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Cannabis is the most frequently used illicit drug worldwide. In the United States, changing attitudes and cannabis legalization has been associated with increased rates of cannabis use disorder (CUD) among adults. Currently, approximately 17% of patients entering treatment for substance use in the US have a diagnosis of cannabis use disorder, yet their treatment outcome is poor. While psychosocial strategies have been shown to improve treatment outcome, the vast majority of patients fail to achieve continued abstinence.

A major limitation of existing treatment options is the lack of FDA-approved medications to facilitate treatment of CUD. The objective of this presentation is to describe the current state of the field in terms of medications development for CUD. Double-blind, placebo-controlled, laboratory studies of potential pharmacotherapies in combination with active and placebo cannabis will be presented. These laboratory studies test a range of strategies for developing potential treatment medications: One medication strategy is to reduce the direct effects of cannabis, i.e., intoxication. Another strategy is to reduce cannabis withdrawal. Abstinence from daily cannabis smoking can produce irritability, anxiety and disrupted sleep, and the resumption of cannabis smoking alleviates these symptoms. Additional strategies include modelling the effects of medications on ‘abstinence initiation,’ i.e., reducing cannabis self-administration under non-abstinent conditions, and on ‘relapse prevention, i.e., reducing cannabis self-administration in cannabis smokers who have been abstinent from cannabis for several days.

In summary, more treatment options for cannabis use disorder are needed. Although cannabis has lower abuse liability than most other abused drugs, its prevalence results in a subset of individuals who are unable to achieve abstinence without treatment. Medications are one option needed to improve treatment outcome.
PL 2
Morpho-functional imaging: connecting a single neuron to whole brain

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We are interested in the correlations between morphology of brain connections and functionality, which is one of the major issues in neuroscience in the comprehensions of many pathologies and mechanisms of behavior and computation. Elucidating the neural pathways that underlie brain function is also one of the greatest challenges in neuroscience.

Nowadays, there are several imaging techniques offering a complementary approach to visualize intact neural networks. Each of those offers a different strategy and furnish complementary information on the role of neural components.

We will describe different approaches enabling to move from single neuron details to whole brain imaging both on functional and morphological point of view. Some examples of correlative microscopies, combining linear and non linear techniques will be described. Particular attention will be devoted to neural plasticity after damage as neurobiological application.

PL 3
The dopamine transporter: a key player in psychostimulant addiction and dopaminergic pathologies

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Dopamine dysfunction is of central importance in neuropsychiatric diseases, such as schizophrenia, affective disorders, ADHD and autism, as well as in neurodegenerative parkinsonism. The presynaptic dopamine transporter (DAT) mediates reuptake of dopamine and thereby plays a key role in regulating dopamine homeostasis by terminating dopamine signaling and ensuring maintenance of reusable pools of transmitter. As target for cocaine and amphetamine, DAT is also of major interest in relation to drug addiction. My laboratory wishes to gain insight into the molecular mechanisms underlying drug action at DAT, to reveal mechanisms governing the activity and availability of DAT in the synaptic terminals and to understand how alterations in these processes contribute to psychostimulant addiction and neuropsychiatric diseases. There is accumulating evidence that rare genetic variants in the DAT gene, including de novo mutations, can play a hitherto unknown key role in the pathophysiology of both neuropsychiatric and neurodegenerative disorders. Most recently, we have identified multiple novel DAT coding variants in patients with neuropsychiatric disorder and/or early-onset neurodegenerative parkinsonism. The coding variants are subject to detailed molecular and cellular analysis, and for selected mutants we have generated knock-in mouse models. Specifically, we have generated mice expressing two DAT coding variants found in an adult male diagnosed with both neuropsychiatric disorder and early-onset neurodegenerative parkinsonism. The variants include Ile312Phe in transmembrane segment 6 and a presumed de novo mutant Asp421Asn in the second sodium site. Behaviorally, the mice
show face strong validity towards ADHD as exemplified by remarkably strong hyperactivity that can be reversed by amphetamine. Altogether, our data support that coding variants in DAT can cause or contribute to both neuropsychiatric diseases and movement disorders, and that studying these variants might represent a novel path towards an improved understanding of pathologies where dopamine dysfunction is of central importance.

**PL 4**  
**Single-cell transcriptomic survey of neural identity and circuit connectivity**

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Next-generation single-cell sequencing and cell type-specific genetic approaches are accelerating progress towards a comprehensive understanding of the molecular basis of circuit assembly and function. This lecture highlights recent insights using these approaches in defined neural circuits specifically focusing on cell adhesion molecules (CAMs). CAMs play critical roles in neural circuit assembly and are frequently associated with neurodevelopmental and psychiatric disorders. Because hundreds of CAMs exist in the brain, their functional analysis has been challenging, but now, developments in single-cell RNAseq, gene isoform-, and synapse-specific analyses are now breaking barriers. This talk will present the most recent findings on the role of CAMs in defining cell type identity, circuit connectivity and function.
S. 01-1
Estrogen and brain inflammation

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**Background:** Neuroinflammation triggers brain damage and cell death. Inflammasomes are key cellular multiprotein platforms sensing neuropathological conditions and propagating inflammation. Activation of inflammasomes causes the proteolytic cleavage of caspase-1 and proteolysis of the pro-inflammatory cytokines IL1ß and IL18 into their active forms. 17ß-estradiol (E2) is well-described as protective agent in the CNS improving the functional outcome after traumatic brain injury and chronic degenerative diseases.

**Material and methods:** Activation and regulation of inflammasomes were studied at the protein/gene expression level in animal models related to acute neurological damage such as stroke and spinal cord injury and to chronic neurodegeneration, i.e. ALS. We focused on the chronological sequence of the appearance of different inflammasome components and their cellular assignment.

**Results:** In a transient ischemic rat model, the temporal course of distinct inflammasome components in the peri-infarct zone reached a maximum 24h post-ischemic and mainly involved NLRP1, -3 and ASC. In consequence, we simultaneously found 100times elevated IL1ß levels. Neurons, astro- and microglia expressed inflammasome components early after stroke. At later stages, mainly micro- and astroglia were positive. Different brain cell types contained individual subsets of inflammasome components. In an experimental rat spinal cord injury, we demonstrated a similar expression course and distribution of inflammasomes. In both models, the systemic application of E2 significantly suppressed the inflammasome induction and prevented the IL1ß rise.

In the chronic SOD1 mouse ALS model, we detected moderate NLRP3 expression, caspase-1 activation and IL1ß in the ventral horn of the spinal cord in pre-symptomatic animals which strongly increased later in fully symptomatic mice. This time course correlated well with motoneuron cell death. The application of E2 again significantly reduced inflammasome activation and cell death.

**Conclusions:** Our studies reveal that inflammasome activation occurs after acute brain damage and in chronic brain disease models and involves a brain-intrinsic inflammatory network of neurons, micro- and astroglia. Data also show that systemic E2 significantly dampens the activation of inflammasomes in the brain, thereby reducing tissue damage and neuronal death.
Hyperbaric and hypobaric treatment prevents radical oxygen species toxicity in brain asphyxia

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Background: Perinatal hypoxia-ischemia is one of the main cause of brain injury in neonates. An increased release and extracellular retention of glutamate leads to an excessive activation of ionotropic glutamate receptors, especially N-methyl-D-aspartate (NMDA) receptor (excitotoxicity), and the accumulation of toxic products such as reactive oxygen species (ROS). Increased level of ROS resulting from insufficient oxygen and glucose supply is one of the most important factors involved in hypoxia-ischemia (H-I) brain injury. Hypothermia initiated shortly after birth, is the only intervention clinically available and thus the challenge to establish new effective therapies remains a priority in neuroscience. Promising results have been observed using individual treatments of hyperbaric oxygen (HBO) or mild hypobaric hypoxia (HH). Presented study shows the effect of HBO and HH on ROS accumulation observed after H-I in experimental model of birth asphyxia.

Materials and methods: We used hypoxia-ischemia (H-I) on 7-day old rats, the left common carotid artery was permanently occluded and then the pups are subjected to hypoxia (7.4% oxygen in nitrogen for 75 min). 1 or 6 h after H-I rats were treated with HBO (compression for 60 min to 2.5 ATA in 100% oxygen) or HH (decreasing the pressure to 0.47 ATA for 60 min). Treatments were repeated for the next 2 days in 24 h intervals. The levels of brain damage, ROS accumulation, changes in antioxidant enzymes activity and expression of SOD1/2 were measured.

Results: Both HBO and HH treatment resulted in decrease in the brain damage and reduction in ROS level. HH resulted in additional increase in superoxide dismutase (SOD) and glutathione peroxidase activity, whereas HBO significantly reduced the activity of these enzymes compared to H-I. HH did not changed increased by H-I SOD1 expression, whereas HBO reduced it. Both treatments reduced by H-I SOD2 expression. Both treatments restored decreased by H-I concentration of glutathione but had no effect on catalase activity.

Conclusions: HBO and HH treatments reduce ROS level and alter activity of selected antioxidant enzymes – HH mobilizes antioxidant enzymes activity whereas HBO decreases. This indicates on different mechanism of neuroprotection. HH neuroprotective effect is probably related to additional activation of antioxidant enzymes and rapid neutralization of ROS, whilst HBO protection may be associated with mechanism that reduce ROS formation.
Astrocytic SN1 glutamine transporter in hyperammonemic encephalopathies

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**Background:** Ammonia is a well-documented neurotoxin, and its increased entry to brain is a primary cause of neurological disorders associated with hyperammonemia, including acute (acute liver failure; ALF), chronic forms of hepatic encephalopathy, congenital deficiencies of urea cycle enzymes, and several metabolic or toxic encephalopathies.

An excessive accumulation of glutamine, product of ammonia detoxification in astrocytes, is held responsible for brain edema associated with ALF. The N-system transporter SN1 specifically mediates glutamine efflux from astrocytes. We hypothesized that inefficient SN1-dependent efflux may cause increased glutamine retention in astrocytes, further ensuing astrocytic swelling and impaired glutamatergic neurotransmission typical of ALF.

**Materials and methods:** Astrocytic swelling in cerebral cortex of mice with azoxymethane (AOM)-induced ALF was visualized by electron microscopy (EM), and brain edema by apparent diffusion coefficient (ADC) measurement with NMR. SN1 expression in the cortex of AOM mouse was collated with ex vivo analysis of system N-mediated [3H]glutamine transport in cerebral cortical slices from AOM mouse. Extracellular and total glutamine content was determined by HPLC and by 1H spectrometry, respectively. The mechanistic relation between cerebral edema and SN1 loss was analyzed in a cerebral cortical region of mouse in which SN1 was knocked down (by ~50%) using vivo morpholino technique (SN1 VM).

**Results:** Decreased extracellular glutamine level, in conjunction with unaltered total glutamine content appeared to manifest inefficient glutamine efflux from astrocytes and its diminished conversion to glutamate in SN1 VM mouse. Swollen astrocytes were documented by EM, and cerebral cortical edema by biophysical and biochemical markers: i) decreased ADC, ii) increased extracellular taurine and iii) phosphocholine. Space-restricted electrophysiological manifestations of glutamatergic impairment in the pertinent cortical region of SN1 VM measured ex vivo (cerebral cortical slices) included i) reduced field potential; ii) tendency towards decrease of LTP, both compatible with glutamate loss in ALF-affected cerebral cortex.

**Conclusions:** Collectively, the study documents SN1 deficiency-related impairment of glutamine transport from astrocytes as a contributing factor to brain edema and related neurological manifestations of ALF.

**Acknowledgements:**
Steroid and xenobiotic receptor signaling in pathologies of the nervous system

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Exposures to environmental organic pollutants, which are classified as Endocrine Disrupting Chemicals (EDCs) due to their abilities to alter the hormone-dependent processes, have been reported to cause long-term alterations in functioning of neural transmission, formation of neural networks, and methylation of specific gene regions in the mammalian brain. Epidemiological data showed strong correlations between exposures to EDCs and increased risks of neuropsychiatric disorders as well as neural degenerations. Intensification of apoptosis and impairment of autophagy, except for acute neural injury where both phenomena are stimulated, are the fundamental processes underlying neural degeneration. Apoptosis is particularly responsible for neural pathologies that depend on mitochondrial dysfunctions, whereas autophagy is deleterious in acute neural disorders such as stroke and hypoxic/ischemic injury. In chronic neurodegenerative diseases such as Parkinson’s and Alzheimer’s diseases, autophagy causes neuroprotection via degrading misfolded proteins. Apoptosis and autophagy are regulated by steroid and xenobiotic receptors. For example, estrogen receptors (ERs; ESR1, ESR2, GPER1) often mediate the anti-apoptotic signaling and promote the maturation of autophagosomes, whereas the aryl hydrocarbon receptor (AhR) mediates the induction and propagation of apoptosis. The retinoid X receptor (RXR)-related receptors, except for the peroxisome proliferator-activated receptors (PPARs), stimulate apoptotic processes that accompany neural pathologies. Recently, we demonstrated that the EDCs-related xenobiotics, such as the pesticide DDT, its metabolite DDE, and antimicrobial agent triclocarban inhibited GPER1 but stimulated the AhR, RXR, and constitutive androstane receptor (CAR) signaling in mouse neurons. We also showed that a UV filter benzophenone-3 attenuated PPARγ and dysregulated the ER and RXR pathways in the neuronal cells. In addition, we provided evidence for EDCs-evoked apoptosis, impairment of autophagy, and epigenetic modifications that involved the global DNA hypomethylation and altered methylation levels of specific genes i.e., Esr1, Esr2, Gper1, Ahr and Car. Recognition of mechanisms of EDCs actions at early developmental stage is particularly important because these agents, via dysregulation of the steroid and xenobiotic receptor signaling, could disrupt epigenetic status which may substantiate a fetal basis of the adult onset of neurological diseases.

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What can we offer patients with rare vascular diseases?

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The group of rare vascular diseases includes clinical entities with different pathophysiologic background and usually grave prognosis. There are disorders related to certain mutations in genes coding vascular structural proteins like Marfan or Ehlers-Danlos syndrome, immune-mediated - like Takayasu disease or inflammatory aneurysm as well as thrombangiitis of unknown mechanism (Buerger’s disease) but inextricably associated with tobacco use. In genetic disorders early diagnosis is essential since many patients have a normal life expectancy with proper management (Marfan syndrome) or mortality can be significantly reduced by treatment with high-dose beta-blockers (Ehlers-Danlos syndrome). Introduction of modern diagnostic modalities like CT or MRI and particularly PET, increased specificity and sensitivity of the diagnosis of large-vessel vasculitis and can be used for monitoring of disease activity. Different anti-inflammatory and immunosuppressive drugs can be used in therapy, from monoclonal antibodies to IL 6 receptor to colchicine. In many patients long-term remissions can be achieved. In our series of subjects with inflammatory aneurysm, we have only two patients with aneurysm-related mortality.

Awareness about rare vascular diseases in the medical community is essential for early diagnosis and referral to specialized centers for appropriate care improves long-term prognosis.
Inborn errors of metabolism as rare diseases

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Inborn errors of metabolism (IEMs) comprise metabolic medicine and are genetically determined disorders, in which specific enzyme defect results in abnormal metabolic pathway of protein, fat or carbohydrate, generally. According to the European definition of “rare disease” (every disease with prevalence lower than 5:10 000) IEMs belong to this category. As ultrarare diseases they represent some of the rarest, but most life-threatening unmet medical needs.

Treatment in IEMs depend on a disorder. In disorders of metabolic intoxication special diets and products (food of special medical purpose), orphan drugs and technologies, are used and in emergency treatment - extracorporeal detoxification. In macromolecule disorders (e.g. lysosomal storage disorders) enzyme replacement or substrate reduction therapy is the most frequent. On two ends of all therapeutic options are: symptomatic (i.e. supportive) therapy and advanced innovative therapeutic methods. The latter may be based on tissue, cell or gene engineering. It allowed for some attempts to correct a genetic defect at patients affected by IEMs. Recently such gene therapy (using various viral vectors) has been efficiently performed in: lipoprotein lipase deficiency (the first gene therapy approved in 2012), adenosine deaminase deficiency (ADA-SCID), Leber’s amaurosis or X-linked adrenoleukodystrophy. Currently several trials are being undertaken in Pompe disease, metachromatic leukodystrophy, Sanfilippo syndrome and familial hypercholesterolemia. Nowadays gene therapy is prepared for severe neurological condition caused by AADC deficiency (neurotransmitter disorder). The initial results of the first five patients treated in USA by MR-guided convective infusion of adeno-associated virus encoding human aromatic L-amino acid decarboxylase (AAV2-hAADC) administered into the midbrain, are promising (K. Bankiewicz, personal information).

IEMs share all specific features of rare diseases, not only in terms of therapeutic issues, but also diagnostic aspects. Due to a limited knowledge of professionals in the field of rare diseases, delayed diagnosis is always taking place and results in a delayed treatment, so worse prognosis. To overcome it, an idea to screen newborns in order to identify affected persons in a presymptomatic period.
Autoimmune encephalopathies: rare, but treatable neurological disorders

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Autoimmune encephalopathies are the heterogeneous group of rare disorders with the presence of circulating autoantibodies against either intracellular antigens (e.g. Hu, GAD) or synaptic receptors (NMDA, AMPA, GABA) and ion channels (LGI1, CASPR2). Two potential triggers of autoimmune encephalopathies are tumors and viral encephalitis. Common clinical features include a change in behaviour, psychosis, seizures, memory and cognitive deficits, involuntary movements, disturbances of autonomic system, and a decreased level of consciousness. These disorders occur in persons of all ages, with some types affecting predominantly children and young adults. Certain syndromes are recognizable on clinical grounds, and their autoimmune etiology can established with laboratory tests (western-blot, cell-based assay). In patients with autoantibodies against neuronal cell-surface antigens prompt treatment leads in most of cases to improvement or full recovery despite of severity of symptoms. In addition to supportive therapies, first-line treatment of these disorders includes a combination of steroids with either plasma exchange or intravenous immunoglobulins. Second-line treatments are cyclophosphamide and rituximab.
Adrenoleukodystrophy (ALD) is the most common peroxisomal disorder characterized by impaired peroxisomal beta-oxidation of very long-chain fatty acids (VLCFA; ≥ C22). That leads to an accumulation of VLCFA in different tissues including the white matter of the brain and spinal cord, adrenal gland, and plasma. ALD is caused by mutations of ABCD1 gene located on the X-chromosome. Mutations of this gene cause ALDP, a peroxisomal transmembrane protein involved in the transport of VLCFA-CoA-esters from the cytosol into the peroxisome. More than 1,200 different mutations of the ABCD1 gene has been identified. There are no known geneotype-phenotype correlations. The range of phenotypes is wide and cannot be predicted through levels of VLCFA or family history. Some individuals with ALD remain asymptomatic until their adult years. The most common presentation forms are: i) childhood cerebral forms (~35% of affected individuals; first symptoms most commonly between age four and eight years); ii) adrenomyeloneuropathy (~40%-45%; typically a man in his twenties or middle age); iii) Addison disease only (~10%; signs of adrenal insufficiency). Moreover, one fifth of female carriers develop mild to moderate spastic paraparesis in middle age or later.

The neuropathological hallmark of the disease is an axonopathy with microgliosis. It is likely that the primary consequence of VLCFA accumulation impairs the function of oligodendrocytes and Schwann cells. However, in up to 60% of affected males, ALD converts to rapidly progressing cerebral inflammation and demyelination. Recent studies showed that brain endothelial dysfunction with an impairment of the blood-brain barrier plays an important role in this conversion. Moreover, ABCD1-deficiency causes impaired plasticity of monocytes/macrophages what plays a negative role in cerebral inflammatory process.
Recent developments in the new psychoactive substances market

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Background: The global drug market changed significantly in the last 10 years. The appearance of new psychoactive substances (NPS) and new ways of distribution have caused greater availability of psychotropic substances and narcotic drugs. Therefore it is reasonable to monitor the substances which are illegally marketed, because they may pose serious risk to people, especially minors.

Material and methods: Presented data were obtained from different sources, including analysis of samples seized from the market and analysis of biological materials collected from intoxicated people, victims of vehicle accidents and drivers stopped for routine checks. A variety of sophisticated analytical techniques, mainly gas and liquid chromatography coupled to mass spectrometry, was used for the analysis of samples.

Results: In the first ‘era’ of NPS, that is in 2008 – 2012 period, stimulants were the most popular. Many NPS were derivatives of phenethylamine or cathinone. Recently, other types of psychoactive substances have increasingly been popular. New ‘generation’ of synthetic cannabinoids has been marketed. Newly developed cannabinoids have significantly lower affinities for CB1 receptor compared to delta-9-THC or synthetic cannabinoids marketed 8-10 years ago. Their administration can cause a huge variety of symptoms, including agitation, vomiting, seizure, tachycardia, hypokalemia, chest pain, cardiac problems, stroke, kidney damage, acute psychosis, and death. Even more severe symptoms are observed after administration of newly marketed narcotic drugs, mainly being derivatives of fentanyl. The United States National Center for Health Statistics (NCHS) indicated that more than 55% of the opioid overdose deaths involved synthetic opioids in the 12-month period ending November 2017, accounting for more than 27,000 deaths. Many deaths were also reported in Poland and other European countries. Among the fentanyl analogues, carfentanil was the most popular in 2016-2017, while methylfentanyl, furanylfentanyl, and acrylfentanyl have also been rising in prominence. By the end of 2017, 34 synthetic opioids were registered in the UNODC Early Warning Advisory (EWA). Fentanyl analogues have been also mixed with other drugs, mainly heroin. This phenomenon increases significantly the risk of overdosing, as the toxic and lethal doses for many fentanyl analogues are much lower than for heroin.

Conclusions: Marketing of substances for which any toxicological or clinical studies were performed poses a serious threat to health and life of humans. Although the number of NPS identified in the last years has decreased, the toxicity of newly detected substances is higher. Growing popularity of agonists of opioid receptors, mainly fentanyl analogues, has been recently the main challenge not only for clinicians or forensic toxicologists, but also legislators.

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Pharmacology of cathinones and related psychostimulants

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Background: Since the early 21st century a large number of novel psychoactive substances (NPS) has emerged. Many NPS are psychostimulants with cathinone- or amphetamine-type chemical structures. Drug users often consume NPS despite the absence of pharmacological and toxicological information. \textit{In vitro} pharmacological screenings of NPS can provide fast estimates on health risks posed by NPS abuse. The translational interpretation of \textit{in vitro} data is based on a large body of animal and human research on well-known, comparative compounds such as cocaine and 3,4-methylenedioxymethamphetamine (MDMA).

Material and Methods: In my presentation, I will review major findings from our work on NPS pharmacology that investigate the contributions of dopamine, serotonin, and norepinephrine transporters (DAT, SERT, and NET respectively) in psychostimulant actions. We screened a large set of NPS, including mephedrone and 3,4-methylenedioxypyrovalerone (MDPV), using \textit{in vitro} pharmacological assays that target interactions with DAT, SERT and NET. Based on the ability of some NPS to interact with SERT, we pursued \textit{in vivo} experiments, here assessing the effects of cocaine as model psychostimulant in transgenic mice with a cocaine-insensitive SERT (Ile172Met substitution; SERT Met172 mice).

Results: Most NPS with a cathinone- or amphetamine-type chemical structure are potent inhibitors of DAT, SERT and NET. Many compounds also act as substrate-type releasers. The comparison of potencies for DAT and SERT inhibition, expressed as DAT/SERT ratio, reveals substantial differences in pharmacological profiles among different NPS. For example, MDPV shows high preference for DAT over SERT whereas mephedrone has a DAT/SERT ratio comparable to cocaine. Our findings with SERT Met172 knock-in mice demonstrates a higher abuse liability of cocaine when SERT interactions are removed, a condition of unopposed DAT inhibition. They also reveal gene networks that are specific to the consequences of cocaine’s action at SERT both with acute and chronic administration.

Conclusions: Pharmacological \textit{in vitro} profiles provide information about possible acute intoxication and health risks of NPS. Furthermore, the comparison of DAT \textit{versus} SERT inhibition may be used to predict the abuse liability of substances, implying that selectivity at DAT over SERT poses higher risk for abuse.

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STUDIES ON THE KETAMINE-LIKE COMPOUND METOXETAMINE (MXE), A DISSOCIATIVE NEW DRUG

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Background: Methoxetamine (MXE) is a dissociative drug structurally similar to ketamine (KET) and phencyclidine (PCP) that was created to purposely mimic the psychotropic effects of its “parent” compounds. We have recently shown that MXE induces KET-like discriminative and rewarding effects, stimulates the mesolimbic dopaminergic (DAergic) transmission and alters emotional states and behavior in rats. In light of the increasing use of MXE and the renewed interest in KET and PCP analogs, we decided to deepen the investigation of MXE-induced effects by means of an acute study in mice and a chronic study in rats.

Materials and methods: Study 1: neurological/sensorimotor functions, cardiorespiratory parameters and blood pressure were evaluated in freely moving mice after an intraperitoneal (i.p.) injection of MXE (0.01-30 mg/kg) in comparison with KET (1-30 mg/kg i.p.) and PCP (1-10 mg/kg i.p.). Study 2: MXE (0.1-0.5 mg/kg, i.p., × 5) was repeatedly administered every other day, and 7 days later rats were challenged with MXE. Emission of ultrasonic vocalizations and locomotor activity were measured after each administration; behavioral effects were evaluated starting 8 days after the challenge through a battery of test including the elevated plus maze (EPM), spontaneous alternation task (SA), novel object recognition (NOR) and marble burying (MB) tests. Finally, DA transporter, tyrosine hydroxylase, and serotonin transporter were measured in various brain regions to evaluate MXE neurotoxic effects.

Results: MXE induces acute neurological, sensorimotor and cardiorespiratory effects in mice qualitatively similar to PCP and KET. Yet, quantitative differences were noted, with PCP typically producing more robust effects than MXE and KET, and MXE producing the most long-lasting effects. Chronic MXE modified neither calling nor locomotor activity of rats but induced behavioral alterations in the EPM, NOR and MB tests, suggestive of increased anxiety and impaired non-spatial memory. Noteworthy, the same rats displayed DAergic damage in several brain areas, and accumbal serotonergic damage.

Conclusions: Altogether, these findings provided the first direct comparison of the in vivo effects of MXE with the two parental compounds (PCP, KET) and demonstrated that MXE induces persistent behavioral abnormalities and neurotoxicity in rats, indicating the need for more research in the field of dissociative drugs and for more information about the consequences of their use.
Hallucinogens of natural origin such as mescaline, psilocybin, DMT (component of ayahuasca) have been used by humans for thousands of years. These substances powerfully alter perception, mood and cognition. The discovery of LSD adverted interest of western countries to these compounds. More recently many new synthetic substances emerged on the market and became popular among young people. They are considered as safe and not subject of abuse. It is believed that hallucinogens stimulate 5-HT2A receptors, especially those located on neocortical pyramidal cells and increase glutamate levels. However, the hallucinogen’s mechanism of action in the brain is still incomplete.

Classical hallucinogens are divided in two main classes: indoloamines and phenylalkylamines. Indoloamines include the tetracyclic ergoline (+)-lysergic acid diethylamide (LSD) and many other tryptamine analogs such as DMT, 5-MeO-DMT, 5-MeO-DIPT. The phenylalkylamines can be subdivided into phenylethylamines such as mescaline and phenylisopropylamines, including “amphetamines” DOM, DOI or DOB. The addition of a N-benzyl group to phenylethylamines markedly increased their activity and this group of compounds (25I-NBOMe, 25B-NBOMe) became a new class of hallucinogens. Phenylethylamines are selective for 5-HT2A/2B/2C receptors. Tryptamines are more potent at 5-HT1A and 5-HT2A receptors. LSD displays high affinity for several types of 5-HT receptors, dopaminergic D1/D2 and adrenergic receptors.

The objective of this presentation is to show the differences between tryptamine derivative 5-MeO-DIPT, N-benzylphenylethylamine 25I-NBOMe and ergoline LSD in their effects on dopamine (DA), serotonin (5-HT) and glutamate release in the rat frontal cortex. 5-MeO-DIPT and 25I-NBOMe increased DA, 5-HT and glutamate release. LSD potently increased glutamate release, was weaker in elevation of DA and decreased 5-HT release. Hallucinogenic activity of animals measured as number of “wet dog shakes” demonstrated a marked effect of 5-MeO-DIPT and 25I-NBOMe with LSD being the weakest in this test.

It is concluded that hallucinogenic activity may be related to an increased level of cortical glutamate. The differences in hallucinogenic potency seems to be related to their 5-HT2A/5-HT1A receptor affinity.
The diagnosis of Parkinson’s disease (PD) is based on typical motor features that mainly depend on dopamine (DA) depletion in the basal ganglia and therefore respond to dopaminergic agents (particularly to L-DOPA). These agents however induce long-term complications, the commonest being abnormal involuntary movements (dyskinesia) and motor fluctuations. Moreover, PD patients suffer from a number of non-motor symptoms which are largely unresponsive to dopaminergic medications.

Therapeutic research for PD actively pursues all these different medical needs with varying degrees of success. Translation of novel therapies from experimental models to patients has been very successful within the area of dopaminergic treatments for PD motor symptoms, while proving very difficult within other areas. By drawing examples from several clinical trials, this lecture will provide a reflection on what factors increase or reduce the likelihood of successful therapeutic translation in PD. Examples will be presented for distinct therapeutic domains, including treatments for motor symptoms, L-DOPA-induced dyskinesia, and neuroprotection/disease modification. The lecture will also review the opportunities afforded by the improved understanding of PD pathogenesis and pathophysiology gained during the past 15 years. This scientific progress has yielded clues about new therapeutic targets and has provided a basis to generate an articulate repertoire of experimental models for preclinical therapeutic research. Finally, opportunities for repositioning drugs will be illustrated using examples from ongoing or imminent clinical trials in PD patients.
Astrocytes and brain compensation potential in Parkinson’s disease

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Slowly progressing degeneration of dopaminergic neurons in the substantia nigra (SN) is the underlying cause of movement disorder observed in Parkinson’s disease (PD). Importantly, the first motor deficits are observed only after at least 70% of SN neurons already die. Therefore, the preclinical phase of the degeneration and possibility of early diagnosis of PD is masked by compensatory mechanisms. By upregulating local neuronal activity and network adaptations compensatory mechanisms maintain the near normal functioning of the dopaminergic system. What do we know about those mechanisms and what cells are involved in it?

Our recent studies showed that besides neurons also astrocytes take an important part in the degeneration and compensation processes. We demonstrated that prolonged dysfunction of astrocytes increased neuronal vulnerability and accelerated their death in the SN. As the effect, lack of the astrocyte support diminished the compensatory potential of the dopaminergic system to maintain normal functioning in rats after selective toxin insult. Prolonged dysfunction of astrocytes influenced also brain energy metabolism showing its extensive adaptive ability.

Introduction of astrocytes as novel targets for protection is a vital option for future therapy. The therapeutic potential of astrocytes in PD will be addressed during the lecture. In summary, the late detection of PD pathology in patients definitely prohibits introduction of any therapies for neuroprotection or modifying the disease progress. Studying the compensatory mechanisms present at the presymptomatic PD stages could strongly benefit in discovery earlier disease markers.

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Translating neurorestorative approaches targeting astrocytes as drivers of neurorepair for next generation therapies in Parkinson’s disease.

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Background: Parkinson’s disease (PD) is the most prevalent central nervous system (CNS) movement disorder and the second most common neurodegenerative disease (ND). PD is characterized by the progressive loss of midbrain dopaminergic neurons (mDA) of the substantia nigra pars compacta (SNpc) and astrogliosis. Current evidence indicates that multiple mechanisms interact and contribute to PD onset and progression via a complex interplay between genetic and environmental factors impacting on astrocyte (AS) biology. AS play integral roles in brain function for normal neurodevelopment and provide neurotrophic and protective support in the adult brain via bidirectional crosstalk with neurons, microglial and neural stem/progenitor cells (NSCs). AS are pivotal in creating the stem cell niche and promote neurogenesis, including the survival and identity of NSC-derived mDA. Our most recent findings suggest the possibility to reprogram dysfunctional AS for neuroprotection and neuroreplacement, while evidence on glial-derived exosomal trafficking of miRNA cargoes as novel biomarkers is being unveiled.

