 and Gal R3 colocalised with neuronal tissue, but in pituicytes the expression of all determined receptor subtypes was very weak.

Conclusions: In our study using the immunohistochemical procedures, we demonstrated the expression of Gal, GalLP and all three GalRs subtypes in NH. We found the colocalisation of Gal, GalLP with oxytocin, pituicytes and neuronal tissue. Our results suggest that galaninergic system can participate on modulation of oxytocin release at the level of NH.

P.1-12
The effect of chronic treatment with amitriptyline and L-dopa in unilaterally 6-OHDA-lesioned rats on dopamine and serotonin transporter binding in substantia nigra

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Background: The objective of the present study was to examine the effects of chronic treatment with amitriptyline and L-DOPA on binding to dopamine (DAT) and serotonin (SERT) transporters in the substantia nigra (SN) of the unilaterally 6-OHDA-lesioned rats.

Material and methods: Male Wistar Han rats received unilaterally 16 µg/4µl of 6-OHDA into the medial forebrain bundle (MFB). Two weeks later, the animals were tested for the rotational behavior induced by apomorphine. Rats exhibiting more than 100 contralateral turns/1h were treated with amitriptyline (10mg/kg) and L-DOPA (12mg/kg), alone or in combination, once daily for 21 consecutive days. The rats were sacrificed 1h after the last injection, and the whole brains were frozen and dissected. The binding of [^{3}H] GBR 12,935 to DAT and [^{3}H] citalopram to SERT was assayed on nigral tissue sections.

Results: Injection of 6-OHDA into MFB caused a dramatic decline in [^{3}H] GBR 12,935 binding to DAT in the ipsilateral SN while on the contralateral side inconsiderable up-regulation of this binding was observed. On the contralateral side, L-DOPA and amitriptyline did not change the increased by lesion [^{3}H] GBR 12,935 binding to DAT. In the SN, the unilateral lesion of dopaminergic innervation caused a significant up-regulation of [^{3}H] citalopram binding to SERT on both sides. In this structure, amitriptyline alone or jointly with L-DOPA decreased significantly the up-regulated [^{3}H] citalopram binding to SERT on both sides.

Conclusions: The present study suggest that amitriptyline modulates the release of 5-HT in the SN what may have functional implications. The obtained data are discuss in the context of motor disturbances observed in Parkinson's disease.

P.1-13

The effect of treatment with perampanel on neurotoxicity of dexamethasone – behavioral study

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Background: The chronic elevation of circulating levels of glucocorticoids (GCs) can result in neuronal degeneration of the hippocampal piramidal neurons or striatum, which are paralleled by cognitive deficits, such as the impairment of memory and learning. Moreover, GCs potentiate stress or ischemia-induced accumulation of excitatory amino acids (EAA) in the extracellular space of hippocampus and by facilitating the glutamate/Ca^{2+} cascade engender hippocampal neurons damage.

Antiepileptic drugs have been shown the neuroprotective effect by potentiating γ-aminobutyric acid (GABA) neurotransmission or by reducing brain excitatory amino acids levels. The purpose of this study was to investigate the effect of perampanel (Fycompa - a new antiepileptic drug, non-competitive α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) receptor antagonist) in animal model of dexamethasone induced-neurotoxicity.

Materials and methods: The experiments were carried out on male Albino Swiss mice (25-30 g). Perampanel (2 or 8 mg/kg/day) was administered ip, 30 before DEX (16 mg/kg/day), ip for 14 days. The long-term memory acquisition, the motor performance, the locomotor activity, as well as the body weight and the lethality were evaluated after 14 days of drugs administration.

Results: The results of our study have shown that DEX evoked deterioration of all parameters in behavioral tests. In mice treated with DEX, perampanel at the dose of 2 mg/kg/day deteriorated behavioral parameters. Perampanel, administered at the dose of 8 mg/kg/day, slightly improved acquisition of memory but it had no impact on other behavioral parameters and influenced neither the lethality nor body weight in mice subjected DEX for 14 days.

Conclusions: Further studies need to be carried out to explain the effect of perampanel in dexamethasone-induced neurodegenerative processes.

P.1-14

Role of adenosine A_1 and A_{2A} receptors in rodent models of parkinsonian and essential tremors

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Background: Harmaline (HARM) is a tremorgenic compound used for modelling essential tremor (ET) in animals. The mechanism underlying HARM tremor is based on activation of olivo-cerebellar climbing fibers and excessive glutamate release but also involves GABAergic and glutamatergic connections to the ventral motor thalamic nuclei. Tremulous jaw movements (TJMs), defined as rapid vertical deflections of the lower jaw that are not directed at any particular stimulus, are used as a rodent model of parkinsonian (PD) rest tremor and can be induced by striatal dopamine depletion or typical antipsychotics.

Materials and methods: To examine the involvement of adenosine receptors in models of ET (HARM) and PD (TJMs) tremors in rats we used two selective ligands: ENBA (A_1 agonist) and SCH58261 (A_{2A} antagonist). Tetrabenazine (TBZ) and pimozide (PIM) were used for induction of TJMs. The expression of zif-268 mRNA and vGluT1, vGluT2, GAD65 and GAD67 proteins in various brain structures were measured.

Results: Administration of TBZ (2 mg/kg, *ip*) and PIM (1 mg/kg, *ip*) led to a significant induction of TJMs and increased expression of zif-268 mRNA and GAD67 and vGLUT2 proteins in the striatum. SCH58261 (5 mg/kg, *ip*) reduced TJMs in TBZ-treated rats. HARM (15 mg/kg, *ip*) induced generalized tremor, measured in the Force Plate Actimeters. ENBA (0.5, 0.1, 0.05 mg/kg, *ip*) in a dose dependent manner reversed harmaline effects and lowered locomotor activity. In addition, HARM raised the expression of zif-268 mRNA and GAD67 protein in the cerebellum and reduced the expression of GAD65 in the thalamus.

Conclusions: The present results suggest different role of adenosine A_1 and A_{2A} receptors in ET vs PD tremors and involvement of GABA and glutamatergic transmission in both types of tremors.

P.1-15

Effect of combined paroxetine and low-dose risperidone treatment on the heterodimerization of dopamine D2 and serotonin 5-HT1A receptors in mice brain cortex

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Background: The aim of the study was to investigate the heterodimerization of dopamine D2 and serotonin 5-HT1A receptors after paroxetine and risperidone treatment, which are often combined in the therapy of obsessive-compulsive disorder (OCD).

Material and methods: Mice were treated acutely or repeatedly (21 days) with paroxetine (10 mg/kg) and risperidone (0.05 mg/kg) given separately and in combination. Locomotor activity with marble burying test was performed as animal model of OCD. Expression of 5-HT1A and D2 mRNA was measured with *in situ* hybridization. Receptor autoradiography with [3H]8-OH-DPAT was performed to quantify 5-HT1A protein level. Heterodimerization of receptors was evaluated by Proximity Ligation Assay (PLA), which enables to visualize and quantify heterodimers in brain. As a negative control of PLA, D2 expression was silenced with siRNA in neural primary culture. Additionally, immunocytochemistry with markers for astrocytes and neurons (i.e. GFAP and NeuN) was performed.

Results: Behavioral tests confirmed the anti-OCD activity of paroxetine, i.e. reduced marble burying behavior with no change in locomotor activity. Risperidone in combination with paroxetine further reduced compulsive behavior. The co-localization of dopamine D2 and 5-HT1A receptors was observed in mice brain cortex. Chronic paroxetine treatment increased level of D2/5-HT1A heterodimers in the ventromedial orbital cortex. Counterstaining after the PLA of astrocytal and neuronal markers enabled to visualize the cell-type specific localization of heterodimers.

Conclusions: D2 and 5-HT1A receptors can form heterodimers in brain cortex. Paroxetine and risperidone seem to act synergistically on the behavioral level. On the molecular level, paroxetine and risperidone influenced the level of D2/5-HT1A heterodimers.

P.1-16

Positive allosteric modulators of α7 nicotinic acetylcholine receptor enhance memory processes

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Background: The alpha 7 acetylcholine nicotinic receptors (α7 nAChRs) are promising drug target for diseases involving cognitive impairment such as Alzheimer's and schizophrenia. Positive allosteric modulators of α7 nAChRs (α7 PAMs) are novel class of compounds offering advantages over α7 nAChRs agonists. Based on the functional properties of modulation, α7 PAMs are divided into two groups, type I and type II. Both appear to increase receptor sensitivity to endogenous agonist. However, type I PAMs have little or no effect on desensitization processes, while the action of type II PAMs is accompanied by a retardation of the kinetics of desensitization.
The aim of the present study was to evaluate the effects of two functionally different α7 PAMs on recognition memory in rats.
Materials and methods: The novel object recognition task (NORT) were used to evaluate the effect of acute and sub-chronic treatment of α7 PAMs (i.e., CCMI and PNU120596) in rats. Memory impairments were induced by long inter trial interval (24 h, natural forgetting) or by administration of the muscarinic antagonist, scopolamine.
Results: Acute and sub-chronic treatment of type I of α7 PAMs CCMI and type II PNU120596 resulted in improvement of recognition memory tested 24 h following last drug administration. In addition, both types of α7 PAMs were effective in scopolamine-based model of NORT deficit.
Conclusions: The present study demonstrates the procognitive activity of α7 nAChRs PAMs in rats. Therefore, our results support the notion that α7 nAChRs allosteric modulation, may constitute a potential procognitive therapy of cognitive disorder.

P.1-17
UV filter used in cosmetics and neurotoxicity

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Background: Evidence suggests that early life exposures to environmental contaminants are associated with increasing risk of the nervous system disorders including Parkinson's and Alzheimer's diseases. Benzophenone-3 (BP-3) is a chemical sunscreen agent, commonly used in cosmetics. The industrial use of BP-3 increased over the past decade and nowadays 10 000 tons of chemical UV filters are produced annually for the global marked. Knowledge about the impact of BP-3 on the nervous system is scarce. Especially worrying is insufficient data about an involvement of BP-3 in apoptotic and autophagic processes which occur during neurodegeneration.

Material and methods: Primary neuronal cell cultures, assessment of DNA methylation, measurements of caspase-3 and LDH activities, ROS formation as well as data analysis were performed as previously. Hoechst 33342/calcein AM stainings were employed to visualize apoptotic bodies and cell death, and LC3 was used to detect autophagosome formation. Mouse primary neocortical cell cultures were exposed to BP-3 (1-100 μM) for 6-24 h.

Results: BP-3 induced neurotoxic effects in the mouse neocortical cells as evidenced by increased LDH release and ROS formation, the features of neurodegeneration. These effects were accompanied by activated caspase-3 and apoptotic fragmentation of nuclei. BP-3 also interfered with autophagic processes and inhibited global DNA methylation in primary neuronal cells.

Conclusions: This study provided evidence on involvement of apoptosis and autophagy in BP-3-induced neurotoxicity. Since BP-3 also altered epigenetic status of DNA, understanding the pathomechanisms of BP-3 actions is important not only in respect of etiology of neurodegenerative but also neurodevelopmental diseases.

P.1-18

The role of BDNF in adverse effects of corticosterone and/or glutamate in organotypic hippocampal cultures

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Background: It is known that an increased level of glucocorticoids can enhance synaptic glutamate concentration, affect synthesis and action of pro-inflammatory cytokines, as well as attenuate synthesis of brain-derived neurotrophic factor (BDNF). What is more, these changes are likely to be responsible for the nerve cell damage and also seem to be involved in the pathogenesis of affective disorders.

The aim of this study was to evaluate the effect of corticosterone, glutamate and prenatal stress on the alterations in BDNF and its receptor (tropomyosin receptor kinase B; Ntrk2) in the hippocampal organotypic cultures.

Materials and methods: The hippocampal organotypic cultures were prepared from brains of 7-day old offspring of the control and stressed mothers. Corticosterone and/or glutamate were added to the culture medium for 24 or 72 h following by the determination of *Bdnf* and *Ntrk2* mRNA levels, as well as extracellular BDNF quantification in the culture media.

Results: In the 24 h culture corticosterone and glutamate increased *Bdnf* and *Ntrk2* mRNA mainly in hippocampal cultures obtained from the prenatally stressed rats. In contrast, corticosterone and glutamate significantly reduced *Bdnf*, but not *Ntrk2* mRNA after 72 h. Interestingly, it has been found that extracellular BDNF levels were lower in 24 h of untreated hippocampal culture obtained from prenatally stressed rats, as well as in the control hippocampal slices incubated jointly with corticosterone and glutamate.

Conclusions: These data indicate that prenatal stress and prolonged action of the glucocorticoid and glutamate inhibits synthesis or release of BDNF in the organotypic hippocampal cultures, which however in prenatally stressed rats is partially compensated by an increase in the *Ntrk2* mRNA.

P.1-19

Neuroprotection of 1MeTIQ in reserpine model of PD

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Background: Abnormalities in monoaminergic neurotransmission are associated with a number of neurological disorders such as Parkinson's disease (PD). Reserpine is a vesicular monoamine re-uptake blocker which depletes monoamines in the. 1-Methyl-1,2,3,4-tetrahydroisoquinoline (1MeTIQ) is an endogenous compound, which exhibits the neuroprotective, antidepressant-like effect and MAO-inhibiting properties.

Materials and methods: The aim of the present study was to investigated the impact of acute dose of 1MeTIQ on the disturbances in dopamine metabolism and release evoked by chronic treatment of low dose of reserpine (0.2 mg/kg i.p. during 14 days); 1MeTIQ was administered in a dose 50 mg/kg once 20 minutes before last dose of reserpine. We performed both *ex vivo* and *in vivo* microdialysis study. The dopamine and its metabolites were assayed in dialysates or in the tissue using HPLC with ED.

Results: The present *ex vivo* study showed that chronic treatment with reserpine caused significant increase the concentration of DOPAC and this effect was completely inhibited by acute dose of 1MeTIQ. Moreover, the *in vivo* microdialysis study indicated that chronic treatment of reserpine produced decrease the release of dopamine into the extracellular space of about 30% ($P<0.05$). Reserpine-induced depression of dopamine release in striatum was completely antagonized by acute treatment with 1MeTIQ.

Conclusions: 1MeTIQ has shown the neuroprotective activity in animal model of Parkinson's disease. The mechanism responsible for the neuroprotection of 1MeTIQ against reserpine neurotoxicity may be connected with the antagonism to reserpine-induced oxidative stress and reduction of free radicals production in the rat brain structures.

P.1-20

Impact of hypoxia-ischemia on oligodendrocyte survival, maturation and myelinating potential

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Background: Oligodendrocyte progenitor cells (OPCs) are precursors of the cells capable of myelinating the CNS and engaged in providing myelinated axons with energy substrates. Unfortunately, OPCs are extremely sensitive to pathological insults, including the neonatal hypoxia-ischemia (H-I). To date, the processes initiated by temporal H-I has remained unclear.

To address the issue, we have designed comparative studies based on in vivo and vitro models to evaluate the H-I effects on OPC survival, proliferation and maturation.

Materials and methods: The organotypic hippocampal slices were exposed to oxygen and glucose deprivation (OGD) which allowed mimicking the ischemic injury ex vivo, while the neonatal H-I was induced in rats for in vivo studies. Two weeks after the H-I brains were extracted and the nervous tissue slices were prepared. An engagement of gelatinases in the OPC recruitment and differentiation was assessed by in situ zymography combined with immunohistochemical analysis with the antibodies relevant for subsequent stages of cell maturation.

Results: The data obtained from both ex vivo and in vivo studies clearly indicate that neonatal H-I strongly affects OPC survival and their subsequent differentiation process. The observed significant decrease in the OPC number is partially compensated by the increased rate of OPC proliferation, however oligodendrocyte maturation and their ability to express myelin components is significantly slowed-down. **Conclusions:** The neonatal H-I resulting in the short-time limitation of oxygen and metabolic substrates leads to a significant decrease in the number of gelatinase-expressing OPCs, which most probably contributes to the altered oligodendrocyte maturation. The impaired oligodendrocyte differentiation and their myelinogenic capability might be the reason of the resulting neurodegeneration.

P.1-21

Up-regulation of inflammatory factors in the hippocampus and frontal cortex of young prenatally stressed rats

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Background: Several lines of evidence suggest that the dysregulations of the immune system play an important role in the development of depression. Among them the disturbances in the expression of pro-inflammatory factors in central nervous system are a particular interest. On the other hand, multiple data show that early experiences can be crucial for neuronal development and consequently for the health in adulthood. Accordingly, the aim of this study was to determine the impact of prenatal stress procedure (the animal model of depression) on the expression of some pro-inflammatory factors: the cytokines and the chemokine in both hippocampus and frontal cortex of young offspring rats.

Materials and methods: Pregnant rats were subjected to restraint stress from 14th day of pregnancy until the delivery. At 7 days of age, male offspring animals were sacrificed. Subsequently the expression of IL-1β, IL-18, IL-6, TNF-α, and CCL2 in the hippocampus and frontal cortex of young offspring rats was determined using qRT-PCR, as well as ELISA assays.