Material and methods: Because aging is the chief risk factor for PD development, we focused on the aged male midbrain microenvironment to address the ability of NSC grafts to activate intrinsic cues that may instruct midbrain AS to implement mDA neurorepair in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced long-lasting mDA neurotoxicity.

Results: Wnt/β-catenin signalling (WβCs), a chief actor for mDA development, was uncovered as a critical AS-derived signal orchestrating mDA neuron plasticity, neuroprotection and neurorepair. Conversely, with age, RAS dysfunction, may act as a chief “hit”, interacting with inflammatory factors to increase mDA vulnerability to PD. Notably, within the aged MPTP-lesioned SNpc, NSCs grafts and RAS-derived factors, especially Wnt1, acted at different levels to rejuvenate the host microenvironment and promoted mDA neurorepair and regeneration.

Conclusions: Identifying the key AS-derived molecular signatures/mechanisms boosting mDA neurogenesis/mDA neuron survival, repair and regeneration have therapeutical implications for developing innovative and high clinical impact biotechnological therapeutics for neurological disorders, including PD.
**S. 05-1**
**Direct recordings from small axon terminals reveal specialized roles of axonal signaling mechanisms**

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To measure the neuronal mechanisms of fundamental hippocampal and axonal functions we use direct patch clamp recordings from the major glutamatergic efferent and afferent fibers of the dentate gyrus region.

First, we compared the intrinsic properties of large and small terminals and axonal shafts of hippocampal mossy fibers. These morphological diverse axonal structures contribute differentially to the output of dentate gyrus granule cells, by forming synapses onto pyramidal cells, GABAergic cells or by allowing action potential propagation, respectively. We asked the question whether their functional heterogeneity is reflected at the level of their intrinsic signaling. The shape of the action potential and its short-term dynamics in the three mossy fiber elements were surprisingly similar. Furthermore, outside out patch recordings from the different axonal membranes revealed that all three types of mossy fiber structures have substantial amount of sodium and potassium currents, supporting local regulation of firing properties. Voltage-sensitive dye imaging and modelling verify the structure-specific properties (or lack of thereof) within mossy fibers. Nevertheless, the similar action potential properties of the small and large mossy fiber compartments verify that even these fast properties can be reliable obtained by direct recordings from small axonal structures.

In contrast to the observed similarities of the different of mossy fiber structures, direct recordings from small terminals of the major glutamatergic afferent fibers to the dentate regions revealed substantial functional heterogeneity. Specifically, action potential shapes and the underlying ionic currents in axon terminals originating from the medial and lateral entorhinal cortices are substantially different, in spite the morphology of these anatomically identified axons are similar.

Thus, axonal signaling is determined not only by the morphology but the active local properties may also support pathway-specific functions.

**S. 05-2**
**Neuronal regulation of (re)myelination**

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Myelin is essential for normal brain function, as it confers fast information transmission and trophic support for axons. The importance of myelin becomes evident in diseases where myelin is lost or damaged, as it leads to both mental and physical disability. Oligodendrocyte progenitor cells (OPCs), which remain in the adult CNS respond to the demyelinating injury by migrating to the lesion site where they differentiate into new myelinating oligodendrocytes to restore the lost myelin sheaths and thus neuronal function. But, this regeneration process often fails, notably in MS, leaving axons vulnerable to atrophy. Failure of remyelination is primarily due to a failure of OPC differentiation rather than a depletion of OPCs, as many are present in chronic demyelinated lesions. To promote endogenous myelin repair it is essential to understand the mechanism that can regulate myelin as well as remyelination.
Neurotransmitters, growth factors and electrical activity influence OPCs in normal development and perhaps in disease. We, and others, have shown, that OPCs receive synaptic input from axons in the white matter and both OPCs and mature oligodendrocytes respond to glutamate via AMPA and NMDA receptors. Given that the glutamate receptors on OPCs are ideally situated to sense neuronal activity they may signal to them to initiate myelination.

Using an in vitro assay of myelination, we have identified that there are two modes of myelination one that is independent of axonal activity and one that depends on neuronal activity releasing glutamate to activate NMDA receptors on oligodendrocytes. The growth factors neuregulin and BDNF switch myelination from a default programme that is independent of neuronal activity, to a mechanism that is regulated by glutamate released from active axons.

Currently little is known about the role neuronal activity and glutamate signalling play in remyelination. Thus we examined this in an in-vivo model of myelin regeneration. We identified that OPCs recruited to the demyelinated lesion expressed glutamate receptors, and that demyelination axons can still conduct action potentials but with latencies similar to those in unmyelinated axons during development. Critically, the demyelinated axons establish synapses with recruited OPCs. These synaptic inputs had identical decay times to those recorded during developmental myelination. Pharmacological manipulation of neuronal activity or glutamate signalling significantly reduced remyelination by affecting OPCs differentiation.

These findings reveal how neuronal activity and release of glutamate instruct OPCs, via AMPA receptors, to differentiate into new oligodendrocytes that restore myelin and recover lost function.

**S. 05-3**

**Principles of Neural Stem Cell Lineage Progression in Cerebral Cortex Development**

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The concerted production of the correct number and diversity of neurons and glia is essential for intricate neural circuit assembly. In the developing cerebral cortex, radial glia progenitors (RGPs) are responsible for producing all neocortical neurons and certain glia lineages. We recently performed a quantitative clonal analysis by exploiting the unprecedented resolution of the genetic MADM (Mosaic Analysis with Double Markers) technology and discovered a high degree of non-stochasticity and thus deterministic mode of RGP behavior. However, the cellular and molecular mechanisms controlling the precise pre-programmed RGP lineage progression through proliferation, neurogenesis and gliogenesis remain unknown. To this end we use quantitative MADM-based experimental paradigms at single RGP resolution to define the cell-autonomous functions of candidate genes and signaling pathways controlling RGP-mediated cortical neuron and glia genesis and postnatal stem cell behavior. Ultimately, our results will translate into a deeper understanding of brain function and why human brain development is so sensitive to disruption of particular signaling pathways in pathological neurodevelopmental and psychiatric disorders.
Oxidative DNA damage: a novel player in the etiology of obesity

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Obesity and related metabolic pathologies represent a significant public health concern. Obesity is associated with increased oxidative stress that damages genomic and mitochondrial DNA. Oxidatively-induced lesions in both DNA pools are repaired via the base-excision repair (BER) pathway, initiated by DNA glycosylases such as Neil-like endonuclease (NEIL1) and 8-oxoguanine DNA glycosylase (OGG1). Global deletion of these enzymes in rodent models, as well as common human polymorphisms, result in increased susceptibility to metabolic disease, underscoring the importance of oxidative DNA repair to whole body energy balance. In probing the mechanisms by which BER may influence susceptibility to metabolic syndrome, we have discovered that mitochondrial localization of BER glycosylases such as OGG1 is critical to their role in metabolism. For instance, transgenic targeting of OGG1 to mitochondria confers significant protection from diet-induced obesity, insulin resistance, and adipose tissue inflammation. These favorable metabolic phenotypes are mediated by increases in whole body energy expenditure driven by specific metabolic adaptations, including increased mitochondrial respiration in white adipose tissue. These data demonstrate a critical role for DNA repair in modulating mitochondrial energetics and whole-body energy balance.

Signaling Cascades in Metabolic Diseases

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Perturbations in signaling cascades regulating basic metabolic processes in adipocytes and hepatocytes often result in metabolic imbalance and metabolic diseases. In adipocytes increased lipogenesis and lipolysis in combination with reduced energy dissipation are the hallmarks of obesity and type 2 diabetes (T2D). Increased lipogenesis also contributes to the development of non-alcoholic fatty liver disease (NAFLD). In my research group we aim at understanding of the complex signaling network regulating the above-mentioned basic metabolic processes. For this purpose we combine cell biology, biochemical and omics approaches with mouse genetics. Using high throughput siRNA based screening we identified a number of novel kinases regulating lipolysis. Using targeted mouse genetics approach we identified several members of Protein kinase D family as central regulators of adipocytes and hepatocyte metabolism. In future, we plan to investigate the identified pathways in the context of metabolic diseases. In parallel, we will utilize screening approaches to identify novel, non-canonical signaling modules (phosphatases and components of the ubiquitin system) regulating abundance, localization and phosphorylation of targets of Pkds and, in the long term, also other kinases implicated in regulation of metabolism. By identifying and characterizing the essential signaling networks in liver and adipose tissue we hope to contribute to more targeted pharmacological strategies for treatment of metabolic diseases such as obesity and T2D.
**S. 06-3**

**Lipid mediators in regulation of pancreatic islet function**

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Obesity-related type 2 diabetes develops in individuals with the onset of β-cell dysfunction. Pancreatic islet lipotoxicity is now recognized as a primary reason for the onset and progression of the disease. Such dysfunction is reflected by the aberrant secretory capacity and detrimental loss of β-cell mass and survival. Elevated circulating serum fatty acid levels and disordered lipid metabolism management are particularly interesting in the search for biologically relevant triggers of β-cell demise. The lipotoxic effect on β-cells depends on the type of lipid mediator (e.g., long-chain fatty acids, diacylglycerols, ceramides, phospholipids), cellular location of its action (e.g., endoplasmic reticulum, mitochondria), and associated-organelle conditions (e.g., membranes, vesicles). Recently, growing body of evidence suggest that metabolism-driven changes in expression of specific transcription factors may result in α- and β-cells dedifferentiation and loss of their functional identity via alterations in DNA methylation pattern. Interestingly, stearoyl-CoA desaturase 1 (SCD1), a significant control point in fatty acids metabolism, can regulate gene expression by changing DNA methylation level. Our data demonstrate that SCD1 activity/expression plays important role in maintenance of pancreatic islets functional identity via epigenetic regulation.

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**S. 06-4**

**Healthy adipocyte**

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Understanding the integral control of metabolism in peripheral tissues as well as the mutual interactions between intrinsic metabolism of various tissues remains one of the major challenges for the life sciences research. Thus, e.g., the mechanisms behind development of the harmful consequences of obesity need to be further clarified. Why a sizeable subgroup (10-40%) of obese people remain metabolically healthy while the others develop various diseases – namely type 2 diabetes, dyslipidaemia, cardiovascular disease, and even cancer? It is becoming increasingly evident that metabolic and secretory features of white adipose tissue (WAT) are of key importance for the consequences of obesity. Our results document that beneficial metabolic effects of various interventions, namely life-style changes including physical activity, caloric restriction and dietary omega-3 fatty acids (FA), as well as some pharmaceuticals like thiazolidinediones could reflect induction of “healthy white adipocytes”. These cells are endowed with high activity of (i) futile metabolic cycle, which is based on hydrolysis of triacylglycerols (TAG) and re-esterification of FA in adipocytes (TAG/FA cycling), (ii) in situ FA synthesis (de novo lipogenesis; DNL), and (iii) oxidative phosphorylation, since ATP is required for both DNL and TAG/FA cycle. DNL in WAT correlates with insulin sensitivity as well as resistance to obesity because it might (i) serve as a source of signalling molecules (lipokines, namely branched fatty acid esters of hydroxy fatty
acids) and (ii) be required for sufficient generation of lipid fuels for extra-adipose tissues. Therefore, in spite of its relatively low contribution to metabolic rate, WAT metabolism is essential for adaptive thermogenesis and may affect propensity to accumulation of body fat. Obesity is linked with the deterioration and dysregulation of the above biochemical activities of WAT and leads to low-grade inflammation. In accordance with the emerging concept that the immune and metabolic systems are interconnected, the interventions that exert beneficial effects on WAT metabolism also ameliorate inflammation. It is relatively difficult to reverse obesity but it might be feasible to reduce its adverse consequences on health by modulating metabolism of white adipocytes.

S. 07-1
Introduction to pharmacogenetics

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Pharmacogenetics is a scientific discipline at the intersection of pharmacology and genetics which concentrates on genetic variability of drug response. The term “pharmacogenetics” was proposed by Vogel in 1959, soon after observation of the acetylation polymorphism of the antituberculectic drug isoniazid by Böncke and Reif (1953) and the hydrolysis polymorphism of the anaesthetic drug procaine and muscle relaxant succinylcholine by Kalow (1956). In 1962 the first book entitled “Pharmacogenetics: Heredity and the Response to Drug” was published by Kalow (Ed. W.B. Saunders, Philadelphia), stressing that inherited differences in metabolism often lead to variability in drug response.

The greatest discoveries came later and were related to cytochrome P450 (CYP) that catalyses most of the drug oxidations. Namely, in 1977 Mahgoub et al. in England and Eichelbaum et al. in Germany independently discovered a deficit of debrisoquine/sparteine hydroxylase which 10 years later was ascribed to the lack of cytochrome P450 2D6 (CYP2D6) protein by Gonzalez et al. (1987) and Zanger et al. (1988). Then Küpfer and Preisig as well as Wedlund et al. (1984) discovered a deficit of S-mephenytoin hydroxylase which was ascribed to the lack of CYP2C19 protein by Goldstein et al. and Wrighton et al. (1994). In the next years, the molecular background of the genetic polymorphisms was discovered.

Later studies led to the discovery of genetic polymorphisms of CYP2C9 and other CYPs (CYP2A6, CYP2B6, CYP3A4/5 rare mutations) which are important for drug metabolism. Also many genetic polymorphisms have been discovered in enzymes that catalyse conjugation processes (N-acetyltransferase, glutathione S-transferase, UDP-glucuronosyltransferase, thiopurine S-methyltransferase). Parallelly, mutations of genes encoding drug transporting proteins (ABC, OAT, OATP, OCT) were found. In addition, genetic variants of nuclear receptors involved in the regulation of drug metabolizing enzymes and drug transporters have been reported.

Recent studies indicate that genetically determined response to drugs may also occur at pharmacodynamics stage, involving the genetic polymorphism of receptors (5-HT2A, 5-HT2C, D2, D3, nAch, β, µ), ion channels and neurotransmitter transporters (5-HTT, VMAT2), which may affect therapeutic outcome and/or side-effects. The results of pharmacogenetic studies create now a well-founded basis for personalized medicine in different types of treatments (e.g. psychiatry, oncology, cardiology).
Mindful pharmacogenetics: the role of cytochrome P450 enzymes in drug metabolism and mental health

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Cytochrome P450 enzymes are abundant in the human body, and maintain a constitutive role as well as a xenobiotic defense role by catabolism and detoxification of drugs, procarcinogens, endogenous as well as xenobiotic compounds. In the brain, CYP enzymes are expressed in a different pattern than in hepatic tissue or intestine. In neuronal cells, drug metabolizing enzymes CYP2D6, CYP2B6 and 1A1 are expressed, which are the main enzymes responsible for the metabolism of antidepressants, antipsychotics, and centrally acting opioids. CYP2D6 and CYP2C19 are genetically polymorphic enzymes with 2-7% in Caucasians carrying a poor metabolizer genotype leading to completely absent enzyme activity, and for both enzymes about 3-5% individuals with ultrarapid metabolism activity. Both enzymes are prone to enzyme inhibition due to interacting drugs and CYP2C19 can also be induced by the common mechanism of nuclear receptors. Interestingly, apart from drug metabolism, the genetic subgroups of poor versus rapid and ultrarapid metabolizers have been associated with affective and psychic behavioral phenotypes, and brain morphological changes. CYP2C19 seems to be expressed in neuronal cells during fetal development, and the genetic polymorphism leads to differences in hippocampus size and anxious-depressive behavior in mice with human CYP2C19 overexpression. Neurodevelopmental influences during fetal development may affect susceptibility to CYP2C19 metabolized antidepressants. Especially since maternal antidepressant drug intake leads to higher drug exposure in amniotic fluid compared to maternal plasma. For CYP2D6, imaging studies point to a functional role of the active enzyme in neuronal brain metabolism of endogenous compounds. Functional brain imaging studies have shown genotype-specific differences in activation patterns in brain regions associated with sustained attention and vigilance. Moreover, CYP2D poor metabolizers seem to be more anxiety-prone and less successfully socialized. In conclusion, drug metabolizing enzymes in the brain may have a constitutive role important during neurogenesis, affecting mental health, and influencing psychiatric diseases. Genetic polymorphisms affect individual enzyme activity, and thereby may lead to different constitutive phenotypes, and different drug exposure in the central nervous system. Therefore, different mechanisms of action of CYP polymorphisms on mental health and adverse drug reactions are existing and potentially overlaying.
Over last decades, substantial progress has been made in identification of genetic biomarkers of drug response. Particular gene variants have been associated with drug inefficacy, hypersensitivity or increased toxicity. The efforts to define the role of genetic polymorphisms in optimizing pharmacotherapy of Parkinson’s disease (PD) were also undertaken. As the mechanism of currently available antiparkinsonians is related to dopaminergic transmission, most studies were focused on variants of genes related to drugs and dopamine metabolism (COMT, MAOB), transport (DAT1- SL6A3, SLC22A1) and action (DRD1-DRD5). Despite significant associations identified in case of some candidate genes, none of the antiparkinsonian drugs have received specified pharmacogenetic recommendations so far. This is partly due to the complexity of disease pathogenesis, common comorbidities in elderly patients, multiple treatment regimens, the lack of dose-response relation in case of many drugs, and many non-genetic factors influencing therapy outcome. New investigations could be performed as hypothesis-free, genome-wide association studies (GWAS), to identify unexpected genetic markers, that could have been omitted during the studies with functional design.

Contrary to pharmacogenetic studies, data on involvement of genetic factors in PD pathogenesis is much more conclusive. Over twenty loci were associated with sporadic PD in GWAS, and subsequently confirmed in meta-analyses. The identified loci segregate with numerous pathways, including protein aggregation, membrane trafficking, lysosomal autophagy, endocytosis, immune response, inflammation and more. That data points to extensive heterogeneity in the PD pathogenesis. Currently, no treatment is available that could stop progressive neurodegeneration in patients, only symptomatic treatment of limited effectiveness is administered. From many compounds with promising disease-modifying potential tested, none have been approved to date, mainly due to insufficient efficacy. However, it is possible that some of drug candidates would turn out to be effective in selected subtypes of PD (with the same underlying molecular defect), which was not addressed in clinical trials. Implementation of available genetic data in recruitment and stratification process, together with large cohorts of PD patients at early stage of disease might overcome that limitation and possibly result in development of novel preventive or disease-modifying therapies.
Genetic variation of response to treatment and toxic effects of FAC chemotherapy of breast cancer

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Background: The differences in patients’ response to the same medication, toxicity included, are one of the major problems in breast cancer treatment. Chemotherapy resistance and/or toxicity makes a significant clinical problem due to shortened survival, decreased quality of life, prolongation of treatment and reinforcement of negative emotions associated with therapy.

Materials and Methods: In this study we evaluated the genetic and clinical risk factors of FAC chemotherapy-related response and adverse effects in the group of 324 breast cancer patients. Selected genes and their polymorphisms were involved in FAC drugs transport (ABCB1, ABCC2, ABCG2, SLC22A16), metabolism (ALDH3A1, CBR1, CYP1B1, CYP2C19, DPYD, GSTM1, GSTP1, GSTT1, MTHFR, TYMS), DNA damage recognition, repair and cell cycle control (ATM, ERCC1, ERCC2, TP53, XRCC1).

Results: We showed that the response to treatment depended of the variability in genes engaged in drugs’ transport (ABCC2c.-24C>T, ABCB1p.Ser893Ala/Thr) and in DNA repair machinery (ERCC2p.Lys751Gln). Furthermore, the growing number of high-risk genotypes was reflected in gradual increase in risk of the non-responsiveness to treatment- from OR 2.68 for presence of two genotypes to OR 9.93 for carriers of all three negative genotypes in the group of all patients. Similar gene-dosage effect was observed in the subgroup of TNBCs. The multifactorial risk models that combine genetic risk modifiers and clinical characteristics were constructed for 12 toxic symptoms. The majority of toxicities was dependent on the modifications in components of more than one pathway of FAC drugs, while the impact level of clinical factors was comparable to the genetic ones. For the carriers of multiple high risk factors the chance of developing given symptom was significantly elevated which proved the factor-dosage effect. We found the strongest associations between concurrent presence of clinical factors, overall and recurrent anemia, nephrotoxicity, early nausea and genetic polymorphisms in genes responsible for DNA repair, drugs metabolism and transport pathways.

Conclusions: Our results demonstrate that outcome of cancer treatment is the effect of many clinical and genetic factors. It seems that multifactorial polymorphic models could be a potentially useful tool in personalization of cancer therapies. We observed also the over representation of triple negative breast cancer (TNBC) patients among the carriers of all unfavorable polymorphic variants.

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Genetic predictors of bleeding in patients treated with vitamin K antagonists: new insight

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Background: Single nucleotide polymorphisms (SNP) in genes encoding proteins involved in metabolism and action of vitamin K antagonists (VKA) affect anticoagulation stability. We investigated how those polymorphisms influence bleeding rates in patients following venous thromboembolism (VTE).

Materials and methods: In 324 patients following unprovoked VTE, 143 (44%) on warfarin and 181 (56%) on acenocoumarol, we recorded bleeds within the preceding 24 months. We assessed eight SNP, including those in cytochrome P450 isoform 2C9 (CYP2C9) and isoform 4F2 (CYP4F2), vitamin K epoxide reductase complex subunit 1 (VKORC1), gamma-glutamyl carboxylase (GGCX), apolipoprotein E (APOE) and multidrug resistance gene 1 (MDR1).

Results: Within 48 months before enrolment, bleeding events occurred in 80 (25%) patients, including 14 (4%) major bleeds. Patients with bleeds had 16.2% lower median time in therapeutic range (TTR) and were more often carriers of CYP2C9*3 variant (26 [33%] vs. 19 [8%], p < 0.001) compared with the remainder. Bleeding occurred more frequently in patients with ≥4 SNP compared with the remainder (27 [34%] vs. 47 [19%], p = 0.009) with no intergroup differences of TTR. Number of SNP was one of the predictors of any bleeding. The regression model for major bleeding including factors such as CYP2C9*3 c. 1075 C, VKORC1 c. -1639 A and APOE c. 388 C showed good predictive ability (area under the curve - 0.79).

Conclusions: In VTE patients on the maintenance treatment with VKA, bleeding episodes are associated with CYP2C9 gene variations and increased number of SNP of genes involved in the action and metabolism of VKA.
Hydroxyapatite nanoparticles: past, present, and future in bone regeneration

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Hydroxyapatite (HAP), is a mineral with chemical formula usually written as Ca_{10}(PO_4)_6(OH)_2. Up to 50% by volume of human bone consists of hydroxyapatite. Carbonated calcium-deficient hydroxyapatite is also a component of dental enamel. Understanding that HAP is a major component of bones and teeth enamel stands from the XVIII century. Understanding the fundamentals of its structure and basic synthesis methods stand from XIX century. Calcium deficient HAP, as present in human bones was first time synthetized also in this period. The phase diagram, e.g., crystalline structure of HAP and the whole family of calcium phosphates as a function of composition and temperature of annealing is well established.

The market for various HAP based products for medicine is huge. The main application is to promote bone regrowth in cases a large gap in the bone exists due to trauma or cancer operation, as well between an orthopedic implant and the bone. Nevertheless, still regrowth of critical sized bone gaps is a challenge, and healing of patients after trauma where bone was broken into small debris, or bone was removed after cancer operation, is not solved satisfactorily.

Nanotechnology offers new opportunities to overcome these barriers. Nanotechnology exploits size effects in materials. It was discovered that particles with size less than usually 100 nm may display different properties than the same material with larger size. The properties change is mainly caused by strong contribution of surfaces to the materials properties, which can be frequently neglected for large particles. Further, the electronic and atomic structure of particles with size of a few nanometers may be different from that of conventional materials. Thus, HAP particles with nanometer size may have higher biological activity than their micron sized counterparts.

In order to exploit these potential advantages, it is important to precisely regulate their size. Recently technologies have been developed to produce nano-HAP with precisely regulated size and stoichiometry. Thus, the way becomes open to exploit nanotechnology in service of bone regrowth technologies.

However, nano-HAP itself is of little use in such medical applications. The next step will be nano-architecture, i.e., building from nan-HAP and other materials, e.g., collagen, or resorbable polymers, active structures, which will promote bone regrowth, fight cancer, or fight inflammatory diseases. Progress in that respect will be shortly reviewed in this paper.

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Cancer nanotechnology: Beyond the horizon

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The old dream of humanity, to live longer and in the perfect health makes thousands of doctors and scientists working hard. The results are very impressive, world average life span doubled during the last 100 years. The old diseases which agonized humanity are no longer dangerous, but new problems appeared. Cancer is slowly taking a leading position on the infamous death statistics of human kind. Despite the enormous scientific and economical effort new drugs and therapies are few and expensive, what makes them unavailable for the majority of human population, and frequently ineffective in the long range treatment. One of the possible ways to fight cancer is reaching for the help of nanotechnology.

Polysaccharide nanoparticles are obtained in water solution without the use of organic solvents, the process employs self-assembly phenomena of matter, is simple and cheap. First polysaccharide is partially oxidized. This leads to breaking of some glucose rings without breaking of the polysaccharide backbone. Obtained carbonyl groups, are then reacted with aliphatic amine, hydrophobic amino-acids and amine group containing drugs. This leads to Schiff base formation and bonding of reagents to the polysaccharide backbone. Highly hydrophilic polysaccharide with hydrophobic side groups are then forming nanoparticles due to hydrophobic-hydrophylic interactions. Nanoparticles have 60-150 nanometers diameter, depending on the polysaccharide type and molecular weight and on the number and type of hydrophobic side groups. Nanoparticles are formed of 10-12 chains of polysaccharide chains and have a high water content – above 95%. Nanoparticles can be freeze-dried and stored, upon immersion in water they reassemble and form nanoparticles again regaining the same size distribution. Pure nano-carrier is completely nontoxic and can be administered in high doses, what was confirmed on rodents.

In vitro research shows that nanoparticles are quickly absorbed by cancerous cells entering cytosol probably by clathrin dependent endocytosis. In endosomes drug is released from nanoparticles and transported to the nucleus. In vitro tests reveals that encapsulated drugs are more potent than pure one, what can help breaking drug resistance of some cancer cell lines. In vivo research, conducted on rodents with epirubicine containing nanoparticles, reveals decrease of side effects of the treatment and also quicker tumor size decrease, as compared to the non-encapsulated drugs. Polysaccharide nanoparticles loaded with fluorescent agent or radioactive isotope can be employed in early diagnosis of cancer, what was also confirmed during animal trials. Polysaccharide nanoparticles can also serve as nanoreactors for fluorescent nanocrystals precipitation, such nanoparticles accumulate in the esophageal cancer and makes it fluorescent and detectable with confocal endo-microscopy in very early stage.
Nanomaterials (NMs) are defined as engineered or natural objects having at least one dimension less than 100 nm, whereas nanoparticles (NPs) are the objects having all dimensions less than 100 nm. NPs are a product of natural processes or are man-made. Anthropogenic NPs can be divided into two groups: (1) unintentionally created NPs that include diesel engines exhaust, ashes and other products of combustion, such as PM2.5 and PM10, or (2) NPs intentionally designed by man for different applications in medicine, industry and everyday life, i.e., the so-called “engineered NPs”. A small size of NPs, comparable with the size of biomolecules, favours their interaction with biological systems. In addition, a large surface to volume ratio implies different physical, chemical and biological properties, as compared to a bulk material.

Nanotechnology is one of the most rapidly developing fields of science. Here, we are going to describe the use of nanomaterials in medicine, especially in radiotherapy and radioimaging. Different aspects of the use of NPs in radiotherapy and imaging will be described, including direct use of NPs made of radioisotopes, the use of NPs as a radioisotope carriers and the use of non-radioactive NPs as a radiosensitizers. Special attention will be paid to the use of theranostic radionanopharmaceuticals. Theranostic approach combines functionality of both, therapy and diagnostics, and have shown potential in cancer nanomedicine. Radionanotherapy, e.g. nano-sized drug and contrast agents carriers that labelled with radionuclide will facilitate individualized treatment guided by specific, personalized diagnosis. With a multifunctional nanoparticle system, the diagnostic (imaging) methods and the therapy (delivering drug, antitumor agent, antibiotics or radionuclides in their payload) can be carried out using the same biological and pharmacological mechanism. Application of radionanotherapy would enable earlier detection and optimized treatment in several disease areas.

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Over the last decade, we have witnessed the tremendous advances in next generation diagnostics which are a result of developments in modern nanotechnology. The rapid detection of molecular biomarkers near to the patient, which facilitates better disease diagnosis, monitoring and management is especially a major challenge in the field of personalized medicine, matching patient’s needs with appropriate therapeutic strategies that improve the health outcomes due to proper treatments. In modern nanomedicine, a number of expectations is approaching to the point-of-care (POC) diagnostics that allow to identify some valuable biomarkers e.g. proteins, enzymes, DNA as well as pathogenic microorganisms at a very early stage enables quick medical decisions. Note that existing protocols for determination of diagnostic biomarkers in some body fluids and/or tissues are time consuming, in consequence, the risk of patients spreading disease whilst awaiting results increases. In clinical laboratories, a large number of analytical methods are mainly based on spectrophotometry, turbidimetry, nephelometry and immunoassays. However, most of these methods are really time-consuming, expensive, prone to false negative results, sometimes not enough sensitive, requiring skillful operations, qualified personnel and sophisticated instrumentation. Therefore, rapid and selective ultrasensitive protocols for determination of novel biomarkers at the trace levels are still needed. The electrochemical nanosensors are the most promising tools in the terms of low cost, low sample volume, high sensitivity, wide dynamic concentration response range and versatility. The modern electrochemical bioassays are mainly based on the antigen-antibody specific interactions, aptamer-protein interactions or hybridization processes between two complementary DNA fragments. To enhance the sensitivity of such novel devices, different nanomaterials including metal nanoparticles, quantum dots, dendrimers, polymer-metal nanoparticle composites, carbon-based nanomaterials are applied as a new carriers for immobilization of biomolecules or targeted signal molecules. Nevertheless, the application of nanomaterials does not meet all analytical requirements due to multiple preparation steps in bioengineering designs. Therefore, there is still much work to be done to update these novel nanosensors to speed up the real progress of POC diagnostics in personalized nanomedicine.
We report here that acute exposure to Valproate (VPA) during early embryonic stages has long lasting effects on zebrafish brain development. Treated larvae develop an epileptic phenotype along with other structural and behavioral deficits, including a deflated swim bladder, lack of posture and non-responsiveness to a shift in bright/dark field stimuli. Since Valproate also acts as a sodium channel blocker, we observed a phenotypic correlation with a genetic model for Dravet syndrome (DS), a severe early onset epileptic encephalopathy. We found that mutation of the sodium channel Nav1.1 induced the same phenotypic alterations. To validate the idea that perturbed sodium channel activity in the developing brain might lead to long lasting, structural changes in the brain, we characterized a new zebrafish DS model generated by CRISPR-CAS targeting of the zebrafish scn1lab gene. In homozygous (scnlab-/-) larvae, we analyzed dendritic arborization of GABAergic neurons and identified a structural deficit that could account for an inhibitory/excitatory neurotransmitter imbalance, which was also reflected in enhanced electrical brain activity, in particular, in the range of high frequencies. In order to restore normal neuronal branching, we tested the serotonergic drug Fenfluramine, which is currently in stage III clinical trials as an antiepileptic drug candidate for DS. Serotonin plays a key role in neuronal morphogenesis, in particular during the critical period of brain development when inhibitory networks are formed. Chronic treatment of scnlab-/- larvae with Fenfluramine completely restored the observed arborization defects to normal. In summary, our findings: 1) suggest a crucial role for sodium channel activity in establishing neuronal morphology during early brain development, 2) provide insight into the neuroanatomical pathology underlying Dravet syndrome, and 3) present a new genetic epilepsy model for elucidating the mechanisms of epileptogenesis.
Novel approaches to discovery of future antiepileptic drug targets

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Background: Recent decades of antiepileptic drug (AED) discovery led to the development of many compounds providing significant therapeutic benefit for patients. This was largely due to generally high predictability of preclinical seizure models, which were instrumental for drug discovery approaches based on phenotypic in vivo screening. However, a large proportion of patients still remain without adequate seizure control driving the need to further improve the AED discovery process. Next generation sequencing studies continue to produce valuable data sets, but still provide rather reductionist mechanistic insights for identification of novel drug targets. System genetics integrates many sources of genetic information allowing better understanding of the broad molecular underpinnings and genetic architecture of complex human diseases such as epilepsy.

Materials and methods: We have developed a new computational approach to drug target identification that combines gene-regulatory information with causal reasoning (“causal reasoning analytical framework for target discovery” – CRAFT). Using a systems genetics approach and starting from gene expression data from human and rodent epileptic hippocampi, CRAFT provided a predictive framework for identifying cell membrane receptors with a direction-specified influence over disease-related gene expression profiles.

Results: We have constructed a number of epilepsy-related gene modules that were enriched with specific biological functions and associated with specific cell types. This led to the identification of several drug targets predicted to control gene networks driving epilepsy. One potential target, the Csf1R microglial membrane receptor, turned out to be particularly promising. We have predicted that Csf1R blockade would significantly reduce the number of epileptic seizures, which was subsequently confirmed in three experimental animal models of epilepsy using a compound that has been in clinical trials for other non-epilepsy related indications.

Conclusions: Moving from traditional drug screening approaches into computational identification of key disease drivers through systems genetics approaches should allow a better match with future, more effective therapies targeting specific subpopulations of patients with epilepsy.
The role of the GPR39 receptor in animal models of seizures and epilepsy

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Background: Zinc is necessary for maintaining the balance between neuronal excitation and inhibition, while an imbalance between excitation and inhibition underlies seizures. G-protein coupled receptor 39 (GPR39) constitutes an important target for extracellular zinc. It was demonstrated that zinc-enriched mossy fiber stimulation-dependent up-regulation of potassium-chloride co-transporter 2 (a neuron-specific chloride extruder, which is fundamental for the proper function of the GABA_A receptor) is not observed in slices from GPR39 knockout animals. Furthermore, GPR39 knockout animals were characterized by increased susceptibility to seizures induced by a single dose of kainic acid. Therefore, GPR39 has been proposed as a novel target for dampening seizures. To assess the effects of GPR39 activation in vivo, we utilized its agonist – the compound TC-G 1008.

Material and methods: Liquid chromatography tandem mass spectrometry was used to assess the brain and serum concentration of TC-G 1008 following its i.p. administration in Swiss mice. Maximal electroshock seizure threshold (MEST) test and pentylentetrazole (PTZ) kindling model were employed as an acute seizure test and a model of epilepsy, respectively. In the MEST test, TC-G 1008 was administered i.p. acutely 30 min before the electroshock. In the PTZ kindling model, subconvulsive doses of PTZ were administered on alternate days for a total of 19 injections, and TC-G 1008 was administered i.p. 30 min before every PTZ injection. Moreover, chronic (a 4-week) zinc deficient diet (containing 3 mg zinc/kg vs. zinc adequate diet containing 50 mg zinc/kg) was administered to explore whether the effects observed in the MEST test following treatment with TC-G 1008 depend on the dietary zinc supply.

Results: TC-G 1008 was found to be brain-penetrant. In the MEST test, TC-G 1008 dose-dependently decreased the seizure threshold. In the PTZ kindling model, it facilitated kindling development. Furthermore, the effects of TC-G 1008 in the MEST test were dependent on the amount of zinc in the diet.

Conclusions: Our preliminary data obtained using TC-G 1008 argue against GPR39 activation as a therapeutic strategy for alleviating seizures/epilepsy.

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Neurobiochemical mechanisms of ketogenic diet: simple rules in complex reality?

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Ketogenic diet (KD) appears to be one of the most effective therapeutic approaches to drug-resistant epilepsy in the pediatric population. Its efficacy in controlling seizure activity has been well documented in several retrospective, prospective, and randomized clinical studies. There are many hypotheses regarding the plausible mechanisms of the KD anticonvulsant action. The crucial probable mechanism involve an increase in production of ketone bodies, the direct inhibitory actions of fatty acids, stabilization of glucose metabolism, enhancement of tricarboxylic acid cycle (TCA) and mitochondrial function as well as influence on amino acids and neurotransmitter systems (including adenosine) that are involved in regulation of neuronal excitability. Considering the multi-modal nature of the KD effects, there arises the question whether there is a common biological background for these effects.

From a biochemical perspective, the KD mimics the effects of starvation that on the one hand, forces the body cells to seek new sources of energy and, on the other hand, forces energy efficiency to be improved. This may be manifested by the decoupling of mitochondrial TCA activity from the reduced NADH equivalents derived from glycolysis in order to improve the effectiveness of the TCA by supplying the alternative sources of energy. As a consequence, these changes may be accompanied by the reversibility of “near equilibrium” NAD\textsuperscript{+} kinase reactions e.g. being a part of malate-aspartate and astrocyte-neuron lactate shuttles.

Within a broader perspective, it appears that the KD-induced change in the distribution of NAD\textsuperscript{+}/NADH ratio between mitochondria and cytosol may also lead to a shift in balance between processes of anaplerosis and cataplerosis as well as changes in the subcellular distribution of Ca\textsuperscript{2+} between cytosol and mitochondria.

Conclusion

It seems that the biochemical changes in redox state fit well the complex nature of the KD action, suggesting that they may play an important role in this phenomenon. Finding new binding sites for the substances regulating those processes may help to explain those dependencies, thus increasing the likelihood of replacing KD with pharmacological agents.
Enhancing mGlu5 signaling for the treatment of cognitive impairment associated with schizophrenia: importance of signal bias and crosstalk with mGlu3

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Background: The metabotropic glutamate (mGlu) receptors are being investigated as novel drug targets for disorders that display cognitive impairment, and have shown promise in both preclinical and clinical studies. While activation of mGlu5 receptors may be efficacious for cognitive function, the development of mGlu5 targeting drugs has been stifled by preclinical toxicity profiles associated with some mGlu5 activators. We therefore tested the hypothesis that altering signal bias of mGlu5 positive allosteric modulators (PAMs) may confer both desired safety and efficacy drug profiles. Additionally, our recent discovery that mGlu3 and mGlu5 act as signaling partners to modulate synaptic plasticity led us to hypothesize that mGlu3 may serve similar functions to those of mGlu5 in cognition.

Methods: We directly tested this hypothesis using acute slice electrophysiology to investigate synaptic plasticity. Cognitive behavioral tests included the novel object recognition assay and associative fear learning paradigms. Male wild-type C57bl/6 mice or CaMKII-cre;mGlu5−/− mice were used in all studies.

Results: Results demonstrate that mGlu5 PAMs can be segmented based on signal bias. Biased mGlu5 PAMs also maintain efficacy in cognitive assays. Interestingly, mGlu2/3 activation by the orthosteric agonist LY379268 enhances LTP in a mGlu3 and mGlu5 dependent manner. Behaviorally, selective activation of mGlu3 or mGlu5 causes an enhancement in trace-fear conditioning. Further testing of mGlu3 activation to rescue cognitive impairment in models of schizophrenia-related pathology is currently underway.

Conclusions: These results taken together demonstrate mGlu3 activation boosts neural plasticity and cognition in mice. This work provides a basic biological mechanism and preclinical therapeutic validation for mGlu3 as a cognitive enhancer.

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Allosteric mGlu4 receptor modulators as antipsychotic-like drugs acrosstalk with 5-HT1A receptors

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**Background:** The aim of our studies was to further investigate the antipsychotic potential of glutamate metabotropic receptor 4 (mGlu4) agonists and positive allosteric modulators (PAMs) as well as to investigate the interactions between mGlu4 receptor stimulators and 5-HT1A serotonin agonists. The possibility that the mGlu4 receptors and 5-HT1A receptors may form dimers was also investigated.

**Material and methods:** Several rodent tests were used to study antipsychotic-like activity of drugs. Interactions of mGlu4 receptor agonists with serotonergic system was also evaluated. Moreover the in vivo microdialysis, the electrophysiological studies were also conducted. To analyze distribution of both receptors in the mouse brain, a double immunofluorescence with DAPI on cortical section was performed. To study possible interaction between mGluR4 and 5-HT1A receptors SNAP or HALO–tag were used. Potential oligomerization was measured by HTR-FRET assay in presence agonists L-Glu, 8-OH DPAT or both. The pharmacological response of mGlu4 and 5-HT1A receptors by measuring cAMP level upon stimulation with reference compounds was evaluated.

**Results:** The mGlu4 receptor agonists/PAMs were effective in all preclinical tests of schizophrenia applied. The co-administration of sub-effective dose of the 5-HT1A agonists enhanced the activity of ineffective doses of mGlu4 receptor agonists/PAMs, while the 5-HT1A antagonist blocked the effects. In the microdialysis studies MK-801 increased cortical DA, 5-HT and Glu levels, mGlu4 agonists reversed this effect. In the electrophysiological studies mGlu4 agonists reversed the DOI-induced changes. When mGlu4 and 5-HT1A receptor were co-expressed in the T-Rex 293 cells, the fluorescence resonance energy transfer (FRET) studies revealed the close vicinity of both receptors. The experiments with the use of proximity ligation assay (PLA) conducted on a primary cultures of astrocytes or neurons have shown a positive PLA signal, which indicated that both receptors are placed in a close vicinity, which enables the potential dimerization of both receptors.

**Conclusions:** The efficacy of mGlu4 receptor activators in mechanistic and behavioral models of schizophrenia provides evidence for a potential role played by this receptor in the pathophysiology of this disease, combined treatment based on the simultaneous stimulation of 5-HT1A and mGlu4 receptors may be considered as combination active in psychosis. MGlut4 and 5-HT1A receptor may dimerize.
IMPORTANCE OF HETERO-DIMERIZATION OF GPCRs IN THE NERVOUS SYSTEM: DOPAMINE RECEPTORS AS EXAMPLE

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The G protein-coupled receptors (GPCR) family is represented by over 800 members, 350 of which constitute potential drug targets, and many of them play an important role in central nervous system (CNS) disorders. Drugs targeted at a given receptor are widely used in the clinic, but they are overburdened with serious side effects, therefore there is still a need to search for new therapeutic approaches. Physical interaction of GPCR (dimerization) has been well described in many studies, and it is very important in the CNS due to its complexity – heterodimers of a given pair of receptors can form only if they are colocalized in the same neuronal cell. Thanks to the studies defining the specific pathways dysregulated in a given disease and the colocalization of GPCR important in the CNS functioning, the numerous heterodimers of receptors are constantly being found. Methodology used in GPCRs heterodimerization studies has evolved, from radioligand binding, receptor crosslinking, receptor complementation, or co-immunoprecipitation approach to biophysical techniques based on resonance energy transfer – each having their pros and cons, however their use still provides new exciting data concerning the complexity of GPCRs physical interactions, which broaden basal knowledge as well as offer new targets for pharmacological intervention. This approach is exemplified by dopamine D1-D2 receptor heterodimer, characterized in the nucleus accumbens and caudate putamen, and linked to various neuropsychiatric disorders such as drug addiction, depression and schizophrenia. It has been shown that D1-D2 heterodimer disrupting peptide exhibited anti-depressant like effects upon in vivo injection in a rat model. Our own studies have shown that D1-D2 heterodimers (which, in contrast to individual receptor which are coupled to adenylate cyclase, have been shown to stimulate phospholipase C) increase upon overstimulation of both dopamine receptors, which is linked with schizophrenia. Interestingly, clozapine, antipsychotic drug with not fully understood mechanism of action, uncoupled such heterodimers formation, leading to normalization of dopamine receptors signaling.
S. 11-1
Productive and Safe Cooperation Between Scientists and Pharma Industry - safeguarding intellectual property and confidentiality

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If we want to overcome fears in the cooperation between academic centers and the pharmaceutical industry in Poland, we should establish the common principles of safe cooperation for both the scientist and the pharmaceutical industry. The key question is the moment of establishing cooperation. Is it always better to establish cooperation only after securing the intellectual property by the academic center? Or maybe the greater risk exists in the lack of business experience of academic centers, and thus in the wrong directions of protection of intellectual property? In the leading academic centers in the world there are numerous proven schemes for the legal security of cooperation between science and business, which can be implemented in Polish academic centers. The examples of numerous business successes of the university, e.g. in the USA, show that safe cooperation with the pharmaceutical industry is possible and brings measurable scientific and financial profits.

S. 11-2
Aspects to consider before launching and early on in a drug discovery program – pharmaceutical industry perspective

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It is sometimes said that science is made up of bad years and good moments. This phrase is particularly true for the drug discovery programs where odds of success are statistically very low. Yet there are tools and aspects that when taken into account, utilized or addressed before or early-on in a program may improve chances and mitigate inherent risks. Moreover, such an approach should also facilitate a future dialogue with pharma partners or venture capitalists as it comes to attracting an investment by a given drug discovery project.

The presentation will be given from a non-scientist perspective. It will briefly summarize author’s insights and suggestions that stem from serving different roles at Adamed. They all have been closely associated with an internal drug discovery pipeline and business development efforts around early-stage assets as well as taking part in an evaluation of several external projects. The presenter also reviewed projects in the capacity of the NCRD expert.

Quite to a surprise, what appears to be obvious in theory reflected in handbooks on drug discovery and dedicated courses, is not universally practiced in projects that one may encounter in real life. Besides, there are also commercial and intellectual property rights aspects that require thorough consideration to make a given drug discovery project a viable effort.

Selected issues inspired by the actual examples of the drug discovery programs will be addressed. On a science side, spanning from a biological activity at relevant concentrations, risk of false positives related to PAINS, selection of controls and substance quality through importance of the mechanism of action elucidation, compound selectivity and drug exposure.
As to a business part, concept of the Target Product Profile will be introduced and its implication for an early stage program will be briefly discussed. A snapshot of a competitive landscape in a drug discovery space will be provided as well. Importance of freedom-to-operate analysis will be underlined and a need of detailed research documenting explained.

S. 11-3
From basic research to medicinal product approval – a brief history of a novel formulation development

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Opioids still belong to the most potent analgesics and they are used in the treatment of moderate to severe pain. Despite the high effectiveness, the problem in opioid therapy is their limited efficacy in some types of pain, the development of tolerance and side effects. In clinical practice, to reduce opioid doses, and thereby to avoid the risk of side effects, attempts have been made to introduce co-analgesics, which enhance the analgesic activity of opioid e.g. NMDA receptor antagonists. Magnesium (Mg) ions belong to the physiological antagonists of these receptors.

We have demonstrated that magnesium salts (in micronized and crystalline forms) increased analgesia after opioids, while this effect was much more marked for micronized form (results protected by patents). In preclinical studies of effectiveness and safety the selected micronized Mg salt, i.e. Mg lactate administered via gastric tube (i.g.) increased analgesia and delayed a development of tolerance after administration (i.g.) of the selected opioid, i.e. tramadol in different experimental models of pain in rats. Moreover, Mg lactate did not modify a respiratory depression after tramadol intake (a serious opioid side effect) and did not change pharmacokinetics of tramadol and its metabolite O-desmethyltramadol.

Our next aim was to check whether similar effect would be observed in humans. In clinical studies a new formulation (tramadol+micronized Mg lactate) did not change the pharmacokinetics of tramadol and its metabolite in healthy volunteers. Moreover, the new combination alleviated chronic pain to a greater extent compared to the reference product (tramadol). In the study arm, in which subjects received our composition, constipation events occurred statistically significantly less frequently than in control arm (with referent tramadol).

Benefits of administration of a new complex analgesic preparation
- Conducting more effective analgesic therapy because Mg ions potentiate opioid analgesia.
- Reducing the daily dose of opioid and consequently reducing the risk of side effects, e.g. constipation.
- Delay of a development of tolerance on analgesic activity of opioid.
- The emergence of oral tablets expands the availability of treatment (e.g. not only in inpatient conditions but also in outpatient care).
- Improving patient comfort (one pill instead of two).

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Discovery of novel therapeutic antibodies using Bioceros CASH™ Platform

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Background: During the last decades therapeutic antibodies (Mabs) have conquered their clinical position as illustrated by the fact that 6 therapeutic Mabs are listed in the top 10 selling drugs in 2017. Due to the high investments during the development of such Mabs, patent protection is crucial. The new guidance for patenting Mabs limits broad claims on their antigens and means that patentees could now be less likely to obtain broad claims to Mabs without disclosing any structural limitations. The strategic approach we have followed to generate, select and protect one of our checkpoint modulator Mabs against CD134 (OX40) will be used as an example to validate the CASH™ platform for the discovery and protection of new innovative therapeutic Mabs.

Material and methods: To accommodate the discovery of patentable novel Mabs, we developed our proprietary CASH™ platform in which we use mouse immunization combined with fit-for-purpose designed bio-assays. Flow-cytometric binding studies were performed using a high-expressing CD134 cell and functional bio-assays were performed using either primary PBMC or isolated human CD4+ effector and regulatory T cells (Teffs and Tregs, respectively). Deletion mutants of membrane human OX40 were expressed on cell lines to locate the antibody binding domains.

Results: After immunization and generation of hybridomas, IgG positive supernatants were tested for binding to CD134 using ELISA and flow cytometry. More hybridomas were positive on ELISA compared to binding to CD134 expressed on cells. Two lead Mabs were selected which were tested for their binding and their potency on Teffs and Tregs. It was observed that these Mabs activated Teff proliferation and inhibited Treg suppressive capacity and were therefore extremely suited for immune-oncology (IO) applications or boosting vaccination. Both Mabs bind to distinct parts on CD134, which were different from the CD134L binding domain.

Conclusion: The CASH™ technology enabled selection of Mabs with unique biological activity, structural binding properties coupled to unique sequences. The custom bio-assays leverage our expression experience with extensive antibody knowledge resulting in novel patentable Mabs. Our current innovative programs focus on novel checkpoint inhibitor Mabs for IO and novel Mabs that can prevent tissue damage caused by autoimmunity.
p53 responds to cellular stress by regulating the transcription of numerous genes that determine cells fate. In stress conditions p53 can trigger cell cycle arrest and DNA repair processes or cell death programs like apoptosis or senescence. The specific outcome depends on the type and intensity of stress signals. In human cells p53 activity is strictly controlled by its negative regulator the protein named Mdm2. Mdm2 forms a tight complex with the p53 trans-activation domain, blocking its ability to regulate target genes and to exert antiproliferative effects. Being a key player in the cellular response to stress, p53 serves as the major obstruction for tumorigenesis. Patients with Li-Fraumeni syndrome which inherit mutated p53 are very susceptible to cancer. Mice with damaged p53 gene appear normal but are prone to the spontaneous development of a variety of neoplasms by 6 months of age. This prominent tumor suppressive role of p53 causes that its function is disabled in virtually all human cancers, either through mutation of the p53 gene or through aberrant expression of proteins acting as its negative regulators such as Mdm2.

Functional genetic studies on mice have shown that restoration of inactivated p53 is sufficient to cause rapid regression of several tumor types. Following this line, targeting the p53-Mdm2 interaction by small molecules to release and reactivate p53 has emerged as promising therapeutic strategy to treat human cancers that are p53 wild-type.

Herein, I will present an approach that resulted in novel class of p53-Mdm2 inhibitors named AD-O21 that selectively induce cell death in p53wt cancers. Extensive medicinal chemistry optimization supported with molecular modelling allowed us to design large group of compounds binding to Mdm2 pocket with nanomolar potency. Well-understood SAR inferred from functional analysis of over 500 AD-O21 derivatives enabled subsequent optimization of metabolic and pharmacokinetic properties. Features that are the most challenging for small molecular protein-protein interface inhibitors. This extensive development resulted in compound named AD-O21.32 that combines very high in vitro activity with favorable PK profile which translate to unprecedented in vivo efficacy.
Many scientists and inventors are starting their research projects, but when it comes to the commercialization phase they fail quite often. On the one side it is absolutely normal (failure) but on the other sometimes there may be a way to shorten and smoothen the predefined way to the market.

A right chosen acceleration program may be the great support. When should you consider to take part in the acceleration program? How should you choose one? What can you expect and what will you never get?

We will try to find answers to those questions together.

CEO at Idea2Business Leader and Co-founder of the Warsaw Technology Accelerator WAW.ac.

He focuses on supporting innovation in science (i.e. datascience, lifescience, energy, engineering and IT) by cooperating with scientists interested in changing their ideas into businesses.

Mentor in the International Startup Contests i.e. Challenge Up, Intel Business Challenge, Intel Global Challenge and acceleration program WARP by Hub:Raum.

Gives lectures, provides trainings and acts as a consultant on business models, leadership and e-business strategies. Alumni of Leadership Academy for Poland, Warsaw University Management Department graduate, Assistant Professor in Strategy Department at the Kozminski University and member of the Top500 Innovators program at the Stanford University.
CaMKII phosphorylation regulates the BDNF expression evoked by fluoxetine in hippocampus of mice

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Background: The level of brain derived neurotrophic factor (BDNF) is known to increase after antidepressant treatment particularly in the hippocampus. This effect may be in part correlated with activation of cAMP-related binding protein (CREB) as Bdnf-gene promoter site contains binding sequence specific for this transcription factor, and chronic antidepressant administration affects also CREB phosphorylation or activity. Moreover, BDNF binding to its receptor TrkB may affect the activity of CREB thus creating a positive feedback loop. This effect is mediated by the activity of intracellular kinases i.e. CaMKII, CaMKIV or ERK1/2. Our previous studies carried out in transgenic mice with CREB deletion restricted only to serotonergic neurons (Creb1TPH2CreERT2 mice) revealed that CREB resident in serotonergic cells directly regulates the BDNF expression in hippocampus after fluoxetine treatment. To investigate this further, we assessed the level and the phosphorylation of kinases important for BDNF-CREB positive feedback loop.

Materials and methods: Mice were given fluoxetine (10mg/kg ip., 1x daily for 21 days), and 24h after last injection animals were decapitated. Using Western Blot method with specific antibodies we assessed the level of phosphorylated and total proteins of CaMKII, CaMKIV and ERK1/2 in the hippocampus and prefrontal cortex of mice.

Results: Fluoxetine in wild type mice had no effect on the phosphorylation or total protein level of neither kinases studied. What is more, there was no effect of genotype or drug on ERK1/2 or CaMKIV parameters in CREB-deficient mice, however these animals exhibited significantly reduced phosphorylation of CaMKII in the hippocampus and prefrontal cortex (fluoxetine had no influence on this effect). Nevertheless, fluoxetine increased the ratio of pCaMKII/CaMKII in the hippocampus of wild type mice, while CREB-deficient animals showed opposing tendency.

Conclusions: The results of our studies indicate that CaMKII phosphorylation may play an important role in the mechanism of BDNF upregulation observed in the hippocampus after fluoxetine treatment as no changes in other kinases studied were found. Moreover, diminished phosphorylation of CaMKII caused by CREB ablation in serotonergic neurons could be one of the factors responsible for attenuation of fluoxetine effects on BDNF expression in Creb1TPH2CreERT2 mice. Nevertheless, this effect needs further studies.

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Levodopa-induced gene expression in the prefrontal cortex of rats with lesions of dopamine neurons

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Background: Parkinson’s disease (PD) is associated with a progressive impairment of motor control and also non-motor symptoms, including cognitive deficits and affective disorders. The effects of PD on cognitive functions are attributed to dopamine depletion in the prefrontal cortex (PFC), though the exact mechanisms involved remain only partly understood.

Materials and methods: Male Wistar Han rats weighing approx. 300 g received 6-hydroxydopamine (6-OHDA, 8 µg/4 µl) or vehicle, infused through cannulas inserted into the left medial prefrontal bundle. Two weeks after the surgery the rats were tested for apomorphine-induced rotations and only the animals with more than 98 contraversive rotations were selected for further procedures. Then, rats were treated i.p. with levodopa (12.5 or 25 mg/kg) supplemented with benserazide (6.25 mg/kg) once daily for 14 days. An hour after the last dose was administered, animals were decapitated, brains were extracted, and the PFCs from both hemispheres were isolated. Analysis of gene expression (Arc, Atf3, Drd1, Drd2, Egr2, Fos, Gfap, Hprt1, Htr1b, and Sgk1) was performed by quantitative PCR based on total RNA derived from each tissue sample.

Results: We find that unilateral 6-OHDA lesions decreased the abundance of most of the transcripts measured, including: Arc, Drd1, Drd2, Egr2, Fos, Gfap, and Sgk1, both in ipsi- and contralateral parts of the PFC. In all cases treatment with levodopa reversed this effect in both hemispheres. The higher dose of levodopa tested (25 mg/kg) increased the abundance of Arc, Egr2, Fos, Htr1b and Sgk1 transcripts above control levels. Differences in transcript levels after levodopa treatment between the lesioned and non-lesioned sides were observed in case of Atf3 and at the lower dose also the activity-regulated transcripts.

Conclusions: Unilateral lesions of dopaminergic neurons affected abundance of transcripts of the selected activity-regulated genes and receptors in both sides of the PFC. Levodopa increased the abundance of the majority of the transcripts assayed in a dose-dependent manner.

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Repeated mephedrone and amphetamine exposure in adolescence rats affects spatial memory in adults

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Background: Amphetamine and its analog - mephedrone (4-methylmethcathinone) are the most common recreational psychostimulants abused by young people. Herein, the use of psychoactive substances can negatively influence the development of the brain, leading to changes in the hippocampus, prefrontal cortex and striatum. The aim of this study was to investigate the consequences of repeated exposure to mephedrone and amphetamine in adolescence on spatial learning and memory in adult rats.

Material and Methods: The experiment were carried out on male Wistar rats. Mephedrone (30 mg/kg, ip) and amphetamine (2.5 mg/kg, ip) were administrated once a day for 10 consecutive days, between the 40th and 50th postnatal days (PND). Next, in adulthood, the influence of such repetitive drug administrations on the acquisition of spatial learning and memory, memory retrieval and reversal learning in the Barnes maze (PND 75-97) task was explored.

Results: At PND 75-97, during the acquisition trial, the animals receiving mephedrone and amphetamine in adolescence made more errors and did not differ on primary latency to find the escape box. Furthermore, during the memory retrieval (13 days after the acquisition) and reversal learning, animals in both drug treatment groups made more errors and spent longer times in finding the escape box.

Conclusions: Our results demonstrated that recreational psychostimulants such as mephedrone and amphetamine, when used in adolescence, attenuate learning and memory processes, impair the retention of long-term memory and negatively affect memory flexibility in adults.

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MRI-guided total fat mass determination in obese rats

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Background: Non-alcoholic fatty liver disease (NAFLD) is difficult to diagnose as its symptoms tend to be non-specific. In addition, the diagnosis mainly depends on liver biopsy and serum aminotransferase assays or imaging modalities with relatively low specificity in the liver such as ultrasound and computed tomography. An application of more specific imaging techniques such as magnetic resonance imaging (MRI) with the implementation of diffusion weighted imaging (DWI) and water-fat separated MRI based on Dixon protocols, have a potential for the non-invasively analyse of NAFLD and is capable of selectively distinguish the adipose tissues from water fluids. The aim of this study was to setup preclinical MRI-based diagnostics for NAFLD in high-calorie diet-induced rat models.

Material and methods: Spraque Dawley rats were fed under standard calorie diet for 2 weeks following 12 weeks of high-calorie diet. Before the end of each stage of the experiment the animals were fasted for 12 hours with water available ad libitum aimed at subsequent subjection to MRI using a 1.5 T scanner. The analysis of whole body fat and liver steatohepatitis was performed based on T2-weighted images (TSE), DWI and Dixon techniques due to in-phase and out-of-phase protocols. A total whole body fat volumetry was performed using Dixon images based on the auto-segmentation of proton signals in adipose tissues.

Results: The application of different modes of MRI enabled to determine the profile of fat distribution in the whole-body organs and liver parenchyma (p=0.0115 for SI INDEX and p=0.00002 for SI from Dixon(fat)) resulting from the high-calorie diet in obese rats. The MRI scans indicated on prominent fat increase, especially for the fraction of the visceral fat. The decrease of cellular water diffusion due to high-calorie diet were noted in the liver (p=0.00003).

Conclusions: The study shows that the application of DWI and Dixon techniques enables to distinguish the proton signals addressing to fat and water fluids enabling determination of fat distribution in the whole body and liver parenchyma due to NAFLD in obese rats.

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The effects of prenatal stress and high-fat diet on oxidative phosphorylation in the brain

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Background: Recently, metabolic brain dysfunction has been postulated as an relevant factor in the development of depression. In addition, major depression often co-occurs with obesity, in which disturbances in energy metabolism are present not only in the periphery but also in the brain. In the present study, we investigated whether in animal model of the co-occurrence depression and obesity there are quantitative and functional changes in respiratory chain complexes and ATP production.

Materials and methods: Pregnant Sprague-Dawley rats were subjected to stress sessions in the third trimester of pregnancy. After behavioral verification adult males (offspring of control and stressed dams) were divided into prenatal stress – reactive (PS-R) and prenatal stress non-reactive group (PS-NR). Then, stressed groups and control were divided into dietary groups: standard laboratory chow (STD) and the receiving food with increased unsaturated fat content (HFD, 45% energy derived from fat). After 16 weeks of diet animals were sacrificed under non stress conditions to dissect frontal cortices. Immunoblotting methodology, high resolution respirometry and luminescence assays were applied to investigate the changes in the concentration and activity of respiratory chain complexes and the level of ATP. The results were analyzed using the STATISTICA software. A p-value <0.05 was considered to be significant.

Results: Immunoblotting revealed that prenatal stress enhanced expression of complexes II, IV and V in the frontal cortex mitochondria. Respiration study, conducted in Oxygraph-2k demonstrated intensification by stress all measured steps of oxidative phosphorylation. In mitochondria respiration study, also influence of high-fat diet on OXPHOS capacity, uncoupled and leak state was showed. There was no significant effect of prenatal stress, diet and their interaction on the ATP concentration in the examined brain structure.

Conclusions: In conclusion, it seems that prenatal stress could uncouple oxidative phosphorylation in the frontal cortex and shift ATP production from mitochondrial oxidative phosphorylation to glycolysis, whereas the HFD acted in an opposite manner and weakened this prenatal stress effect. The exact functional consequences of the revealed alterations require further investigation.

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Protein Kinase D2 promotes intestinal fat absorption and contributes to diet induced obesity

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Background: Imbalance between food intake and energy expenditure is central to the development of obesity and related metabolic diseases. In our study, we focus on the diacylglycerol and PKC target: Protein Kinase D2 (Pkd2). This kinase is part of the Pkd family, which has been characterized before because of their role in cell motility and proliferation, angiogenesis, trans-Golgi network trafficking among others. However the impact of specific PKD isoforms on development of metabolic diseases has not been investigated so far.

Materials and methods: To unravel the function of PKD2 in development of obesity we have utilized mice carrying mutation in the activatory loop of this kinase, which results in the inactivation PKD2-dependent signaling in the whole body (PKD2ki/ki mice). This mice and corresponding wild type control animals were maintained on normal (ND) or high fat diet (HFD) and the series of metabolic test were performed on both cohorts (including metabolic cages, glucose and insulin tolerance, lipids in circulation and intestinal lipids absorption). Finally, we have utilized classical biochemical and histological methods to study PKD2 on the development of obesity.

Results: Our results indicate that mice with inactive PKD2 are resistant to HFD induced obesity and present an improved response to insulin and better glucose uptake from circulation. These mice, not only gain less weight, but also present lower levels of lipids in circulation. Histology analysis of the white adipose tissue of mice with inactive form of PKD2 show that the size of the adipocytes is smaller to the controls. However, PKD2ki/ki mice present normal energy expenditure, food intake and voluntary movements. Importantly, mice with the inactive form of PKD2 show decreased absorption of intestinal fat expressed as triglycerides in circulation. As a result of the decreased intestinal fat absorption, PKD2 knock-in mice excrete higher amount of feaces which also show different physical characteristics including a yellow color and lower density of stool.