Results: We demonstrated that prenatal stress up-regulated only IL-6 gene expression in frontal cortex, and did not affect the tested parameters in hippocampus. On the other hand, we found

disturbances in the concentration of some inflammatory factors in the brain areas of young offspring rats after prenatal stress procedure. Biochemical study showed the higher hippocampal levels of IL-1β, IL-18, IL-6 and CCL2, while in frontal cortex of prenatally stressed offspring IL-18, IL-6 and TNF-α concentrations were increased.

Conclusions: In summary, our study presented that prenatal stress enhanced the inflammatory processes in the brain of young offspring rats. Nevertheless the exact role of these malfunctions in the pathogenesis of depression requires further research.

P.1-22

Inhibition of kynurenine 3-monooxygenase significantly reduces neuropathic pain and intensifies its pharmacological treatment

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Background: Recent studies have highlighted the participation of the kynurenine system in the pathology of neurodegenerative diseases (i.e., Huntington's disease, migraines and multiple sclerosis), but the role of this system in neuropathic pain has not been assessed. The aim of our studies was to exam the role of kynurenine 3-monooxygenase (Kmo), an enzyme that is important in kynurenic pathway in a rat model of neuropathic pain.

Material and methods: Chronic constriction injury (CCI) of the sciatic nerve was performed according to Bennett and Xie (1988). Behavioral studies consisted of the allodynia/hyperalgesia measurements, biochemical studies comprised the RT-PCR and/or Western blot analysis in the tissue (spinal cord, DRG) and primary glia cultures. The experiments were carried out according to IASP rules (Zimmermann, 1983).

Results: We demonstrated that the chronic Kmo inhibitors (Ro61-6048 and JM6) administration significantly reduced allodynia and hyperalgesia 7 day after CCI. Our results indicate that the increased *Kmo* mRNA levels were reduced in the spinal cord and DRG by chronic administration of microglia inhibitor - minocycline and the effect paralleled the decreased intensity of neuropathic pain. Moreover, minocycline administration improved the lipopolysaccharide (LPS)-induced upregulation of *Kmo* mRNA expression in microglial cell cultures. Chronic Ro61-6048 administration diminished the mRNA and protein levels of IBA-1, IL-6, IL-1beta and NOS2 in the spinal cord and/or the DRG.

Conclusions: Our data suggest that in neuropathic pain model, inhibiting Kmo function significantly reduces pain symptoms. The results of our studies indicate that the kynurenine pathway is an important mediator of neuropathic pain pathology.

P.1-23

The efficacy of antidepressants in the olfactory bulbectomy model in rats is associated with alterations in MeCP2 protein

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Background: The growing number of evidence indicate that the epigenetic mechanisms may play significant role both in the pathophysiology and treatment of depression. The methyl-DNA binding protein MeCP2 has a critical role in activity-dependent neuronal plasticity and transcription during brain development. Furthermore, recent studies suggest that various posttranslational modifications, notably phosphorylation, may regulate MeCP2's functions in learning and memory, depression-like behavior, and the response to antidepressant treatment.
The aim of present study was to investigate the effect of olfactory bulbectomy (OB) procedure (animal model of depression) and drugs (with different mechanisms of action) administration on the total MeCP2 and phospho-S421-MeCP2 protein level in the frontal cortex of rats.
Materials and methods: Male Sprague Dawley rats were subjected to the OB procedure and treated with amitriptyline (AMI 10mg/kg), fluoxetine (FLU, 10mg/kg), venlafaxine (VLX, 10mg/kg) or olanzapine (OLZ, 2mg/kg) for 14 days. The levels of proteins were determined in nuclear fraction by Western blotting.
Results: Our analysis revealed a statistically significant increase in the total MeCP2 and decrease in p-S421-MeCP2 protein level (by 83% and 70%, respectively) in OB rats. Fluoxetine (but not other drugs) reduced the total MeCP2 in OB rats. Interestingly, VLX, AMI and FLU (but not OLZ) administration resulted in an increase in S421 phosphorylation. These changes were associated with behavioral alterations observed in open field test.
Conclusions: Our findings confirm the role of MeCP2 (especially S421 phosphorylation) in the development and treatment of depression. Observed changes seems to be a drug-specific and characteristic only for antidepressants (not antipsychotics).

P.1-24

AM 251, CB1 receptor antagonist reverses the psychotic-like effects in mice in the animal model of schizophrenia

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Background: It has been known that the endocannabinoid system, via cannabinoid (CB) receptors, is involvement in major psychiatric disorders, including schizophrenia. The aim of the present research was to evaluate the impact of CB1 receptor antagonist, AM 251 on the symptoms typical for schizophrenia.

Material and methods: We provoked psychosis-like effects in mice by an acute administration of N-methyl-D-aspartate (NMDA) receptor antagonist, MK-801 (0.1-0.6 mg/kg), an animal model of psychosis. An acute administration of MK-801 induced psychotic symptoms, manifested in the increase in locomotor activity (hyperactivity), measured in actimeters.

Results: First we evaluated the influence of an acute administration of AM 251 (0.25; 0.5; 1 and 3 mg/kg) on the locomotion of mice. An acute administration of the doses of 1 and 3 mg/kg of AM 251 had antipsychotic effects, causing the decrease in locomotion of mice in comparison to the vehicle-treated control group. Next, we revealed that hyperactivity induced by an acute injection of MK-801 (0.3 and 0.6 mg/kg) was attenuated by an acute administration of non-effective dose of AM 251 (0.5 mg/kg).

Conclusions: The present findings confirm that endocannabinoid system is able to modify a variety of schizophrenia-like responses in mice, including the hyperlocomotion in mice. Antipsychotic-like effects induced by CB1 receptor antagonist, obtained in our research, provide evidence that this type of CB receptors is involved in the psychosis-related behavior. This knowledge may open in the future new possibilities for the development of CB-based therapies for psychiatric human disorders.

P.1-25

Assessment of the influence of nicotine on depression-like behavior and oxidative stress in mice exposed to the chronic mild stress

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Background: Nicotine acting through central mechanisms, exerts influence on mood and emotional tension, and contributes to physical and psychological dependence. Additionally, in some experimental animal models chronic and acute stress aggravated both behavioral as well as biochemical effects caused by administration of nicotine.

Material and methods: Taking into account concomitance of nicotine abuse and stress accompanying daily life we aimed our research at assessment of behavioral effects accompanying acute administration of nicotine in combination with chronic mild unpredictable stress (CMUS) in mice. In experiments, mice were submitted to the procedure of CMUS for 4 weeks, 2 hours per day (e.g., wet litter or the lack of it, tilting the cage, limitation of water or food, an electric sound signal). The depressive effects were measured in the forced swim test (FST). Additionally, the effect of metyrapone was described. We measured the effects of the CMUS and nicotine administration in stressed and unstressed mice on parameters of antioxidant barrier and process of lipid peroxidation. **Results:** An acute treatment with nicotine (0.2 mg/kg) significantly decreased the immobility duration in both unstressed and stressed mice compared to saline-treated groups. Administration of metyrapone (50 mg/kg) alleviated this depressive-like effect induced by stress, which suggests influence of glycocorticosteroids in anhedonia caused by stress. Additionally, our biochemical study further confirmed increase in oxidative stress parameters after an acute nicotine administration in stressed mice submitted to the CMUS protocol, i.e., decreased TAS, SOD and GPx activity and increased MDA concentration.

Conclusions: The results can increase our knowledge of the pathogenesis of the nicotine-stress interactions on the basis of the development of nicotine dependence and ongoing tobacco abuse.

P.1-26

Different mechanisms are involved in ATM kinase inhibitor mediated protection against the H_2O_2- and doxorubicin-induced neuronal cells damage

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Background: The involvement of ATM (Ataxia telangiectasia mutated) kinase in DNA damage repair response is well established, however its role in regulation of cellular response to oxidative stress, especially in neuronal cells, is less recognized. In the present study we investigated the mechanisms of neuroprotective effects of ATM kinase inhibitor, KU-55933 in models of oxidative stress- and DNA-damaging factor-induced cell death in retinoic acid-differentiated human neuroblastoma SH-SY5Y cells.

Materials and methods: SH-SY5Y cells were treated with oxidative stress inducer, hydrogen peroxide (H_2O_2, 1mM) and DNA-damaging factor, doxorubicin (Dox, 1µM). The cell viability after 24h of treatment was measured by MTT reduction and LDH release assays, whereas the activity caspase-3 was evaluated by the fluorogenic method (after 9h). The level of phosphorylation of proteins involved in DNA damage response (p53 and H2AX) was confirmed by Western blot.

Results: KU-55933 (0.1-10 µM) was neuroprotective in both models of cell damage, however an attenuation of caspase-3 activity by this inhibitor was shown only in Dox model. Moreover, despite showing the induction of DNA damage response after exposure of cells to Dox and H_2O_2, only in the former one we observed the reduced level of phosphorylated H2AX and p53 proteins after KU-55933 treatment.

Conclusions The data showed that KU-55933 protects neuronal-like cells against oxidative stress- and DNA-damage induced cell death via different mechanisms. Protection mediated by KU-55933 in Dox model acted by inhibition of DNA-damage response and caspase-3 activity, but not in H_2O_2 model. These data suggest the existence of other, DNA-damage and caspase-3-independent mechanisms in protection mediated by ATM kinase inhibitor at least in oxidative stress model which explanation needs further research.

P.1-27

The evidence of oxidative DNA damage in the rat brain by illicit drug 5-MeO-DIPT given chronically in adolescent animals

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Background: 5-Methoxydiisopropyltryptamine (5-MeO-DIPT) is an illicit drug with a street name 'foxy' or 'methoxy'. It belongs to several compounds closely related to tryptamine and shows psychoactive properties. It acts as a competitive serotonin transporter (SERT) inhibitor and has agonistic activity to several serotonin receptors, mainly to 5-HT1A and 5-HT2A/2C. It increases DA, 5-HT, glutamate release in various brain regions when given in single doses. Euphoria, disinhibition, visual and auditory hallucinations, anxiety, insomnia, myoclonus, all of which were reported by users indicates serious effects on the central nervous system. Our present study was undertaken to find out whether repeated doses of 5-MeO-DIPT given in adolescence have impact on brain neurotransmission in adult animals.

Material and methods: Rats were treated with 5-MeO-DIPT (2,5 mg/kg/day x 4) for two weeks starting at age of 30 days. The release of DA, 5-HT and glutamate were measured in striatum (STR), nucleus accumbens (NAS) and frontal cortex (FCx) using microdialysis in freely moving animals two month later.

Results: The release of DA was increased in NAS, FCx while was decreased in STR in response to challenging dose of 5-MeO-DIPT (2,5 mg/kg). 5-HT and glutamate levels were also enhanced in all studied brain regions after administration of 5-MeO-DIPT; however the increase in DA was smaller in comparison to single dose effect in STR and FCx and glutamate in NAS. The marked outflow in basal DA and glutamate but not 5-HT was observed in all brain regions. The oxidative damage of cortical DNA was found as single and double-strand breaks in comet assay.

Conclusions: These data indicates possible damage of pre- and postsynaptic cellular elements in the adult rat brain after 5-MeO-DIPT administration during adolescence.

P.1-28

Mapping of metabolism, thermoregulation and analgesia QTLs in a cross between mouse lines divergently selected for high and low swim stress-induced analgesia

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Background: Exposure of an animal to stressful stimuli elicits a transient decrease in pain sensitivity, which often affects thermoregulatory mechanisms in the threatened organism. In the present study we performed a genome-wide quantitative trait locus (QTL) analysis in two mouse lines from a long-term divergent selection experiment. Briefly, we found that a SIA (stress-induced analgesia) is inversely related to PMR (peak metabolic rate) and thermogenic capacity. This relationship was strong enough to permit the claim of the existence of a negative genetic linkage between SIA and PMR as well as thermogenesis.

Materials and methods: The parental lines were outbreed lines divergently selected for 60 generations for high- (HA line) or low- (LA line) swim stress-induced analgesia (SSIA) elicited by a 3-min swim in 20°C water. An F2 population of 267 mice was used for the QTL analysis with 40 microsatellite markers distributed across the first five chromosomes.

Results: The analysis revealed significant QTLs for SIA, PMR and hypothermia (Hyp). Some of these QTLs map to regions of known gene mutations influencing these traits, thus indicating the previously described QTLs as candidate genes. Apart from QTLs affecting single traits, we identified QTLs common for examined traits (SIA-PMR-Hyp), (SIA-PMR), (SIA-Hyp) and (PMR-SIA) explained phenotypic correlations between them.

Conclusions: Identification of candidate genes may help to elucidate the fundamental mechanisms underlying the development of stress. In humans, such knowledge would be valuable in the development of drugs or gene therapy approaches to treat stress-related disorders, or establish personalized preventive strategies.

P.1-29

Low-anxiety rats are more sensitive to amphetamine in comparison to high-anxiety rats

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Background. Understanding the neurobiological factors that contribute to emotional disorders and sensitisation to psychostimulants will be essential for developing future effective psycho- and pharmacotherapies. The aim of our study is to assess the individual differences in motivation and susceptibility to psychoactive substance.

Material and methods. We used low-anxiety (LR) and high-anxiety (HR) rats that are known to have different fear-conditioned response strengths, different susceptibility to amphetamine in the TIPS procedure and different amphetamine-dependent frequency modulated (FM) 50-kHz ultrasonic vocalisation (USV) responses. This study utilised the conditioned place preference test (CPP) to assess the behavioural effects of amphetamine and the two injection protocol of sensitisation (TIPS). We recorded and analysed appetitive 50-kHz ultrasonic vocalisation (USV) as well. We also used immunocytochemistry to examine changes in the expression of c-Fos and NMDA receptor 2B (GluN2B) subunit in the cortical and subcortical brain regions of HR and LR rats in response to two injections of amphetamine.

Results. We have discovered that in comparison to HR rats, LR rats vocalised much more intensively and spent more time in the amphetamine-paired compartment. In TIPS protocol after the second dose of amphetamine, the LR rats showed more c-Fos and GluN2B neuronal activation in layers II and III of the M1/M2 motor cortex area and prefrontal cortex (PRE, PRL, IL) and also showed more GluN2B neuronal activation in the basal amygdala.

Conclusions. These data reveal that HR and LR rats are characterised by different levels of reactivity in the cortical-limbic pathway, which controls reward-related motivational processes. These findings contribute to the hypothesis about the role of heterogeneity in emotional processes as one of the causes of sensitization to amphetamine and drug addiction.

P.1-30

Maraviroc by polarization of microglia and astroglia reducing pain in rat model of neuropathy evidences from *in vivo* and *in vitro* studies

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Background: Recent studies suggest that CCR5 signaling pathway is crucial in neuropathy development and its modulation can have some beneficial properties. The aim of our study was to investigate the influence of maraviroc (MVC; CCR5 antagonist) on neuropathic pain symptoms, changes in spinal glial cells activation and polarization 7 days after chronic constriction injury (CCI) to the sciatic nerve. Additionally, we exam MVC influence on primary glial cultures after LPS stimulation.

Materials and methods: Intrathecal (*i.t.*) catheters were implanted in male Wistar rats and a Bennett's CCI model was established. MVC (20μg/5μl) preemptively administered *i.t.* 16h and 1h before CCI and then once daily for 7 days. Two behavioral tests were conducted to measure allodynia (von Frey test) and hyperalgesia (cold plate test). Primary glial cell cultures were treated with MVC (100 nM) 30 min before LPS stimulation (100 ng/ml).The proteins levels were examined by Western blot.

Results: We provide evidence that MVC attenuated the neuropathic pain symptoms in CCI-exposed rats. Biochemical analysis showed that MVC diminished the level of glial markers (IBA1 and GFAP) and reversed CCI-induced upregulation of CCR5 and "classical" pronociceptive activation markers (IL-1β, IL-18, IL-6, NOS2) in the spinal cord. In opposite, MVC upregulated „alternative" antinociceptive activation markers (IL-1RA, IL18BP, IL-10). Similarly, MVC reduced pronociceptive and enhanced the antinociceptive factors after LPS stimulation in primary astroglia and/or microglia cell cultures.

Conclusion: Our studies give a new evidence that the MVC attenuates neuropathy symptoms, promotes spinal glial „alternative" polarization and restores the balance between pro- and antinociceptive factors. Our results suggest the modulation of CCR5 by MVC as a novel therapeutic approach for neuropathy.

P.1-31

The CCR2 antagonist, RS504393, attenuates neuropathic pain symptoms and enhances opioid effectiveness

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Background: Recent studies suggest the key role of neuroimmune interactions in neuropathic pain development. The CCR2 is suggested to be an important regulator of nociception. Therefore, the aim was to investigate the influence of RS504393 (CCR2 antagonist) on neuropathic pain symptoms development and opioid effectiveness in rats after sciatic nerve injury. Simultaneously, we studied changes in CCR2 expression, microglial activation and mRNA level of IL-1β, IL-18 and NOS2 in the spinal cord and DRG.