Conclusions: Our data indicate that PKD2 determinate intestinal lipid absorption, but does not directly affect the metabolism of other organs. Importantly, inactivation of PKD2 prevents lipid absorption but does not affect the uptake of other nutrients, which results in resistance of PKD2ki/ki mice to HFD induced obesity. Therefore, inhibition of PKD2 represents an attractive target for treatment of obesity and related diseases.
S. 12-7

Neuroprotective capacity of DIM against ischemia involves inhibition of AhR, but not ERα signaling pathways

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Background: According to the latest statistics, stroke is the 2nd leading cause of death worldwide. The only pharmacological treatment approved by the Food and Drug Administration (FDA) for acute ischemic stroke is recombinant tissue plasminogen activator (rt-PA). However, rt-PA has a very narrow therapeutic window and long list of contraindications. Therefore, there is an urgent need to find the effective therapy with an extended therapeutic window and minimized side effects to treat the patients undergoing stroke. Our previous study has shown that plant-derived 3,3’-diindolylmethane (DIM) protects neurons undergoing hypoxia via inhibition of aryl hydrocarbon receptor (AhR), but not estrogen receptor ERβ signaling pathway. Despite the evidence of the interaction between AhR and ERβ, AhR is also known to interact with estrogen receptor ERα. However, there are no data on neuroprotective action of DIM in hippocampal cells subjected to ischemia and an involvement of AhR/ERα signaling pathways in DIM action. Therefore, the aim of the present study was to assess neuroprotective capacity of DIM in mouse hippocampal cells exposed to oxygen and glucose deprivation (OGD) with special focus on AhR/ERα signaling pathways.

Material and methods: The experiments were performed on mouse primary hippocampal cell cultures. On 7 day in vitro (DIV) the cells were treated with DIM (0.01-10 µM) and subjected to 6 h of OGD (Neurobasal without glucose, hypoxic modular incubator chamber 95% N₂/5% CO₂). Caspase-3 and lactate dehydrogenase (LDH) activities as well as protein expression levels were measured after 18 h of reoxygenation.

Results: We have shown that 6 hours of OGD increased LDH and caspase-3 activities to 265 and 129% of the normoxic control level, respectively. OGD also increased the protein level of AhR and AhR-related CYP1A1, but it did not change the protein level of ERα and ERα-related CYP19A1. DIM (0.1-10 µM) inhibited the effects of ischemia i.e., it reduced LDH release to levels ranging from 182-197% and caspase-3 activity to 108% of the ischemic control value. DIM also strongly decreased the protein expression levels of AhR and CYP1A1, but it did not affect the levels of ERα and CYP19A1.

Conclusions: These data demonstrated that neuroprotective capacity of DIM in hippocampal cells undergoing ischemia involves inhibition of AhR signaling pathway which may have implication for identifying new DIM-related agents that could protect neurons against ischemia by targeting AhR.

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Role of circularRNAs in microRNA-induced endothelial dysfunction

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Background: Circular RNAs (circRNAs) is a large class of noncoding RNAs which form covalently closed single stranded loops. They were found in nearly all tissue and recently have been reported to regulate gene expression by sponging miRNA. However, their role in specific human conditions is barely known. Thus, the aim of our study was to explain the potential role of circRNA in endothelial dysfunction using in-vitro model.

Materials and methods: Current study was performed using iCell endothelial cells (Cellular Dynamics, Madison, USA) transfected with miR-21, miR-126 and miR-191 as a model of endothelial dysfunction. Total RNA was isolated from both miRNA transfected and non-transfected cells using TRIzol reagent and adsorption minicolumns. Transcriptome analysis was performed using total RNA-sequencing (Illumina, HiSeq 2500) with ribosome depleted RNA libraries. Obtained raw .bcl files were converted to .fastq, and then RNA reads were mapped to the reference genome (STAR pipeline). Salmon and deseq2 R package were used to screen for the differentially expressed genes between transfected and control endothelial cells. CircRNA were extracted by filtering splice-junctions with find_circ pipeline followed by characterization using DCC package with 1000 reads as threshold. Possible associations between differential expression of protein-coding genes induced by three selected miRNA and circRNAs were analyzed using Spearman’s rank-order correlation.

Results: Total RNA sequencing revealed transcripts for 14957 circRNAs in endothelial cells and there were no significant correlations between circRNAs and its host genes. 1358 circRNAs were significantly correlated with at least one protein coding gene (p = 10−6, R > 0.8). 12 selected circRNAs (hsa_circ_0012223, hsa_circ_0034515, hsa_circ_0076992, hsa_circ_0003382, hsa_circ_0008576, hsa_circ_0003581, hsa_circ_0009129, hsa_circ_0078905, hsa_circ_0026132, hsa_circ_0035983, hsa_circ_0055538, hsa_circ_0064574) potentially explain 7.3% variability in endothelial transcriptome.

Conclusions: CircRNAs are a class of RNAs which probably act as a trap for microRNAs and inhibit their functions. Altered expression of microRNAs was found in various human diseases. Our results showed that single microRNAs profoundly change transcriptome of endothelial cells. This is accompanied by a prolific and coordinated production of circRNAs. Synthetic circRNA could be promising method of treatment targeting disease-associated microRNAs.

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Response of glial cells to temporal deprivation of oxygen and glucose in model perinatal asphyxia

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Background: Leukodystrophic disorders developing as fatal consequences of the experienced hypoxic-ischemic (HI) episode by neonates is thought to be associated with the neuroinflammatory process and alterations in oligodendrocyte differentiation. Temporal deprivation of oxygen and trophic support seems to be the major cause of either the cell death or the arrested maturation of the myelin-forming cells. Mutual interaction between oligodendrocytes (myelin generating cells) and microglia (associated with immunological response) are hypothesized to play a major role in initiating the endogenous neuroreparative processes aimed at overcoming the nervous tissue crisis.

Materials and methods: To verify this hypothesis, the co-culture experiments were established for the purpose of applying in vitro model of oxygen-glucose deprivation (OGD). Accordingly, homogenous fractions were separated from the primary cultures of glia isolated from the brains of neonatal Wistar rats. Subsequently, the cells (either oligodendrocytes or microglia) were subjected to OGD procedure, which mimics in vitro the HI episode typical for the perinatal asphyxia. In order to examine proliferation and polarization of microglial cells in the nervous tissue ex vivo, the hippocampal organotypic slices were prepared from 7-days old rats. The applied schedule made it possible to evaluate interactions between cells, which contribute either to initiating neurorestorative processes or to triggering mechanisms leading to development of leukodystrophic diseases. The cell phenotype was determined by specific antibodies like ED1, Iba1 and anti-arginase for microglia and anti-NG2, anti-O4, anti-GalC and anti-MBP for oligodendroglia. The migratory potential of the examined cells was assessed by means of live recording, using Cell Observer SD (Zeiss).

Results: The obtained results indicated that even a short deprivation of oxygen and trophic support affects microglial polarization, as indicated by a changed proportion of phenotype-specific cell markers. This effect is also exerted in a paracrine manner by the OGD-subjected oligodendrocytes.

Conclusion: To summarize, determined in vitro interaction between neural cells might indicate the future targets for pre-clinical studies aimed at pharmacological modulation of processes triggered by hypoxic-ischemic episodes.

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Hepatic Pkd3 provides negative feedback on Chol. and TAG synthesis by suppressing insulin signaling

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Background: Hepatic diacylglycerol (DAG), an intermediate product of triglyceride synthesis, is markedly elevated in livers of obese and insulin-resistant patients as well as in multiple mouse models of obesity and diabetes. In liver, accumulation of DAG activates protein kinase C isoforms to promote insulin resistance. DAG can bind and activate another closely related group of kinases, namely protein kinase D (PKD) family members.

Material and methods: Adenoviral overexpression and silencing was used to study PKD3 signaling in vitro. Conditional mice lacking PKD3 in liver were subjected to high fat diet feeding in order to analyze the impact of PKD3 deletion on metabolic and physiological parameters.

Results: We showed that PKD3 is the only member of PKD family expressed in hepatocytes. Moreover, PKD3 is activated in hepatocytes loaded with DAG as well as in livers from obese and diabetic subjects. We showed that deletion of PKD3 specifically in hepatocytes results in resistance to obesity-induced insulin insensitivity and glucose intolerance in mice. However, at the same time, lack of PKD3 promotes accumulation of triglycerides and cholesterol in liver. Biochemical analysis revealed that PKD3 suppresses activity of downstream insulin effectors including AKT and mechanistic target of rapamycin complex 1 and 2 (mTORC1 and mTORC2).

Conclusions: Taken together, our results indicate that PKD3 provides the feedback on the hepatic lipid production and suppresses insulin signaling. Therefore, manipulation of PKD3 activity might be a valid strategy to improve hepatic lipid content or insulin sensitivity.

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The effect of prenatal immune challenge in rats on the maternal-care behavior and on monoamine neurotransmission in offsprings

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Background: It was previously reported that prenatal infection increases the risk of various brain disorders in offsprings, such as depression and schizophrenia (Brown, Front Psychiatry 2:63, 2011, Markham and Koenig, Psychopharmacology 214:89, 2011). It was also found that prenatal lipopolysaccharide (LPS) administration induced depression-like behavioral abnormalities in offsprings (Bakos et al Ann N Y Acad 1018:281, 2004). The aim of this project was to investigate the effect of prenatal LPS on the maternal-care behavior of female Wistar rats and on monoamine neurotransmission in their male and female offsprings.

Materials and Methods: LPS was administered to the gestating Wistar rats, during the last trimester of the gestation. During the first week after the delivery, the following characteristic of maternal care behavior were assessed: passive nursing duration, off nest time, licking nursing time, licking time, blanket nursing time, nest building time, and arched back nursing time. When the offsprings reached the age of two months, they were anesthetized and mounted in stereotaxic frame. Glass electrodes were inserted into the dorsal raphe nucleus (DRN), locus coeruleus (LC), and ventral tegmental area (VTA) for in vivo assessment of the excitability of serotonin (5-HT), norepinephrine, and dopamine neurons, respectively.

Results: No significant differences in the maternal care behavior between the dams administered vehicles and dams administered LPS were observed. Prenatal immune challenge with LPS resulted in a significant inhibition of the spontaneous firing activity of 5-HT neurons in both male and female offsprings. Prenatal LPS did not alter the spontaneous firing activity of NE neurons in male or in female offsprings. However, it resulted in a significant increase in the spontaneous firing activity of DA neurons in male, but not in female offsprings.

Conclusion: Since 5-HT and dopamine systems are involved in pathophysiology depression and schizophrenia, it is possible that prenatal infection triggers these disorders in offsprings via suppression of hippocampal and/or 5-HT neurons and/or activation of DA transmission.
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P. 1-02
Repeated exposure to the predator scent stress has an impact on the excitability of brain serotonin neurons in rats

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Background: Rodents’ exposure to the predator scent stress (PSS) has been used as an animal model of post-traumatic stress disorder (PTSD). The aim of this study was to investigate the effect of chronic exposure to the PSS on the firing activity of serotonin (5-HT)-secreting neurons and plasma corticosterone concentrations in rats.

Methods: Adult Male Sprague-Dawley rats (250-300 g) were exposed to the PSS (sand containing cat urine) for ten minutes daily on ten consecutive days. Control rats were exposed to the sand containing clean water. After the last exposure, the rats were left intact for fourteen days. On the fifteenth day after the last exposure, part of the animals was euthanized, their blood was collected, and serum/plasma? corticosterone concentrations were analyzed using spectrofluorimetry. Another part of the animals was anesthetized with chloral hydrate (0.4 g/kg, i.p.) and glass electrodes filled with 2M NaCl were inserted into their dorsal raphe nuclei (DRN). The excitability of 5-HT neurons was assessed using single-unit extracellular in vivo electrophysiology.

Results: The excitability of 5-HT neurons (2.74±0.52 Hz) in the DRN of PSS rats was significantly higher (p<0.01, two-tailed Student’s t-test) compared to that in the control group (1.34±0.15 Hz). A significant (p<0.05, two-tailed Student’s t-test) decrease in plasma corticosterone (126.84±34.25nM) was observed in PSS rats in comparison with the values in controls (333.28±43.69 nM).

Conclusions: Observed increase in the basal firing activity of 5-HT neurons is likely to be an adaptive mechanism designated to diminish anxiety and depression-like symptoms usually induced by chronic stressors. A contributing mechanism may be the PSS-induced reduction in corticosterone secretion, since corticosterone was found to tonically inhibit the excitatory glutamatergic input to 5-HT neurons of the DRN (Wang et al J Physiol 15:5795, 2012). Further studies need to be performed to test this hypothesis.

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PZKKN-94 – a novel and potent dually acting 5-HT₆R antagonist/5-HT₁⁹R agonist with pro-cognitive, antidepressant and antiparkinsonian properties in animal models


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Background: A number of preclinical and clinical studies indicate the therapeutic potential of 5-HT₆ receptor modulators in the treatment of cognitive disorders associated with Alzheimer's disease and behavioral and psychological symptoms of dementia. Several studies revealed that 5-HT₁⁹R agonist may play a role in modulating the mood, extrapyramidal motor functions and motor symptoms related to Parkinson’s disease (PD) dyskinesia. In the present study we assessed drug-like properties and pharmacological properties of a novel dually acting 5-HT₆R antagonist/5-HT₁⁹R agonist – PZKKN-94.

Materials and methods: In vitro experiments revealed that PZKKN-94 behaved as very potent 5-HT₆R antagonist (Kᵢ = 2.2 nM, Kᵦ = 1 nM) and 5-HT₁⁹R agonist (EC₅₀ = 30 nM). The compound is characterized by satisfactory in vitro metabolic stability (Clᵢᵣᵢᵦ = 25 l/min/mg) and good oral bioavailability in rats with C(max) of 116 ng/ml and ca. 3 folds preference for CNS at C(max). Behavioral studies showed ability of PZKKN-94 (0.3 or 0.1 mg/kg), also in combination with donepezil, to reverse the cognitive impairment in Novel Object Recognition (NOR) test caused by scopolamine or phencyclidine. Furthermore, PZKKN-94 (0.3 mg/kg) displayed pro-cognitive effect in aged animals (20 months) and no tolerance was developed after 14-day BID administration. PZKKN-94 (1 mg/kg) was able to improve attention in Attentional Set Shifting test (ASST), and showed antidepressant activity at a dose of 0.1 mg/kg in Force Swim Test (FST). Finally, PZKKN-94 decreased the haloperidol-induced catalepsy (at a dose of 3 mg/kg) in rat model of PD.

Conclusion: A new approach, based on dually acting 5-HT₆R antagonist/5-HT₁⁹R agonist – PZKKN-94, for the treatment of CNS disorders was proposed. Evaluation of PZKKN-94 properties revealed its high activity potential in in vitro and in vivo studies and pro-cognitive, antidepressant, and antiparkinsonian properties. Preferential drug-likeness profile and promising behavioral results justify further mechanistic studies of complementary modes of action of PZKKN-94 and its development as a potential therapy in CNS disorders.

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A Genetically Encoded Fluorescent Sensor Capable of Detecting Intracellular Hydrogen Peroxide in Human Lung Cancer Cells

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Background: Nowadays different nanomaterials are used in the field of personalized nanomedicine to trigger oxidative stress in targeted cancer cells. Hydrogen peroxide (H₂O₂) is an important mediator in cell signalling and cell death. Traditional fluorescent probes used for detection of H₂O₂ are not specific and have a tendency to react with a wide variety of reactive oxygen and nitrogen species. The aim of this study was to use genetically encoded pHyPer vector capable of detecting intracellular hydrogen peroxide in adenocarcinomic human alveolar basal epithelial cells.

Material and methods: pHyPer plasmid cloning vector containing immediate early promoter of cytomegalovirus (PCMV IE) for protein expression, SV40 origin for replication in mammalian cells expressing SV40 T-antigen, pUC origin of replication for propagation in E. coli, and f1 origin for single-stranded DNA production was used to encode a fluorescent sensor HyPer in A549 lung cancer cells. To increase mRNA translation efficiency, Kozak consensus translation initiation site was generated upstream of the pHyPer coding sequence. The pHyPer-vector was transfected into A549 lung cancer cells using lipofectamine protocols. Two days after lipofection the A549 cells were exposed to graphene-encapsulated magnetic nanoparticles (GEMNS).

Results: HyPer demonstrated submicromolar affinity to hydrogen peroxide in A549 cells. GEMNS enhanced vector expression and increased the fluorescence ratio of the cytosolic HyPer. The localization of the HyPer protein was confirmed by confocal microscopy and flow cytometry in transfected A549 cells.

Conclusions: The pHyPer vector is a precious molecular sensor for detection of hydrogen peroxide in adenocarcinomic human alveolar basal epithelial cells.

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Effects of LSD on dopamine, serotonin and glutamate release in the rat brain

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Background: Lysergic acid diethylamide (LSD) is a semisynthetic, indolamine hallucinogen stimulating central serotonin receptors: 5-HT_{1A/1B/1D}, 5-HT_{2A/2C/5A}, 5-HT_{6}, 5-HT_{7}, dopamine D_{1}, D_{2} and adrenergic α1 and α2 receptors. The consumption of LSD in Europe is small but stable in the long run. LSD has strong psychoactive properties but does not induce addiction, serious toxicity and fatalities. With multiple applications tolerance develops very quickly. The aim of this study was to find out the effect of LSD on dopamine (DA), serotonin (5-HT) and glutamate extracellular levels in the rat frontal cortex and striatum. The ability of LSD to evoke wet dog shakes (WDS) as indicator of 5-HT_{2A} receptor activation in rats was also examined.

Materials and methods: Rats were administered with a single doses of LSD (0.1 mg/kg ip). The striatal and cortical release of DA, 5-HT and glutamate was measured using microdialysis in freely moving animals and HPLC. Wet dog shake (WDS) movements as indication of hallucinogenic activity was examined.

Results: LSD administration evoked a robust increase of extracellular glutamate level in the frontal cortex but not in the striatum. It also increased DA extracellular level, while 5-HT release was decreased in both studied brain regions. It is suggested that increase in cortical extracellular glutamate level may be mediated by activation of serotonin 5-HT_{2A} receptors located on cortical pyramidal cells. Alternatively, this effect may be also caused by stimulation of inhibitory 5-HT_{1A} receptors expressed in GABAergic interneurons. The LSD effect on DA release may be exerted via dopamine D_{2} or D_{1} receptors. Decrease in 5-HT release from 5-HT terminals in cortex and striatum seems to occur by activation of 5-HT_{1A} receptors in the nucleus raphe.

Conclusions: LSD by having a high affinity for several serotonin and dopamine receptors displays mixed effect on brain neurotransmission. Induction of WDS seems to depend on negative interaction between 5-HT_{1A} and 5-HT_{2A} receptors.

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The study of interaction between temozolomide and lamotrigine on the level of blood-brain barrier

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**Background:** Treatment of neurodegenerative diseases, including brain tumors and correlated with them seizures, is very complicated. Anti-cancer drugs need to penetrate blood-brain barrier (which is located between circulatory system and brain tissue) and reach therapeutic concentration in the brain. This barrier is a specific cell membrane system, built of various types of cells. Its main physiological function is to inhibit the penetration of toxic endogenous substances from circulation to the brain.

**Materials and methods:** In this study we utilized in vitro blood-brain barrier (BBB) model consisting of human derived endothelial cell hCMEC/D3 and normal human astrocytes-nHA which were growing in co-culture system. Before each experiment we measure the trans-endothelial electrochemical resistance (TEER) to confirm integration of the cell model. The main purpose of the research was to measure the penetration speed through the BBB of temozolomide (TMZ) in combination with the antiepileptic drug - lamotrigine (LTG). Although temozolomide is an effective drug used in the therapy of glioblastoma, its therapeutic concentration in the brain tissue is sometimes non-adequate. In combination with LTG, temozolomide can increase the penetration through BBB and at the same time reduces the frequency of seizure episodes during anti-cancer pharmacotherapy.

**Results:** The studies have shown that TMZ is relatively well distributed across the blood-brain barrier. The transport of temozolomide through BBB occurs fastest at a concentration of 50 micro moles (permeability coefficient value - 2.26x10^-6 cm/sec). The penetration of the chemotherapeutic TMZ in combination with the antiepileptic LTG, was the fastest (5.12 x10^-6 cm/s) for TMZ at concentrations of 150 micro moles, and for LTG at 100 micro moles.

**Conclusions:** The studied interaction between these two drugs gives the greatest hope for the effectiveness of the treatment of convulsions, coexisting with brain tumors, in particular with glioblastoma. Furthermore we detected that the combination of these drugs in a lower micro molar concentration penetrates the blood-brain barrier much slower and is unable to achieve therapeutic effects in the CNS.

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None
CACNA1A partial loss-of-function in larval zebrafish (Danio rerio): a new model of ataxia?

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Background: The CACNA1A gene encodes the α1A subunit of a neuronal voltage-gated Cav2.1 P/Q type calcium channel. The Cav2.1 is critically important in mediating neurotransmission and is highly expressed presynaptically, especially in the cerebellar Purkinje and granule cells and at the neuromuscular junction. The mutations in this gene are associated with episodic ataxia type 2 (EA2) with/without concomitant absence seizures. In zebrafish, there are two splice variants encoding CACNA1A protein.

Materials and methods: In order to induce partial loss-of-function of CACNA1A, an antisense morpholino oligomer (MO) designed to target the ATG (translational start) site of one CACNA1A splice variant was utilized (Gene Tools, USA). The MO (9 ng) was injected into fertilized one- to two-cells stage zebrafish embryos. Automated locomotor activity tracking and touch response assessment was performed at 5 days post fertilization. The MO-injected zebrafish larvae were imaged and electrographic (EEG) analysis of brain activity was performed.

Results: The data revealed that MO-injected larval zebrafish phenotypically are distinguishable from wild-type siblings. However, knockdown larvae displayed decreased locomotor activity as well as absent or delayed touch response. We did not observe any changes between tested groups when conducting EEG analysis.

Conclusions: Although the CACNA1A gene partial loss-of-function in larval zebrafish might mimic some symptoms of ataxia in humans, further validation is needed for it to be considered as a new animal model of this neurological disorder.

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The influence of the NMDA receptor antagonist on the activity of cytochrome P450 in the liver

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Background: Cytochrome P450 (CYP) isoenzymes are members of the superfamily of heme-containing monooxygenases that play an important role in the oxidative metabolism of endogenous and exogenous substrates. Hepatic cytochrome P450 is under hormonal control of growth hormone, glucocorticoids, thyroid, and sex hormones. The main role in the central neuroendocrine regulation of liver CYP is played by the hypothalamus. This structure is heavily innervated by glutamatergic system, where signals are transmitted through different glutamate receptors. However, a detailed engagement of individual glutamate receptors in hormonal regulation has not been fully recognized so far. The aim of the present study was to test the effect of selective GluN2B subunit NMDA receptor antagonist (potential drug) on the cytochrome P450 activity in the liver.

Material and method: The studies included five-day administration of a selective GluN2B subunit NMDA receptor antagonist – CP 101606 (20 mg/kg, i.p.), followed by determination of cytochrome P450 activity in the liver microsomes using CYP isoform specific reactions (high-performance liquid chromatography).

Results: The repeated, intraperitoneal injection of CP 101606 significantly decreased the activity of CYP2A, 2B, 2C11 and CYP3A measured by the testosterone hydroxylation in specific positions. The activity of the CYP1A (caffeine 3-N-demethylation) and CYP2D (bufuralol hydroxylation) was also significantly lower compared to the control group. Whereas the activity of CYP2C6 (warfarin hydroxylation) was not changed.

Conclusions: The selective GluN2B subunit NMDA receptor antagonist CP 101606 produced significant decreases in the activities of hormone-dependent cytochrome P450 isoforms and of CYP2D in the liver. Further molecular and hormonal studies are in progress to explain the mechanism of the observed changes in cytochrome P450 activity. The obtained results should help to predict the effect of future drugs acting via NMDA receptor on cytochrome P450 function.

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The influence of asenapine on cytochrome P450 expression and activity in human hepatocytes

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Background: Asenapine is a novel atypical antipsychotic drug approved for the treatment of schizophrenia and bipolar disorders. It has a unique human receptor-binding profile characterized by high affinity for serotonergic, dopaminergic, α-adrenergic and histaminergic receptor subtypes. Inhibition or induction of cytochrome P450 (CYP) isoenzymes are the most common causes of undesired drug–drug interactions. The aim of the present study was to estimate the effect of asenapine on the main CYP isoenzymes in human liver.

Methods: Experiments were performed in vitro using inducible-qualified human cryopreserved hepatocytes from three different donors. Asenapine was added to the culture medium at therapeutic concentrations of 0.01, 0.025 and 0.1 μM. CYP isoenzyme activities were determined in the culture medium using CYP isoform-specific reactions: caffeine 3-N-demethylation (CYP1A1/2), diclofenac 4’-hydroxylation (CYP2C9), perazine N-demethylation (CYP2C19) and testosterone 6β-hydroxylation (CYP3A4). The concentrations of CYP-specific substrates and their metabolites formed in the culture medium were measured by HPLC with UV detection. The level of CYP isoform proteins in hepatocytes was measured using human CYP isoform-specific ELISA kits. The expression of CYP genes (mRNA levels) was determined by qRT-PCR.

Results: The obtained results showed that at the tested concentrations asenapine significantly decreased the activity of CYP1A1/2 (to 67-78% of the control value) and mRNA level of CYP1A2 (to 57-72% of the control value) in the cultures of human hepatocytes. However, the neuroleptic exerted no statistically significant effect on the protein level of CYP1A1/2 and mRNA level of CYP1A1. Moreover, asenapine did not produce any significant changes in the activity and expression (mRNA and protein levels) of CYP2C9, CYP2C19 and CYP3A4.

Conclusion: By inhibiting the activity of CYP1A2 asenapine may diminish the metabolism of CYP1A2 substrates (e.g. caffeine, theophylline, phenacetin, tricyclic antidepressants, propranolol, fluvoxamine, clozapine) and lead to an increase in their concentrations when co-administered to patients.

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Venlafaxine and Escitalopram: effects on rat hepatic CYP isoforms in the chronic mild stress

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Background: Depression is associated with changes in brain neuronal transmission and in the function of endocrine system, which may lead to molecular alterations in the brain and periphery. Venlafaxine (VEN) and escitalopram (ESC) belong to most often used antidepressants. Venlafaxine is a serotonin-noradrenaline reuptake inhibitor. Escitalopram is a very selective serotonin reuptake inhibitor. The regulation of CYP expression is under control of the nervous system. The aim of the present study was to characterize the effect of ESC and VEN on rat hepatic CYP isoforms in the chronic mild stress (CMS), a paradigm of depressive-like behaviour in rodents.

Material and methods: Male Wistar Han rats were subjected to the CMS procedure and/or chronic treatment with VEN (10 mg/kg, i.p.) or ESC (10 mg/kg, i.p.) for 5 weeks. The activities, protein levels and gene expression of CYP isoforms were estimated in the liver by HPLC, Western immunoblot and Real-Time analyses, respectively. Serum hormone levels were measured using ELISA kits.

Results: 1. The antidepressant VEN: a) positively regulated the CYP1A2 mRNA expression and increased the CYP2C6 activity in non-stressed rats; b) positively regulated the CYP1A1/2 protein level, but decreased the CYP1A2 activity and CYP2C6 protein level under stress conditions (CMS) by the modulation of the hypothalamic-pituitary-gonadal (HPG) axis and/or hypothalamic-pituitary-adrenal (HPA) axis.
2. The antidepressant ESC: a) enhanced the CYP2C11 activity in non-stressed animals; b) the increase of CYP2C11 activity was prevented in stress conditions (CMS) by the modulation of the hypothalamic-pituitary-gonadal (HPG) axis and/or hypothalamic-pituitary-adrenal (HPA) axis.
3. VEN and ESC did not change the activities of CYP2A, CYP2B or CYP3A.
4. Alterations in the noradrenergic and/or serotonergic transmission by VEN or ESC modulated the hypothalamic-pituitary-thyroid (HPT) axis.
5. The level of growth hormone was not changed at 24 h after the last dose of VEN or ESC.

Conclusions: 1. The action profile of VEN suggests a significant in vivo interaction between VEN and substrates for CYP1A2 and CYP2C6.
2. The action profile of ESC suggests a significant in vivo interaction between ESC and substrates for CYP2C11.
3. The modified function of the HPG and HPA axes is involved in the action of antidepressants.

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The influence of the novel atypical neuroleptic iloperidone on the expression and activity of cytochrome P450 isoenzymes in human hepatocytes

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Background: Inhibition or induction of cytochrome P450 (CYP) isoenzymes are the most common causes of undesired drug–drug interactions. Iloperidone is a novel atypical antipsychotic drug approved for the acute treatment of schizophrenia in adults. The aim of the present study was to evaluate the influence of iloperidone on the expression and activity of CYP isoenzymes in a culture of human hepatocytes.

Materials and methods: Experiments were performed in vitro using inducible-qualified human cryopreserved hepatocytes from three different donors. Iloperidone was added to the culture medium at therapeutic concentrations of 0.025, 0.075 and 0.25 μM. Treatments lasted for 72 h each and were renewed every 24 h when the culture medium was changed. CYP isoenzyme activities were determined in the culture medium using CYP isoform-specific reactions: caffeine 3-N-demethylation (CYP1A1/2), diclofenac 4’-hydroxylation (CYP2C9), perazine N-demethylation (CYP2C19) and testosterone 6β-hydroxylation (CYP3A4). The concentrations of CYP-specific substrates and their metabolites formed in the culture medium were measured by HPLC with UV detection. The level of CYP isoenzyme proteins in hepatocytes was measured using human CYP isoform-specific ELISA kits. CYP isoenzyme mRNA levels were determined by qRT-PCR.

Results: The obtained results showed that iloperidone at the tested concentrations potently decreased the activity and mRNA level of CYP3A4, but it did not produce any significant changes in the CYP3A4 protein level. Moreover iloperidone at its highest concentration only significantly increased the activity, protein and mRNA levels of CYP2C9. On the other hand, iloperidone exerted no statistically significant effect on the activities, protein and mRNA levels of CYP1A1/2 and CYP2C19.

Conclusions: The presented findings may have clinical implications for the prediction of potential drug-drug interactions involving iloperidone and CYP3A4 substrates (e.g. antidepressants, benzodiazepines, calcium channel antagonists, macrolide antibiotics, testosterone) or CYP2C9 substrates (e.g. diclofenac, S-warfarin, tolbutamide, fluoxetine, amitriptyline, fluvastatin, losartan).

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Effect of antidepressants and aripiprazole on the levels of monoamines in the rat frontal cortex

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Background: Treatment of schizophrenia is a serious clinical problem. Classical antipsychotic drugs, i.e. antagonists of dopamine D2 receptors alleviate positive symptoms but they do not affect the negative symptoms and the impaired cognitive functions. Although the atypical antipsychotics have some efficacy in alleviating social dysfunction in schizophrenic patients, this effect is small and mechanisms of this action are still unknown. Moreover, preclinical and clinical studies have suggested that the antidepressant-induced augmentation of the activity of atypical antipsychotics may efficiently improve the treatment of negative and some cognitive symptoms of schizophrenia. Thus, in the present study, we aimed to evaluate the effect of the antidepressant escitalopram (ESC) or mirtazapine (MIR) and atypical antipsychotic drug aripiprazole (ARI), given separately or jointly, on the extracellular levels of monoamines in the rat frontal cortex.

Material and methods: The experiments were conducted on male Sprague-Dawley rats (270-300 g). The animals were given a single intraperitoneal injection of ESC (5 and 10 mg/kg) or MIR (10 and 20 mg/kg) and ARI (0.3, 1 and 3 mg/kg). The release of monoamines and their metabolites in the rat frontal cortex was investigated using a microdialysis in freely moving rats, and monoamines were assayed by HPLC with coulochemical detection.

Results: The present results showed that ARI (0.3 mg/kg) increased the cortical extracellular levels of dopamine (DA) only (but not serotonin (5-HT) and noradrenaline (NA). Co-treatment with ARI (0.3 mg/kg) and an ineffective dose of ESC (5 mg/kg) significantly increased the level of 5-HT and decreased the level of 5-HIAA with no change in the level of DA, however, it decreased the levels of HVA and DOPAC and did not affect the level of NA. Moreover, co-treatment with ARI (0.3 mg/kg) and an ineffective dose of MIR (10 mg/kg) significantly increased the level of NA but did not change the level of DA and 5-HT while elevating the levels of their metabolites.

Conclusions: The obtained results suggested that the increase in cortical extracellular levels of 5-HT or NA by combined administration of antidepressants and ARI may be of crucial importance to the pharmacotherapy of negative and some cognitive symptoms of schizophrenia.

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Antidepressants with aripiprazole reversed the MK-801-induced deficit in the recognition memory

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Background: Schizophrenia is a devastating psychiatric disorder that impairs mental and social functioning and affects approximately 1% of the world’s population. Although the atypical antipsychotics have some efficacy in alleviating social dysfunction in schizophrenic patients, this effect is small and mechanisms of this action are still unknown. Moreover, preclinical and clinical studies have suggested that antidepressants are able to augment the activity of atypical antipsychotics which may efficiently improve the treatment of negative and some cognitive symptoms of schizophrenia. Thus, in the present study, we aimed to evaluate the effect of the atypical antipsychotic drug aripiprazole (ARI) and the antidepressant escitalopram (ESC) or mirtazapine (MIR), given separately or jointly, on the MK-801 (a NMDA receptor antagonist) -induced deficit in the novel object recognition test (an animal test modeling some cognitive symptoms of schizophrenia).

Material and methods: The experiments were conducted on male Sprague-Dawley rats (220-240 g). On the experiment day (T1), the animals were placed in a box with two identical objects for 5 min. Then, one hour after T1, the rats again were placed in the box (T2) with two different objects one from the previous session (old) and the other, a new one, for 5 min. The time exploring the objects was measured for each of the two objects separately (sniffing, touching or climbing). The deficits in the recognition memory induced by MK-801 (0.1 mg/kg), were measured in T2 session. ESC or MIR and ARI were administered 60 and 30 min before MK-801 injection, respectively, and MK-801 was administered 30 min before T1 session.

Results: The present results showed that MK-801 (0.1 mg/kg) induced deficit in memory retention in rats. ARI at a higher dose (0.3 mg/kg) reversed this effect. Co-treatment with an ineffective dose of ARI (0.1 mg/kg) and ESC or MIR (5 mg/kg) abolished the deficits evoked by MK-801, and this effect was blocked by a 5-HT₁A receptor antagonist (WAY 100635, 0.1 mg/kg) or a dopamine D₁ receptor antagonist (SCH23390, 0.25 mg/kg).

Conclusions: The obtained results suggest that the enhancement of antipsychotic-like effect of ARI by antidepressants on the MK-801-induced deficit in the memory retention in rats was associated with serotonin 5-HT₁A and dopamine D₁ receptors.

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Cysteine metabolism in the rat kidney following active versus passive cocaine administration

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Background: The effects of cocaine self-administration on cysteine (Cys) metabolism in peripheral tissues have not been studied so far. This issue is especially interesting because the products of Cys metabolism, such as sulfane sulfur possess antioxidant and redox regulatory properties as well as are a potent source of hydrogen sulfide (H₂S). Thus, they can modulate the level of reactive oxygen species (ROS) in the studied tissues, affect redox signaling via the S-sulfhydration and mitochondrial oxidative phosphorylation via oxidation of sulfide to sulfate. The aim of our study was to assess the impact of cocaine on Cys metabolism in the rat kidney following cocaine self-administration and extinction training procedures with a yoked triad.

Materials and methods: The whole pool of sulfane sulfur, its bound fraction and H₂S were evaluated as markers of anaerobic Cys metabolism while the sulfate was a measure of its aerobic metabolism. The total- and non-protein- SH group (T-SH and NPSH, respectively) levels were assayed as indicators of the redox status of thiols. The activities of enzymes involved in H₂S formation (cystathionine γ-lyase, CSE; 3-mercaptopyruvate sulfurtransferase, 3-MST; rhodanese, TST) and GSH metabolism (γ-glutamyl transpeptidase, γ-GT; glutathione S-transferase, GST) were also determined in the kidney homogenates.

Results: Cocaine self-administration and yoked procedure did not change the whole pool of sulfane sulfur but they increased the levels of the bound sulfane sulfur, H₂S T-SH and NPSH simultaneously maintaining sulfate at control levels. Both procedures during maintenance decreased CSE, 3-MST and γ-GT activity. GST activity was decreased only in the yoked cocaine group. During the extinction phase, both in rats previously self-administering cocaine and in the yoked cocaine group, the levels of bound sulfane sulfur and TSH were still increased while sulfate was maintained at the control levels. In these groups the activities of 3-MST and GST returned to the control values while TST decreased.

Conclusion: Our results show that cocaine evoked long-lasting changes in Cys metabolism and activities of the related enzymes.
The effect of 25-I-NBOMe on rat's brain neurotransmitters and motor activity

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Background: Recently the novel psychoactive substances (NPS) have become popular as recreational drugs of abuse. Hallucinogens, a class of NPS, powerfully alter perception and mood but do not produce dependence and addiction. 4-Iodo-2,5-dimethoxy-N-(2-methoxybenzyl)phenethylamine (25-I-NBOMe) is a N-benzylated derivative of the 2C family of hallucinogens. 25-I-NBOMe shows high affinity at 5-HT2A/2C and 5-HT1A receptors. It also binds to the a2-adrenergic receptors. As other hallucinogens it induces its hallucinogenic effect through the activation of the 5-HT2A receptors. The aim of this study was to find out the effect of 25-I-NBOMe on dopamine (DA), serotonin (5-HT), noradrenaline (NA) and their metabolites tissue content in the rat hippocampus (HP), striatum (STR), frontal cortex (FCX) and nucleus accumbens (NAS). Additionally, the extracellular levels of DA, 5-HT and glutamate in NAS were assessed using microdialysis in freely moving animals. Furthermore, the exploratory behaviour in rats was measured in the open field test.

Materials and methods: The study was conducted on male Wistar-Han rats (Charles River, Germany) weighing 280-300 g. The experiments were approved by the II Local Bioethics Commission (Institute of Pharmacology, Polish Academy of Sciences, Kraków). The animals were treated with single doses of 25-I-NBOMe 1, 3 or 10 mg/kg. The tissue level of NA, DA, 5-HT and their metabolites was assessed with HPLC 45 minutes after drug injection. The accumbal extracellular DA, 5-HT and glutamate levels were measured using microdialysis in freely moving animals following the administration of aforementioned doses. In addition, the effect of 25-I-NBOMe administration at doses of 1, 3 and 10 mg/kg on rat’s locomotor activity was measured using the open field test.

Results: 25-I-NBOMe increased DA, DOPAC and HVA content the most potently in FCX, while weaker in the NAS and HP; it did not affect the DA, DOPAC, and HVA tissue level in STR. 5-HT and 5-HIAA content was markedly increased in FCX, but not in the other studied brain regions. NA content was not affected by 25-I-NBOMe. The extracellular level of DA, 5-HT and glutamate was significantly increased in NAS by all 25-I-NBOMe doses. The locomotor activity of rats was decreased when measured in the open field test.

Conclusions: The increase of extracellular DA levels in NAS and DA content in NAS, HP and FCX may result from activation of cortical 5-HT2A receptors. This leads to enhanced glutamate release and stimulation of dopaminergic cell bodies in ventral tegmental area. Similarly, the increase in cortical 5-HT content may be due to stimulation of raphe nuclei by glutamatergic efferents descending from cortex. The sedative effect that 25-I-NBOMe exerted on locomotor activity may occur via the activation of the a2-adrenergic receptors.

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The antidepressant-like effects of polyphenols extracted from Impatiens glandulifera in mice

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Background: Impatiens glandulifera Royle (Balsaminaceae) is an annual herb native to the Western Himalaya region growing in riparian zones. In Poland, it is one of the top 20 invasive alien plants. Phytochemical studies conducted on Impatiens glandulifera have revealed the presence of significant amounts of polyphenols. The previous in vitro study confirmed that phenolic acids and flavonoids from the plant possessed antioxidant activity. Moreover, results of our behavioral experiments showed antinociceptive and antianxiety activity of extracts of I. glandulifera. In this study, the potential antidepressant-like effects of polyphenol (protocatechuic acid - PCA and hyperoside - Hyp) and its effects on monoamine neurotransmitter systems were assessed in different behavioral procedures.

Materials and methods: The experiments were performed on male albino Swiss mice. The forced swimming (FST) and tail suspension (TST) tests were used to evaluate the antidepressant-like activity. To assess the possible involvement of monoamine neurotransmitter systems in antidepressant effects of PCA and Hyp, p-chlorophenylalanine methyl ester (PCPA) and α-methyl-p-tyrosine (AMPT) were administered. Fluoxetine (a selective serotonin reuptake inhibitor) was used as a reference drug.

Results: Hyp and PCA at the same doses 1.875, 3.75 and 7.5 mg/kg significantly decreased the immobility time in the FST and TST, without producing locomotor alterations. Pretreatment with PCPA (100 mg/kg) prevented the antimobility effect of Hyp (3.75 mg/kg) in the FST. Thus, pretreatment with AMPT (100 mg/kg) was able to prevent the antidepressant-like effect of both polyphenols, at the dose of 3.75 mg/kg in the FST.

Conclusions: The results showed that polyphenols extracted from I. glandulifera exert antidepressant-like effects in FST and TST. These effects are supposed to be mediated by monoaminergic system.

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The effects of indirect cannabinoid receptor ligands on cognitive symptoms of schizophrenia in mice

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Background: Schizophrenia is a serious mental disorder in which people interpret reality abnormally. One of the symptoms of schizophrenia is memory-loss or other cognitive-related disorders. Many different drug targets and strategies for drug development have been employed for enhancement of cognition in schizophrenia; the relative lack of success to-date indicates the difficulty of this therapeutic area. Thus, there is intense research into developing new treatments for these diseases. One of the possible strategies for the modulation of cognitive symptoms of schizophrenia is connected with endocannabinoid system. Endocannabinoids are endogenous cannabinoid mediators which act via cannabinoid receptors (CB). So far, the best known endocannabinoids are anandamide (N-arachidonoylethanolamine, AEA) and 2-arachidonoylglycerol (2-AG) which are the main endogenous agonists of CB. The activity of AEA and 2-AG at their receptors is limited by cellular uptake, followed by intracellular degradation. A fatty acid amide hydrolase (FAAH) is the main AEA hydrolase, whereas a monoacylglycerol lipase (MAGL) is critical in degrading 2-AG.

Materials and methods: We examined the impact of inhibitors of the enzymes mentioned above (FAAH inhibitor: URB597 and MAGL inhibitor: JZL184) on cognitive symptoms of schizophrenia in mice, assessed in the passive avoidance test (PA). We provoked memory impairment by an acute injection of N-methyl-D-aspartate (NMDA) receptor antagonist: MK-801 (animal model of schizophrenia).

Results: An acute administration of JZL184 (at the doses of 8-40 mg/kg) as well as of URB597 (at the doses of 0.3 and 1 mg/kg) had no influence on memory-related effects in the PA test in mice, whereas administration of URB597 (0.1 mg/kg) and JZL184 (4 mg/kg) enhanced acquisition of memory processes. Next, we evaluated the influence of non-effective doses of inhibitors on the MK-801 (0.6 mg/kg)-induced memory impairment. Both, JZL184 (20 mg/kg), as well as URB597 (0.3 mg/kg) potentiated memory dysfunctions provoked by MK-801 in PA test in mice.

Conclusions: Through indirect modulation of endocannabinoids concentration level we can affect memory processes, what may provide an evidence of endocannabinoid system engagement in memory impairment in schizophrenia patients.

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Anticonvulsant activity of new hybrid compounds based on the pyrrolidine-2,5-dione scaffold

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Background: The aim of this study was the evaluation of anticonvulsant activity in the group of new derivatives of N-benzyl-2-(2,5-dioxopyrrolidin-1-yl)-2-phenylacetamide in models of seizure in mice. The tested compounds have been developed as integrated hybrid molecules derived from the pyrrolidine-2,5-dione ring. These hybrids join the chemical fragments of well-established anticonvulsant drugs: ethosuximide, levetiracetam and lacosamide.

Materials and methods: The experiments were performed using adult male CD-1 mice weighting 20-26 g. Antiseizure activity was examined in the maximal electroshock seizure (MES) test, and for selected compounds also in the psychomotor seizure (6 Hz), as well as in the subcutaneous pentylenetetrazole (scPTZ) tests. The initial pharmacological studies included also neurotoxicity screening in the rotarod test (NT). Moreover, median effective dose (ED₅₀), median toxic dose (TD₅₀) and protective index (PI) were determined for active compounds. The experimental protocol was approved by the I Local Ethical Committee in Kraków, Poland.

Results: In the MES test the highest activity showed compound WO-19 (a derivative with the diphenylacetohydrazide moiety), for which the ED₅₀ value was 101.32 mg/kg. In the 6 Hz test three compounds revealed prominent activity, namely WO-1 (basic structure without substituents), WO-6 (derivative with a chlorine atom in the position 3 of benzyl moiety) and WO-10 (derivative with a trifluoromethyl group in the position 3 of benzyl moiety), for which the ED₅₀ values were 62.95 mg/kg, 89.89 mg/kg and 57.69 mg/kg, respectively. No significant activity was observed in the scPTZ test. For active compounds no acute neurotoxicity was observed in the rotarod test even at the highest used dose of 300 mg/kg.

Conclusions: Among tested compounds, WO-1 and WO-10 were more potent in the 6 Hz test and provided better safety profile in the rotarod test compared to valproic acid.

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1MeTIQ reverses the behavioral schizophrenia-like symptoms produced by ketamine in rats

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Background: Schizophrenia is a chronic neuropsychiatric disorder characterized by positive and negative symptoms and cognitive impairments. Ketamine administration induces schizophrenia-like symptoms in rodents therefore it is used to model schizophrenia in animals. Olanzapine is an antipsychotic which is used in the treatment of schizophrenia. 1-Methyl-1,2,3,4-tetrahydroisoquinoline (1MeTIQ) is an endogenous compound, which exhibits the neuroprotective, antidepressant-like effect and MAO-inhibiting properties [Wąsik et al. 2015].

Methodology: The aim of the present study was to investigated the impact of acute dose of 1MeTIQ or olanzapine on the behavior of animals measured in elevate plus maze (EPM) test and disturbances in monoamine metabolism evoked by low dose of ketamine (10 mg/kg i.p.); 1MeTIQ (25 and 50 mg/kg i.p.) or olanzapine (3 mg/kg i.p) was administered once 20 minutes before ketamine injection. We performed behavioral EPM test. Moreover, in the ex vivo biochemical experiment the levels of dopamine (DA), noradrenaline (NA), serotonin (5-HT) and its metabolites were assayed in the hippocampus using HPLC with ED.

Results: The data received from behavioral EPM test indicated a significant decrease of the time spent in the open arms after ketamine administration. This effect was completely reversed by both 1MeTIQ (50 mg/kg) and olanzapine injections. Simultaneously, both 1MeTIQ (50 mg/kg) or olanzapine given alone significantly reduced motor activity measured as the distance travelled. The present ex vivo study showed that treatment with ketamine caused a significant decrease the concentration of NA and 5-HT in rats hippocampus and what more both olanzapine and 1MeTIQ did not antagonize this effects. In the same time, 1MeTIQ given with ketamine significantly increased the concentration of DA in the hippocampus.

Conclusion: The present study demonstrated the ability of 1MeTIQ to reverse ketamine-induced behavioral changes similarly to olanzapine. The anxiolytic effect of 1MeTIQ observed in EPM behavioral test will be discuss in the context of its possible mechanism of action in CNS.

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Drug-induced diabetes risk reduction – in silico study involving gut hormone GPCR receptors

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Background: Among severe side effects which occurs during prolonged pharmacotherapies type 2 diabetes mellitus (T2DM) is one of the most common new-onset disease. There are certain drug classes which are associated with the increased risk of developing T2DM, e.g., statins, diuretics and beta-blockers. Incomplete or unavailable description of molecular and/or genetic mechanisms of diabetes caused by each of those drug classes makes the T2DM risk reduction impossible in most cases. In this in silico study we proposed a way to reduce the T2DM risk, at least in some cases, via enhancing the compensating, glucose-regulating incretin effect. The incretin effect involves signaling pathways of three gut hormone GPCR receptors: GIPR, GLP-1R and GCGR.

Material and methods: We prepared models of gut hormone GPCRs using GPCRM - our web service for structure prediction of GPCRs. The models were validated by the enrichment study using known active ligands and DUD-E decoys. Theoretical binding affinities between selected drugs and models of gut hormone GPCRs were obtained from the virtual screening against the ZINC15 FDA-approved drugs library.

Results: We observed that certain drugs (e.g. beta-blockers and statins) exhibited exceptionally strong binding affinities towards gut hormone GPCRs. Interestingly, the strongest binding affinities towards selected receptors exhibited these drugs which also exhibited decreased risk of the new-onset T2DM in the clinical trials. In the blind test, the recently discovered beta-blocker (compound no 15) was nominated by our approach as the least harmful beta-blockers when drug-induced T2DM was taken into account.

Conclusions: Provided our in silico study was confirmed with experimental and/or clinical studies, the drug-induced diabetes risk could be significantly reduced by a thorough inspection of possible off-target interactions of a certain drug with gut hormone GPCRs. Such off-target interactions lead to enhancing of the incretin effect which restores the glucose and insulin serum levels balance disturbed by either on-target or off-target interactions of a certain drug on other metabolic pathways.

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Evaluation of the impact of six synthetic cathinones on the spontaneous locomotor activity of mice

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Background: Novel psychoactive substances (NPS) appeared on the world-wide market in the first decade of twenty-first century and gained significant popularity, especially among young users. Synthetic cathinones are one of the most prominent NPS group consisting of more than 100 compounds. Based on their chemical structures, synthetic cathinones can act as potent methamphetamine-like psychostimulants by enhancing dopaminergic (DA) and noradrenergic (NE) neurotransmission or as MDMA-like empathogens by enhancing DA, NE and serotonergic (5-HT) neurotransmission. As each year new substances are introduced into the drug market, reliable data on the pharmacology of NPS are limited. In this work we analyzed psychostimulant activity of 6 synthetic cathinones classified basing on their in vitro pharmacological profile into two subgroups:

a) potent DA and NE uptake inhibitors containing pyrrolidine ring (pyrovalerones): PVP, PV8 and PV9

b) substances enhancing DA, NE and 5-HT signaling: methcathinone and its two substituted analogs, 3-fluoromethcathinone (3-FMC) and 4-chloromethcathinone (4-CMC)

Materials and methods: Horizontal and vertical spontaneous locomotor activity was assessed in male C57BL/6J mice using Opto-Varimex Auto-Track system during 120 min following the s.c. injections with saline, PVP, PV8, PV9, methcathinone, 3-FMC and 4-CMC. Data were analyzed using two-way ANOVA followed by post-hoc test.

Results: All tested compounds produced time- and dose-dependent increase of horizontal locomotor activity in mice. Among pyrovalerones (PVP, PV8 and PV9), PVP was found to produce significantly more pronounced effects. Interestingly, only pyrovalerones, but not methcathinone and its substituted analogs produced significant increase of vertical locomotor activity.

Conclusions: Basing on the obtained results, we conclude that increase of horizontal locomotor activity in mice is a valuable tool which may be utilized to confirm in vivo drugs’ DA and NE stimulating activity, while increase of vertical locomotor activity seems to be associated with pure psychostimulants, lacking 5-HT enhancing properties.

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Metformin and sphingolipids in high fat diet

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Background: Development of type 2 diabetes is strongly related with high fat diet (HFD) intake. Moreover, it is well recognized that short term HFD can induce systemic changes leading to peripheral insulin resistance (IR).

Materials and methods: Male Wistar rats were divided into five groups. Control rats were fed with regular chow and received intragastrically 0.9% NaCl. Second group was fed with standard diet and received intragastrically metformin of daily dose 300 mg/kg. Third and fourth groups were fed daily ad libitum high fat diet for 3 or 6 weeks, respectively. Fifth group was fed with HFD for 6 weeks, after 3 weeks of HFD, rats were administrated a daily dose of metformin (300 mg/kg) for next 3 weeks. Thin-layer chromatography was used for isolation of ceramides.

Results: We showed in this study that short term HFD evoked alterations in ceramide (Cer) and sphingomyelin (SM) levels in prefrontal cortex (PFC) and hippocampus in rats. 3-week HFD increased concentration of total Cers as well as several Cer and SM species (Cer: -C14:0, -C16:0, -C18:0, -C20:0, -C22:0, -C16:1, -C18:1, and -C24:1 SM:-C18:1) in PFC. Prolongation of HFD feeding to 6 weeks resulted in subsequent rise of the content of these Cers, and most SM species. Changes in SF concentrations induced by HFD reported in hippocampus were less profound than in PFC. Thus, we found rise of several Cer and SM species (Cers: -C18:0, -C20:0; SMs: -C18:0, -C20:0, -C16:1 and -C18:1) and reduction of Cer C16:1 in rats fed with HFD for 3 weeks. Continuation of HFD resulted in further increment of several Cer and SM species (Cers: -C18:0 and -C20:0; SMs: -C14:0, -C16:0, -C18:1), and reduction of several Cer species (Cers: -C24:0, -C16:1 and -C18:1).

Conclusions: Importantly, administration of metformin to rats fed with HFD reduced concentrations of both Cers and SMs in studied brain structures. To our knowledge this is the first report showing a protective activity of metformin in the CNS. The most important observation of this study is that even short term HFD induces changes in lipid metabolism in CNS, and metformin effectively abrogates this changes.
Effects of synthetic cathinones on viability of cortical astrocytes in culture

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Background: Novel psychoactive substances are gaining in popularity worldwide. Among them synthetic cathinones constitute one of the largest group with an increasing number of chemical modifications. As little is known about their toxicity, the present work assessed effects of α-pyrrolidinophenones: α-PVP (α-pyrrolidinovalerophenone), PV8 (α-pyrrolidinoheptanophenone) and their fluoro-substitute derivatives (4-F-α-PVP and 4-F-PV8) on viability of primary cultures of rat cortical astrocytes.

Materials and methods: Primary astrocyte cell cultures were prepared from cerebral cortices isolated from 1-day old Wistar rat pups. Effects of α-pyrrolidinophenones (10-300 µM) on cell viability were evaluated by the MTT assay.

Results: α-PVP and 4-F-α-PVP showed a weak or moderate cytotoxic effects on astrocyte cell cultures. After 24 h 300 µM α-PVP evoked a slight reduction in cell survival by 9%. More pronounced effects were observed at concentrations of 50, 100, 200 and 300 µM after 72 h incubation, with a max. decrease (by 37% of control values) at 300 µM. Exposure to 200 and 300 µM 4-F-α-PVP for 24 h decreased astrocytes viability by 10% and 19%, respectively. Prolongation of incubation to 72 h produced a significant decline in cell survival at concentrations of 25, 50, 100, 200 and 300 µM, with a max. effect (decrease by 29% of control values) at 300 µM. After 24 h 100 and 200 µM PV8 evoked a slight increase in astrocytes viability (by 15% and 17%, respectively) in comparison with the control group. On the contrary, exposure of cells to 300 µM PV8 for 72 h resulted in a decrease (by 22%) in their viability. Incubation of astrocytes with 300 µM 4-F-PV8 for 24 and 72 h reduced their survival by 80% and 92% of control values, respectively.

Conclusions: The α-pyrrolidinophenones, α-PVP and PV8 and their fluoro-substitute derivatives affected viability of rat astrocyte cell cultures in a time- and concentration manner. Cortical astrocytes were more resistant to cytotoxic activity of the tested cathinones than neuroblastoma SH-SY5Y and hepatoma Hep G2 cell lines.

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The role of cannabinoid receptor ligands on the scopolamine-induced cognitive impairment in mice

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Background: The endocannabinoid system (ECS) is a widespread neuromodulatory system which is involved in a variety of physiological processes, including neurogenesis, synaptic plasticity, memory formation and learning. The ECS contains of two-types cannabinoid (CB) receptors, CB1 which are abundant located in the brain structures such as hippocampal cells, cerebellum, striatum, cerebellar cortex and substantia nigra. CB2 receptors are mainly expressed in immune system, and to a lesser extent in the brain on microglia, astrocytes and subpopulations of neurons.

The interaction between cholinergic system which is mainly engaged in memory formation, and the ECS in the context of cognitive impairments remains poorly understood. Scopolamine is a muscarinic blocker in the CNS (central nervous system) which disrupts learning and memory functions and represents commonly accepted amnestic model in mice. Here, we examined the influence of CB receptors selective and non-selective ligands on memory consolidation impaired by acute scopolamine administration.

Materials and methods: We examined an impact of cannabinoid receptor ligands: selective CB1 receptor agonist: oleamid, non-selective mixed CB1/CB2 receptors agonist: WIN 55-212, and selective CB1 receptor antagonist: AM-251 on memory processes. Then we evaluated an influence of these ligands on cognitive impairment caused by scopolamine using passive avoidance (PA) test.

Results: An acute administration of oleamid (10,20 mg/kg) and WIN 55-212 (1 mg/kg) impaired memory processes, whereas administration of AM-251 (1 mg/kg) enhanced memory in mice. Next, we examined the influence of non-effective doses of cannabinoid ligands on the cognitive impairment induced by scopolamine (1 mg/kg). Co-administration of oleamid (5 mg/kg) and scopolamine worsen cognition, on the other hand injection of AM-251 and scopolamine resulted in memory enhancement. If it comes to effects of WIN-55-212 and scopolamine co-injection, there was not statistically significant results, but it showed tendency towards memory improvement.

Conclusions: Our results demonstrated that, the ECS interacts with cholinergic system and modulates the memory and learning processes. These findings could be helpful in further research concerns the enhancement of pharmacotherapy in diseases which are associated with cognitive impairments, especially referred to cholinergic system dysfunction, such as Alzheimer’s disease (AD).

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The involvement of glucagon-like peptide receptors in morphine rewarding effects in rats


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Background: Literature data inform that glucagon-like peptide receptors occur in ventral tegmental area and they are involved in the activity of mesolimbic system. Our previous results showed that inhibitor of dipeptidyl peptidase-4, linagliptin, reduced morphine withdrawal signs in rats. In the present study we undertook to investigate the role of linagliptin in morphine rewarding effect in rats.

Material and Methods: The conditioned place preference (CPP) test was used to studying the morphine rewarding effect in rats. The experiment was divided into several phases: pre-conditioning, conditioning and post-conditioning, extinction and reinstatement. Morphine rewarding effect was obtained by administration of morphine (5 mg/kg, ip) for three days during post-conditioning. The extinction phase lasted five days and priming dose of morphine (1 mg/kg, ip) was given during reinstatement. Linagliptin was used at doses of 10 and 20 mg/kg, ip.

Results: We demonstrated that linagliptin (10 and 20 mg/kg, ip) reduced morphine rewarding effect in CPP test. Linagliptin at dose of 10 mg/kg, ip, (but not 20 mg/kg, ip) significantly inhibited morphine-induced reinstatement and has an effect on extinction of morphine rewarding effect.

Conclusions: Results demonstrated that that glucagon-like peptide receptors are involved in to morphine reinforcement.

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Does sex matter for development of diabetes and obesity? Lessons from rat model

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Background: Neurons expressing kisspeptin (KP), neurokinin B (NKB), and dynorphin A (DYN A), so called KNDy neurons, located in the arcuate nucleus of the hypothalamus (ARC), are important regulators of reproduction. Moreover, the ARC is a region involved in energy homeostasis, and expression of KP, NKB and DYN A is dependent on both hormonal and metabolic status.
We hypothesized that male and female rats with high-fat diet-induced obesity (DIO) and/or streptozotocin (STZ)-induced diabetes mellitus type 2 (DM2) will have differential alterations in the number of KP-ir and/or NKB-ir, and/or DYN A-ir neurons in the ARC of the hypothalamus.

Materials and methods: Rats were assigned to 3 groups: 1) control (C) - fed a regular chow diet; 2) obese (DIO) and 3) diabetic (DM2) - both fed a high-fat diet (50% calories from fat). To induce DM2, low doses of STZ were injected. Vaginal smears were taken daily from female rats for 2 consecutive weeks to detect the stage of the estrous cycles. Metabolic and hormonal profiles were assessed (insulin, glucose, leptin, triglycerides, cholesterol, estradiol in females testosterone in males), and immunocytochemistry for KP, NKB and DYN A was performed in sections containing the ARC.

Results: We have found that: 1) DIO and DM2 males had a decrease in testosterone levels. 2) Both DIO and DM2 females had an increased incidence of irregular estrous cycles, shift in length of phases of the cycle and increased estradiol levels. 3) Male rats with experimentally induced diabetes type 2 (DM2), but not obese (DIO) had increased numbers of KP-immunoreactive (-ir), NKB-ir and DYN A-ir neurones in the ARC (Dudek M. et al., J. Neuroendocrinology, 2017). 4) In contrast, DM2 did not alter numbers of KP-ir, NKB-ir and DYN A-ir neurons in the ARC of females. Similar to male rats, DIO did not influence number of KP-ir, NKB-ir and DYN A-ir neurons in the ARC of females.

Conclusions: Although male and female rats burdened with DIO and DM2 both show significant alterations in the metabolic and hormonal profiles, only male rats seem to respond to diabetes type 2 with altered number of KNDy neurons. Understanding the mechanism(s) of these sex differences could help in developing new strategies to treat reproductive disorders in male and female diabetic patients.

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Depressive-like behavior is related to blood-brain barrier dysfunction and BDNF leakage

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Background: Observations from animal and clinical studies provide evidence that depressive disorders are linked with decreased brain-derived neurotrophic factor (BDNF) levels in the central nervous system (CNS). Additionally, we propose that blood-brain barrier (BBB) integrity constitutes an important factor in regulation of CNS BDNF concentration. Mice selected for high (HA) or low (LA) swim stress-induced analgesia (SSIA), differ both in the intensity of depressive-like behaviors and BBB permeability. Our hypothesis assumes that the development of depressive-like behaviors in HA mice depends on the loss of BDNF from the CNS through the leaky BBB. Thus, we aimed to assess the influence of pharmacological BBB tightening on depressive-like behaviors in HA and LA mice, and correlating this data with the CNS concentration of BDNF.

Methods: Mice from each line were treated subcutaneously with dexamethasone for seven days in order to facilitate BBB closure. Depressive-like behaviors were evaluated in the tail-suspension test (TST). BDNF levels in the prefrontal cortex and hippocampus were measured in the ELISA assay.

Results: As anticipated, HA mice showed more pronounced depressive-like behaviors in the TST test, that was associated with compromised BBB integrity. This corresponded with lower baseline BDNF levels in the prefrontal cortex seen in HA mice. Dexamethasone produced an anti-depressive effect in the HA, but not in the LA line. Surprisingly, dexamethasone treatment significantly augmented BDNF levels in the prefrontal cortex in both lines of mice. No differences were found in baseline or dexamethasone-induced BDNF levels in the hippocampus in either line.

Conclusions: Depressive-like behaviors in HA mice are related to increased BBB permeability. The intensity of depressive-like behaviors is associated with lower BDNF levels in the prefrontal cortex, but not in the hippocampus of HA mice. The possible anti-depressive effect of dexamethasone in HA mice is mediated through the prevention of BDNF efflux from the prefrontal cortex. An increase of BDNF levels in the prefrontal cortex of LA mice is not correlated with a decrease in depressive-like behaviors, due to the lack of BBB modification by dexamethasone. This implies a specific influence of BDNF only on pathological behaviors generated as a result of OUN dysfunction leading to depression.

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Effects of the mTOR kinase inhibitor, rapamycin, on cognitive deficits in adult rats with FASD

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Background: Ethanol is well known for its teratogenic effects during fetal development. It bring about many physical, behavioral and cognitive abnormalities in children whose mothers consumed alcohol in pregnancy. This group of disorders are known as Fetal Alcohol Spectrum Disorders (FASD), the effects of which could be seen immediately after birth, in adolescence but also in adulthood. Especially dangerous for the fetus is maternal alcohol consumption during 3rd trimester of pregnancy. This leads to serious brain damage, including executive and adaptive functions, impairment in memory, attention and cognition. Recent reports have shown that the mammalian target of rapamycin (mTOR), a serine-threonine specific protein kinase, could participate in neurotoxic effects of ethanol. The aim of the present study was to investigate whether rapamycin, the mTOR inhibitor, can attenuate learning and memory impairment in rats with FASD.