Material and methods: All experiments were conducted according to the IASP rules. Firstly, rats were implanted with intrathecal catheters and then were followed chronic constriction injury (CCI) of the sciatic nerve. RS504393 was administered 16h and 1h before CCI and then once daily for 7 days. Selected rats received also a single injection of morphine/buprenorphine in 7ᵗʰ day of experiment. Behavioral tests (von Frey, cold plate) were conducted 3 and 7 days post-CCI. The qRT-PCR was used to measure the mRNA level of studied factors.

Results: We provide evidence that chronic administration of RS504393 attenuated neuropathic pain symptoms and enhanced the analgesic properties of opioids in CCI-exposed rats. The qRT-PCR analysis showed that RS504393 decreased the level of CD40-positive cells and reversed CCI-induced upregulation of CCR2 in the spinal cord and DRG. Additionally, the mRNA level of IL-1β, IL-18 and NOS2 was increased after nerve injury in the spinal cord and/or DRG, and RS504393 significantly reduced those changes.

Conclusions: Our results suggest pharmacological modulation of CCR2 by RS504393 as a novel therapeutic approach for combination therapy with opioids in patients suffering from neuropathic pain.

P.2 Preclinical research – Neuropsychiatry

P.2-01

Endogenous amines with antidepressant-like effects

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Background: Animal models are widely used to study antidepressant-like effect in rodents. Reserpine as well as 1-benzyl-1,2,3,4-tetrahydroisoquinoline (1BnTIQ), an endogenous substance which is considered as an etiological factor of idiopathic Parkinson`s disease led to a pharmacological model of depression. Presently, we investigated antidepressant-like effect of two neuroprotective endogenous amines: 1,2,3,4-tetrahydroisoquinoline (TIQ) and its close methyl derivative, 1MeTIQ. The question arises, whether TIQ and 1MeTIQ are able to prevent the behavioral and biochemical depression produced by reserpine and 1BnTIQ?
Materials and Methods: Behavioral forced swim test (FST) with high predictive validity for antidepressant efficacy was used to examine the antidepressant properties of TIQ and 1MeTIQ. Additionally, we carried out also the neurochemical *ex vivo* studies in the rat brain structures to determine the levels of monoamines and their metabolites by HPLC after chronic administration of investigated substances. The rate of monoamines metabolism, and the indices of neuronal activity was also presented.
Results: TIQ and 1MeTIQ in doses 25 and 50 mg/kg completely antagonized reserpine (0.2 mg/kg) as well as 1BnTIQ (25 mg/kg) produced depression assessed in the behavioral (FST) and biochemical studies (monoamines levels and their metabolism in the brain structures).
Conclusions: The present data demonstrate that investigated endogenous amines, TIQ and 1MeTIQ used in the models of depression elicited the antidepressant-like activity, and we suggest that both compounds may be effective for the therapy of depression in clinic.
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P.2-02

The effect of the novel atypical atipsychotic drug lurasidone on cytochrome P450 isoenzyme activities in human liver

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Background: Inhibition of cytochrome P450 (CYP) isoenzymes is the most common cause of harmful drug–drug interactions. Lurasidone is a novel antipsychotic drug approved for the treatment of schizophrenia. The aim of the present study was to estimate the inhibitory effect of lurasidone on the main CYP isoenzymes in human liver.

Materials and methods: Experiments were performed *in vitro* using pooled human liver microsomes and cDNA-expressed human CYP isoforms (Supersomes 1A2, 2C9, 2C19, 2D6 and 3A4). CYP isoenzyme activities were determined using CYP-specific reactions: caffeine 3-N-demethylation (CYP1A2), diclofenac 4'-hydroxylation (CYP2C9), perazine N-demethylation (CYP2C19), bufuralol 4'-hydroxylation (CYP2D6) and testosterone 6β-hydroxylation (CYP3A4). The rates of CYP-specific reactions were determined by HPLC, in the absence and presence of lurasidone (0,01-50 μM). The inhibition constant (K_i) for the inhibition of CYP-specific metabolic reactions by lurasidone was obtained using a non-linear regression analysis (Program Sigma Plot 8.0; Enzyme Kinetics).

Results: The obtained results showed that lurasidone moderately and to a similar degree inhibited CYP1A2 in human liver microsomes and Supersomes (K_i = 18 and 17 μM, respectively). Moreover, lurasidone weakly diminished the activity of CYP3A4 in liver microsomes (K_i = 45 μM) and Supersomes (K_i = 39 μM). On the other hand, the neuroleptic did not affect the activity of CYP2C9, CYP2C19 and CYP2D6, neither in liver microsomes, nor in Supersomes.

Conclusions: The inhibition of CYP1A2 and CYP3A4 by lurasidone, demonstrated *in vitr*o in the present study, seems to be of limited significance *in vivo*, since the calculated K_i values are above the presumed concentration range of lurasidone in human liver during pharmacotherapy.

P.2-03

The involvement of mTOR pathway in the sustained antidepressant-like effect of a joint administration of ketamine and a group II mGlu receptor antagonist, LY341495

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Background: Numerous studies have reported the rapid and sustained antidepressant effects of the NMDA receptor antagonist ketamine. Because ketamine induces several undesirable effects, a variety of strategies have been suggested to avoid such effects. We have recently proposed to enhance the sub-effective doses of ketamine by co-administration with the group II mGlu receptor antagonist LY341495. Our results indicated that this drug combination is not only effective but also safer than the use of solely ketamine. The aim of the current study was to investigate a possible mechanism of these behavioral effects.

Material and methods: To investigate the sustained antidepressant-like effects of a joint administration of ketamine and LY341495 , both compounds were administered individually or in combination, 24 h before the forced swim test (FST). The same time schedule for drug treatment was used in the second part of the study, which was aimed to investigate the role of mTOR pathway in antidepressant effects, using Western blotting technique.

Results: We found that sub-effective doses of ketamine and LY341495, given jointly, induce significant sustained antidepressant-like effects 24 h after drugs administration. The results obtained using Western blot technique indicate that mTOR pathway activation in the frontal cortex and hippocampus may be involved in the mechanism of this action. Furthermore, an increased level of PSD-95 protein in the hippocampus was observed in rats administered with ketamine and LY341495, thus suggesting the enhanced synaptogenesis in this brain structure.

Conclusions: Altogether, these data suggest that the joint administration of ketamine and LY341495 might be a noteworthy alternative to the use of solely ketamine in the therapy of depression and that mTOR pathway activation may be involved in this effect.

P.2-04

Endogenous opioid system activity determines the severity of memory impairments after mild traumatic brain injury (mTBI) in mice selected for high (HA) and low (LA) swim stress-induced analgesia

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Background: Mild traumatic brain injury (mTBI) is one of the most frequent neuropathological conditions that lead to the development of persistent, burdensome cognitive deficits. Endogenous opioid peptides play a pivotal role in the modulation of mTBI pathology as their levels correlate with the severity of head trauma. Thus, an endogenous neuroprotective mechanism associated with the activation of the opioid system was proposed. We therefore hypothesize that a genetically determined divergence in opioid system activity facilitated by stress influences the intensity of spatial memory deficits.

Materials and methods: Endogenous β-endorphin levels in brain and plasma were assessed in mice bidirectionally selected for high (HA) and low (LA) swim stress-induced analgesia with the ELISA assay before and after a swim stress challenge in 20°C water. Mechanical mTBI was induced in both lines with a weight-drop device. The effect of mTBI and naloxone on spatial memory performance was evaluated 7 days after the impact in the Morris Water Maze test.

Results: Higher baseline concentrations of β-endorphins were found in the brain and plasma of HA than LA mice. Swim stress facilitated a profound increase in β-endorphin plasma levels only in the LA line. Mice from the HA line developed less severe spatial memory impairments than their LA counterparts. Naloxone pretreatment profoundly exacerbated behavioral manifestations of memory deficits in HA mice, while it was ineffective in the LA line.

Conclusions: Elevated levels of β-endorphins in the brain produce a naloxone-reversible neuroprotective effect in mice with a hyperactivated opioid system. Endogenous opioid peptide leakage to the periphery in LA mice causes an exacerbation of mTBI-induced memory impairments.

P.2-05

Effects of acute and repeated administration of antidepressants and drugs with antidepressant activity on [³H]ketanserin binding to serotonin (5-HT)$_{2A}$ receptors in rat brain structures

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Background Pharmacological research has yielded some insights into the role of 5-HT$_{2A}$ receptors in major depression and in mechanism of action antidepressant drugs. These receptors are very sensitive to changes in 5-HT neurotransmission, and most antidepressants control over the disturbed 5-HT levels in depression.

Material and methods The aim of this study was to investigate the effects of antidepressant drugs: imipramine (IMI, 15 mg/kg), escitalopram (ESC, 10 mg/kg), tianeptine (TIA, 10 mg/kg) and the drugs with antidepressant activity: N-acetylcysteine (NAC, 100 mg/kg) and URB597 (a fatty acid amide hydrolase inhibitor, 0.3 mg/kg) on the 5-HT$_{2A}$ receptor labelling pattern in selected rat brain structures.

Male Wistar rats received drugs i.p. acutely or chronically (14 days). 24 hours after the last drug administration the animals were decapitated and their brain were analyzed by autoradiography with the 5-HT$_{2A}$ receptor antagonist [³H]ketanserin.

Results In rat brain areas analysed, we found that studied drugs bi-directly altered 5-HT$_{2A}$ receptor labelling pattern. Both acute and repeated administration of IMI decreased the level of [³H]ketanserin binding in striatum and cortical areas. The level of [³H]ketanserin binding either increased or decreased in cortical structures after acute NAC and URB597 administration, respectively. The reduction in the [³H]ketanserin binding was seen in the nucleus accumbens shell after acute treatment of IMI, ESC, NAC and URB597, as well as after chronic administration of IMI, TIA and URB597.

Conclusions To summarize, the present data indicate different 5-HT$_{2A}$ receptor sensitivity to antidepressant drugs depending on drug schedule of administration and rat brain areas. The 5-HT$_{2A}$ receptors localized to the nucleus accumbens shell seem to be an interesting target down-regulated by drugs having different mechanism of action.

P.2-06

Enhancement of the antidepressant- and anxiotytic-like action of escitalopram by low dose of risperidone in rats

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Background: Several clinical reports have documented a beneficial effect of the addition of a low dose of risperidone to the ongoing treatment with antidepressants, in particular selective serotonin reuptake inhibitors (SSRI), in the treatment of drug-resistant depression and treatment-resistant anxiety disorders.

Materials and method: In the present study, we investigated the effect of treatment with the antidepressant escitalopram (SSRI) given separately or jointly with a low dose of risperidone (an atypical antipsychotic) in the forced swimming test and in the elevated plus-maze test in rats.

Results: The obtained results showed that escitalopram at doses of 2.5 or 5 mg/kg evoked antidepressant-like effect in the forced swimming test. Moreover, risperidone at the low doses (0.05 or 0.1 mg/kg) enhanced the antidepressant-like activity of escitalopram (1 mg/kg) in the forced swimming test by increasing the swimming time and decreasing the immobility time in those animals. WAY 100635 (a 5-HT_{1A} receptor antagonist) at a dose of 0.1 mg/kg abolished the antidepressant-like effect induced by co-administration of escitalopram and risperidone. Escitalopram (5 mg/kg) and mirtazapine (5 or 10 mg/kg) or risperidone (0.1 mg/kg) induced an anxiolytic-like effect in the elevated plus-maze test, and the combined treatment with an ineffective dose of risperidone (0.05 mg/kg) enhanced the anxiolytic-like effects of escitalopram (2.5 mg/kg) or mirtazapine (1 and 2.5 mg/kg) in this test.

Conclusions: The obtained results suggest that risperidone applied at a low dose enhances the antidepressant-like activity of escitalopram in the forced swim test, and that 5-HT_{1A} receptors may play some role in these effects. Moreover, a low dose of risperidone may also enhance the anxiolytic-like action of antidepressants studied.

P.2-07

Antidepressant drug treatment alters the level of expression of synthesis and degrading enzymes in the brain endocannabinoid system

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Background: Our previous study showed that the level of endocannabinoids (eCBs) was changed in several brain structures after antidepressants treatment. The aim of the study was to investigate the effects of some antidepressant drugs on the level of expression of eCB synthesizing enzymes (N-acyl phosphatidylethanolamine phospholipase D (NAPE-PLD) and diacylglycerol lipase (DAGL)) and degrading enzymes (fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL)).

Materials and methods: Male Wistar rats received imipramine (IMI, 15 mg/kg), escitalopram (ESC, 10 mg/kg) or tianeptine (TIA, 10 mg/kg) acutely or chronically (for 14 days). Twenty four hours after the last injection the animals were decapitated. Brain structures were analyzed using Western Blot.

Results: Increased levels of AEA observed after chronic IMI and ESC treatments may be linked with reduced FAAH protein expression level in the dorsal striatum, as well as with increased NAPE-PLD level in the hippocampus and dorsal striatum following chronic ESC treatment. Increased 2-AG level after acute and chronic IMI treatment corresponds with increased DAGL and decreased MAGL protein expression levels in the frontal cortex, while a decrease of cerebellar 2-AG level may be caused by a decreased DAGL protein level after chronic IMI treatment. Increased 2-AG levels observed after chronic ESC treatment may be induced by increased DAGL protein level in the dorsal striatum and hippocampus, at the same time chronic TIA treatment increased DAGL protein level in the frontal cortex and dorsal striatum which correlate with raised 2-AG level in these structures.

Conclusions: Our data support the engagement of metabolic enzymes in the effects of antidepressant drugs on the altered levels of eCBs in rat brain.

P.3 Preclinical research – Other themes

P.3 -01
The effect of dapagliflozin on selected indicators of not advanced atherosclerosis in an animal model of type 2 diabetes

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Background: The aim of the study was to investigate the impact of dapagliflozin (DAPA) – a novel inhibitor of renal sodium-glucose cotransporter 2 (SGLT2) – on selected indicators of not advanced atherosclerosis: circulating endothelial cells (CEC) and circulating progenitor cells (CEPC) in animal model of type 2 diabetes.
Material and methods: 59 male Wistar rats were divided into 4 groups: DAPA-treated animals with type 2 diabetes and glucose fluctuations (n = 15), placebo-treated animals with well controlled diabetes (n = 14), placebo-treated rats with type 2 diabetes and glucose fluctuations (n = 15) and a healthy control (n = 15). The vascular endothelial dysfunction and its regeneration capability (CEC and CEPC amount in peripheral blood) were evaluated by flow cytometry.
Results: The percentage of CEC in DAPA-treated group was significantly lower than in diabetic rats with blood glucose fluctuations and comparable to the control. The percentage of CEPC in animals receiving DAPA was higher comparing to the other groups.
Conclusions: Dapagliflozin has a beneficial effect on the markers of non-advanced atherosclerosis in diabetic rats glucose fluctuations in blood. The results can contribute to the identification of appropriate place of this drug in the treatment of type 2 diabetes.

P.3-02

Purinergic P2 receptors are under negative control of P1 receptors in the regulation of renal hemodynamics in diabetic rats

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Background: Symptoms of developing diabetic nephropathy include disorders of intrarenal hemodynamics and abnormal renal function. Additionally, we have shown a lowered concentration of ATP and an increased concentration of adenosine in the interstitial fluid of the renal cortex. ATP and adenosine are natural agonists of purinergic receptors that regulate intrarenal microcirculation. Thus, alltered ATP and adenosine concentrations would be expected to influence purinergic signaling.

Materials and methods: In order to test this hypothesis, a series of experiments were performed using anaesthetized rats with severe hyperglycemia and an appropriate control group. We examined the effect of Ap4A, a natural agonist of P2 receptors, on intrarenal hemodynamics cortical (CBF) and medullary (MBF) blood flow and glomerular filtration rate (GFR) during intravenous infusion of DPCPX, an antagonist of P1 – A1 purinergic receptors.

Results: In control rats, intravenous infusion of Ap4A, similarly to β,γ-meATP, a synthetic P2X1 receptor agonist, increased CBF, while simultaneously reducing GFR. In contrast, in diabetic rats no significant changes in renal hemodynamics were observed. However, during intravenous infusion of DPCPX, Ap4A significantly increased CBF in diabetic rats, whereas in control rats, intravenous infusion of DPCPX completely attenuated the hyperaemic effect of Ap4A. GFR reduction was unaffected by DPCPX in both groups.

Conclusions: These findings lead to the conclusion, that in diabetic kidneys the Ap4A-dependent purinergic system is under the inhibiting influence of adenosine A1 receptors. Future studies are warranted to address possible interaction mechanisms between A1 and P2X receptors in kidneys.