Material and methods: The experiment was performed with male and female Wistar rats. FASD model rats were intragastrically intubated with ethanol (5g/kg, 11.33% v/v), once a day over postnatal day (PND) 4 to 9. Rapamycin was given intraperitoneally at the dose of 3mg/kg or 10mg/kg, 1 h before ethanol treatment. The Novel Object Recognition (NOR) test was conducted in young adult (45 PND) rats.

Results: Our results demonstrated that ethanol impaired short (after 2-) and long (24-h delay) term memory in the NOR test by decreasing discrimination index (DI) when compared to control animals. Pre-treatment with rapamycin during FASD development increased the DI in the NOR task in the FASD male and female rats.

Conclusions: Our results show that rapamycin pretreatment ameliorated the development of cognitive and memory impairment in rats with FASD.

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MH-76, a novel α₁-adrenoceptor blocker, ameliorates altered metabolism in fructose-fed rats

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Background: Metabolic syndrome associated with visceral adiposity, hyperlipidemia, hyperglycemia and hypertension is often caused by excessive fructose consumption. Treatment of hypertension in patients with metabolic syndrome is a difficult task as many antihypertensive drugs have adverse effects on the metabolic profile. We investigated if MH-76, a novel α₁-adrenoceptor antagonist with an additional ability to stimulate NO/cGMP/K⁺ pathway ameliorates metabolic syndrome in fructose-fed rats. Prazosin was used as a reference compound.

Materials and methods: Male Wistar rats were divided into four groups (n=8) and studied for 18 weeks: group control: standard chow diet and drinking water; group Fructose: high-fructose diet (20% fructose in drinking water); group Fructose+MH-76: high-fructose diet with subsequent MH-76 (5 mg/kg ip) treatment 12 weeks later, group Fructose+prazosin; high-fructose diet with subsequent prazosin (0.2 mg/kg ip) treatment 12 weeks later.

Results: Fructose-fed rats presented increased body weight with markedly increased visceral adiposity, insulin resistance and hyperinsulinemia, hyperglycemia, elevated triglyceride concentration, mild hypertension and impaired endothelial function. Moreover fructose feeding increased TNF-α concentration in adipose tissue. MH-76 in addition to its antihypertensive effect, reversed endothelial dysfunction, reduced insulin resistance and hyperinsulinemia and decreased hypertriglyceridemia. Moreover, MH-76 prevented excessive body weight gain and visceral adiposity. It also decreased TNF-α concentration in adipose tissue. Prazosin showed antihypertensive effect but had no impact on endothelium dysfunction. Furthermore prazosin exacerbated hyperinsulinemia and did not prevented excessive body weight gain and visceral adiposity.

Conclusions: Fructose induced hypertension, endothelial dysfunction, visceral adiposity and altered metabolism in rats was alleviated by MH-76 whereas prazosin exerted only antihypertensive effect. The therapeutic potential of MH-76 may be attributed to its α₁-adrenoceptor blockade, endothelial activity and anti-inflammatory properties. The use of multiple-targeted, improved α₁-blockers may be an interesting option in pharmacotherapy of hypertension, especially in case of metabolic complications such as dyslipidemia or the metabolic syndrome.

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Effect of maternal carbohydrate diet on MC4 receptors in rat brain after cocaine self-administration

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Background: Substance use disorder is a disease of the central nervous system related to the physiological, behavioral and cognitive disorders, among which the intake of a substance (e.g. cocaine) dominates over other behaviors that were previously valuable for patient. Previous research suggests that the melanocortin (MC) system and its receptors may play a role in cocaine abuse. Moreover, increasingly data indicate that maternal diet can increase the risk of psychiatric disorders in offspring in adulthood. The aim of this study was to investigate the effect of cocaine self-administration on the level of MC4 receptors in the selected brain structures in male rats which mothers were fed a modified diet.

Material and methods: Wistar rat dams were maintained ad libitum either on high carbohydrates (HCD, carbohydrates 70%) or standard rodent chow (SD) during gestation and lactation. At 63 postnatal day male offspring was introduced three weeks of cocaine self-administration protocol (daily 2 h sessions) - stable cocaine dose (0.5 mg/kg/inf.) with increasing schedule of reinforcement fixed ratio (1-5). The animals were sacrificed through decapitation immediately following the last 18. experimental cocaine session and the prefrontal and frontal cortex, hippocampus, nucleus accumbens, dorsal striatum, ventral tegmental area, amygdala and hypothalamus were dissected for biochemical experiments. In the isolated synaptosomal fraction changes in MC4 receptors level were evaluated using an enzyme-linked immunosorbent assay.

Results: We observed that in rats self-administered cocaine the MC4 receptor level significantly increased as compared to SD group in the ventral tegmental area, amygdala and hypothalamus. We also found the decreased MC4 receptor level in the prefrontal and frontal cortex, nucleus accumbens and dorsal striatum in HCD offspring following cocaine self-administration.

Conclusions: Our data suggest that self-administered cocaine induces adaptive changes in MC4 receptors level in brain. Male offspring which mothers were fed a modified diet respond to other molecular changes to exposure to cocaine relative to the control group. It suggest that maternal diet may affect the susceptibility of offspring to cocaine addiction, and MC4 receptors can be recognize as a potential target in the search drug for drug addiction.

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Is irisin the new biomarker player in brain disorders?

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Background: Irisin is a novel myokine and adipokine that has gained much attention recently due to its mechanisms of action. Irisin can cross the blood brain barrier and act as a neurokine to protect brain function. Irisin has received great attention because of its capability to treat diabetes, obesity, mental disorders and may act as a potential biomarker. Irisin is involved in regulation of neuronal differentiation, learning process, metabolism. It is still necessary to deepen in several aspects in order to clarify its full potential as a meaningful drug target in human disease states. The irisin/BDNF axis is a pathway that influences several neurobehavioral mechanisms involved in the pathogenesis of disorder. Current studies strongly indicates that the maternal exposure to high fat diet exert irreversible impact on the structure and function of offspring's brain and also affect the immune system which can predispose to many diseases, including brain disorders. The aim of the present study was to quantify in male and female rat serum irisin and the hippocampal BDNF, irisin, IL-1α, IL-6 and TNFα concentrations in order to investigate the possible link between the irisin/BDNF axis and brain disorder.

Material and methods: Wistar rat dams were maintained ad libitum either on high fat diet (HFD, crude fat 35%) or standard diet (SD) during gestation and lactation. At 28 and 63 postnatal day (PND) the male and female offspring was sacrificed through decapitation for biochemical analyses. The serum irisin concentrations were evaluated by enzyme-linked immunosorbent assay and hippocampal irisin, BDNF, IL-1α, IL-6, TNFα using a Multiplex Assays using Luminex.

Results: Our data indicated that in SD and HFD groups irisin level were almost twice higher in younger (28 PND) animals in both serum and brain. However, no significant changes were observed between diets. In addition, data showed an inverse correlation between irisin and BDNF concentration in the rat hippocampus. Further, significant increased level of interleukin was seen in all individuals at 63 PND.

Conclusions: Our data indicated significantly elevated irisin level both in male and female young rats. The explanation of the observed differences in the level of the examined factors depending on the age as well as the potential regulation of the irisin/BDNF connection require further investigation.

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Changes in the brain expression of NR2B subunit in different conditions of cocaine forced abstinence

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Background: NMDA receptor-dependent mechanisms seem to be critical for the disturbances in synaptic plasticity occurred during cocaine abstinence and may be new critical biomarkers to drive cocaine seeking and relapse. The aim of this study was to investigate the changes in the expression of the NR2B subunit in different brain structures of animals following different conditions of cocaine forced abstinence.

Material and methods: The expression of the NR2B subunit receptor was determined with using Western blot assays in the total homogenate and post-synaptic density (TIF) fractions of the dorsal (dHIP) and ventral (vHIP) hippocampus, dorsal (dSTR) and ventral (vSTR) striatum, infralimbic (ilCTX) and prelimbic (plCTX) cortex in rats with the history of cocaine (0.5 mg/kg/inf.) self-administration. Data were analyzed by using factorial ANOVAs.

Results: In rats housing in an enriched environment an increase in the NR2B expression was seen in the total homogenate of dHIP, while a decreased NR2B expression in TIF fraction of plCTX was reported. In rats housing in isolated condition a rise in the NR2B expression was observed in the dHIP, ilCTX (homogenate) and plCTX (TIF). Extinction training in drug-associated environment induced either an increase in the vHIP or a decrease of the NR2B expression in the dSTR in the TIF fraction. An increase of this protein expression in the homogenate of vHIP and in the TIF fraction of dSTR was presented in rats following extinction in drug-associated environment without instrumental task.

Conclusion: These findings seem to suggest that different condition of abstinence from cocaine change the expression of NR2B subunit receptor.

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Antinociceptive and antipruritic effects of the histamine H₃ receptor antagonists

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Background: The expression of histamine H₃Rs in the thalamic areas, dorsal root ganglia or spinal cord suggests their potential involvement in the two closely related, however distinct sensations such as pain and itch. We assessed the analgesic and antipruritic activity of the compound DL76, which is the new non-imidazole H₃R antagonist showing high affinity towards the H₃R (hKᵢ 1/4 22 ± 3 nM) and the high brain cumulation.

The goal of our study was to evaluate the activity of compound DL76 in the models of acute, inflammatory and neuropathic pain and histamine-dependent and histamine-independent itch. Moreover, we attempted to assess the involvement of histamine H₁R, H₂R and H₄R receptors in the overall effect.

Materials and methods: We examined anti-inflammatory, analgesic and anti-oedematous effects of the compound in the in vivo model of inflammation induced by carrageenan in rats. Moreover, we evaluated the analgesic activity in the formalin test, capsaicin test and hot plate test. We also assessed the impact on tactile allodynia in oxaliplatine-induced neuropathic pain. While the antipruritic activity of the test compound was tested in the histamine, chloroquine and SLIGRL-NH₂ induced model of itch. We compared all results with the effect of the reference H₃R antagonist Pitolisant. Moreover, we assessed the interaction of DL76 and Pitolisant with H₁R, H₂R and H₄R antagonists.

Results: The investigated compound reduced paw oedema (which was particularly interesting), mechanical and thermal hyperalgesia in the carrageenan-induced acute inflammation. DL76 was also active in the second phase of formalin test and in the model of neuropathic pain. DL76 showed antipruritic activity in all models of itch, while H₁R blockade resulted in decreased histamine-induced itch but not that induced by chloroquine or SLIGRL-NH₂ indicating that H₃R antagonism may be beneficial in the pruritus, that is resistant for the classic antihistamines. The centrally acting H₂R antagonist significantly attenuated the antipruritic effect of DL76, as well as the analgesic activity in the model of neuropathic pain.

Conclusions: These results show that the blockade of histamine H₃Rs might have therapeutic utility for the treatment of inflammatory and neuropathic pain as well as in the treatment of histamine-dependent and histamine-independent itch. Our results also indicate that the stimulation of the postsynaptic H₂R (secondary to histamine release) is associated with analgesic and antipruritic activity of the H₂R antagonists.
Effects of exposure to nanoparticles or airborne particulate matter (PM) on behavior in mice

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Background: Deficits of learning and memory, depressive-like behavior and increased biomarkers of inflammation in the brain as the result of exposure to airborne particulate matter (PM) have been increasingly reported. Chemical composition of PM varies across the region, source of emission and season. Silica and iron form a large part of PM mass. Thus, in the present study, we assessed the effects of long-term exposure to SiO$_2$ or Fe$_2$O$_3$ nanoparticles or to crude PM and that with reduced content of organic matter on selected parameters of behavior in mice.

Materials and methods: Standard reference material NIST 1648a was used as crude PM. NIST 1648a was treated with a low-temperature plasma for 120 min. (referred to as LAp 120) in order to decrease the organic carbon content. Four groups of C57BL/6J male mice were exposed to SiO$_2$ or Fe$_2$O$_3$ nanoparticles or to NIST 1648a or LAp 120 for 14 weeks at the concentration of 1 mg/m$^3$ of air, for 6 h a day and 5 days per week. Starting from 6 weeks of exposure selected behavioral tests were conducted once a week: measurement of locomotor activity, rotarod test, open field test, cliff avoidance test and marble burying test. Body weight gain was also measured.

Results: The body weight gain of mice was decreased after exposure to all selected compounds. Locomotor activity was increased after exposure to SiO$_2$ nanoparticles, NIST 1648a and LAp 120. Differences in time spent on a platform in the cliff avoidance test did not reach statistical significance but the number of fecal bolii was increased in the group exposed to SiO$_2$ nanoparticles which is indicative for stress-related behavior. The behavior of animals assessed with open field, rotarod and marble burying tests was not affected by the exposure.

Conclusions: Obtained results showed that the exposure to nanoparticles and airborne PM negatively affected some of physiological and behavioral parameters. These changes may be related to proinflammatory and prooxidative action of the compound under study. Surprisingly, chemically inert material, SiO$_2$, applied in the form of nanoparticles exerted visible negative physiological and behavioral effects.

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The effect of 25-I-NBOMe on rat brain neurotransmission

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Background: The term “novel psychoactive substances” (NPS) refers to newly used designer drugs that may pose a threat to public health comparable to classic previously listed classic psychotropic substances. Typically NPS are analogues of controlled compounds designed to produce effects similar to the substances they mimic. A new group of toxic phenylethylamine derivatives named NBOMe are frequently bought using the internet and have similar effects to other hallucinogenic drugs. 4-Iodo-2,5-dimethoxy-N-(2-methoxybenzyl)phenethylamine (25-I-NBOMe) is a N-benzyalted derivative of the 2C family of hallucinogens. Serious toxicity and fatalities have been reported after use of 25-I-NBOMe. The knowledge on central nervous system effect of 25-I-NBOMe is very limited. Therefore, the aim of this study was to find out the effect of 25-I-NBOMe on dopamine (DA), serotonin (5-HT) and glutamate extracellular levels in the rat frontal cortex and striatum.

Material and methods: Experiments were conducted on male Wistar-Han rats (Charles River, Germany). The experiments were approved by the II Local Bioethics Commission (Institute of Pharmacology, Polish Academy of Sciences, Kraków). The rats were treated with acute doses of 25-I-NBOMe and the release of DA, 5-HT and glutamate was measured using microdialysis in freely moving animals. The extracellular level of DA and 5-HT was measured with HPLC with coulochemical detection while glutamate level was measured with HPLC with electrochemical detection after reaction with OPA/sulphate to form a derivative prone to detection.

Results: 25-I-NBOMe at all studied doses (1, 3 and 10 mg/kg) increased the release of DA, 5-HT and glutamate in frontal cortex and striatum.

Conclusions: The presented data provides a fundamental knowledge on neurochemical mechanism of the first representative of NBOMe hallucinogens. 25-I-NBOMe markedly affects dopaminergic, serotoninergic and glutamatergic neurotransmission increasing extracellular level of neurotransmitters after acute treatment.

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Effects of exposure to nanoparticles or airborne particulate matter (PM) on thymus and thymocytes in mice

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Background: Exposure to airborne particulate matter is regarded as potential health risk and literature data showed disturbances in the immune system and proinflammatory tendency especially in the lung. Some literature data reported also negative effects of PM exposure on thymus and thymocyte subpopulations. Chemical composition of PM varies across the region, source of emission and season. Silica and iron form a large part of PM mass. Thus, in the present study, we assessed the effects of long-term exposure to SiO$_2$ or Fe$_2$O$_3$ nanoparticles or to crude PM and that with reduced content of organic matter on thymus and thymocytes in mice.

Materials and methods: Standard reference material NIST 1648a was used as crude PM. NIST 1648a was treated with a low-temperature plasma for 120 min. (referred to as LAP 120) in order to decrease the organic carbon content. Four groups of C57BL/6J male mice were exposed to SiO$_2$ or Fe$_2$O$_3$ nanoparticles or to NIST 1648a or LAP 120 for 14 weeks at the concentration of 1 mg/m$^3$ of air, for 6 h a day and 5 days per week. Then the mice were sacrificed and spleens and thymuses were dissected and weighted. Thymic cell populations (CD11c, CD3, CD4 and CD8) were assessed with flow cytometry. Relative spleen and thymus weight and cellularity of these organs were also calculated.

Results: The body weight at the end of exposure was decreased only after exposure to NIST 1648a. There were no differences in the relative thymus and spleen weight as well as in the cellularity of these organs. Composition of main thymic cell populations was not affected by the exposure to compounds under study.

Conclusions: Obtained results showed that the exposure to crude PM (NIST 1648a) negatively affected general metabolism as reflected by terminal body weight. None of the components tested had an effect on the thymus or on the percentage of the major thymic cell populations.

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Assessment of ultrasonic vocalizations of playing juvenile rats in the valproic acid model of autism

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Background: Autism spectrum disorder (ASD) is most likely of a neurodevelopmental nature and its symptoms are often evident from the early age. Its core symptoms include disturbed social behaviour, disturbed social communication and stereotypical behaviour. Prenatal exposure to the anticonvulsant valproic acid (VPA) medication is associated with an increased risk of ASD in humans and yields an autistic-like phenotype in rodents. The aim of the present study was to examine the effects of prenatal valproic acid (VPA) treatment on the level of communication in juvenile rats during the social play (SP).

Materials and methods: Pregnant Sprague-Dawley rat dams received a single, intraperitoneal injection of VPA (0 or 500 mg/kg) on GD 12.5. The offspring were subjected to SP test on PND 30. SP behaviour and associated ultrasonic vocalisations (USVs) were recorded during the test lasting for 10 minutes.

Results: Social play behavior was significantly reduced in VPA-treated rats. The analysis of USVs features in the first minutes (1-5 min) of the test did not reveal differences between control and VPA groups. However, when second block of test (6-10 min) was studied, comparing to controls, the VPA animals displayed: i) a greater number of the USVs, ii) the broader USV bandwidth and iii) the increased number of frequency modulated calls.

Conclusions: The present study demonstrates reduced social behavior and abnormal pattern of ultrasonic vocalizations in VPA-exposed rats.

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Importance of kynurenic pathway enzymes in neuropathic pain development in rats

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Background: Neuropathic pain caused by a primary injury or dysfunction in the peripheral or central nervous systems is a tremendous therapeutic challenge. Treatment of neuropathic pain remains a major challenge; therefore, essential mechanisms remain to be elucidated. The participation of the kynurenine system in the pathology of neurodegenerative disorders and autoimmune diseases was studied, but the significance of this pathway have been poorly studied in neuropathic pain. Here, we collected the first evidence revealed in one study on the potential contributions to neuropathic pain development by enzymes in the kynurenine pathway [tryptophan 2,3-dioxygenase (Tdo), indoleamine 2,3-dioxygenase (Ido1/2), kynurenine 3-monooxygenase (Kmo); kynureninase (Kynu), 3-hydroxyanthranilate-3,4-dioxygenase (Haoo)] at the spinal cord and DRG levels.

Method: Chronic constriction injury (CCI) of the sciatic nerve was performed according to Bennett and Xie (1988). The mechanical and thermal hypersensitivity was measured using von Frey test and cold plate test, respectively. Biochemical studies comprised the qRT-PCR and/or Western blot analysis in the tissue [spinal cord, the dorsal root ganglia (DRG)] and primary glia cultures. The experiments were carried out according to IASP rules (Zimmermann, 1983).

Results: Spinal mRNA level of Ido2, Kmo and Haoo were elevated as measured on day 7 after CCI, parallel to the C1q-positive cell activation. In the DRG, Ido1, Ido2, Kmo and Haoo mRNA levels were raised, as we demonstrated on day 7 after CCI. According to our data obtained from primary microglial cell cultures, all enzymes of the kynurenine pathway except Tdo are derived from these cells. Our pharmacological studies give evidence that repeated intraperitoneal administration of minocycline, a microglia/macrophage inhibitor, not only attenuated tactile and thermal hypersensitivity but also diminished the levels of Ido2 and Kmo mRNA.

Conclusions: The results of our studies show that the kynurenine pathway is an important mediator of neuropathic pain pathology. Interestingly, it also appears that pharmacological indirect modulation of the kynurenine pathway by microglia/macrophages inhibitors might provide satisfactory therapeutic effects in the future.

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Rat brain NMDA receptor subunit expression changes after mephedrone and amphetamine withdrawal – effects on memory

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Background: Our experiments indicated that binge pattern of amphetamine (2.5 mg/kg, ip) and its analog - mephedrone (4-methylmethcathinone, 30 mg/kg, ip) administration (once a day for 10 consecutive days) in adolescence (PND 40-50) rats induced spatial learning and memory (Barnes maze task) deficits in adults (PND 75-97). The NMDA receptors (NMDAR) are involved in learning and memory processes that play important roles in drug abuse. The purpose of this study was to examine the participation of NMDA receptor subunits in different brain structures in mephedrone and amphetamine-induced memory impairment.

Material and methods: At PND 97, the animals were decapitated and their brain structures were removed. The prefrontal cortex, hippocampus and striatum were immediately dissected and all samples were frozen on dry ice and stored at -80 °C until analyses. Western blotting was used for protein fraction analysis. The expression of NMDA receptor subunits (GluN1, GluN2A, GluN2B) and scaffolding protein (PSD95) were evaluated relative to that of b-actin control protein using mouse monoclonal antibody. Blots were washed and incubated with donkey goat anti-rabbit secondary antibody or goat anti-mouse, and visualized with a fluorescence detection.

Results: The experiments indicated that mephedrone withdrawal (7 weeks) significantly enhanced expression of the GluN2B subunit of the NMDA receptor in the striatum (P<0.01) and decreased expression of the PSD-95 scaffolding protein in this brain structure. In contrast, amphetamine withdrawal decreased only the GluN2A subunit expression of the NMDA receptor in the hippocampus.

Conclusions: Our results demonstrated that withdrawal-induced spatial memory impairment in adult rats exposed to mephedrone during adolescence is associated with up-regulation of the GluN2B subunit of the NMDA receptor in the striatum. Amphetamine withdrawal was without effect on this subunit. Because in our study the expression of PSD-95 was decreased, and this protein is able to increase receptor stabilization in the membrane, our results suggest that up-regulation of the GluN2B is transient, and may be correlated with drug-seeking behaviour.

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Dry powder inhalation as an alternative administration route for esketamine – pharmacokinetics study in rats

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Background: Ketamine is known and used as anesthetic for over 50 years and is getting more and more attention as a very rapid acting antidepressant. However, the ketamine undergoes a strong first-pass metabolism effect, excluding the oral administration of the drug and infusion of the ketamine requires an outpatient support. In order to develop a reliable and comfortable delivery method, we explored the pharmacokinetic characteristics in rats of one of esketamine and ketamine racemate after dry powder inhalation, intravenous (I.V.) and intratracheal (I.T.) administration.

Materials and methods: Esketamine hydrochloride at the target level of 10 mg/kg or ketamine hydrochloride racemate at the target level of 20 mg/kg were administered to male Wistar rats. Administration procedures included acute I.V. or I.T. (only esketamine) routes or 30-min long dry powder inhalation (INH.). Blood samples were collected 5, 10, 30, 60, 120 and 240 min after I.V. and I.T. administration and 6, 12, 24 and 30 min after the start of inhalation and 5, 15, 20, 30, 60, 120, 150 and 240 min after the end of administration (at least n=3/timepoint). Brain samples were collected 10 or 15, 30, 60, 120 and 240 minutes after the end of all administrations. Enantioselective and racemic ketamine concentration analysis together with its major metabolites (norketamine and hydroxynorketamine) was evaluated by LC/MS/MS method.

Results: Following ketamine administration, ketamine and its metabolites were detected in the blood and brain samples (achieving 2-3 higher concentrations than in plasma). 30 minutes inhalation enabled to successfully deliver a dose of 7.8 mg/kg for esketamine and 19.9 mg/kg for ketamine racemate. I.V. and I.T. administration provided a comparable pharmacokinetic profile with very high, 95%, bioavailability for I.T. route. Dry powder inhalation administration provided successful systemic and brain tissue exposure with the 85% bioavailability for both esketamine and racemate dosing.

Conclusion: Dry powder inhalation was proved as a very potent delivery method of ketamine with high bioavailability – in contrary to available data for oral administration. Therefore, the inhalation route of administration could provide a novel solution for ketamine delivery and offer additional advantages including efficient and precise dosing, comfortable and preferable administration over intravenous route.

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Modified maternal diet changes ceramide levels in the female rat offspring liver

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Background: According to the concept of the developmental origin of health and disease, the mother's diet in the fetus and early postnatal period influences the physical and mental development of the offspring. The mother's diet may be a disease-inducing factor, e.g. obesity in both juvenile and adulthood. Ceramide is an important building block for many complex sphingolipids (sphingomyelin, glycosphingolipids). It regulates many biochemical processes, including apoptosis, aging, insulin signaling, and many other. Dysregulation of the ceramide metabolism (e.g., by the sphingomyelinase pathway and the de novo pathway) has been proposed as an important factor in the pathogenesis of disease e.g. obesity and non-alcoholic fatty liver disease (NAFLD). NAFLD includes changes in many biological processes, e.g., lipid metabolism, lipotoxicity, apoptosis, inflammation in which ceramides are also involved. Ceramides seems to be involved in NAFLD, while their role in the disease is not fully understood.

Materials and methods: Wistar females during pregnancy and lactation had unlimited access to modified types of diets: standard diet (SD), high-fat diet (HFD), high-carbohydrate diet (HCD) or a mixed diet with increased carbohydrate and fat content. Then, on day 28 or 63, the offspring of both sexes were decapitated to obtain liver which was later analyzed with using Western Blot.

Results: We found significant decreases (by 50%) in the protein levels of synthase ceramide 2, synthase ceramide 4, alkaline sphingomyelinase in the females offspring rats after mixed diet and high fat diet. On the other hand, a significant increase (by 90%) was observed in the neutral sphingomyelinase level after the mixed diet in the females offspring.

Conclusions: The results of the research show, for the first time, changes in the expression of enzymes of the metabolism ceramide in the female offspring following exposure to varies diets to mothers.

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An adverse effect of Kv7-channel opener retigabine on the spatial memory in rats

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Background: Retigabine is a novel antiepileptic drug whose mechanism of action is unique and primarily associated with activation of the Kv 7 voltage-gated potassium channels. The drug also inhibits glutamatergic transmission, increases GABAergic transmission and at high doses affects voltage-gated calcium and sodium channels. This mechanism introduces the possibility of using retigabine in treating not only epilepsy. However, multidirectional influence of the drug on various neurotransmission systems may not only induce beneficial effects but also increase the risk of side effects associated with cognitive functions, including disruption of memory processes.

Material and methods: Adult male Wistar rats were treated acutely (10 mg/kg or 20 mg/kg, i.g.) or repeatedly (10 mg/kg, i.g.; once daily for 14 days) with retigabine. Effects of the drug on a spatial memory were assessed using the Morris water maze test.

Results: Acute treatment of rats with 20 mg of retigabine evoked a transient impairment of memory processes in rats. When the drug was administered at 10 mg/kg, this effect was of a lesser degree and delayed in time. Furthermore, the drug administered repeatedly for 14 days disturbed learning processes but memory improvement was noted 48 h after the last dose of the drug, which may indicate a transient nature of the occurring dysfunction.

Conclusions: These results indicate that retigabine may affect memory and learning processes, especially at high doses and in the first phase of the treatment.

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The effects of prenatal exposure to valproic acid on attentional performance in the 5-CSRTT in rats

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Background: Autism spectrum disorders (ASD) is a heterogeneous neurodevelopmental disorder that is characterized by two main groups of symptoms: social/communicative deficits and restricted, repetitive patterns of behaviours, interests or activities. ASD is also accompanied by the diversity of comorbid features, including hyperactivity, impulsivity and inattention. The aim of the present study was to evaluate the 5-CSRTT (5-choice serial reaction time task) performance in a rat model of autism induced by prenatal exposure to valproic acid (VPA). In this task, animals must continuously monitor an array of five holes in order to detect and respond to brief light stimuli that are presented randomly in one of five holes. The animal indicates the detection of a stimulus by performing a nose poke in the illuminated hole. Correct responses result in the delivery of a food pellet, whereas incorrect responses elicit a brief timeout period. The task allows for the simultaneous examination of multiple aspects of performance. The accuracy of attentional processes is reflected as the relation of correct responses to the total responses. Additionally, premature responses (nose pokes made before stimulus presentations) offer a measure of impulsivity.

Materials and methods: Pregnant Sprague-Dawley dams received a single intraperitoneal injection of VPA (0 or 500 mg/kg) on GD 12.5. The male offspring were tested on the 5-CSRTT during adulthood. Additionally, locomotor activity was measured in the Opto-Varimex actometers.

Results: VPA rats demonstrated locomotor hyperactivity and stereotypic-like behavioral patterns. However, the prenatal exposure to VPA did not significantly affect any measure of 5-CSRTT performance, including accuracy, impulsivity (i.e., the premature responding), the speed of processing (i.e., the latency to correct response) or animals’ motivation (i.e., the latency to collect reward).

Conclusion: While prenatal VPA treatment enhanced locomotor and stereotypic-like activities, the attentional performance was not affected in the present experimental setting.

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Importance of α1A-adrenergic receptor subtype for desipramine-evoked CaMKII phosphorylation in mice


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Background: Various mental disorders, including depression, are associated with abnormalities in adrenergic signaling in the brain. Also many antidepressant drugs act on noradrenergic system. Among the adrenergic receptors the α1 family (α1-AR) consists of the α1A, α1B and α1D subtypes. All they are Gq/11 coupled receptors and activate phospholipase Cβ leading to the increase of intracellular Ca²⁺ level. This may cause activation of calcium/calmodulin-dependent protein kinase II (CaMKII), what is crucial for induction and maintenance of synaptic plasticity. However, the α1-ARs subtypes differ in transduction of intracellular signaling events. The study conducted in the model of transgenic mice engineered to express a constitutively active mutant form of α1A-AR or α1B-AR demonstrated that these two receptors differentially modulate antidepressant-like behaviors. Our previous studies also suggested the differential involvement of these adrenergic receptor subtypes in the chronic mild stress model of depression.

The aim of the current study was to evaluate the effects of selective knock-out of α1A- or α1B-AR and chronic antidepressant treatment on phosphorylation at Thr286 of CaMKIIα/β in prefrontal cortex of female mice.

Material and methods: Female knock-out mice devoid of α1A-AR (α1A-KO) or α1B-AR (α1B-KO) and wild type controls (WT) were chronically treated (21 days) with desipramine (20mg/kg) or saline. The protein level and phosphorylation ratio of CaMKII isoforms were analyzed in the prefrontal cortex by Western blot method.

Results: We found that both the deletion of α1A-AR and the deletion of α1B-AR did not affects the level of CaMKIIα/β phosphorylation. However after chronic treatment with desipramine, the deletion of α1A-AR prevents the increase in phosphorylation level of CaMKIIα/β which was observed in WT mice. This effect was not visible in the case of the α1B-AR deletion. There was no statistically significant influence of α1A-KO or α1B-KO or chronic treatment with desipramine on the total level of CaMKII protein.

Conclusions: Our results indicate different involvement of the α1A-AR and α1B-AR subtypes in the mechanism of action of the classical antidepressant drug desipramine. The α1A-AR, in contrast to the α1B-AR, appears to be necessary to obtain the proper effects of chronic desipramine treatment in prefrontal cortex of female mice.

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RELIEF FROM CHRONIC PAIN IN OSTEOARTHRITIC RATS: IMPACT UPON REWARD CIRCUITS AND THE ROLE OF ENDOCANNABINOID SYSTEM

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Background: Chronic pain is a main symptom of osteoarthritis (OA). What is more, a high percentage of OA patients suffer from mental health problems. Consequently, the management of OA remains a significant challenge. The endocannabinoid (EC) system has attracted attention as an emerging drug target for pain treatment together with its activity on mesolimbic reward system. Understanding of circuits that govern the reward of pain relief is crucial for search of effective analgesics. Therefore, we investigated the role of EC system upon dopamine (DA) and noradrenaline (NA) in OA-related chronic pain.

Materials and methods: Animal model of OA was induced through intra-articular injection of sodium monoiodoacetate (1 mg MIA). In order to increase EC tone, animals were injected intra-peritoneally with URB597, an inhibitor of EC degradation enzyme.

Results: OA rats exhibited significant decrease in DA metabolism in nucleus accumbens (NAc), striatum (STR) and hippocampus (HC). NA metabolism was also depleted by MIA injection; however, it was observed in frontal cortex (FCx) and hippocampus (HC). URB597 treatment reversed depression of dopamine metabolism in the reward brain system, potentiated NA depression in FCx and normalized the impairment of NA activity in the hippocampus.

Conclusions: Our results showed that chronic pain in OA is reflected by inhibition of mesolimbic dopaminergic transmission. It is possible that hypodopaminergia in reward circuits may be responsible for depression observed in OA patients. We also observed decrease in DA and NA levels in HC that was reversed following URB597 treatment. Similar effect is observed during neuropathic pain, where NA decrease is driven by proinflammatory cytokine TNFα (Ignatowski et al., 2005). On the contrary, NA release in FCx is able to facilitate chronic pain perception. In our study, we observed depletion in NA levels in FCx, which may serve as a homeostatic mechanism aimed at inhibition of pain perception. Increase in EC tone reversed MIA-elicited changes in neurotransmitter levels. To conclude, our data add-up to the understanding about changes in neurotransmission in chronic pain states and may explain the clinical improvement in perceived life quality following cannabinoid treatment. Increased mechanistic studies in preclinical models of the intersection between chronic pain and reward circuits may also offer new approaches for improvement of therapy.

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The effects of AGH-194, the 5-HT7 receptor agonist, in a ketamine-based rat model of cognitive dysfunction


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Background: Pharmacological tools, including selective antagonists and agonists of 5-HT7 receptor have revealed the involvement of this receptor in central nervous system processes. Recent reports suggest the efficacy of 5-HT7R ligands in overcoming cognitive impairments in animal models (Nikiforuk A., 2015 CNS Drugs 29:265–275).

One of the new synthesized compound, AGH-194 (4-fluoro-5-iodo-3-(1-methyl-1H-imidazol-5-yl)-1H-indole), is a highly potent (Ki ~ 2 nM) and selective 5-HT7 receptor agonist.

Materials and methods: The aim of the present study was to evaluate potential pro-cognitive efficacy of AGH-194, against 10 mg/kg ketamine-induced cognitive deficits in the attentional set-shifting task (ASST) in rats. In this paradigm, rats must select a bowl containing a food reward based on the ability to discriminate the odours or the media covering the bait. The ASST requires rats to initially learn a rule and form an attentional “set” within the same stimulus dimensions. At the extra-dimensional shift (ED), animals must switch their attention to a new, previously irrelevant stimulus dimension and, for example, discriminate between the odours and no longer between the media covering the bait. The ED phase, regarded as an index of cognitive flexibility, is impaired in animal models of schizophrenia, including those based on administration of antagonists of the N-methyl-D-aspartate receptor (NMDAR).

Results: The administration of ketamine (KET) significantly and specifically impaired rats’ set-shifting ability, manifested as an increased number of trials to criterion during the ED phase of the ASST.

The administration of AGH-194 (0.3 and 1 mg/kg) facilitated set-shifting performance in vehicle-treated controls and reversed set-shifting impairment in ketamine-treated animals.

Conclusions: These results support the therapeutic potential of 5-HT7 receptor agonists in the treatment of cognitive decline associated with schizophrenia.

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Antagonism of histamine H3 receptor reduced neuropathic pain in mice and enhanced morphine analgesia

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Background: Neuropathic pain is often refractory to usually effective pain relief drugs. The weakening effect of the analgesics has led scientists to seek new drugs and therapies. The expression of histamine H3R receptor in regions related to nociceptive transmission suggest a role of this receptor in the modulation of pain. The aim of our studies was to determine the analgesic effect of H3R antagonist in preclinical model of neuropathic pain (CCI, chronic constriction injury) in mice.

Materials and methods: We investigated the impact of newly synthesized H3R (E-162; 1-(5-(naphthalen-1-yloxy)pentyl)piperidine) antagonist on mechanical (von Frey) and thermal (cold plate) stimuli in CCI-exposed males and/or females 7 days post-injury, along with the possible participation of H1R in those effects. We carried out experiments to investigate the participation of H3R antagonist in morphine analgesia. To assess the in vivo metabolic stability of E-162 we used an in vitro model with mouse liver microsomes. We analyzed in vitro pharmacological profile of E-162 in binding and functional assays (cAMP) at H3R. We also revealed the presence of H3R in microglia and astroglia using primary cell cultures.

Results: We showed that E-162 reduced pain-related behavior and profound morphine analgesia in males. Interestingly, E-162-induced analgesia was reversed by pyrilamine (an H1R antagonist), pretreatment. Moreover, antagonist produced prolonged analgesia in neuropathic females. The analysis of the metabolic stability showed the presence of trace amounts of two metabolites of tested compound. E-162 demonstrated good affinity for H3R (K_i= 55nM) and it blocked the decrease in cAMP (IC_50= 165nM) and therefore was classified as an antagonist at H3R.

Conclusions: We provide the first evidence for the analgesic potency of newly synthesized H3R antagonist and its beneficial properties for morphine effectiveness during neuropathy. E-162 is promising drug candidate because it is metabolically stable and its structural homologue of pitolisant (Wakix™) is already used in the therapy of narcolepsy. Moreover, we have shown sex-related differences in pain sensation after administration of H3R antagonist, which is an important issue from a clinical point of view.

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Antinociceptive effects of zaprinast and its influences on opioids analgesia in neuropathic rats

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Background: According to IASP (International Association for the Study of Pain), neuropathic pain is caused by lesion or disease of somatosensory nervous system and affects 7-8% of adults worldwide. Management of neuropathic pain remains an important clinical problem because of resistance to opioid analgesic drugs and accompanying adverse effects. G protein-coupled receptor 35 (GPR35) recently has garnered interest as a potential therapeutic target for pain control, due its association in immune modulation in the nervous system. Therefore, the aim of our research were to investigate how zaprinast, that acts both an agonist of GPR35 and inhibitor of cyclic GMP (cGMP)-specific phosphodiesterase (PDE) impacts neuropathic pain.

Materials and methods: Chronic constriction injury (CCI) was performed in rats, according to Bennet and Xie (1998). Repeated intrathecal administration of zaprinast were conducted 16 h and 1 h before injury and then after nerve ligation daily for 7 days. The mechanical (von Frey test) and thermal (cold plate test) hypersensitivity were measured on day 7 post-CCI animals. Moreover, using Western blot we analysed the changes in glial cell markers in the spinal cord and DRG (dorsal root ganglia) tissue and measured protein levels of selected pronociceptive factors.

Results: Zaprinast attenuated the mechanical and thermal hypersensitivity on 7 day after CCI by reducing microglia activation. We demonstrated that zaprinast potentiated the analgesic properties of morphine and buprenorphine and inhibits the CCI-upregulated of IL-1β, IL-6, IL-18 and NOS2 in the spinal cord and in the DRG.

Conclusions: Our study extends the knowledge of the effects of zaprinast on neuropathy and highlights that modulation of Gpr35 could represent a promising target for neuropathic pain treatment in clinic.

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New 5-HT\textsubscript{7} receptor agonist has analgesic effect and enhances morphine analgesia in neuropathic pain

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Background: Neuropathic pain is initiated by a lesion or disease of the somatosensory system and affects 7-10\% of the general population. Conventional analgesics, such as opioids, not fully satisfy the clinical needs, because of modest efficacy and undesirable side effects. Therefore, there is a strong need to develop novel analgesics. A promising target for the development of a new analgesics is 5-HT\textsubscript{7} receptor, which is a member of the serotonin receptor family. The aim of our studies was to determine the analgesic effect of newly synthesized 5-HT\textsubscript{7} agonist in animal model of neuropathic pain.

Materials and methods: We investigated the effect of newly synthesized 5-HT\textsubscript{7} receptor agonist (AGH-194) on mechanical (von Frey) and thermal (cold plate) stimuli in chronic constriction injury to the sciatic nerve (CCI model) in mice. Single intraperitoneal injection of AGH-194 (5, 10, 20 mg/kg, i.p.) was performed at day 7 after CCI and then behavioural test were assessed 30, 90 and 180 min after drug administration. We also investigated the body temperature of mouse via a rectal procedure after AGH-194 administration. We analysed the influence of single AGH-194 (10 mg/kg) injection on morphine-induced analgesia.

Results: We showed that AGH-194 strongly diminished symptoms of neuropathic pain in CCI-exposed mice. The analgesic effect was time- and dose-dependent. What is also important, we did not observed any influence of AGH-194 on body temperature. Moreover, AGH-194 enhanced morphine-induced analgesia.

Conclusions: Our studies give evidence that newly synthesized 5-HT\textsubscript{7} agonist diminished neuropathic pain symptoms and has beneficial properties for morphine-induced analgesia. Our data indicate that 5-HT\textsubscript{7} receptor is as a promising molecular target in neuropathic pain treatment.

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Pharmacological blockade of CXCR3 by (±)-NBI-74330 reduces neuropathic pain

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Background: Millions of people worldwide suffers from neuropathic pain. There is still no efficient way of treat this pathology. It has been noticed that chemokines system plays a significant role in the pathophysiology of the neuropathic pain. The aim of the study was to investigate the impact of CXCR3 and its ligands (CXCL4, CXCL9, CXCL10, CXCL11) on the development of neuropathy and checked whether inhibition of the receptor might bring some beneficial properties.

Materials and methods: CXCR3 ligands were administrated intrathecally and behavioral tests (von Frey/cold plate) were performed in naïve mice. Using RT-PCR and Western blot, time-dependent changes of levels of CXCR3 and its ligands were measured after chronic constriction injury (CCI) of rats sciatic nerve. The CXCR3 antagonist [(±)-NBI-74330] were injected intrathecally (preemptively 16h and 1h before CCI and then once daily for 7 days) to CCI-exposed rats. Then the hypersensitivity were measured, using von Frey and cold plate tests. 6h after the last (±)-NBI-74330 administration, spinal cords were collected to perform the Western blot analysis.

Results: In naïve mice after all CXCL3 ligands injections, pain related behavior was observed. In CCI-exposed rats the time-course changes showed on the day 2nd upregulation of CXCR3, CXCL10 and CXCL11 and on the day 7th increased levels of CXCR3, CXCL4, CXCL9 and CXCL10. What is more the level of CXCL9 persisted higher until the day 28th. Our results indicate that (±)-NBI-74330 repeated injection reduced mechanical and thermal hypersensitivity development on the day 7, and in parallel diminished levels of IBA-1 positive cells and pronociceptive factors (IL-1beta, IL-18).

Conclusion: Our results proof that all CXCR3 studied agonists (CXCL4, CXCL9, CXCL10, CXCL11) have strong pronociceptive properties. It seems, that under neuropathic pain conditions CXCL10 and CXCL11 are important in the early phase of hypersensitivity development, in contrast CXCL9 might be responsible for the neuropathy persistence. Our data provides new evidence that CXCR3 may indeed play a significant role in the neuropathic pain development by modulating spinal neuroimmune interactions, suggesting that its blockade may have potential therapeutic utility.

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Maternal immune activation causes disturbances in IL-2 and IL-4 levels in the frontal cortex of young offspring rats.

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Background: Based on the epidemiological studies in humans, the immune hypothesis of schizophrenia has gained an increasing attention of researchers. Also, animal models of maternal infections (e.g. with bacterial agents) support the conclusion about the link between maternal immune activation during pregnancy and schizophrenia-related changes in the offspring. The crucial factor involved in the immune disturbances seem to be cytokines axis malfunction. Therefore, the presented study was designed to examine the impact of maternal immune activation on the protein levels of interleukin-2 (IL-2) and IL-4 in the frontal cortex (FCx) and hippocampus (Hp) of young offspring rats in the neurodevelopmental animal model of schizophrenia, based on the administration of lipopolysaccharide (LPS).

Materials and methods: Pregnant Wistar rats were injected subcutaneously with LPS (2 mg/kg of body weight) every second day from the 7th day of pregnancy until the delivery. The control group received the appropriate amount of vehicle. At the age of 7 days, the offspring male rats from both control and LPS-treated mothers were sacrificed and frontal cortices and hippocampi were dissected. The levels of IL-2 and IL-4 in FCx and Hp were measured using RayBio® Cytokine Antibody Array.

Results: The obtained results revealed that the young offspring rats after prenatal exposure to LPS exhibit decreased concentrations of both IL-2 and IL-4 in the frontal cortex. In the hippocampus, the protein levels of examined cytokines were not affected, however, the overall amounts of IL-2 and IL-4 were much higher than in the FCx.

Conclusions: In summary, our study indicated that prenatal exposure to LPS causes disturbances in some cytokines levels in the frontal cortex of young offspring rats. Nevertheless, the exact role of these malfunctions in the development of schizophrenia in the adult life requires further investigation.

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Fractalkine modulates neurons activity in the rat amygdala in neuron- and microglia-dependent manner

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**Background:** Beyond their classical chemotactic functions, chemokines, including fractalkine, have been implicated in many neurobiological processes potentially relevant to psychiatric disorders. One of their potential mechanisms may be disturbed neuron-microglia crosstalk. Yet, recognition and characterization of chemokine effects on neurophysiology are still lacking. Therefore, this study aimed to evaluate the effects of fractalkine on basal membrane neurons properties, their excitability and synaptic transmission in the rat basolateral amygdala.

**Materials and methods:** Whole-cell patch-clamp recordings were made from principal neurons in the rat basolateral amygdala using acute brain slices (300\textmu m). After recording a baseline, fractalkine (2nM) was bath-applied. Both inhibitory and excitatory synaptic transmission were measured by recording spontaneous (sIPSC/sEPSC) and miniature (mIPSC/mEPSC). Specificity of observed effects was investigated using the same experimental protocol with additional incubation in a CX3CR1 antibody or minocycline (inhibitor of microglia activation).

**Results:** Fractalkine increased the frequency of spontaneous EPSC in the CX3CR1-dependent action. Incubation in minocycline did not abolish this effect, indicating that microglia was not involved in this phenomenon. Interestingly, the miniature excitatory transmission was not altered, suggesting indirect, action potential-dependent mechanism of fractalkine. The spontaneous IPSC amplitude was decreased by fractalkine application, which was CX3CR1- and microglia- dependent.

**Conclusions:** Our results suggest multifaceted effects of fractalkine application in the basolateral amygdala, indicating that this protein can be an active modulator of neuronal activity in the fear-related response circuitry, which may have significant scientific and therapeutic implications.

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Hyperforin potentiates antidepressant-like activity of NMDA receptor antagonist – lanicemine

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Background: Ketamine induces rapid and sustained antidepressant effects in humans. Unfortunately, ketamine evokes unwanted side effects. Therefore, alternative strategies for the treatment of depression are sought. The objective of the present study was to evaluate the antidepressant effects of hyperforin or lanicemine given alone and in combined treatment in the naive female and male mice and in male mice exposed to chronic corticosterone treatment. Moreover, the effects of hyperforin, lanicemine and combined administration of hyperforin and lanicemine on the expression of selected proteins in the frontal cortex were determined. Finally, the involvement of Ca²⁺ signaling pathway in the hyperforin’s antidepressant-like activity was studied.

Methods: To evaluate the antidepressant-like activity of hyperforin and lanicemine in vivo (tail suspension test, TST; forced swim test, FST and splash test) and in vitro (western blotting, Ca²⁺ imaging studies) methods were employed.

Results: Combined administration of hyperforin and lanicemine evoked long-lasting antidepressant-like effects in both male and female naive mice and in male mice subjected to chronic corticosterone administration. Joint administration of hyperforin and lanicemine enhancing the expression of the synapsin I, GluA1 subunit and BDNF proteins in the frontal cortex. In Ca²⁺ imaging studies, lanicemine enhanced Ca²⁺ influx induced by hyperforin.

Conclusion: The results obtained in this study showed that combined treatment with hyperforin and lanicemine induces a long-lasting antidepressant-like activity in mice. The mechanism of action of hyperforin + lanicemine indicates a Ca2+-dependent process.

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Big Data Challenges and Opportunities for Precision Medicine

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The massive generation of sequencing data around specific diseases and clinical phenotypes is driving biomedical research into characterization and deeper understanding of common biological changes at the molecular and cellular level. Advances in genomics, transcriptomics, and the availability of Big Data has transformed the medical sciences. New high-throughput data analysis allows to obtain, manage and analyze many datasets. The bioinformatics methods and tools significantly contribute to the better understanding of the diseases and their mechanisms, the discovery of more effective treatment, based on the patient’s genetic profile, which has a significant impact whether patients will respond well to specific treatment. These approaches that combine the discovery and the functional interpretation of the genomic variability associated to disease are generating the basis of Personalized Medicine. In order to accomplish the objectives, the bioinformatics has to face several challenges. Currently, personalized medicine is based on the identification of biomarkers that mostly consist on individual mutational events, based on statistical associations to disease progression or treatment responses. Moreover, it is crucial in the early and precise diagnosis of serious diseases and the development treatment, and include study on tackling mental illness, intestinal bacteria, RNA therapeutics, gene therapies, and immunotherapy.

Here we will discuss how low informative, gene expression and gene variation data can be integrated and transformed into mechanism-based biomarkers containing higher-level information on the molecular mechanisms that determine disease outcome or drug response. In next step, how these models can be used to find systemic disease drivers and to propose the development of knowledge-based treatment, which include RNA therapeutics, gene therapies, and immunotherapy.
Mesoporous silica nanocarriers conjugated to folic acid for enhancing cancer targeting with anticancer natural prodrugs

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Background: Thousands of naturally derived prodrugs show pharmacological activities (i.e. anticancer, antioxidant, anti-inflammatory and antiviral effects). However, there are significant limitations that inhibit their use in medicine (e.g. hydrophobicity, low bioavailability, lack of specific targeting, etc.). We developed a simple and effective nano-delivery system consisting of conjugated with folic acid (FA) mesoporous silica nanoparticles (MSNs), loaded with three anticancer natural prodrugs, to enhance cellular uptake, cancer targeting, and anticancer activities.

Materials and Methods: Two types of MSNs investigated: KCC-1 type, and MCM-41 type, were functionalized with amino-groups, and further conjugated with FA. They were loaded with three anticancer prodrugs: curcumin, quercetin, and colchicine. Several characterization techniques (FE-SEM, HR-TEM, XRD, FTIR, TGA-DSC, nanoparticles tracking analysis, and zeta potential) were employed. Human hepatocellular carcinoma cells (HepG2) and HeLa cancer cells were used for in vitro tests. The cellular uptake and sustained drug release were observed by SEM and confocal laser scanning microscopy. The mechanism of anticancer effects was explored by evaluating signaling pathways (caspase-3, H2O2, c-MET, and MCL-1).

Results: The most important finding was that FA-conjugated MSNs showed efficient cellular uptake, sustained intracellular release, and anticancer activity. Folic acid conjugation plays a crucial role and their efficiency was high in comparison to non-conjugated MSNs and amine-functionalized MSNs. The amine-functionalized and FA-conjugated curcumin-loaded KCC-1 MSNs especially efficiently penetrated all cells organs and steadily released curcumin compared to MCM-41 MSNs. The KCC-1 type MSNs loaded with curcumin prodrug indicated the highest anticancer activity against HepG2 cells, which was confirmed also on HeLa cells. The main anticancer effect is apoptosis induced through specific signaling molecular pathways: caspase-3, H2O2, c-MET, and MCL-1.

Conclusions: Conjugated with folic acid nano-porous silica mesospheres carrying curcumin and other pro drugs are efficient anti-cancer targeted drugs during in-vitro studies. The proposed safe and inexpensive natural prodrugs offer new possibilities for targeted cancer therapy.

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Expression of serotonin 5-HT2C receptors in animal model of comorbid depression and cocaine addiction
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Background: Several clinical reports indicate a high comorbidity between depression and substance use disorders (SUD). 5-HT2C receptors play an important role in the regulation of monoaminergic transmission, modulate behavioral effects of abused drugs and alterations in their functional status have been detected in depressive states. The 5-HT2C receptors are currently considered as a promising target for improved treatments of cocaine abuse with comorbid depressive disorders.

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 animals underwent cocaine self-administration followed by 10-days extinction procedures during which the animals received saline instead of cocaine; there was no presentation of the conditioned stimuli. To distinguish the effects of cocaine, the yoked procedure was used. Yoked controls received saline infusion each time that their active cocaine counterpart received a cocaine infusion. Immediately after the last session, rats were decapitated their brain were quickly removed and selected structures were dissected according to the Rat Brain Atlas. The expression of 5-HT2C receptors was analyzed using Western blot.

Results: Removed olfactory bulbs caused higher expression 5-HT2C receptors in ventral hippocampus, but cocaine self-administration did not change the expression of this receptors in the SHAM and OBX group. After extinction phase the expression of 5-HT2C receptors was significantly higher in the dorsal medial striatum only in OBX group which had previously been self-administered cocaine.

Conclusions: These results suggest that 5-HT2C receptors play an important role in drug relapse in rats with depression and cocaine use disorders. In addition, these receptors may be the target for new therapeutic strategies.

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Effect of reboxetine treatment in lipopolysaccharide (LPS)-stimulated microglial cells

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Background: The implication of the immune system and its mediators, cytokines, in etiology of depression has been investigated since many years. Microglia cells are the main immunocompetent cells in the central nervous system. They are capable of migration, proliferation and phagocytosis, participate in antigen presentation, synthesize nitric oxide (NO), and are the main source of pro-inflammatory cytokines (IL-1β, IL-18, IL-6 or TNF-α) in the brain. As they are the main source of inflammatory factors, it is plausible that the regulation of their activation may be a potential therapeutic target. Reboxetine, a widely used antidepressant, has been shown to have potential antiinflammatory activity, but the underlying mechanisms remain obscure. So, the aim of this study was to assess the effect of antidepressant drug - reboxetine - on the chosen parameters of microglial activity in basal conditions and after lipopolysaccharide (LPS) stimulation.

Materials and methods: Female Sprague-Dawley rats were paired with males. Pregnancy was confirmed the next morning. Pregnant rats were subjected to three stress sessions from 14th day of pregnancy until the delivery. Primary microglia cultures were prepared from cortices of 1-2 day old offspring. Reboxetine were added at a dose of 0,1-50 μM for 24 hours. Moreover, the microglia were exposed to nonspecific immune system activator - LPS in the concentration of 100 ng/ml. Cell viability was determined by MTT test, while cell death by LDH test. Nitric oxide (NO) synthesis was assessed by Griess reaction.

Results: Administration of reboxetine at a dose of 5 μM resulted in an increase of cell viability measured by MTT test and decrease mortality measured by LDH test of the LPS-stimulated microglia cells. We noticed also an increase in NO synthesis in cells stimulated with LPS, which was also normalized by administration of reboxetine at a dose of 5 μM

Conclusions: Obtained results indicate that antidepressant – reboxetine may have an important influence on microglia cells activation. They modify the viability and death processes of these cells and can decrease their proinflammatory profile.

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Glucocorticoid receptor translocation in frontal cortex of mice in model of fear devaluation in PTSD

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Background: Posttraumatic stress disorder (PTSD) appears through sensitization of fear response to neutral sensory stimuli associated during traumatic experience. Stress increases the release of corticosteroids which transmit and regulate stress signal by binding to glucocorticoid receptors (GR). At the cellular level regulation of stress response seems to rely on proper functioning of GR translocation from cytoplasm to nucleus where GR act as a transcription factor. Hsp90 chaperone protein link GR to transporting machinery and was shown to be engaged in mechanism of stress resilience. Previous study in PTSD model revealed that sensitized behavioral stress response (freezing) was normalized when mice were re-exposed to less intense footshock and this restoration required intact frontal cortex. To assess whether changes in GR translocation are involved in the restoration of fear regulation, in the current study we measured cytosolic and nuclear level of GR and Hsp90 in frontal cortex in mouse model of PTSD and after fear devaluation procedures.

Material and methods: Male C57BL/6N mice were submitted to a fear conditioning (FC) training. After 21 days break mice went through post-conditioning treatment as follows: 2 groups FC 1d, FC 5d had recalled traumatic memory by FC application for 1 or 5 days; next PT 1d, PT 5d groups were treated with less intense footshock (pain threshold); last Ext 1d, Ext 5d groups went to standard extinction procedure. Naive mice served as a control group. Biochemical analyses were performed 1 h after last treatment. Serum corticosterone was assessed by ELISA. Frontal cortex proteins were fractionated and standard Western Blot was performed to measure GR, Hsp90 level in each fraction.

Results: Increased corticosterone vs control level was observed only in FC 5d group. There were no changes in the expression of GR among studied groups in cytosolic and nuclear fraction. However, cytosolic level of Hsp90 was decreased in Ext 5d and PT 5d groups.

Conclusions: The decrease in the expression of Hsp90αβ in the cytosolic fraction of frontal cortex of mice with devalued fear suggests a decreased availability of this protein to interact with GR and its translocation to nucleus.

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Changes in phentolamine binding potency in mice with α1A– or α1B–adrenergic receptors knock-out

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**Background:** All three subtypes of α1–adrenergic receptor (AR), α1A, B and D, are widely distributed in the central nervous system. They all function as a stimulatory receptors coupled to Gq/11 and phospholipase Cβ, but are encoded by a separate gene. Each subtype displays distinct pharmacological properties. The discrimination between receptor subtypes is difficult because of the lack of sufficiently specific ligands. In this study we used genetically modified mice to determine if the lack of one receptor subtype, α1A- or α1B-AR, may influence binding potency of phenylephrine (an agonist) and phentolamine (an antagonist) to the remaining pool of α1–ARs.

**Material and methods:** The ex vivo experiment was conducted on C57Bl6/J male mice (n = 4 / group) with knock-out of α1A- or α1B-AR (KO) and on their wild type (wt) littermates, respectively. The cerebral cortices were isolated and the membrane preparations (P2 fraction) were prepared. To characterize interaction of phenylephrine or phentolamine with the pool of α1–ARs, nine concentrations of the drug (10^{-11} M to 10^{-4} M) were incubated in the presence of radioligand [3H]prazosin (0.9 nM). All measurements were carried out in duplicate and the experiment was repeated two times. The results as the binding affinity (K_i) and half maximal inhibitory concentration (IC50) were calculated from a sigmoid curve using GraphPad Prism.

**Results:** We found phenylephrine to be equipotent in displacing of specific ligand for remaining α1-AR, in both types of mutants. The difference was observed in case of phentolamine binding parameters. In α1B-KO, the IC50 for phentolamine was decreased compared to wt littermates (0.48 nM vs. 7.99 nM) and K_i values were 0.29 nM and 4.87 nM, respectively. In α1A-KO, the changes were opposite and an increase of IC50 (6.35 nM vs. 2.21 nM) and K_i values (3.87 nM vs. 1.35 nM for wt) were observed.

**Conclusions:** The mutation changes the binding potency of phentolamine to cerebral cortical α1–ARs. In the case of α1B-KO, the antagonist showed higher affinity to the remaining pool of α1-ARs (α1A- and α1D-ARs) while the α1A-KO resulted in a reduced affinity of phentolamine to the remaining α1B- and α1D-ARs. The results support our previous observation regarding differences in the affinity of another α1-AR antagonist, WB4101, to the cerebral α1A- and α1B-ARs subtypes in unmodified rats.

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Brain serotonin deficiency alters social behaviours in social interaction test

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Background: Brain serotonin levels affect central nervous system processes, including social behaviour. Tryptophan hydroxylase 2 (TPH2) is a rate-limiting enzyme of serotonin synthesis. Therefore, TPH2 - deficient (Tph2/-) rats represent a valuable model to study the consequences of central serotonin depletion. The goal of the current study was to examine two groups of rats Tph2/- (KO) and Tph2+/+ (WT) in the social interaction test (SIT).

Materials and methods: Social behaviour was characterized in two groups of male Tph2+/+ (N=16) and Tph2/- (N=15) rats, created using zinc-finger nuclease technology. Social interaction test was performed in an open field arena. The behaviour of the rats was recorded using the camera placed above the arena and connected to a Noldus MPEG recorder 2.1. On the test day, two unfamiliar rats of matched genotype and body weight (± 5 g) were placed in the open field arena, and their behaviour was recorded for 10 min. The social interaction time was measured for each rat separately. The following active social behaviours were scored: sniffing (the rat sniffs the body of the conspecific), anogenital sniffing (the rat sniffs the anogenital region of the conspecific), social grooming (the rat licks and chews the fur of the conspecific), following (the rat moves toward and follows the other rat), mounting/climbing (the rat stands on the back of the conspecific/the rat climbs over the back of the conspecific). The time of active social behaviours was summed to yield a total score. Additionally, copulatory-like behaviours were observed in Tph2/- animals.

Results: This study shows that Tph2/- rats spent less time in social interactions and demonstrated less episodes of social behaviours. Additionally, Tph2/- animals demonstrated sexual, copulatory-like behaviours. During the social interaction test there were no episodes of aggressive behaviours (fighting) in either Tph2/- or Tph2+/+ rats.

Conclusion: The present study further confirms the role of serotonin in the regulation of social behaviour.

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Influence of antimicrobial plant root extracts on activity of teeth-derived stem cells

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Background: After caries, paradontosis is the most frequently occurring disease of the oral cavity. It is defined as a chronic inflammation of teeth supporting tissues - gums, periodontal ligaments and alveolar bone. Almost 700 species of bacteria have been identified in the oral cavity; many of them seem to be responsible for periodontitis. Extracts from Pelargonium sidoides roots is used in paramedicin to treat many diseases. Studies have shown antimicrobial properties of the extracts preventing adhesion of microbes to mucous membranes of a host. However, little is known about influence of such extracts on adult or stem cells present in teeth tissues. The aim of the study was to establish effects of P. sidoides root extracts on proliferation, metabolism and cell cycle of teeth stem cells in vitro.

Materials and methods: Periodontal Ligament Stem Cells (PDLSC) and Dental Pulp Stem Cells (DPSC) were obtained from healthy third molars and cultured in Complete Growth Medium (CGM). Next, the population was divided into the following experimental groups: I) cells cultured in fresh CGM (Control), II) cells treated with 0.01% P. Sidoides Root Extract (PSRE) and III) cells treated with 0.01% ProAnthoCyanidinS (PACS) dissolved in CGM. Principally, the cells have been observed for a week using phase-contrast microscopy followed by MTS viability test. Next, flow cytometry analyses of cell cycle and mitochondrial membrane potential (MMP) were performed to define PSRE and PACS influence on growth and metabolism of PDLSCs and DPSCs after 24h of treatment.

Results: Cell viability test has shown strong cytotoxicity of 0.01% PSRE and PACS after 7 days of treatment. 24h culture of both cell types in 0.01% PSRE and PACS revealed cell cycle arrest in G0/G1 or G2/M phase. Concomitantly, examination of MMP has shown its strong increase in relation to cells of Control group.

Conclusions: The results revealed P. sidoides root extracts active in relation to PDLSCs and DPSCs proliferation and metabolism. Short time exposure of cells to the extracts caused inhibition of cell growth due to arrest of their cell cycle. However, their metabolism increased in relation to untreated cells. Little is known about the mechanism of PSRE and PACS activity but due to their cytotoxicity, the principal strategy of cells treated with P. sidoides root extracts might be stimulation of their anti pathways.

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Behavior, enrichment and transport, the breeder's strengths

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The behavior of the animal reflects its adaptation to the environment. A scrupulously observation, on a daily basis, makes possible to determine, at least in a part of this, the level of comfort and the level of adaptation the animal can feel into the environment it is asked to live in.

The environment enrichment provided to our lab animals not only allows the animal to better reproduce its natural instincts, but also avoids the appearance of typical pathologies, especially pathologies related to stress. A change of environment is therefore experienced by lab animals as a stressful situation that alters its vital functions and thus affects its metabolism.

Each of these stages is fully experienced by laboratory animals during their life cycle. We now know the harmful effects that stressful situations can generate in laboratory animals. Also, researchers fear concrete consequences on the results they can collect from animals involved into such conditions.