P.3-03

Influence of metformin on mitochondrial subproteome in the brain of apoE knockout mice

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Background: Neurodegenerative diseases are the set of progressive brain disorders characterized by an excessive accumulation of mutant proteins in the brain. Such changes (causal factors of neurodegeneration) all impact mitochondria, immanently leading to their dysfunction. These observations predestine mitochondria as an attractive drug target for counteracting degenerative brain damage. The aim of this study was to assess the changes in mitochondrial proteome in the brain of apoE-knockout mice (apoE$^{-/-}$) and to investigate the influence of prolonged metformin treatment.

Material and methods: The study was performed on female C57BL/6J control (n=6), apoE$^{-/-}$ mice (n=6) and apoE$^{-/-}$ mice treated with metformin (n=6) at a dose of 10 mg per kg of body weight per day for 16 weeks. At the age of 6 months mice were killed, the brains were dissected and subjected to subcellular fractionation for mitochondria enrichment followed by 2DE-LC-MS/MS proteomic analysis.

Results: The quantitative histological analysis of the neurodegeneration degree indicates, that treatment with metformin markedly attenuated changes in the neocortex. The proteomic quantitative assessment of the brain mitoproteome in apoE$^{-/-}$ revealed the changes in 10 proteins expression as compared to C57BL/6J mice and 25 proteins expression in metformin-treated apoE$^{-/-}$ mice. Identified proteins mainly included apoptosis regulators, metabolic enzymes and structural proteins.

Conclusions: Presented study provided proteomic characteristics suggesting a decrease of antioxidant defense and structural disturbances in the brain mitochondria of apoE$^{-/-}$ mice. In our setting, the use of metformin changed the expression of several proteins primarily involved in metabolic processes and the structural maintenance of mitochondria as well as in the regulation of apoptosis, what could potentially restore their native functionalities.

P.3-04

AA3052, and opioid and neurokinin-1 receptors structurally related hybrid peptide exerts antinociceptive effects with lack of development of tolerance during its intracerebroventricular injection

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Background: Opioids and substance P are known to play a great role in pain transmission and perception. Therefore, specific compounds being a chemical composition of both these neurotransmitters and/or their analogs are consequently developed. In line with this, we would like to present synthesis and evaluation of biological activities of a novel chimera, AA3052. This hybrid peptide consists of an opioid pharmacophore DALDA and an modified fragment of substance P. Studies on analgesic properties of AA3052 were conducted after using mechanical and thermal pain stimuli and compared to DALDA's response.

Material and methods: Both substances were administered intracerebroventricularly. The mechanical stimulus was applied using analgesimeter, whereas thermal stimulus and thus Hot-plate test was used in order to measure analgesic responses mediated by compounds examined. Additionally, tolerance development (within 6 days) and impact on locomotor activity was measured.

Results: Presented herein chimeric compound was reported to possess dose- and time-dependent pain-relieving abilities after its i.c.v administration into rats. Interestingly, although only the high dose of 100 μg/kg in comparison to DALDA (10 μg/kg) exerted noticeable antinociceptive effects after a single injection, no analgesic tolerance developed over the entire period of repeated treatment with a test compound as opposed to DALDA.

Conclusions: AA3052 occurred to be an interesting drug candidate for pain management. However, due to lack of an opioid moiety's involvement in hybrid-induced analgesia, further studies need to be performed, as the mechanism of its action may be more complex than it was suggested.

P.3-05

Histamine in inflammation – pharmacological study of histamine receptors ligands on human eosinophils activity

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Background: Histamine is having an effect on immune cells through chemotactic activity, contributing to the cellular migration and trafficking into the sites of inflammation. This process requires leukocytes interaction with blood vessel cells, including endothelium. Despite general knowledge on leukocytes adhesion to endothelium, the role of histamine in adhesion still remains elusive. Therefore the aim of the study was to examine the effect of histamine receptors ligands on human eosinophils adhesion to endothelium. Furthermore developed cellular adhesion model was used in newly synthesized histamine receptors ligands activity evaluation.
Material and methods: Highly purified eosinophils have been isolated from the human peripheral blood, using immunomagnetic cell sorting methods. Cellular adhesion was evaluated during eosinophils co-culture with human Ea.hy.926 endothelium cell lines, under static conditions. During the adhesion assays cells were exposed to: fMLP, histamine and selective histamine receptors ligands. Pharmacological study also included newly synthesized compounds: TR-18, MWJ-3, and JNJ10191584.
Results: Histamine and fMLP significantly upregulated the number of adherent eosinophils to endothelium. Among the selected histamine receptors ligands only selective histamine H4 receptors antagonists (JNJ7777120 and thioperamide) decreased the number of adherent cells in presence of histamine. Furthermore selective H4R agonist (4-methylhistamine) could also upregulated the number of adherent eosinophils. Interestingly newly synthesized histamine receptors ligands had different effect on eosinophils adhesion.
Conclusions: Histamine is having a direct effect on human eosinophils adhesion. Histamine H4 receptor is involved in histamine dependent cellular adhesion. Presented cellular adhesion model is suitable for pharmacological evaluation of newly synthesized compounds.

P.3-06

Novel protocol for differentiation of induced Pluripotent Stem Cells (iPS) into dopamine and melanin producing cells

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Background: Induced Pluripotent Stem cells (iPS) are unlimited source of different cell types. Thanks to their traits they can found application not only in clinic but also as *in vitro* models of diseases. One important type of cells which can be generated from iPS cells are dopamine producing cells. They are particularly vulnerable to degeneration in Parkinson's disease (PD). IPS-derived dopamine producing cells *in vitro* comprise perfect model not only for drug testing but also for elucidating pathogenesis of PD.

Materials and methods: Here we present distinct protocol for differentiation of human iPS cells in a multi-step procedure. Firstly, iPS cells cultured on feeder layer were transferred to suspension culture. In next step progenitor cells were selected and expanded in serum-free medium. In final step cells were terminally differentiated into dopamine-producing cells. In characterization of acquired cells following methods were applied: RT-PCR, immunocytochemistry, HPLC, EPR and Fontana-Masson staining.

Results: On each step of differentiation protocol cells expressed specific markers (embryonic –OCT 3, NANOG - or neuronal - Nestin, Tuj-1, TH, DAT, Tyrosinase) on the level of mRNA and protein. Dopamine production was proved by HPLC with MS and appearing black pigment was identified as melanin by EPR and Fontana-Masson staining.

Conclusions: We show unique protocol for iPS cells differentiation into dopamine and melanin producing cells. Our model is useful for pharmacological tests both *in vitro* and *in vivo* but moreover it is valuable in establishing the role of melanin in pathogenesis of neurodegenerative diseases.

P.3-07

Properties of immune cells isolated from mice with selective ablation of glucocorticoid receptor in the adrenergic cells

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Background: Noradrenergic neurons with terminals in the hypothalamus are known to regulate activity of the hypothalamic pituitary adrenal axis. The aim of the study was to evaluate whether glucocorticoid receptor (GR) ablation in adrenergic cells affects immune system.

Materials and methods: The experiment was carried out on male and female C57BL/6N GR^{DBHCre} and littermate wild type mice. Conditional ablation of GR was achieved using the Cre/loxP system. Mutant mice showed degeneration of chromaffin cells and lack of adrenaline. We examined response of macrophages and thymocytes to selected stimuli and expression of cytokines in hippocampus and hypothalamus.

Results: Mutation changed in macrophage's properties, i.e. an increase in NO production and arginase activity after the interferon gamma stimulation *in vitro*. We found also sex-dependent differences, i.e. higher basal metabolic activity, NO production and arginase activity in non-stimulated macrophages obtained from female mice.

Mutation changed in thymocyte' properties, i.e. decreased a dynamics of *in vitro* inhibition of the mitogen-induced proliferative response by corticosterone, lower synthesis of interleukin-6 by concanavalin A-stimulated cells and attenuate of noradrenaline-induced increase of interleukin-10 synthesis by thymocytes. We did not found any differences in expression of cytokine's mRNA in hippocampus and hypothalamus.

Conclusions: Thus, obtained results suggest that the ablation of GR in adrenergic cells cause only slight pro-inflammatory tendency in the immune system.

P.3-08

Telomere length as a potential marker of osteoarthritis

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Background: Idiopathic form of osteoarthritis is one of the most common disorders, which severely limits mobility in the elderly population. The main cause of osteoarthritis is unknown; however, epigenetic factors including changes in telomere stability may predispose to osteoarthritis. Telomeres are specialized nucleoprotein complexes located at the ends of chromosomes and under normal conditions telomere length shortens with successive cell division. Oxidative stress, which could be increased in a cartilage of a knee joint, can additionally accelerate the rate of telomere shortening.

Materials and methods: We performed telomere length analysis on genomic DNA obtained from 100 patients with primary form of knee osteoarthritis and 100 control individuals, who were not affected by osteoarthritis. We analyzed telomere length in 50 unaffected and 50 affected cartilage samples collected from the same joint of each patient with osteoarthritis. Additionally, we measured telomere length in blood leukocytes collected from 100 patients and 100 control individuals. Relative telomere length measurements were completed via qPCR and analysis of results was performed with comparative Ct ($2^{-\Delta\Delta Ct}$) method. To assess statistical significance of the results we used dependent *t*-test for paired samples.

Results: Severe shortening of telomeres in affected cartilage samples was detected. This study also revealed telomere shortening in leukocytes of patients with osteoarthritis comparing to the control group without osteoarthritis.

Conclusions: Our data indicate that telomere shortening could be a new important mechanism contributing to cartilage aging and osteoarthritis pathology. Measurement of relative telomere length in leukocytes could be potentially used as a marker of osteoarthritis severity.

P.3-09
Ahr-targeting rescues neurons from hypoxia

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Background: Stroke is the 3rd leading cause of death worldwide. The only approved therapy against ischemic stroke is rt-PA. However, it has limitations related to narrow therapeutic window and risk of hemorrhage. Therefore, scientists are prompted to find more effective compounds to cure neuronal degenerations induced by hypoxia/ischemia. The latest study has shown that experimental stroke is followed by an increase in expression of aryl hydrocarbon receptor (AhR). 3,3'-diindolylmethane (DIM) is a plant-derived compound exhibiting properties of selective AhR modulator. Our recent data demonstrated that treatment with DIM and simultaneous exposure to hypoxia led to neuroprotection. However, there are no data on neuroprotective capacity of DIM used after episode of hypoxia which is clinically most relevant.

Material and methods: Primary neuronal cell cultures, hypoxia (18 h), caspase-3 and lactate dehydrogenase (LDH) activities, and expression of AhR, ARNT and CYP1A1 were performed as previously. Hippocampal cultures were subjected to hypoxia that was followed by treatment with DIM (0.1-10 μM) within 1-6 h.

Results: Hypoxia stimulated caspase-3 and LDH activities which was accompanied by an increase in expression of hypoxia-inducible factor-1α. DIM in a concentration- and time-dependent manner inhibited hypoxia-induced parameters. Moreover, DIM substantially reduced expression of AhR, its nuclear translocator ARNT as well as AhR-regulated CYP1A1.

Conclusion: Our study provided evidence on strong neuroprotective capacity of DIM against hypoxia. Furthermore, it demonstrated that DIM-evoked neuroprotection was retained till 5th h after hypoxic insult which may contribute to development of effective therapeutic strategies targeting AhR signaling pathways.

P.3-10

Disadvantages of using heterologous mammalian cells with tetracycline induced expression GABA(B) receptor in pharmacological studies

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Background: GABA(B) receptor is a heterodimer formed by two subunits, which both are necessary for receptor functionality. GABA(B1) subunit includes an extracellular domain which binds GABA or other ligands and GABA(B2) subunit is a transmembrane domain responsible for activation of G-protein. Expression of GABA(B1) and (B2) subunits in heterologous mammalian cells is a tool for pharmacological studies searching new agonists, antagonists and allosteric effects.

Material and methods: HEK293 T-REx cell line expressing GABA(B1) and (B2) subunits was obtained through cloning GABA(B1) sequence into pcDNA5/FRT/TO vector, stable transfection and induction of protein expression with tetracycline. The GABA(B2) constitutive expression was obtained by stable transfection with pcDNA3.1 vector coding GABA(B2) sequence. GABA(B1) and (B2) expression were confirmed by standard molecular biology techniques (qRT-PCR and Western blotting).

Results: HEK293 T-REx parental cell line expressed endogenous GABA(B) receptors that undergone tetracycline induction. That suggests the presence of tetracycline-responsible elements in the transcription regulatory sequences of GABA(B) gene.

Conclusions: In some cases the system used in pharmacological studies - mammalian cells with heterologous tetracycline induced expression of different metabotropic receptors need to be reevaluated. Endogenous expression level of some metabotropic receptors in HEK293 T-REx cell line could increase after tetracycline induction and could influence the results of pharmacological studies.

P.4 Preclinical research – Biotechnology

P.4-01
The new bioactivities of modified pullulan

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Background: It has been suggested that INSIG1 plays a central role in the feedback control of lipid synthesis regulating the synthesis of cholesterol due to binding in sterol-dependent way to HMG-CoA reductase and causing proteosomal degradation of this enzyme.

Materials and methods: Cationically modified Pullulan-GTMAC (Pull-GTMAC) was synthesized by us by covalent attachment of glycidyltrimethylammonium chloride (GTMAC) and was investigated using Real-time PCR and extraction of lipids by Soxhlet method.

Results: It was shown that Pull-GTMAC affects the expression of lipid metabolism genes by statistically significant up-regulation of both INSIG1 and LDL receptor gene in brown fat tissue of apoE-knockout mice after oral administration with feed at a dose 300 mg/kg b.w./day for 18 weeks. Both pullulan-GTMAC and dextran cationically modified with GTMAC (Dex-GTMAC) induced statistically significant decrease of raw fat content in the feces of apoE-knockout mice fed with these two polysaccharides at a dose of 300 mg/kg b.w./day for 16 weeks as compared to control. Dex-GTMAC caused higher reduction of excreted fat than Pull-GTMAC. In addition, Pull-GTMAC exhibited an antiproliferative properties in MTT test on HepG2 cell line. There was statistically significant decrease of HepG2 cells viability induced by Pull-GTMAC at concentrations of 0.1, 0.3, 1.0, 3.0 µg/ml of cell culture medium after 24 h incubation as well as after 48 incubation at concentrations of 0.3, 1.0, 3.0 µg/ml. However, Pull-GTMAC increased cell viability after 48 h incubation at concentration of 100 µg/ml. Similar varied effects occurred in rat SMCs.

Conclusions: Results indicate that cationically modified polysaccharides (Pull-GTMAC and Dex-GTMAC) affect efficiently systemic metabolism of lipids and exhibit (Pull-GTMAC) antitumor potential.

P.4-02

Genome and transcriptome profiling of leukemic blasts in context of *in vitro* resistance towards 11 anticancer drugs

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Background: In this study we analyzed the gene expression and cytogenetic profiles in correlation with the profile of *ex vivo* resistance to 11 antileukemic drugs in pediatric acute leukemias.

Materials and methods: We tested leukemic blasts, obtained from 166 patients, for *in vitro* sensitivity to idarubicin, cyclophosphamide, daunorubicin, doxorubicin, mitoxantrone, etoposide, cytarabine, fludarabine, treosulfan, clofarabine and busulfan. Samples from patients were designated as sensitive or resistant. Gene expression profiles were obtained by interrogating Affymetrix U133A arrays. Genomic signatures were performed with using the Agilent SurePrint G3 Human CGH arrays. Key results for selected genes were verified using qRT-PCR with UPL probes.

Results: An individual genomic and transcriptomic profiles for the resistance against each drug were constructed, containing 33-466 genes and several characteristic cytogenetic changes. The most of them include genes classified as: enzyme modulator, transcription factor, transferase, as well as involved in cell communications. The most numerous in individual resistance profiles were represented genes of the following pathways: inflammation mediated by chemokine and cytokine signaling, Wnt or integrin signaling and angiogenesis. A list of the top 20 multidrug resistance candidate genes for analysed antileukemic drugs was identified.

Conclusions: The validated genetic patterns provide new gene candidates for multidrug resistance. Overexpression of *ANXA1*, *CDKN1A*, *FGR*, *HK3*, *SERP1*, *DUSP2* and *ITGAM*, as well as a down regulation of *RETN*, are most correlated with *in vitro* resistance. Although many potential mechanisms may contribute to the lack of sensitivity to therapy, there is accumulating evidence that changes in expression level of genes and cytogenetic alterations from multiple biologic pathways are involved.

P.4-03

The release and action of irisin in human adipocytes

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Background: Irisin is one of the polypeptides responsible for the browning of white adipocytes in response to chronic exercise. Recent study reports that recombinant irisin regulates the thermogenic program in fat through ERK and p38 pathways. The study was aimed to investigate intracellular mechanisms of irisin action in human adipocytes and to study regulatory factors of irisin secretion.

Material and methods: Human cell line CHUB-S7 were differentiated to mature adipocytes and than exposed for 24hrs to irisin or GLP-1 (glucagon-like peptide-1) or GLP-1R agonist, exendin-4. NAD+ and irisin in medium were measured by ELISA; mitochondrial respiration was measured by high resolution respirometry, gene expression was determined by RT-PCR.

Results: GLP-1 and exendin-4 lead to mild uncoupling of mitochondrial electron transport from ATP synthesis, slightly decreasing mitochondrial membrane potential ($\Delta\psi$) analyzed by JC-1 fluorescence (flow cytometry). Basal respiration rate and the relative contribution of uncoupled respiration was also significantly higher compare to control cells. The level of NAD^+ was elevated and the same effect was observed in irisin treated cells. The concentration of native irisin released from adipocytes was measured in culture medium. Secretion of irisin to the culture medium were higher in GLP-1 or exendin-4 treated human adipocytes. The FNDC5 (encoding irisin) gene expression was also up-regulated by GLP-1. Enhanced expression of PGC1alpha, UCP2 genes suggest change in cells phenotype towards beige adipocytes.

Conclusions: The stimulatory effects of GLP-1 or its analog exendin-4 on mitochondrial bioenergetics in mature CHUB-S7 adipocytes was observed. Increasing of energy expenditure by GLP-1 may be associated with up-regulation of irisin synthesis, secretion and autocrine action in adipocytes.

P.4-04

Palmitic acid and arachidonic acid induce mitochondrial dysfunction in beta cells and change the expression of miRNA related to insulin signaling pathway

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Background: One of the reason of β-cell failure and the development of diabetes is mitochondrial dysfunction, which may lead to impaired glucose stimulated insulin secretion (GSIS) and β-cell apoptosis. Elevated level of free fatty acids (FFA) is implicated in the etiology of insulin resistance, obesity and T2D. MicroRNA are small non-coding RNAs, relevant for the regulation of gene expression. A specific set of miRNA plays an important role in β-cell differentiation, insulin secretion, as well as compensatory β-cell mass expansion in response to insulin resistance.

Methods: The mouse pancreatic β-cells (BTC-6) were incubated for 24h with 0,6mM palmitic (PA) or arachidonic (AA) acid. Mitochondrial functions were monitored by mitochondrial oxygen consumption rates (high resolution respirometry), ATP content (luminescent assay), changes in the mitochondrial membrane potential (JC-1 staining). Apoptosis was identified by activity of the caspase-9 (*R&D Systems*) and annexin V (flow cytometry). GSIS was measured using ELISA test. Changes in the miRNA expression were investigated by real time PCR using TLDA MicroRNA Panel (AB).

Results: PA and AA increased the percentage of cells in the early phase of apoptosis and the activity of the caspase-9. PA stimulated oxygen consumption, without increased ATP production, what suggests mitochondrial uncoupling. Incubation with fatty acids decreased GSIS and regulated miRNA expression. PA up-regulated miR-375, miR-181c, miR-24 and down-regulated miR-128a. AA down-regulated miR-93, miR-130a, miR-222 and miR-181c.

Conclusions: Both PA and AA induced β-cells apoptosis involves pathway associated with mitochondria and decrease GSIS. Palmitic acid disrupts the function of mitochondria by OXPHOS uncoupling. Both studied fatty acids change the expression of miRNA related to insulin signaling pathway, glucose uptake (miR-93, 130a, 128a, 375) and energy metabolism (miR-181c).

P.5 Preclinical research – Medicinal Chemistry

P.5-01

Influence of polymyxin B nonapeptide on the antibacterial activity of metallic and bimetallic nanoparticles-colloids

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Background: Some metal-containing nanoparticles (NPs), including bimetallic NPs are interesting antimicrobial agents. The combination of the substances exhibiting a synergistic effect with the metal NPs seems to be a new strategy in searching for an innovative antimicrobial preparations.
The investigation whether polymyxin B nonapeptide (PMBN) destabilising the membrane permeabilisation affects the antibacterial activity of metal NPs, was performed.

Materials and methods: The antibacterial activity of 3 metallic NPs: AgNPs, PdNPs and CuNPs, their mixtures and 2 bimetallic NPs: Ag/PdNPs (50% 50%) and CuZnNPs (63% 37%) was evaluated. All studied NPs were prepared in the colloid state in the concentration 400 ppm by NanoKoloid factory, Poland. The antimicrobial activity was screened by the cylinder-plate diffusion test and the MIC values ± PMBN (1 mg/L) were estimated according to CLSI recommendations. The 11 Gram-positive and Gram-negative ATCC bacterial strains were applied in this study.

Results: The highest activity against all bacteria has been observed in the case of NPs containing silver - colloids of AgNPs, Ag/PdNPs, mixtures of AgNPs and PdNPs (1:1), AgNPs and CuNPs (1:1), as well as colloids of bimetallic Cu/ZnNPs (MICs 12.5-50 mg/L and 31.5/18.5-126/74 mg/L, respectively). PdNPs, CuNPs and their mixture (1:1) did not show the antibacterial activity (MIC >200 mg/L and >100/100 mg/L, respectively). Importantly, the synergistic effect of PMBN on the antibacterial activity of bimetallic Ag/PdNPs against all studied strains was obtained. The MIC values decreased from 12.5/12.5 – 50/50 mg/L to 1.56/1.56 – 12.5/12.5 mg/L. This influence was not observed in the case of other studied NPs and their mixtures.

Conclusions: Presented data indicated the structure-activity relationship among tested NPs. Bimetallic NPs containing silver applied together with a substance destabilising the membrane permeabilisation may be consider as an effective antimicrobial preparation.

P.5-02

Towards new 5-HT₇R ligands with improved metabolic stability – synthesis of LP-211 derivatives and their comprehensive evaluation *in silico* and *in vitro*

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Background: Compounds modulating activity of serotonin receptor 5-HT₇ are very important in terms of the therapy of disorders that are crucial from the social point of view, such as depression, cognitive disorders, anxiety and Alzheimer disease. However, the activity itself is not sufficient for a compound to constitute a promising drug candidate – not less relevant are also its physicochemical and pharmacokinetic properties, lack of toxicity and metabolic stability.

Materials and methods: A series of derivatives of the selective 5-HT₇R agonist: LP-211 was synthesized and evaluated *in vitro*. In order to facilitate and support the design of new stable 5-HT₇R ligands, *in silico* model for the metabolic stability evaluation was developed, based on the Support Vector Machines algorithm.

Results: The study led to identification of the derivative TP22 that showed affinity and selectivity profile comparable to that of LP-211, 3-fold higher *in vitro* metabolic stability, and ability to stimulate neurite outgrowth *in vitro* and to cross blood-brain barrier. The promising properties of TP22 determined its selection for further *in vivo* studies. The analysis of the accuracy predictions of the *in silico* model revealed that they strongly depended on the chemical substituents introduced to compounds and the set of descriptors used for compounds representation.

Conclusions: In further *in silico* research, it is important to focus on the provision of the proper representation of data, especially description of the compounds lipophilicity, crucial for the proper metabolic stability prediction.

P.5-03

Aminergic GPCRs from a site-directed mutagenesis perspective – analysis and prediction

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Background: Aminergic subfamily of class A G protein-coupled receptors (GPCRs) is one of the main targets of drug discovery campaigns, comprising proteins participating in the staggering range of interrelated physiological processes in human organism. The determination of structural drivers for ligand affinity within aminergic proteins has long been supported by extensive mutagenesis studies.

Materials and methods: All mutagenesis data referring to ligand affinities to aminergic GPCRs were collected and analyzed from various perspectives – the distribution of the mutated amino acid residues, the reference compounds, and the effect of the mutations on ligand binding were examined. Moreover, on the basis of the docking and interaction fingerprints coupling, an *in silico* protocol for mutational effect prediction was developed.

Results: The comprehensive evaluation of mutational data studies provided deeper insight into the structural requirements for ligand activity for aminergic GPCRs and enabled to look at the data from broader perspective. The developed methodology for mutational data prediction enabled not only the correct evaluation of the effect of mutation for particular proteins, but also allowed for predicting the consequence of mutation in the other receptor subtypes.

Conclusions: The extensive mutational study carried out allows for determination of comprehensive interaction patterns which are characteristic for the particular aminergic targets. This will be of great help in the further research on development of ligands modulating the activity of these receptors in the desired way.

P.5-04

***In vitro* and *in silico* studies on zinc interaction with 5-HT₇ receptors**

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Background: Zinc ions play important functions in the central nervous system (CNS) but many aspects of their action is unclear. In recent years, various aspects of GPCR allosterism were intensively studied, because the interaction with the receptor according to other than classical, competitive model of orthosteric ligand binding, creates new possibilities for its regulation. Our interests are focused on Zn^{2+} effects mediated by serotonin receptors, which are key players in the etiology of anxiety and mood disorders.

Materials and methods: The direct influence of Zn^{2+} on compound binding to human 5-HT₇R, stably expressed in HEK293 cells, was investigated by a set of *in vitro* functional cAMP assays and radioligand binding methods (saturation, competition and both association and dissociation kinetic studies) using [^{3}H]5-CT, [^{3}H]SB-269970 and [^{3}H]Mesulergine - an agonist and two antagonists, respectively. The MD simulations were performed on homology models of 5-HT₇R, created on the basis of crystal structure of 5-HT₁ʙR (pdb: 4IAR). Structures of reference agonist (5-CT) and antagonist (SB-269970) were docked into the model (Glide 5.5) and the ligand-receptor complexes were input for the MD with zinc ions. Simulations sytems were constructed with POPC membrane and TIP3P water model.

Results: Results of both types of *in vitro* experiments demonstrated that Zn^{2+} ions act as negative allosteric modulator (NAM) at 5-HT7 receptors. MD shows the differences in interactions with zinc ions between antagonist- and agonist-bound models of 5-HT₇R. In both experiments zinc was contacting with ecl2 (D167 and D168), however in complex with antagonist zinc ion is also observed interacting with D3.32.

Conclusions: Our study extends data regarding the Zn^{2+} action at 5-HT₇R. Both *in vitro* and *in silico* studies suggest that zinc can modulate the 5-HT₇R.

P.5-05

Evaluation of alpha2-adrenoceptor subtypes involved in mydriatic effects of two newly synthesized imidazoline derivatives

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Background: The imidazoline derivatives are able to evoke pupillary dilation after systemic application to laboratory animals (rats, cats and mice). Stimulation of postsynaptic alpha2-adrenoceptors in the brain is assumed to mediate this effect, while imidazoline receptors seem to be not involved. Moreover the engagement of alpha2 receptors subtype(s) in mydriatic activity of imidazolines is still not explained **in detail**. In these study the pupil dilatory effect evoked by two newly synthesized imidazoline-like alpha2-adrenoceptor agonists: marsanidine, and 7-methylmarsanidine were compared. The compounds were tested alone and also in presence of alpha2-adrenoceptor antagonists (nonselective -yohimbine and selective towards the following alpha2-adrenoceptor subtypes:alpha2B - ARC239, alpha2C - JP1302, alpha2D - RX 821002, respectively).

Materials and methods: The compounds studied were administered to anaesthetized male Wistar rats intravenously in cumulative doses and in separate experiments each of the antagonists was given in a single dose prior to marsanidine and 7-methylmarsanidine. Pupil diameter was measured with stereoscopic microscope equipped in green light filter.

Results: Marsanidine and 7-methylmarsanidine exerted marked mydriatic effects (Emax: $3.972 \pm 0,095$ mm , $3.572 \pm 0,078$ mm, respectively). JP1302 and ARC239 caused slight parallel shifts to the right of the dose-effects curves obtained for both imidazolines. In case of yohimbine and RX821002 the marked parallel shifts of marsanidine and 7-methylmarsanidine dose-response curves were observed but the antagonistic effects of RX821002 were more pronounced.

Conclusions: The results obtained may suggest that alpha2D subtype of alpha2-adrenoceptor is mainly engaged in mydriatic effects of imidazoline derivatives.

P.5-06

Identification of new Formyl Peptide Receptor-2 (FPR2) agonists with neuroprotective properties

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Background: The Formyl Peptide Receptor-2 (FPR2), is a G protein-coupled receptors family and can induce pro- and anti- inflammatory as well as pro-resolving effects when activated. It has been suggested that FPR2 may be an innovative target for the development of anti-inflammatory drugs for the treatment of chronic inflammation, an hallmark of several neurodegenerative diseases. Activation of FPR2 receptors may promote the resolution phase of the inflammation, when restoration of tissue occurs. FPR2 receptors interact with a large number of structurally diverse ligands. Recently, we have identified a new class of non-peptidic FPR2 agonists with an ureidopropanamide structure.

Materials and methods: New ureidopropanamide derivatives have been prepared and their agonistic activity has been assessed as ability to induce intracellular Ca^{2+} release in HL-60 cells transfected with human FPR2 and in human neutrophils. In vitro metabolic stability of the new compounds has been evaluated in rat microsomes. Next, the influence of the most promising agonists on viability/metabolic activity, necrotic death, and nitric oxide production in both lipopolisacharide (LPS) stimulated and unstimulated primary microglia cells has been examined.

Results: The newly prepared compounds showed improved agonistic properties and microsomal stability. Some FPR2 agonists exhibited statistically significant neuroprotective properties, mainly on nitric oxide production and lactate dehydrogenase release evoked by LPS stimulation.

Conclusions: New FPR2 agonists with improved pharmacokinetic properties have been identified. Selected compounds showed neuroprotective effects in LPS-stimulated microglia cells, suggesting that targeting FPR2 receptors could be a promising target for the development of new therapeutic approaches in neurodegenerative diseases.

P.5-07

Comparison of structural changes of bovine serum albumin (BSA) upon glycation, oxidation and alkylation processes - *in vitro* studies

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Background: Albumin, the most abundant protein in the blood, plays many important functions in human organism, serving among other as the main antioxidant and transport protein. Some pathophysiological and environmental factors, such as hyperglycemia, reactive oxygen species, or substances derived from processed food may lead to structural changes of albumin molecule, affecting its biological role. The aim of this study was to evaluate changes in the secondary and tertiary structure of BSA, highly homologous to human albumin, caused by exposure to glucose (GLU), chloramine T (CT) and acrylamide (ACR) in concentrations that may occur under *in vivo* conditions.

Materials and methods: BSA solutions (0,6 mM) were treated with: 30 mM glucose, 0,1 mM chloramine T and 90 nM acrylamide. Following the incubation in 37°C samples were taken at different time points: after 1 hour, 1 day and 1 week and were dialyzed. The secondary and tertiary structure of BSA were assessed by the circular dichroism and fluorescence spectroscopy, respectively. The effect of above agents on the structure of BSA was expressed as percent of changes in comparison to the native form of BSA.

Results: All tested agents caused conformational changes in BSA molecule, both in secondary and tertiary structure, which increased progressively with time of incubation. After 1 week, GLU, CT and ACR changed the secondary structure in similar degree, about 10%, while the tertiary structure was the most affected by CT, causing an about 18% change. GLU quenched fluorescence intensities in 8% and ACR only in 0,5%.

Conclusions: All tested agents in concentrations imitating possible *in vivo* condition changed BSA structure. It indicates that exposition to glucose, chloramine and acrylamide occurring in different diseases, or as a result of dietary intake may have adverse effects on biological functions of albumin, in particular its antioxidant and binding properties. Interestingly, ACR, although small amounts, caused comparable to other factors changes in the secondary structure.

P.5-08
Phosphoramidate diesters of zidovudine and their cytostatic activity in vitro

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Background: Some of nucleoside analogues play an important role in anticancer and antiviral pharmacotherapy. Zidovudine (AZT), although originally designed as an antitumor agent, is so far widely used as antiviral inhibitor of HIV-1 reverse transcriptase, that acts as a DNA chain terminator. It is postulated that the mechanism of action of zidovudine may be associated with its conversion to zidovudine-triphosphate , followed by its incorporation by DNA host polymerases. Phosphoramidate pronucleotides seems to be effective for the intracellular delivery of nucleoside 5'-monophosphates (MP) as regards to their in vitro anticancer activity showed in human tumor cell models.

Materials and methods: A series of seventeen newly synthesized: 5'-(4-chloropfenyl)-and 5'-(2-chlorophenyl)- phosphoramidate diesters (7D – 23D) desired as the potentially pronucleotides were studied against five different human cancer cell lines, based on the DR and D, NCI, NIH Bethesda programs and using SRB in vitro method.

Results: Three mostly active compounds: 9D, 11D, 18D and 22D were qualified for in vitro investigation using a mixture of insect cell-expressed human drug metabolizing cytochrome P450 isoforms (CYPs: 1A2,2C8,2C9,2C19,2D6 and 3A4) model as to be related to their activities in human liver microsomes. The results performed with cDNA-expressed cytochrome P450 isoforms showed considerable increase of anticancer activity in vitro against U87 tumor cells for compounds: 9D and 11D with their IC_{50} of 0.83 and 0.39 $\mu g/cm^3$ respectively, in comparison with non-activated control samples.

Conclusions: It is proposed that the influence of drug metabolizing CYP450 isoforms plays important role both in chemical reduction of phoshoramidate diesters of zidovudine with formation to highly reactive intermediate of 3'-amino-derivatives metabolites and enhance their in vitro anticancer activity.

P.5-09
Structural features of arylpiperazine derivatives - selective ligands of the 5-HT₇ receptor

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Background: The search for selective drugs acting on serotonin receptors is actual task, due to increasing number of people suffering from various mental diseases. Arylpiperazine derivatives are well known ligands of the serotonin receptors. Compounds with the arylpiperazine

fragment, which show high affinity to the 5-HT7 receptor, often bind to the 5-HT1*A* receptor as well. Thus, search for structural features responsible for selective binding to the 5-HT7, but not to the 5-HT1*A*, is our main goal.

Materials and methods: Crystal structure analysis was performed for arylpiperazine derivatives with confirmed affinity to the 5-HT7 receptor. Molecular modeling was applied to obtain models of the 5-HT1*A* and 5-HT7 receptors. Docking study was performed in order to define the differences in the binding mode.

Results: Crystal structure analysis of selected arylpiperazine derivatives, substituted in position 2 or 3 of the aromatic ring with different benzene related substituents, showed a various inductive effect on the π-electron resonance. This have direct impact on pyramidal geometry of the substituted nitrogen atom of the piperazine. Ligands were docked in putative binding sites of the receptor models. Selected poses were compared with conformations observed in the crystal structure to find similarity and differences in geometries.

Conclusions: The angular relationship between the planes of the aromatic rings and their orientation relative to the piperazine ring seem to be the most important criterion allowing selective binding to the 5-HT7 receptor.

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P.5-10

Potency of polypirydyl ruthenium complexes as contrast agents for optical imaging

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Background: Optical imaging techniques, in particular those based on luminescence, are very attractive due to being cheap, easy to handle, quite sensitive and harmless for patients. The progress in the development of instrumentation for optical instrumentation has decisively contributed to applicability of this new technique in clinical trials. Currently there is a continuing need for designing of novel probes which enable to monitor disease-specific anatomic, physiological and molecular parameters through their optical signals.

Materials and methods: Polypyridyl ruthenium complexes display interesting photophysical properties such as a high quantum yield and long lifetimes of luminescence (usually phosphoroscence), such that these complexes can be used for cell imaging or as markers in diagnosis based on optical imaging. To gain more insight on how to design such compounds, the influence of substituents on physicochemical and photophysical properties, as well as the impact on cytotoxicity and optical imaging in *in vitro* studies, were analysed for a series of complexes of the type $[Ru(dip)_2(R_1bpy-R_2)]^{2+/+}$ (R_1 = H or CH_3, R_2 = H, CH_3, COO^-, bpy-2-nitroIm or 1,3-dicyclohexyl-1-carbonyl-urea denoted as DCU).

Results: The imaging properties of the studied ruthenium complexes are strongly influenced by the level of internalization and cellular protein interactions. The interaction with proteins can pronouncedly increase the luminescence properties of these Ru complexes. The $[Ru(dip)_2(CH_3bpy-DCU)]^{2+}$ complex, regardless of its weakest luminescence parameters, displays the best imaging properties in cancer cells.

Conclusions: The obtained results pointed out that, while designing cellular imaging probes, it is necessary to consider not only the luminescence parameters of a single substance, but their luminescence expressed in the cellular environment and the extent of its accumulation.

P.5-11

Discovering new allosteric modulators for mGlu7 receptor- characterization of new compound IP562257

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Background: Glutamate, the main excitatory neurotransmitter in CNS exerts its effects trough both ionotropic and metabotropic receptors. One of them is the most highly conserved across species metabotropic receptor isoform 7. It is localized to presynaptic terminals and acts as an important regulator of synaptic plasticity. Interestingly, the receptor has the lowest affinity for glutamate compare to other isoforms. It implicates mGlu7 receptor as an ideal target for a number of pathological conditions such as anxiety and depression. Additionally the fact that mGluR7 activation facilitates extinction of learned fear and aversion, enable to use it as potential therapeutic target in human anxiety conditions as post-traumatic stress disorder and drug abuse.

At present there are known only few compounds that influence the pharmacodynamics of the receptor *in vitro* and *in vivo*: MMPIP a negative modulator, AMN082 a positive modulator and XAP044 antagonist. Thus, there is strong need for developing better compounds which can be used in clinical trials. The most promise could be mGlu7 allosteric modulators due to better selectivity compare to orthosteric ligands. Aim: Identification novel chemical scaffold possessing mGlu7 negative allosteric modulatory effect.

Materials and methods: Human GRM7 was cloned into HEK-293 cells contained T-Rex expression system (Invitrogen). HTRF cAMP dynamic-2 kit was used to determined forskolin-induced cAMP accumulation according to manual (Cisbio) after treatment with agonists and IP562257. The concentration response curves were fitted using the non-linear regression analysis program, GraphPad Prism.

Results: The new chemical scaffold possessing mGluR7 potential NAM activity was obtained by chemical synthesis on the house. IP562257inhibited agonist induced activation of mGlu7 receptor.

P.5-12

Identification of IP340144, a new scaffold for negative allosteric modulators of mGluR5

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Background: mGlu5 receptor has been recognized as the potential target for pharmacotherapy of Parkinson disease and in fragile X syndrome, with the primarily interest on selective negative allosteric modulation.

Material and methods: Stably transfected HEK293 T-REx cells with inducible expression of human mGluR1 or mGluR5 were used in receptor activity assays. Active ligands were selected experimentally from *in house* collection of 718 compounds in a two-step strategy. The initial screen was based on the detection of calcium ions mobilization from intracellular stores upon receptor activation. The cells were exposed to submaximal concentration of receptor agonist L-glutamate in combination with known allosteric ligands or newly synthetized compounds. Then, cytoplasmic calcium ions were quantified based on the fluorescence of cell-permeable calcium indicator, Fluo-8 AM. Fluorescence signal was measured in real-time in a kinetic plate reader that allows simultaneous ligand dispensing and fluorescence detection in 384-well plate format (FDSS/μCell Functional Drug Screening System, Hamamatsu). Candidate compounds were selected for further characterization based on inositol phosphate synthesis. IP1 was quantified in competitive immunoassay, where native IP1 produced by cells displaces labeled IP1 from its complex with specific antibody, followed by a drop in FRET signal (IP-One Homogenous Time-Resolved Fluorescence assay, Cisbio).

Results: 22 compounds were selected in the first screen based on fluorescent detection of calcium mobilization. The promising compounds originated from the library of a novel chemical scaffold. The most potent IP340144 was further characterized to down-modulate mGluR5-induced accumulation of IP1 and to not interact with mGluR1.

Conclusions: We have developed a new mGluR5 NAM scaffold and characterized its activity in *in vitro* receptor activity assays.

P.5-13
IP272050, a novel mGluR8 positive allosteric modulator

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Background: Aberrant glutamatergic neurotransmission may underlie the pathogenesis of schizophrenia. MGlu$_8$ receptor is located pre-synaptically where it acts as an inhibitory autoreceptor in key brain areas related to the pathophysiology of schizophrenia.
While mGluR$_8$ shows great promise as pharmacotherapy target for some psychiatric disorder, especially schizophrenia, we are still lack of selective and active compound.
Methods: Human GRM8 was cloned into HEK-293 T-Rex expression system (Invitrogen). The screening study and activity of potential mGluR$_8$ ligands was determined using forskolin-induced cAMP accumulation, in a HEK-293 T-Rex cell line with iducible expression of mGlu$_8$ or in mock transfected HEK-293 T-Rex cell line. For the functional characterization of mGlu$_8$ we measured the level of cyclic AMP (cAMP) by cAMP dynamic2 kit (Cisbio). All liquid handling operations were performed using EVO 2000 system (Tecan). Data analysis was performed in GraphPad Prism v. 5.03 (GraphPad Software). Social interaction test and DOI-induced head twitches in mice has been performed according to standard procedure.
Results: Via *in vitro* screening of *in house* compound collection we have identified chemical scaffold possessing mGluR$_8$ potential PAM activity. Then, we developed library of active compounds with better pharmacodynamic properties. The most interesting IP272050 interacted more potent with mGluR$_8$ than reference compound AZ12216052. IP272050 have been characterized to:
– activate mGluR$_8$ as a positive allosteric modulator (EC$_{50}$ = 4,4 µM in the presence of 1 µM L-Glu, dose-response L-Glu shift = 1,9)
– be active *in vivo* in: social interaction test and DOI-induced head twitches in mice
Conclusions: We have developed a novel mGluR$_8$ PAM active *in vitro* and *in vivo*.

P.5-14

Novel 1*H*-pyrrolo[3,2-*c*]quinoline based 5-HT₆ receptor antagonists with potential application for the treatment of cognitive disorders associated with Alzheimer's disease

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Background: 5-HT₆ receptor has been proposed as a promising drug target for the treatment of cognitive disorders associated with Alzheimer's disease (AD). Herein we report the design synthesis and pharmacological evaluation of novel class of 5-HT₆R antagonists based on 1*H*-pyrrolo[3,2-*c*]quinoline core.

Materials and methods: Primary evaluation of the obtained compounds consisted in determination of their 5-HT₆R affinity, selectivity and functional profile. The functional properties of the most promising compounds from the preliminary screening were further evaluated as their ability to inhibit cAMP. The influence of selected compounds on the 5-HT₆R constitutive activity at Gs signaling was assessed by BRET using the cAMP sensor CAMYEL. Procognitive effects of selected compounds were assessed in NOR tasks whereas their anxiolytic and antidepressant-like activity was determined in Vogel test and FST test in rats.

Results: The study allowed for the identification of compound **14**, classified as neutral 5-HT₆R antagonist, while SB-742457 used as reference, behaved as an inverse agonist. Compounds **14** and SB-742457 reversed phenyclidine memory deficits and displayed procognitive properties (3 mg/kg) in NOR tasks. Moreover, compound **14** displayed higher anxiolytic effect (MED=3 mg/kg) than SB-742457 in the Vogel test and showed similar antidepressant-like properties in 3-fold higher dose (MED=10 mg/kg) than SB-742457 (MED=3 mg/kg) in FST.

Conclusions: Obtained results support the therapeutic potential of 5-HT₆R antagonists and inverse agonists of 5-HT₆R for the treatment of AD. Presented work suggests that 1*H*-pyrrolo[3,2-*c*]quinoline scaffold is an attractive molecular framework for the development of pro-cognitive agents.

P.5-15

Towards metabolically stable arylsulfonamide derivatives of (aryloxy)ethyl piperidines as potent 5-HT₇ receptor antagonists with antidepressant and anxiolytic properties

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Background: Several preclinical and clinical data support the hypothesis that 5-HT₇R may be regarded as potential target for the treatment of depression, anxiety and cognitive disorders. Continuing our studies in identifying 5-HT₇R antagonists, we synthesized a focused library of arylsulfonamide derivatives of (aryloxy)ethyl piperidines. Structural modifications were aimed to optimize physicochemical parameters towards improvement of metabolic stability.

Materials and Methods: Synthesis of designed compounds was carried out according to a multistep procedure. Radioligand binding assays were performed to determine the affinity for 5-HT₇Rs and selectivity over other related monoaminergic receptors using HEK293 cells. The functional *in vitro* activity were evaluated in cAMP cellular assays using HEK293 cells. The *in vitro* biotransformation studies were performed using mouse liver microsomes. The antidepressant and anxiolytic activity were *in vivo* determined in animal models–forced swim test and four-plate test, respectively.

Results: Evaluated compounds displayed high affinity for 5-HT₇Rs ($K_i < 50$ nM) and selectivity over 5-HT₁ₐ, 5-HT₂ₐ, 5-HT₆, D₂Rs. Having identified potent and selective 5-HT₇R antagonists ($K_b = 1$–40 nM), tested compounds showed high metabolic stability *in vitro* assays ($Cl_{int} = <40$ µl/mg/min). Finally, the most promising compounds exerted antidepressant and anxiolytic properties at doses of 1.25–2.5 mg/kg displaying potency similar to that of the 5-HT₇R antagonist SB-269970 and diazepam used as references.

Conclusion:. The study allowed the identification of potent and selective 5-HT₇R antagonists with high metabolic stability, which displayed significant antidepressant and anxiolytic properties. Further studies would provide additional information regarding pharmacokinetic profile of these derivatives and their potential applications for the treatment of cognitive deficits.

P.5-16

Halogen bonding enhances affinity at 5-HT₇R in a series of N-[2-(dimethylamine)ethyl]-N-(2-phenylehtyl)anilines

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Background: Halogen bonds (XB) are specialized non-covalent interactions known since XIX century, but only recently they were recognized as important in binding of a biologically active molecules. They can be described by general structure $DX\cdots A$, where DX is a halogen bond donor (X = Cl, Br, I), and A is a Lewis base.

Materials and Methods: All of the obtained compounds were obtained in a two step synthesis involving firstly nucleophilic substitution of 2-chloroalkyldimethylamine with aniline and secondly reductive amination of phenylacetaldehyde. Molecular modeling of halogen bonds was performed using combined quantum-polarized ligand docking (QPLD) and Molecular-Mechanics-Generalized-Born/Surface Area (MM/GBSA) free-energy calculation. Affinity values at 5-HT₇R were obtained through a radioligand binding assay.

Results: Here we present a series of N-[2-(dimethylamine)ethyl]-N-(2-phenylehtyl)aniline derivatives that were found in a bioisosteric query designed for creating a dual $D_2R/5\text{-}HT_6R$ ligands. One of the obtained chlorine substituted compounds revealed to possess also a high affinity for 5-HT₇R ($K_i = 4$ nM). A QM/MM docking experiments suggest that halogen bonding with Ser5.42 may be responsible for its high 5-HT₇R affinity.

Conclusions: The chlorine substituted compound, possessing selective activity at both 5-HT₆R and 5-HT₇R, might provide an interesting candidate for an antipsychotic/antidepressant drug with procognitive properties. Analysis of Structure-Activity Relationship enforced with molecular modeling revealed, in the case of obtained series, important role of halogen bonding in receptor binding affinity and selectivity.

P.5-17
Homology modeling of serotonin receptor 5A

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Background: Serotonin receptors belong to Class A of the G Protein-Coupled Receptors (GPCRs), a superfamily of membrane receptors sharing common topology of seven helices penetrating the cell membrane. Serotonin receptor 5A (5-HT_{5A}) is the least known member of the 5-HTRs and its localization within brain structures is speculative. 5-$HT_{5A}R$ is postulated to be involved in mood control and cognitive functions, and its malfunctioning can be linked to many diseases, such as schizophrenia or Huntington's disease. There is only a handful of known 5-$HT_{5A}R$ ligands (~80) and the development of new ones is needed.

Materials and methods: As the crystal structure of 5-HT_{5A} remains unknown, homology modeling is the method of choice for the structural investigation of the receptor. Following previously published research, a series of homology models was generated for a number of available crystal templates of aminergic GPCRs. A set of 5-$HT_{5A}R$ conformations suitable for virtual screening were selected based on docking of selected known ligands of the receptor.

Results: The homology modeling protocol resulted with a set of ten conformations used for structure-based virtual screening. Meticulous visual inspection of the best scored ligand-receptor complexes obtained via docking of commercial libraries allowed selection of compounds for *in vitro* testing.

Conclusions: The applied methodology allowed selection and evaluation of the VS-capable homology models of 5-$HT_{5A}R$, and in the long run, provided structural information essential for effective rational design of novel compounds active towards this challenging pharmacological target.

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P.5-18
Selected ADMETox parameters of new histamine H₄ receptor ligands

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Background: It is assumed, that the histamine H₄ receptor (H₄R), discovered in 2000/2001, is involved in inflammatory processes and immune responses, because of its mainly expression in various cells of the immune system. Potential therapeutic effects of H₄R antagonists/inverse agonists in animal models of acute inflammations, allergic rhinitis, asthma or pruritus were confirmed. As physiological role of H₄R is not yet clear - new, potent and selective ligands are

required to investigate its action. Among H$_4$R ligands already described in the literature there can be found a large group of triazine derivatives. Therefore, the aim of this study was to examine selected ADMETox parameters for the most potent H$_4$R ligands (KB4, TR11 and TR32) chosen from the newly obtained series of (4-methylpiperazin-1-yl)-1,3,5-triazin-2-amine derivatives with different heterocyclic substituents in triazine 6-position.

Materials and methods: Compounds were evaluated for their H$_4$R affinity in radioligand binding assay at the *Sf9* cells stably expressing human histamine H$_4$R. Then, metabolic stability (*in silico* and using HLMs) and the antiproliferative effect (against HEK293 cell lines) were carried out.

Results: Selected derivatives were characterized with significant affinity to the H$_4$R. According to *in silico* results, the *N*-methyl substituent at the piperazine ring was involved in the metabolism of all examined compounds with the highest probability. *In vitro* studies showed that all tested compounds were metabolically stable molecules and showed weak (TR11) or none (KB4, TR32) toxic properties.

Conclusions: The obtained results showed that in the group of (4-methylpiperazin-1-yl)-1,3,5-triazin-2-amines with heterocyclic moiety can be found potent histamine H$_4$ receptor ligands and investigated derivatives can be considered as a promising in further studies.

P.5-19

Structure prediction of G protein-coupled receptors based on low sequence identity

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Background: For many years, a small number of experimental structures of membrane proteins deposited in the Protein Data Bank imposed limitations on the statistical analysis of that distinct protein class. Only few studies referred to usage of membrane-fitted statistical potentials in structure prediction protocols, e.g., Rosetta membrane *ab initio*, BCL and FILM3. On the contrary, quite a few knowledge-based methods for the membrane protein model quality assessment (e.g., ProQM) and prediction of one-dimensional features such as transmembrane topology (e.g., TOPCONS) were recently published.

Materials and methods: Here, we report details of our hybrid modeling protocol (Latek et al. J Chem Inf Model 2016) used in the recent GPCR Dock 2013 competition which involves our web-service GPCRM (http://gpcrm.biomodellab.eu) (Latek et al. PLOS ONE 2013) and knowledge-based potentials (Woetzel et al. PLOS ONE 2012).

Results: The test cases of 5-HT1B and 5-HT2B serotonin receptors demonstrated that both the receptor structure and the ligand binding mode can be predicted with the atomic-detail accuracy as long as the target-template sequence similarity is relatively high. On the other hand, a low target-template sequence similarity observed, e.g., between SMO from the frizzled GPCR family and members of the rhodopsin family, seriously hampers the GPCR structure prediction and ligand docking.

Conclusions: We showed that usage of knowledge-based potentials implemented in BCL together with ligand-based assessment of the GPCR binding site is an efficient way to cope with major bottlenecks in the GPCR structure prediction. We also demonstrated that the

knowledge-based potentials for membrane proteins were significantly improved due to the recent surge in available experimental structures.

P.5-20

A new method of obtaining the alkylarylpiperazines *O*-derivatives of salicylamide in the presence of microwave radiation

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Background: *O*-derivatives of salicylamide are a very interesting research objective. Published so far, data relating to the biological activity of arylopierazynyloalkilo *O*-derivatives of salicylamide apply to their potential use in urology (antagonists of α1-adrenergic receptor), and in the treatment of diseases of the central nervous system (ligands for D_2 and 5-HT_{2A}). Our previous studies have also shown activity of this group of ligands for the 5-HT_{2A} and α1, as well as additionally shown that selected ligands binds to the receptor 5-HT_{1A} and 5-HT_7. Currently known methods for the synthesis of alkylpiperazinyl *O*-derivatives of salicylamide are carried out under conventional conditions, which involve the preparation of a long time reaction and low yield.

Materials and methods: Our research aimed to develop a new method of synthesis of ligands belonging to alkylarylpiperazinyl *O*-derivatives of salicylamide. The synthesis was carried out in a two-stage process, which involves the synthesis of appropriate *O*-alkyl derivatives of salicylamide, which are then subjected to a condensation reaction with the selected arylpiperazines. Reactions were carried out in the presence of microwave radiation with the use of potassium carbonate and solvents such as DMF or acetonitrile.

Results: The results show that in the presence of microwave radiation, both the first and the second process step occur effectively. Reactions are taking place in a few minutes with yield 60-70%.

Conclusions: The reactions under microwave radiation in the presence of solvents such as DMF or acetonitrile are an interesting method for the synthesis of ligands, which are *O*-derivatives of salicylamide. This new method for the synthesis of ligands from the group of alkylarylpiperazinyl allows to obtain expected product with high purity in a short time and with a high yield.

P.5-21

Evaluation of versatility method synthesis of Olanzapine derivatives under microwave conditions

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Background: Benzodiazepines (BZD) are chemical compounds widely used in treatment of CNS - central nervous system diseases. They have show, anxiolytic, sedative and anticonvulsant properties. Recent researches lead that some of them, especially 1.5-benzodiazepines show antiviral, anti-inflammatory, anticancer or antibacterial activity. Because of widely known properties and low addictive potency, many scientists tried to optimized better synthetical approaches to obtain new Olanzapine derivatives, which have less side effects. An example of that compounds is Olanzapine that contain LACP's ligands. LACP (*Long Chain Aryl Piperazines*) are well known ligands, which exhibit high affinity to serotonin receptors. In this paper it was shown synthesis of Olanzapine derivatives using methodology based on microwave irradiation.

Material and methods: Olanzapine was synthetized via microwave irradiation. DOL was placed together with potassium carbonate, and catalytic amount TBAB and DMF. Reaction was carried out over 15 sec in microwave oven. After this time TLC indicated only product, trace amount of starting material and no side products. Synthesis of Olanzapine derivatives was carried out analogously.

Results: In case of synthesis Olanzapine there was obtained desire product with high yield – 65% and purity 95%. According to this procedure a few Olanzapine derivatives were synthetized. Average yield of obtained compounds is 58% and purity 94%.

Conclusion: Synthesis of Olanzapine under microwave irradiation is versatile method also for derivatives. This solvent free approach is quick and required only few seconds – in literature the same reaction is performed in acetone and require 24 hours of stirring. Most of synthesized compounds are pure as crude products. Methodology is ecofriendly and can be developed in industrial chemistry after further optimalizations.

P.5-22

Quantum mechanical study of 1-hexyl-4-(2-methoxyphenyl)piperazine derivatives as new ligands of serotonin receptors

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Background: Long-chain arylpiperazines (LCAP) are one of the commonly studied classes of bioactive compounds due to their potential therapeutic effects caused by interactions with different subtypes of serotonin receptors. A number of studies have been aimed at examining the impact of LCAP structure modifications on the affinity, selectivity and function at a given receptor protein.

Materials and methods: The structure of six new derivatives of 1-hexyl-4-(2-methoxypneyl)piperazine complexed by serotonin receptors ($5-HT_{1A}R$, $5-HT_6R$, $5-HT_7R$) has been investigated by means of quantum mechanical methods. ONIOM (Our own N-layered Integrated molecular Orbital and molecular Mechanics) computation procedures were carried out to optimize molecular geometries of binding sites with complexed ligands. Calculations were performed with the use of B3LYP(DFT):AMBER(MM) approach. The optimized structure of ligands complexed with key residues was used for the *ab initio* FMO (Fragment Molecular Orbitals Method) calculations.

Results: The ONIOM calculations shown that the 2-(piperazin-1-yl)-methoxybenzene moiety of considered ligands form salt bridge between the protonated nitrogen atom of piperazine and Asp3.32, as well as hydrophobic interactions of the methoxybenzene ring with the aromatic cluster of TMH6, for all studied receptors. The orientation of the rest of the ligands structure was determined rather by requirement of hydrophobic interactions.

Conclusions: The performed calculations are helpful in the interpretation of the experimental results concerning the activation of protein receptors, as well as they provide the reasonable binding energies and binding patterns of ligand-protein interactions.

P.5-23

Quantitative structure-(chromatographic) retention relationship study for compounds determined in grasshopper abdominal secretion (*Chorthippus* spp.) by GC/MS

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Background: The quantitative structure-retention relationships (QSRR) are considered as a useful tool for predicting the analyte behavior in a chromatographic system and hence for confirming its structural identification.
In the present work we focused on an application of the QSRR analysis in lipidomics of the grasshopper abdominal secretion components determined by GC/MS. In particular, the correlation between the QSRR- calculated and the experimental. Retention Index for LASSO type and the Stepwise Regression was studied.
Materials and methods: Grasshoppers were collected at two locations: Starogard Gdański and Łubianka meadows. In pretreatment procedure, liquid-liquid extraction and derivatization, was used. Then, samples were analyzed with the use of gas chromatography coupled with mass spectrometry. Obtained data was processed with the use of AMDIS software. We identified 38 compounds and classical 3D-QSRR approach was applied to the structural and the corresponding chromatographic retention parameters. QSRR models were built basing on the LASSO and the LASSO/Stepwise Regression algorithms.
Results: We obtained models based on two algorithms, namely the LASSO and the LASSO/Stepwise Regression. The R^2 and Q^2 values for the developed LASSO model equaled 0.95 and 0.88, respectively (mean squared error equaled 1.22×10^4). In case of LASSO/Stepwise Regression approach the R^2 and Q^2 equaled 0.98 and 0.67 (mean squared error equaled 1.78×10^5), respectively. **Conclusion:** Application of two statistical approaches resulted in simple QSRR models providing reliable predictions of components of lipidome of insects' secretion. The obtained models may be applied as supportive tool in the identification based of the lipidomic profiles of the insects.

P.5-24

GPCRdb homology models - "less model & more crystal"

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Background: Although methods to determine protein 3D structures are improving, from the ~800 GPCRs we still only know the structure of 33 unique receptors. This prompts the need for receptor models that can capture the intricate structural characteristics of GPCRs. GPCRdb now introduces models of human non-olfactory GPCRs built on the principle of "less model & more crystal". This employs a multi-template method that resulted in the best serotonin 5-HT$_{1B}$ receptor model in the latest GPCR Dock assessment.

Materials and methods: The coverage from experimental structures is maximized, while *de novo* modeling is left as a last resort. Specifically, segments that are missing (e.g. loops, helix ends), non-representative (e.g. distorted, deleted or fused segments) or differ (e.g. TM helix bulges and constrictions) are replaced with more optimal local templates. Next, an in-house rotamer library is utilized that has been extracted from all GPCR structures and provides a specific rotamer for each position of the structure (by use of generic residue numbers). Finally, MODELLER is used to model regions where no template was available. The models are automatically updated in new GPCRdb releases, providing increased precision as new templates become available.

Results: Inactive models were built for 278 receptors from class A. All of them are accessible through the gpcrdb.org website along with information about the templates. Models for three recently determined structures were assessed along with corresponding models from other homology modeling services. Root-mean-square deviation calculations were used to compare the performance of the different services.

Conclusions: Our models exhibit a close approximation to the experimentally determined crystal structures mainly excelling in the 7TM RMSD comparison. The modeling of loops needs further improvement.

P.6 Preclinical research – Nanomedicine

P.6-01
Nanoencapsulation of neuroprotective drugs and evaluation of their action in human neuroblastoma cell line

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Background: The purpose of the present study was to evaluate the neuroprotective action of nanoencapsulated inhibitors of intracellular biochemical cascades leading to neuronal cell death.

Materials and methods: The emulsion-core and polyelectrolyte-shell nanocapsules were synthesized using the layer-by-layer (LbL) adsorption method. Hydrophobic cores were stabilized by the surfactant (AOT)-polyelectrolyte complexes. After preparation they were characterized for their size and zeta potential (DLS, NTA, LDE, SEM). Biocompatibility of nanocapsules was evaluated on SH-SY5Y human neuroblastoma cells using various biochemical assays (MTT, LDH). The neuroprotective action of nanoencapsulated model drugs was evaluated in the same cell line using hydrogen peroxide-, staurosporine- and doxorubicin-induced model of neurodegeneration.

Results: The average size of synthesized nanocapsules was around $80 - 100$ nm and the concentration was around 10^{11} particles/ml. Their zeta potential values ranged from less than -30 mV for the ones with external polyanion layers to more than 30 mV for the polycation layers. Zeta potential of PEG-coated nanocapsules was around 0 mV. The cytotoxicity results obtained showed that nanocapsules were non-toxic to SH-SY5Y cells so they were further used as drug-loaded nanocarriers. Moreover, our studies showed that nanoencapsulated forms of neuroprotectants were biocompatible and protected SH-SY5Y cells in a model of cell death induced by cell-damaging agents in lower concentrations than those of the same drug added directly to the culture medium.

Conclusions: Our data demonstrate that designed nanocapsules might serve as a novel solution for the delivery of hydrophobic neuroprotective agents.

P.6-02

Detection of human enterovirus 71 and carbapenamase producing Enterobacteriaceae using surface enhanced raman spectroscopy (SERS) by gold nanostars

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Background: The aim of this research was to develop the rapid and highly sensitive detection system for Human Enterovirus 71 (EV71) causing Hand, Foot and Mouth Disease, and Carbapenamase Producing Enterobacteriaceae (CPE), known as "superbugs", using Surface Enhanced Raman Spectroscopy (SERS) by plasmonic gold nanostars (AuNS).

Materials and methods: AuNS were synthesized by one-pot reduction of gold chloride by ascorbic acid in the presence of silver nitrate. AuNS surface was further modified by the adsorption of SCARB2 protein (EV71 receptor) or β-lactam antibiotics. The detection method was based on the observations of the changes in SERS spectra after binding of EV71 to the AuNS-SCARB2 conjugate and the hydrolysis of the β-lactam ring of the antibiotics conjugated on gold nanostars after incubation with CPE.

Results: When AuNS-SCARB2 was added to cell lysate samples, the presence of 10^7 pfu/ml EV71 caused differences in their Raman spectra. As revealed by dynamic light scattering (DLS) measurements and analysis of the Raman peaks, these differences were caused by the aggregation of AuNS-SCARB2. When CPE sample was added to AuNS-antibiotic, a significant difference in the SERS spectrum of the CPE compared to the non-CPE was observed by 25 minutes. The result was found to be statistically significant at p-value < 0.05 with n= 3.

Conclusions: We developed a Surface Enhanced Raman Spectroscopy based scheme to rapidly detect EV71 and CPE. In contrast with current methods of EV71 and CPE detection, our SERS-based scheme involves minimal sample handling procedures, produces results with a short turnaround time, and utilizes a cheap and easily producible SERS substrate.

P.6-03

Nanoparticles gels for biomedical applications

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Background: Hydrogels nanoparticles have gained considerable attention in recent years as one of the most promising drug delivery systems. They are unique combination of hydrogel properties (hydrophilicity, elasticity, high water content) with nanometer size, therefore, they are desirable materials in design of novel drug delivery devices. Among the many reports, polysaccharide-based nanogels appear to be very attractive due to their natural origin, great biocompatibility, and/or biodegradability. The aim of this study is synthesis and characterization of calcium alginate nanogels and zinc pectin nanogels for biomedical applications.

Materials and methods: Polysaccharides nanogels (alginate and pectin) were formed in microemulsion water-in-oil template (W/O) using FDA approved surfactant (AOT) and easily evaporated oils. Obtained nanogels were modified by the Layer-by-Layer technique. Furthermore, they were characterized by size and zeta potential measurements. Encapsulation of models substances (Rhodamine b and 5-aminofluorescein) in calcium alginate nanogel were performed. We also evaluated cytotoxicity of prepared calcium alginate nanogels by MTT and LDH assays.

Results: We successfully synthesized gel nanoparticles (size <100 nm, zeta potential -30 mV) based on calcium alginate (ALG-NG) and zinc pectin (PEC-NG) crosslinking. Concentration of obtained stock suspension of nanogels was 10^{10} nanoparticles/ml. Nanogels were stable for up to 2 months.

Encapsulation of fluorescent dyes was performed in synthesized nanogels. Surface of nanogels was modified by LbL using natural and synthetic polyelectrolytes. Cytotoxicity tests of calcium alginate and zinc pectin nanogels were performed.

Conclusions: Obtained polysaccharides nanoparticles (size < 100nm) appear to be a promising material for encapsulation of therapeutic agents.

P.6-04

Gadolinium alginate nanogels for theranostic applications

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Background: Theranostics is a promising field that combines therapeutic and diagnostic capabilities into a single multifunctional platform. The majority of the imaging and therapeutic compounds are based on gadolinium complexes used as contrast agents or combined with

anticancer drugs. The main drawbacks of these kind of small-sized molecules are their too short blood circulating times, cytotoxicity, poor biodistribution, etc. For this reason, it is extremely important to involve nanotechnological tools and develop new strategies for diagnosis and treatment, in e.g. anticancer therapies. The aim of this study is synthesis and characterization of gadolinium nanogels for theranostic applications.

Materials and methods: Nanogels were synthesized using reverse microemulsions water-in-oil (W/O). Surface of nanogels was modified by LbL using natural (chitosan, alginate) and synthetic (poly-L-glutamic acid, poly-L-Lysine hydrobromide) polyelectrolytes. The obtained nanogels were characterized by size and zeta potential measurements. Moreover, morphology of carriers was visualized by Cryo-SEM. We evaluated toxicity of prepared carriers by MTT and LDH assays and the MRI of nanogels suspensions was performed.

Results: Gadolinium alginate nanogels were synthesized by the reverse water-in-oil (W/O) microemulsion method. The average size of synthesized nanocarriers was ∼100 nm. We showed, that our nanogels can be easily modified by Layer-by-Layer technique (LbL), which was evidenced by zeta potential measurements. Biocompatibility of nanocapsules was evaluated on SH-SY5Y human neuroblastoma cells. Gadolinium nanogels were successfully imaged by Magnetic Resonance Imaging.

Conclusions: The obtained results indicated that gadolinium alginate nanogels may be considered as novel strategy for therapeutic and diagnostic systems and the applied methodology may be further used of nanotechnology in modern therapies.

P.6-05

Synthesis of water-soluble, intrinsically fluorescent amino functionalized polyhedral oligomeric silsesquioxanes based nanoparticles for biomedical applications

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Background: Nanoparticles developed from amino functionalized polyhedral oligomeric silsesquioxane (POSS) structures and its derivatives open new perspective in the field of drug delivery. Fluorescent dye-labeled particle are required in order to track the uptake of the nanoparticles by the cells. However, conjugation of dyes requires extra efforts. It will be quite useful to prepare biocompatible nanoparticles with intrinsic fluorescence without an extra conjugation of expensive dyes. In this work, we aimed designing biocompatible amino functionalized POSS and to investigate their fluorescent behavior and explore their potential applications in medical applications.

Materials and methods: Amino POSS structure was modified with different hydrophilic molecules in order to increase water solubility of the nanoparticles. Nuclear magnetic resonance (NMR) was used to study the modification of the various POSS moieties. A confocal laser scanning microscopy (CLSM) was used to investigate fluorescence of the nanoparticles.

Results and conclusions: The results from NMR characterisation confirm a complete modification of the nanoparticles. The preliminary investigations by CLSM proved the intrinsic

emission from amino-functional POSS and their derivatives in aqueous solutions in absence of other types of fluorescent groups. The cost-effective synthesis methodology, water-solubility, degradability and hyperbranched backbone make amino functionalized POSS and its derivatives good candidates for fluorescent materials applied in medical applications. Further experiments are planned to assess the functionality of the POSS nanoparticles in biomedical applications.

P.6-06

Biodegradable and non-toxic polymer-based nanocarriers for plasmid DNA delivery

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Background: Gene therapy (GT) is an emerging technology that may have a significant potential for treatment of genetic disorders. These therapeutic approach, based on permanently or transiently replacing genetic defects with exogenous nucleic acids, gives an opportunity to increase the efficiency of treatment. The main challenge in GT is to develop carriers that can efficiently deliver nucleic acids directly through plasma membrane. One of the most promising group of delivery systems are polymeric nanoparticles as their chemical structure can be modified to allow designing nanocarriers with desired physicochemical properties. In the present work we were focused on the preparation of safe, nontoxic and biodegradable polymer-based gene nanocarriers.

Materials and methods: The nanocarriers composed from plasmid DNA (pDNA) and selected polymers (e.g. poly(L-Lysine), PLL; poly-L-glutamic acid, PGA) were synthesized using self-assembly (SA) technique. The encapsulation process were performed using the layer-by-layer (LbL) method. All nanocarriers were characterized by size, size distribution and zeta potential. Cytotoxicity tests and stability in the simulated body fluid was also determined.

Results: The cationic polymer-based pDNA nanocarriers were successfully synthesized (PLL-pDNA) using SA technique. The synthesized nanocarriers were modified by Layer-by-Layer technique (LbL) that allowed to obtain multilayer shells (PLL/PGA) with different number of layers. The LbL process was evidenced by zeta potential measurements. The cytotoxicity tests showed that synthesized nanocarriers are nontoxic.

Conclusions: The obtained results indicated that polymer-based pDNA nanocarriers synthesized by SA method may have a significant potential for therapeutic systems and the applied methodology may be further considered as promising non-viral vectors for gene delivery.

P.6-07

Neuroprotectants-loaded nanoparticles as efficient (brain) drug delivery systems

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Background: Prevention and treatment of stroke and neurodegenerative diseases e.g. Alzheimer's and Parkinson's are major and still unresolved problems of contemporary medicine. In the clinical trials we may find only few neuroprotective substances, however their efficiency in the treatment is not satisfactory. One of the major limitations is an inefficient delivery by the blood-brain barrier (BBB). Therefore, the main aim of the research is to develop a new strategy of delivery of neuroprotectants by the nanocarriers that are able to cross the BBB.

Materials and methods: The neuroprotectants loaded nanoparticles (NP's) were synthesized from nanoemulsion by the Phase Inversion Composition (PIC) technique. The NP's were composed of the biodegradable and biocompatible polymers (polycaprolactone, PCL and/or poly(lactic acid), PLA) containing active neuroprotective agents (Undecylenic acid, MDL28170, Curcumin) as well as model drug (Coumarin-6). All nanocarriers were characterized by size, size distribution, zeta potential, imaged by Cryo-SEM and their stability in the simulated body fluid was also determined. Biocompatibility and neuroprotective action of received nanocarriers were evaluated in the SH-SY5Y human neuroblastoma cell line using cell viability/toxicity assays (MTT reduction, LDH release).

Results: We have synthesized nontoxic PCL and PLA-drug loaded NP's using PIC method. The average size of nanocarriers was ~100nm. Moreover, PCL NP's were stable at least two months that we confirmed by zeta potential measurements. We've also observed neuroprotective effects for PCL/drug-loaded NP's.

Conclusions: We have successfully synthesized stable and nontoxic drug-loaded NP's. We showed that PIC technique is a promising method and may be considered as novel strategy for synthesis of neuroprotectants-loaded nanocarriers as a drug delivery to the brain.

P.7 Clinical research

P.7-01
Suppression of lipid mediators by omalizumab

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Background: Omalizumab is a recombinant humanized monoclonal antibody that selectively binds to free IgE, and has been shown to be efficacious in patients with severe allergic asthma and other allergic diseases. Aspirin-exacerbated respiratory disease (AERD) is characterized by serious eosinophilic airway inflammation and mast cell activation. Therefore, the effects of omalizumab in AERD may be different to those observed in typical severe asthma. This study investigated the efficacy of omalizumab for AERD, including changes in mast cell activation, urinary leukotriene E4 (LTE4) and prostaglandin D2 metabolite 9α,11β-prostaglandin F2 (PGD2M).

Materials and methods: Japanese adults (n = 21) with AERD were enrolled at Sagamihara National Hospital between July 2009 and September 2013. All patients had received their usual asthma medication for at least 12 months, and none had previously received omalizumab. The following parameters were measured at baseline and after 12 months of omalizumab treatment: urinary LTE4 and PGD2M concentrations; peripheral blood eosinophil count; respiratory function; and nasal and asthma-related symptom scores. The number of asthma exacerbations and hospitalizations, and daily systemic corticosteroid doses were also evaluated.

Results: Omalizumab treatment produced a marked decrease in urinary concentrations (pg/mg of creatinine) of LTE4 (703.2 [IQR: 234.9-2245.4] vs 167.7 [IQR: 72.3-296.5], reduction rate 76.2%; p=0.001) and PGD2M (55.5 [IQR: 14.8-96.5] vs 6.1 [IQR: 4.3-13.6], reduction rate 89.0%; p=0.002). Nasal and asthma-related symptom scores, the number of exacerbations and hospitalizations, and daily corticosteroid doses were also significantly reduced after treatment.

Conclusions: Omalizumab suppressed leukotriene overproduction and mast cell activation, and was an effective treatment in AERD patients with severe asthma and nasal symptoms.

P.7-02

Platelet activation markers overexpressed specifically in aspirin-exacerbated respiratory disease

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Background: Aspirin-exacerbated respiratory disease (AERD) is characterized by respiratory reactions upon ingestion of cyclooxygenase (COX)-1 inhibitors and cysteinyl leukotriene (cysLT) overproduction. We tried to explore the role of platelets in the pathogenesis of AERD.
Material and methods: First, platelet activation markers [the expression levels of P-selectin (CD62P), CD63, CD69, and GPIIb/IIIa (PAC-1) in peripheral platelets, the percentage of circulating platelet-adherent leukocytes, and the levels of plasma soluble P-selectin (sP-selectin) and soluble CD40 ligand (sCD40L)] were examined in stable patients with AERD (n = 30), aspirin-tolerant asthma (ATA; n = 21), and idiopathic chronic eosinophilic pneumonia (CEP; n = 10). Furthermore, the levels of plasma sP-selectin and sCD40L in AERD (n = 24) and ATA patients (n = 7), and surface markers on platelets in AERD patients (n = 8) were also assessed during the aspirin challenge test.
Results: In the stable condition, the expression levels of all surface markers on platelets, the percentage of platelet-adherent eosinophils, and the levels of plasma sP-selectin and sCD40L were significantly higher in AERD patients than in ATA patients. In the aspirin challenge test, the levels of platelet activation markers did not change both in AERD and ATA patients.
Among these markers, p-selectin expression and plasma sP-selectin levels positively correlated with urinary leukotriene E_4 concentration. Additionally, plasma sP-selectin and sCD40L levels negatively correlated with lung function.
Conclusions: Peripheral platelets were activated to greater extent in stable AERD patients than in stable ATA patients, CEP patients, and controls. Platelet activation was involved in cysLT overproductions and persistent airflow limitations in AERD in stable disease condition.
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P.7-03

Comparison of DNA methylation profiles between type 2 diabetes and obesity

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Background: The main goal of our study was to compare the status of DNA methylation in patients with diabetes type 2 and patients with obesity. It was postulated that ω-3 fatty acids: EPA and DHA can decrease inflammation associated with obesity and prediabetes. The aim was also to study if the three months n-3 PUFA supplementation could influence epigenetic changes in leukocytes of patients with obesity and patients with diabetes type 2.

Material and methods: blood samples of patients obtained before and after three-month long diet supplementation with ω -3 PUFA (1.8 g/day, capsules DHA:EPA 5:1) were collected. The methylation of DNA was performed using Agilent Human DNA methylation microarrays. Differences in methylation of the promoters of genes were identified to assess pathways and gene interactions which can be important in pathophysiology of diabetes.

The results indicated differences in the methylation of genes related to different signal pathways that may be affected in diabetes. The most significant changes include apoptosis regulation (TNF receptor superfamily genes), autophagy, endoplasmic reticulum stress, oxidative stress response and inflammatory pathways (NK-kB). In next step, the results will be analyzed with histone H2 acethylation status (estimated by ChIP-on-chip technology) to reveal mechanism of epigenetic regulation of selected pathways.

Conclusion: Results from DNA methylation analysis revealed epigenetic regulation of genes, that could play the role in development of diabetes and its complications. Supplementation with n-3 PUFA seems to influence methylation status of genes from NK-kB pathway or glutathione metabolism. Based on microarray analysis, methylation status of selected genes related to insulin resistance, inflammation and other pathways will be validated by bisulfite conversion and Sanger sequencing.

P.7-04

New molecular markers for the early diagnosing, monitoring therapies and in preventive personalized medicine in polish patients diagnosed with Parkinson`s disease

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Background: Parkinson`s disease (PD) is the second most common age-dependent neurodegenerative disorder. There is no single molecular test that is suitable to reliably diagnose PD with adequate specificity and sensitivity. **The aim** of this project is to uncover the sources and understand the factors that contribute to develop of PD and the extraordinary pharmacokinetic and pharmacodynamic variability within and between polish patients with PD. We analyze miRNA expression profile, and genetic variants in *FGF20* that might be associated with with PD. Additionally we examine drug metabolism activity profiles, interindividual variability and regulation of expression, and the functional and clinical impact of genetic variation in drug metabolizing in PD patients. **Materials and methods:** we involved 100 patients diagnosed with PD. The profiling for miRNA expression for all samples was performed using the TaqMan Array Human MicroRNA Panel v3.0 (Applied Biosystems, CA, USA). The genotyping of *FGF20* and other genes linked with drug metabolism was performed by using Sanger sequencing method and 3130xl Genetic Analyzer (Applied Biosystems). **Previous results.** We have linked several SNPs especially in 3'UTR of FGF20 with different clinical type of PD. At this moment we also selected some types of miRNA that seems to play a crucial role in pathomechanism of PD. **Conclusions**: These final results will be used to decipher the molecular events leading to PD and to identify prospective genetic-based neuroprotective therapies. Outcome of the study: search for possible diagnostic tool (in future proposal for the custom chip based on genetic modification–induced changes in gene expression, including miRNA), as well as suggestions for the early diagnostics/intervention markers for diagnosing, monitoring therapies and in preventive personalized medicine.

P.7-05

Preliminary results of the application of mesenchymal stem cells for the post-vaccination encephalopathy in 3 years old child

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Background: In rare cases children might develop post – vaccination encephalopathy, especially after primary vaccination. The current standard of care for these children consists of conservative treatment and rehabilitation. However, there is a lack of the treatment enabling regeneration of central nervous system. The purpose of the study was to assess safety and feasibility of cell therapy in post-vaccination encephalopathy.

Materials and methods: A 3 month old girl, with post vaccination encephalopathy was enrolled to the experimental cell therapy procedure, which consist of single transplantation of autologous bone marrow nucleated cells (BMNC) injected intravenously ($1x10^9$) and via lumbar puncture ($0.5x10^9$) and 4 rounds of mesenchymal stem cells (MSC) administrations via lumbar puncture every 3 months, combined with intense neuro and neuropsychological rehabilitation.

In first hours of life the girl experienced vaccination – induced hemolysis, followed by status epilepticus, resulting in deterioration of neurological status and mental retardation. The patient stayed in a conscious awareness state with tetraparesis, without ability to seat or control trunk. At 9 months she developed drug resistant epilepsy. The first MRI revealed delay in brain myelinization without structural brain alterations.

Results: There were no complications connected with cells transplantation procedures and no side effects in the 2 years follow-up period. First improvement was noticed 6 weeks after the first MSCs transplantation. Psychomotor improvement was observed, both by the mother and in the evaluation tests. 2 years after start of the therapy, she can seat controlling her head. Significant reduction of epileptic episodes is observed. The control MRI reveals improvement in myelination of white matter estimated on 9/10 months of life.

Conclusions: We postulate that the use of a cell therapy can be safe and may have a beneficial effect for post - vaccination encephalopathy pediatric patients.

P.7-06

The cerebral monoamine oxidase activity in experimental posttraumatic stress disorders: Focus on the behavioral disorders and on the oxidative stress

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Background: Posttraumatic stress disorders **(**PTSD) results from an imbalance of the noradrenergic, dopaminergic, and serotoninergic systems. Monoamine oxidase (MAO) plays a key role in metabolism of these neurotransmitters. Therefore, MAO inhibitors and selective serotonin reuptake inhibitors are widely used in the therapy of PTSD. However, MAO catalytic characteristics are exhibited a strong dependence on the lipid microenvironment. Unfortunately, this impartment enzyme property does not taken into account in the appointment of MAO inhibitors. As a result, the effectiveness of therapy significantly reduced. This study focused on the relationships between transformation of the catalytic characteristics of cerebral MAO, oxidative stress, and behavioral disorders in experimental PTSD.

Materials and methods: The study performed on 42 adult Sprague-Dawley male rats. Monoamine oxidase activities, Lipid peroxidation (LPO) intensity, and oxidative modification of proteins in suspension of the brain mitochondria evaluated over the course of experimental post-traumatic stress disorder. PTSD simulated by permanent unavoidable exposure of a strong stimulus.

Results: The most pronounced changes of free radical oxidation products in the suspension of the cerebral mitochondria detected during this period: the increase of the content of primary and secondary LPO products and intensity of metal-catalyzed oxidation of proteins. These changes in the intensity of free radical oxidation led to changes of MAO activity: reduction of serotonin oxidative deamination paralleled with activation of glucosamine deamination.

Conclusions: Our data indicate the presence of situations where inappropriate use of MAO inhibitors in the correction of PTSD because the long-term consequences of stress is a decrease in the activity of MAO

P.7-07

Maternal smoking disturb the antioxidant enzyme system in human milk

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Background: Human milk is rich source of nutrients and contain essential antioxidant compounds. Despite many evidence about the harmful effect of tobacco smoke on foetal and neonatal development, many women continue to smoke whilst gestation and breastfeeding. Maternal smoking disturbs the anti-oxidative/pro-oxidative balance in the milk, which is manifested by a decreased levels of vitamins as also disturbance of enzymes activity.

The aim of this study was to evaluate the effect of smoking on antioxidant enzymes activity in human milk. The study protocol was approved by Resolution No. 593/13, 13 June 2013.

Material and methods: The biological material for the purposes of this study - colostrum (1 ± 1 days after birth) and mature milk (1 month ± 1 week after birth) - was obtained by voluntary consent given by patients (n = 73) from the Department of Newborn Infection, Gynaecology and Obstetrics Hospital, University of Medical Sciences, Poznan, Poland.

The superoxide dismutase (SOD), catalase (CAT) and S-transferase (GST) activity were measured spectrophotometrically. Cotinine concentration (major metabolite of nicotine) in maternal serum was measured by high-performance liquid chromatography with DAD detector detection and norephedrine as an internal standard, following earlier liquid–liquid extraction.

Results: The results of the analysis have shown that catalase activity in colostrum and mature milk among women smokers was statistically significant higher compared to the nonsmokers (by 19.40 and 31,03%, respectively) and positively correlated with cotinine concentration (Rs=0,83; p=0,01). Mean cotinine concentration among female smokers was 197 ± 98 ng/ml and among exposed to second-hand tobacco smoke: 23 ± 11 ng/ml of serum.

Conclusions: Maternal smoking disturb the antioxidant enzyme system in human milk.

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