It is the responsibility of the laboratory animal breeders to implement surveillance measures, provide the necessary enrichment and carry out a transport policy that could meet each of those expectations.
Comparative evaluation of new dihydropyrimidine and dihydro(pyridine) derivatives perturbing mitotic spindle formation

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**Background:** The mitotic spindle plays a key role in cell division which makes it an important target in cancer therapy. In the present study the antiproliferative activity of 4-benzyl-5-phenyl-3,4-dihydropyrimidine-2(1H)-thione (compound 1) and its pyridine bioisostere 4-benzyl-5-phenyl-3,4-dihydropyridine-2(1H)-thione (compound 2) were evaluated and compared with monastrol (MON), the first known cell-permeable small molecule which disrupts bipolar spindle formation by inhibiting Eg5-kinase activity.

**Material and methods:** All *in vitro* experiments were performed in two cancer cell lines: human malignant melanoma (A375) and human breast adenocarcinoma (MCF7). The antiproliferative activity of the compounds were assessed using Cell Proliferation Reagent (WST-1 assay) and analysis of cell cycle arrest by flow cytometry. To confirm the molecular target confocal microscopy imaging of mitotic cells as well as the cell-based and cell-free tubulin polymerization assay were performed. To estimate the selectivity of the tested compounds against kinesins, the microtubule-activated kinesin ATPase assay was performed.

**Results:** Our data revealed that from both tested derivatives the compound 2 showed higher antiproliferative activity than MON against MCF7 and A375 cell lines (IC\textsubscript{50} values 2.5 and 5.8-fold lower in comparison to MON), and comparable reversible cell cycle inhibition in G2/M phase. However, compound 2 produced distinct phenotype from monoastral spindles, inhibited tubulin polymerization and did not affect Eg5 ATPase activity.

**Conclusions:** Our data revealed that not only pyrimidine compounds like MON but also pyridine derivatives have promising anticancer activity. The activity of compound 2 may suggest its new promising anticancer mechanism targeting another than MON component required for spindle bipolarity. Development of a more specific approach in antimitotic treatment, distinct from not efficient enough and broadly toxic conventional drugs, might contribute to improved therapy outcomes and decreased side effects.
Compromised identity of pancreatic islet cells – role of stearoyl-CoA desaturase 1 in epigenetic control of gene expression

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Background: Type 2 diabetes (T2D) is a complex metabolic disorder, the worldwide prevalence of which is growing rapidly. Recent genetic and epigenetic studies indicate that not only β-cell demise, but also the loss of pancreatic endocrine cell identities are major causes of the development of T2D. The identity of insulin- and glucagon-secreting cells in pancreatic islets is maintained by dynamic control of specific transcription factors (TFs) expression. It has been shown that stearoyl-CoA desaturase 1 (SCD1), the rate-limiting enzyme in monounsaturated fatty acids synthesis, can regulate gene expression through changes in DNA methylation level. In the present study we tested the hypothesis that SCD1 affects expression patterns of TFs involved in maintenance of pancreatic islet cells identity via epigenetic mechanisms.

Materials and methods: The experiments were conducted ex vivo on Scd1 knock-out mice (SCD1 KO) and in vitro on INS-1E β-pancreatic cell line. Changes in SCD1 gene expression/activity in INS-1E cells were introduced using the SCD1 inhibitor (A939572), siRNA and plasmid vectors, respectively. Lipotoxicity was induced by palmitic acid (0.4 mM) treatment.

Results: Our data show that pancreatic islets of SCD1 KO mice are characterized by lower insulin secretion and different microarchitecture comparing with wild type mice. Furthermore we observed that SCD1 deficiency decreases protein level of TFs crucial for maintenance of β-cells identity (Pdx1, FoxO1, Isl1), and increases of those characteristic exclusively for α-cells (glucagon, Arx). In contrast SCD1 overexpression increases expression of Pdx1, FoxO1 and Pax6 in INS-1E cells. Furthermore, we noticed that inhibition of SCD1 activity as well as silencing of SCD1 gene expression, lead to global DNA hypomethylation and decrease in methyltransferase 1 (Dnmt1) protein level in INS-1E cells. In addition, we also observed significant changes in methylation pattern within promoter regions of Pdx1 and MafA.

Conclusions: Obtained results suggest that SCD1 affects expression of identity TFs in pancreatic islets via alterations in DNA methylation pattern. Therefore, SCD1 plays an important role in maintenance of pancreatic islet cells functional identities.

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Overexpression of stearoyl-CoA desaturase 1 affects NAD homeostasis and oxygen consumption in skeletal muscles

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**Background:** Stearoyl-CoA desaturase 1 (SCD1) activity is essential for the regulation of lipid-mediated metabolic pathways and energy metabolism. Global Scd1 knockout in mice leads to hypermetabolic profile that results in improved insulin sensitivity and protection from diet induced obesity. The molecular mechanisms underlying this phenomenon remain elusive.

**Materials and methods:** Considering skeletal muscles as the main site of insulin resistance development, we used OMICs approach to examine muscle transcriptome changes upon Scd1 knockout/overexpression in mice skeletal muscle. We performed Gene Set Enrichment analysis on transcriptomics data followed by Cytoscape Enrichment Map visualization. Moreover, we measured AMP/ATP and NAD⁺/NADH turnover in muscles and metabolic performance of animals using PhenoMaster metabolic cages.

**Results:** We observed that oxygen consumption and heat production are decreased in mice with muscle-specific Scd1 overexpression (Scd1Tg) compared to wild type C57BL6 mice. Moreover, transcriptional analyses revealed that Scd1 overexpression leads to downregulation of PI3K, AMPK, insulin signaling and glucose transport pathways in skeletal muscle. In particular, we observed decreased Nampt gene expression in Scd1Tg group and lower NAD⁺/NADH and AMP/ATP ratios in Gastrocnemius muscles.

**Conclusions:** Our results indicate that targeted overexpression of Scd1 in skeletal muscles plays an important role in regulation of nucleotide metabolism and affects total energy expenditure. The plausible mechanism is related to changes in the AMP/ATP and NAD⁺/NADH ratios that are crucial for regulation of intracellular metabolic pathways, including AMPK pathway. Our findings reveal a novel mechanism that links SCD1 with the maintenance of metabolic homeostasis and insulin sensitivity in skeletal muscle.

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Biodegradable medical devices for the controlled release of antimicrobial peptides

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Background: Natural and synthetic antimicrobial peptides (AMPs) are new therapeutic options for treatment of multiple-strain infections. However, clinical and commercial development of AMPs has some limitations due to their limited stability, low bioavailability, and potential hemotoxicity. One particular approach towards an improved use of AMPs for therapeutic applications could be biodegradable macromolecular delivery systems: sustained release matrix tablets and polymeric microparticles.

Materials and methods: The tested polymers were homo- and copolymers of rac-lactide and ε-caprolactone synthesized in various conditions by enzymatic ring-opening polymerization. Microstructural (digital microscope, SEM, non contact laser profilometer), structural (NMR, FT IR, MALDI-ToF MS), thermal (DSC, TGA) and biological (MTT, fluorescent microscopy analysis performed on mouse fibroblast cell line) properties of the obtained materials were evaluated. The peptide-loaded microparticles were prepared from polyesters matrices using the solvent evaporation method. The polymeric tablets were obtained by dissolved the polymer in DCM under argon atmosphere. Then, the AMPs was slowly added to the vigorously stirred polymer solution. The samples were dried and the tablet discs were prepared. The in vitro release kinetics of peptide from the new devices was investigated.

Results: In our studies we obtained short- and medium-term peptide delivery systems. We found that the peptides were released with high control, according to the polymer degradation mechanism with near-zero-order kinetics.

Conclusions: This research provides a basic to development of novel biodegradable medical devices for treatment of local infections.

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Design, synthesis and biological evaluation of dually acting MAO-B/5-HT_6 receptor modulators

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Background: Alzheimer’s diseases (AD) is a progressive neurodegenerative disorder that impairs cognitive function, memory, and leads to dementia in late stage of life. The cause of AD remains not fully understood, and different strategies have been proposed as potential therapies. One of them consists in designing of multi-target compounds. The present study reports a new approach based on dual inhibition of monoamine oxidase type B (MAO-B) and serotonin type 6 receptor (5-HT_6R).

Materials and methods: A hybrid approach, involving in silico structure optimization, was applied for designing of dually acting modulators. Primary evaluation of the obtained compounds consisted in determination of their affinity for 5-HT_6R and inhibition of MAO-B, subsequent selectivity screening for structurally related GPCRs and MAO-A.

Results: The study allowed for the identification of dually acting compounds capable of inhibiting MAO-B and targeting 5-HT_6R with acceptable selectivity over structurally related targets.

Conclusions: Based on a multi-ligand approach, dually acting compounds modulating both symptomatic (5-HT_6R) and disease-modifying (MAO-B)-related targets were successfully developed. A better understanding of effects produced by MAO-B/5-HT_6 modulators may impact future development of neurodegenerative-directed treatment strategies.

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Prenatal exposure to benzophenone-3 induces apoptosis in neocortical neurons of offspring

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Background: Benzophenone-3 (BP-3) is the most commonly used UV filter. In September 2017, according to scientific reports, the European Union has limited the use of BP-3 from 10% to 6% in cosmetic sunscreen products. BP-3 is not only a commonly used sunscreen agent but also an ingredient in plastic, color agents, textiles, inks and lacquers and in many other products such as perfumes. Population studies have shown that at least 97% of human population are exposed to BP-3. What is worse, BP-3 easily penetrates into our body through the skin and is excreted only in a small amount. The cellular barriers in the human body, such as the placental and blood–brain barriers, do not seem to be an obstacle for BP-3. Individual doses of BP-3 from each cosmetic application accumulate and may have an adverse effect on the human body since BP-3 has been found in human fat tissue in high concentration (~ 5 mg BP-3/kg of fat tissue). Particularly sensitive to the harmful effects of BP-3 can be the brain which consists of at least 60% of adipose tissue.

Materials and Methods: To determine the involvement of prenatal exposure to BP-3 in fetal origins of adult nervous system disease, we pretreated embryos with BP-3 and then isolated cells from those embryos for primary neocortical culture. To verify apoptotic and neurotoxic capacity, lactate dehydrogenase (LDH) release, caspase-3 activity, mitochondrial membrane potential and the expression of apoptosis-related factors (by qPCR, microarray, ELISA and western blot) were measured.

Results: Treatment of pregnant mice with BP-3 (50 mg/kg) evoked caspase-3 activation and LDH release as well as substantial loss of mitochondrial membrane potential in neocortical cells from their embryonic offspring. Intensification of apoptosis in embryos treated with BP-3 was also evidenced by an apoptosis-focused microarray analysis, which revealed up-regulation of 22 genes involved in apoptotic cell death. This effect was supported by increased BAX and CASP3 mRNA and protein levels, as evidenced by qPCR, ELISAs and western blots [1].

Conclusions: Taking into account the above data, we hypothesize that BP-3 is prenatal risk factor and may be the fetal basis of the adult onset of the nervous system disease.

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The effect of antidepressants on cytochrome P450 2D in the chronic mild stress model of depression

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Background: Liver cytochrome P450 subfamily 2D (CYP2D) contributes to the metabolism of many drugs, carcinogens and neurotoxins. Brain CYP2D isoforms play an important role in the local metabolism of neuroactive substrates (neurotransmitters, neurosteroids, psychotropics). Physiological regulation of CYP2D differs between organs. Liver CYP2D is non-inducible, while brain CYP2D is induced by different drugs and substances of abuse. Drug- and time-dependent interactions between psychotropics and CYP2D were observed in the liver. Recent studies suggest that psychological stress can modify CYP2D expression in the liver in a stress-specific way. The aim of present study was to investigate the effects of chronic treatment with escitalopram and venlafaxine on the activity of CYP2D in the rat brain and liver, in normal conditions and under chronic mild stress (CMS), an animal model of depression.

Material and methods: The experiment was carried out on male Wistar rats. Escitalopram or venlafaxine (10 mg/kg/day ip) were administered to control and CMS rats for 5 weeks. The activity of CYP2D was studied by measurement of the rate of bufuralol 1’-hydroxylation in microsomes derived from the liver or several brain structures (HPLC). Besides, the protein level (Western blotting) and mRNA level (qRT-PCR) of liver CYP2D were estimated. The data were statistically analyzed using a factorial ANOVA, followed by post-hoc Duncan’s or Fisher’s tests.

Results: CMS caused an increase the brain CYP2D activity in the hippocampus. Chronic escitalopram or venlafaxine raised the enzyme activity in the frontal cortex, hypothalamus and cerebellum of stressed rats without affecting the enzyme in nonstressed animals. CMS had no effect on the liver CYP2D activity, while chronic antidepressants significantly decreased the enzyme activity and protein level in both experimental groups.

Conclusions: The obtained results indicates that CMS increases the CYP2D activity in the hippocampus and triggers the stimulatory effect of antidepressants in other brain structures. Thus the local brain metabolism of CYP2D neuroactive substrates (neurotransmitters, neurosteroids, psychotropics) may be intensified by CMS and/or antidepressants. Whereas in the liver, chronic escitalopram or venlafaxine may slow the metabolism of CYP2D substrates.

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Effectiveness of nanoconjugate of transferrin with alloyed quaternary nanocrystals Ag-In-Zn-S in cancer therapy

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Background: The difficulty issue of cancer treatment consists of a number of factors, including a plurality of reasons (often not fully identified). This fact involves: significant biochemical similarity of tumor cells versus healthy cells, and the resulting low selectivity of therapy and/or high complexity of molecular mechanisms of action described for known therapeutic agents. Drugs applied in chemotherapy often exhibit cytotoxic activity against both tumor and healthy cells. This fact is the main driving force of research aimed at reducing / elimination the cytotoxic activity of the anticancer drugs toward the healthy cells. One way to limit the negative effects of anti-tumor drugs on healthy cells is targeted therapy employing functionalized drug carriers. Tumor cells have a large number of transferrin receptors on the surface of their cytoplasmic membranes, many times greater than in healthy cells. This fact of overexpression of transferrin receptors has made it possible to support the selective transport of anticancer drugs.

Materials and methods: The studies included the synthesis of nanoconjugate doxorubicin-transferrin-quantum dot and its quantitative and qualitative characteristic by using voltammetric and spectroscopic methods.

Results: Here we present a biocompatible and stable nanoconjugate of transferrin anchored to Ag-In-Zn-S quantum dots modified with 11-mercaptoundecanoic acid (Tf-QD) as a drug carrier versus typical anticancer drug, doxorubicin. Detailed investigations of Tf-QD nanoconjugates without and with doxorubicin by fluorescence studies and cytotoxic measurements showed that the biological activity both the transferrin and doxorubicin was fully retained in the nanoconjugate.

Conclusions: The obtained results clearly proved that after binding to the Tf-QD nanoconjugate, the DOX molecules are still capable of intercalating the ctDNA helixes. The cytotoxic studies showed that QD alone and in the form of nanoconjugate with Tf were not cytotoxic towards human non-small cell lung carcinoma (H460 cell line) and the tumor cell sensitivity of the DOX-Tf-QD nanoconjugate was comparable to that of doxorubicin alone.

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Acetylcholine determination in biological samples – new aspects of analytical calibration

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Background: Acetylcholine (ACh) is a neurotransmitter that works in the peripheral and central nervous systems. The ACh is responsible for the activation of muscles in the neuromuscular junction, while in the brain it is involved in processes such as arousal, attention and motivation. The deficiency of ACh results in the development of diseases such as myasthenia gravis or Alzheimer's disease. From a clinical point of view, it is very important to use appropriate methods that enable the most accurate detection and determination of this neurotransmitter in biological samples.

Materials and methods: The determination of ACh in different rat brain structures was carried out with the use of microdialysis in free-moving animals. This method provides small sample volumes with low concentrations of the analyte. The assay was performed using HPLC with electrochemical detection using the Alexys Antec neurotransmitter analyzer. The principle of the analysis consisted of the initial separation of HPLC in ionic vapours and detection of the analyte on the platinum electrode after the previous enzymatic online conversion of ACh to hydrogen peroxide.

Results: The aim of conducted research was checking the accuracy of the obtained results using various approaches to analytical calibration. The standard series method (SSM) with the interpolative way of determining the result and the standard addition method (SAM) with linear and polynomial fit allowing the calculation of the result on the extrapolative way were tested. Obtained analytical results were compared with the apparent concentrations obtained on the basis of the integrated calibration method (ICM) combining features of both SSM and SAM. Tests were carried out for synthetic samples comparing the accuracy of the analytical results as well as the possibility of using the ICM approach as an alternative to traditional calibration methods. The advantages of the integrated calibration method due to the set of obtained estimations and interpretation possibilities were confirmed in the studies of real samples.

Conclusions: The use of the Alexys Antec neurotransmitter analyzer allowed fast and sensitive ACh determination in the real samples. The comparison of obtained results calculated in various ways allowed an effective assessment and compensation of systematic errors as well as an improvement of the accuracy.

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Evaluation of the effects of ACEA 1021, NMDA receptor antagonist, on neurotoxicity of dexamethasone

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Recent studies have shown that long-term treatment with glucocorticoids (GCs) and especially dexamethasone (DEX – a synthetic GCs receptor agonist) prolonged stress or ischemia-induced neurons damage. Furthermore, elevated levels of endogenous corticosteroids are known to be toxic to the CA1 and CA3 subfields of the hippocampus and to the striatum, and to reversibly decrease specific elements of memory performance. GCs potentiate stress or ischemia-induced accumulation of excitatory amino acids (EAA) in the extracellular space of hippocampus and engender hippocampal neurons damage by facilitating the glutamate/Ca\(^{2+}\) cascade.

ACEA 1021 (5-nitro-6,7-dichloro-1,4-dihydro-2,3-quinoxalinedione; licostinel), a selective antagonist at the strychnine-insensitive glycine site associated with the NMDA receptor complex, has been reported to prevent the excitotoxic action of high extracellular glutamate levels.

The aim of this study was to investigate the effect of ACEA 1021 on neurotoxic effect of DEX.

The experiments were carried out on male Albino Swiss mice (25-30 g). ACEA 1021, at the doses: 1.25, 2.5, 5.0 or 10 mg/kg/day, ip, was administered 15 min before DEX (16 mg/kg/day, ip) each day. The long-term memory acquisition (the step-through passive avoidance test), the motor performance (“chimney” test) and the locomotor activity (with DIGISCAN Optical Animal Activity Monitoring System) were evaluated 14 days after the drugs administration. The body weight and the lethality of mice were also controlled each day.

The results of our study have shown the prolongation of climbing time in the “chimney” test, decrease of the retention time in the memory task and the locomotor activity, and reduction of body weight, but did not affect the lethality of mice treated with DEX for 14 days. ACEA 1021, administered alone for 14 days at the dose of 10 mg/kg, increased the locomotor activity, but did not change other parameters of mice behavior in comparison with the control group. In mice treated with DEX, ACEA 1021 at the doses of 5 and 10 mg/kg (but not 1.2 and 2.5 mg/kg) reduced the climbing time in the “chimney” test, improved memory acquisition and locomotor activity, yet it did not have any effect on the body weight in comparison with DEX alone administered for 14 days.

The above findings suggest that ACEA 1021, at higher doses, could prevent the neurotoxic effects induced by DEX, but further study needs to be carried out to explain this effect.

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Evaluation of phosphorylation of selected proteins in neurometabolic disturbances in PC12 cell line

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Background: Diabetes and Alzheimer's disease have been thought to be independent disorders. Interestingly, the results of recent epidemiological studies suggest the existence of the link between both pathophysiological mechanisms. The aim of the study was to determine the influence of hyperglycemia and hyperinsulinemia with coexisting glutamate-induced excitotoxicity, on the effect of cytotoxicity in PC12 cell line. The further studies included evaluation of the intensity of phosphorylation of ERK, p38 and JNK kinases from the group of mitogen-activated protein kinases (MAPK), which contribute to the initiation of complex signaling pathways that play a role in excitotoxicity.

Material and methods: Cytotoxic effects of various concentrations of glucose (G1 and G2), insulin (I1 and I2), glutamate (L1 and L2) and their synergistic action on PC12 cells were assessed using the MTT test. The BCA test was carried out to determine the protein concentration in cell lysates, while the enzyme immunoenzymatic InstantOne ELISA was used to evaluate the activation of selected signaling proteins.

Results: The degree of survival assessed by MTT test, demonstrated increased cytotoxicity in PC12 cells after incubation with the above-mentioned substances. However, ELISA results indicate their differential impact on the MAPK pathway - there is a dissonance between the activation of p38 and JNK pathways and the lack of activation of the ERK pathway under the influence of most combinations of reagents used. Significant activation of the ERK path occurred only under the influence of lower concentration of glucose (G1) or combination of higher glucose and glutamate concentration (G2L1). The only one combination not contributing to the activation of the JNK pathway – it was combination of all three agents in different concentration (G1I2L2), while in the case of the activation of p38 pathway, the exceptions included two of agents in different combinations (G1L2 and G2L2).

Conclusions: In view of the growing number of reports on the relationship between diabetes and excitotoxicity induced – neurodegeneration and still limited pharmacotherapy for neurodegenerative diseases, it is important to conduct further studies on the phosphorylation of MAPKs pathways and estimation its potential role in pathogenesis of this disease.

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The use of transcriptomic biomarkers for prognosis of heart failure development

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Background: Heart failure (HF) is the most common cause of morbidity and mortality (especially in industrialized countries and aging populations) and the incidence of this disorder continues to increase. The development of heart failure after acute myocardial infarction (AMI) is directly related to left ventricular remodelling. Classical risk factors of heart failure (including infarct size, diabetes) only partially explain why some patients suffer later from decompensation of the left ventricle, whereas others function quite well. The aim of our study was to identify distinct biomarkers that correlate with HF development.

Materials and methods: We collected blood samples from AMI patients on admission (1st day of AMI). Clinical evaluation was performed 6 months after AMI and patients were divided into HF (n=37), non-HF (n=26) and moderate-HF groups (n=119). Microarrays were used to analyze individual gene expression profiles of the patients. Microarray results were validated by the ddPCR method using blood RNA.

Results: 7 differentially expressed transcripts that best discriminate between HF, non-HF and moderate-HF patients were selected. Reference values were obtained for all transcripts and all groups of patients and the HF prognosis risk score was calculated.

Conclusions: The obtained results show that the identified gene expression changes at the early phase of AMI allow to differentiate patients who developed HF from those who did not, and that they may serve as a convenient tool contributing to the prognosis of heart failure.

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The effects of maternal separation on blood-brain-barrier permeability during ontogenetic development

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Background: Early-life stress (ELS) is considered as relevant etiological factor in psychiatric and neurodegenerative disorders. Our research is focused on possible mechanism associating ELS and brain-blood-barrier (BBB) disturbance, which may increase the susceptibility of the brain to chronic diseases in adult life. BBB plays a major role in maintaining the stable environment required for normal brain function. During perinatal period, BBB develops along with the brain, therefore any adverse stimulus may disturb its homeostasis. All these facts and insufficient data in this filed inclined us to investigate if ELS influences BBB integrity in defined brain structures - the medial prefrontal cortex, striatum and hippocampus.

Materials and methods: To model ELS we applied maternal separation (MS) procedure in Wistar rats. Briefly, during first two weeks of rodents’ life (postnatal day (PND) 1-14), which are crucial for development of brain and BBB, pups were isolated from dams for 3 hours each day. Then, fluorescent tracer, sodium fluorescein dye (NaF), was used to study quantitative permeability of BBB in important development time points: on juvenile (immature BBB, PND 14), preadolescent (the BBB becomes tight, PND 22) and adult (PND 70) males and females rats.

Results: Our study indicated that the striatum was the region that showed the most significant changes in BBB permeability triggered by MS during early-life period. We found that NaF dye was delivered at a much higher concentration into the striatum of MS juveniles males compared to the control group. On the contrary, MS caused attenuation of NaF dye accumulation in juvenile females striatum. In others brain regions NaF dye content was much evenly distributed in-between groups. Only for adult male rats the amount of NaF dye which enter into the medial prefrontal cortex was significantly lower in MS group. In the hippocampus we did not observe any significant effects of MS at any development time point.

Conclusions: The results strongly imply that MS affects BBB maturation and in consequence may disturb functions of selected brain regions in developing central nervous system.

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Edaravone altered expression of Comt, Adora 1 and Slc6a15 genes in corticosterone-induced depression in mice

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Background: The pathophysiology of depression is poorly understood. Studies have shown that chronic stress leads to oxidative stress and neurodegeneration. The current pharmacotherapy of depression is still not satisfactory; research is being conducted to find new medicines and treatments. Edaravon (3-methyl-1-phenyl-2-pyrazoline-5-one), a free radical scavenger, was applied in the treatment of amyotrophic lateral sclerosis, acute cerebral infarction and ischemic stroke. In several animal models it was revealed also neuroprotective effect of edaravone attributed to the potent free radical scavenging activity. Its mechanism of action is not well understood. The evaluation of the antidepressant-like activity of edaravone was based on the analysis of the expression of selected genes related to the DOPA system, 5-HT and adenosine system in the prefrontal cortex of mice. The expression of Comt, Adora1 and Slc6a15 genes was marked. These genes are responsible for neuronal transduction and for excitability changes in nerve cells.

Materials and methods: Mice were injected subcutaneously (sc) with 40mg/kg corticosterone chronically for 21days. Paroxetine (10 mg/kg) and edaravone (10 mg/kg) were administered ip 30 min prior to the corticosterone injection. After 21-days treatment with respective drugs, mice were sacrificed by decapitation and the prefrontal cortex was rapidly dissected and used for the real-time PCR analysis.

Results: In the group of mice receiving the corticosterone, a statistically significant decrease in Comt, Adora 1 and Slc6a15 genes expression was observed as compared to the vehicle control group. The significant increase in Comt, Adora 1 and Slc6a15 genes was observed in the group of animals pretreated with corticosterone and edaravone in comparison to the group received corticosterone alone.

Conclusions: The findings of this work confirm the role of reactive oxygen species in the pathophysiology of depression and indicate a possible role of Comt, Adora 1 and Slc6a15 genes in mediating antidepressant-like effects of edaravone under chronic stress.

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KETAMINE, DEVIL AND/OR ANGEL – INFLUENCE ON COGNITIVE FUNCTIONS

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The glutamate N-methyl-D-aspartate receptor non-selective antagonist - ketamine - was recently repurposed as a rapidly acting antidepressant. Valuable properties of this medication in the quick-acting treatment of depression are documented. The long-term effects of ketamine use have not been known, including the cognitive sphere. It is well known that prolonged exposure to stress induces depression and cognitive impairment.

It seems reasonable to ask how long-term ketamine administration will affect stressed animals in aspects of cognitive functions? In this study we tested this working hypothesis, is it possible that ketamine, used in prolonged-regimen in rats could alleviate some aspects of stress-evoked memory deficits?

Material and methods: Stressed (by restraint) and non-stressed rats were treated with ketamine (4 mg kg⁻¹, s.c.) for 21 days and next followed by a battery of behavioural tests: for assessment of working and reference spatial memory (Morris water maze (MWM) and Barnes maze (BM)), stereotypy (stereotypy test - ST), locomotor functions (Open field - OF) and anxiety behaviour (Elevated plus maze - EPM).

Results: Stressed rats displayed significant decline in the spatial working and reference memory. The effect of chronic ketamine administration depended on the type of test and differed between control rats and animals simultaneously exposed to chronic stress. While in MWM the impact is quite unequivocal, because we have observed an enhancement in spatial memory in stressed animals, and a worsening in animals not stressed after ketamine administration. In the BM, the effect of ketamine changes in successive attempts, from favourable in the initial period to the negative at the end of the test (72 hours later) in the group of animals stressed and without significant impact on the control animals. The data obtained from the auxiliary tests did not show the effect of administration (ketamine or solvent) or used procedures on loco-motor efficiency (OF) or the level of anxiety (EPM) or stereotypy behaviour (ST) in tested rats.

Conclusion: These findings demonstrate that ketamine potently abolishes or prevent some kinds of stress-induced memory impairments and cognitive decline in rats, but in some circumstances, it could even increase damage of memory, especially in unstressed animals.
Influence of xanthotoxin on kynurenic acid production in rat brain cortex

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Background: Xanthotoxin (also called methoxsalen or oxsoralen) is a furanocoumarin occurring in medical plants, especially from seeds of parsley family (Apiaceae). Its molecule is highly sensitive to visible and ultraviolet light, inducing sunburns in persons whose skin was exposed to this compound. Moreover xanthotoxin was shown to display potent anticoagulant, antimicrobial, cytotoxic, photosensitizing and antioxidant properties. It has been reported that xanthotoxin can cross the blood-brain barrier and may cause central nervous system effects including neuroprotection. Kynurenic acid (KYNA) is the only known endogenous broad-spectrum antagonist of excitatory amino acid receptors, displaying the highest affinity towards the glycine site of N-methyl-D-aspartate (NMDA) receptor. Brain synthesis of KYNA from its bioprecursor L-kynurenine occurs due to the activity of kynurenine aminotransferases (KAT I and KAT II). Its impaired production was implicated in the pathogenesis of epilepsy and neurodegenerative disorders. Additionally its antiinflammatory and antioxidant properties are suggested. The aim of the research was to check the influence of xanthotoxin in vitro on the production of KYNA in the rat brain and on the activity of KAT I and KAT II enzymes, converting L-kynurenine to KYNA.

Material and methods: The research was carried out on male Wistar rats. The effect of xanthotoxin on KYNA production was tested in rat brain slices and KATs activity was examined in rat brain homogenates. KYNA was quantified using HPLC system with fluorimetric detector (Thermo Fisher Scientific). The statistical comparisons were made using one-way analysis of variance (ANOVA), with the post-hoc Bonferroni test. Differences among values were considered statistically significant if \( p < 0.05 \).

Results: In cortical slices xanthotoxin at the concentration of 0.5 and 1 mM significantly decreased KYNA synthesis. The activity of KAT I was decreased by xanthotoxin at the concentration of 0.1; 0.5 and 1 mM to 63%. Xanthotoxin did not influence the activity of KAT II.

Conclusion: Our data suggest that xanthotoxin might decrease KYNA production in rat brain cortex, most probably via influence on KAT I activity.

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Preparation of new dressing improving of wound healing in streptozotocin-induced diabetic rats

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**Background:** Delayed wound healing in diabetic patients is one of the most difficult complications in clinical medicine. Therefore, a novel method to promote the healing of a diabetic wound is now very important. It is know that neuropeptides improve wound healing by modulating inflammation. Also, keratin materials accelerate wound healing by stimulating proliferation of fibroblasts and keratinocytes. The main goal of our study was to prepare a new dressing consisting of a lanolin ointment, keratin scaffolds and one of the opioid neuropeptides (biphalin or hybride compound 3106) to improve wound healing in diabetic rats.

**Material and methods:** Animals were divided into 3 groups: group with untreated wounds as a negative control (NC), and two groups with topically treated wounds by daily treatment of the wound with an ointment containing keratin scaffolds incrusted with compound 3106 (3106) or biphalin (BIF). The diabetes in rats were induced by intraperitoneal injections of streptozotocin. The skin wounds were created according modified procedure description in Konop et al., (2016). Skin samples were collected on 4, 7, 14, and 21 day after wounding. The healing process was assessed by hematoxylin-eosin (HE), Masson’s Trichome stain and immunohistochemical analysis of the blood vessels and macrophages (CD34 and CD68 antibodies). The expression of inflammatory factors (TNF-α, IL-10) in the supernatants was measured by ELISA. Based on photographic documentation, the healing process was quantified by measuring the wound area and percentage of healing was calculated.

**Results:** The histological analysis HE and Masson’s trichome staining of skin sample on 14 day after wounding demonstrated well-formed and more organized granulation tissue with collagen deposition and covered by newly formed epithelial layer in 3106 and BIF groups than in NC group. At 7 day the wound was reduced about 50% in 3106 and BIF groups while the wound in NC group was reduced only about 17%. At 14 day the neovascularization was greater and better in 3106 group than in NC group. The immunohistochemical analysis of macrophage demonstrated about 100% more cells in 3106 group at 4 and 7 day and 100% at 4 and 38% at 7 day in BIF group compared with NC group. The expression level of TNF-α and IL-10 was similar in all group.

**Conclusion:** Our work provides evidence that a dressing containing keratin scaffolds and tested neuropeptides could have the potential to become a new therapeutic treatment for diabetic chronic wound healing.
Urinary proteins in HIV-infected patients treated with antiretroviral therapy – retrospective study

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**Background:** Applying of combined antiretroviral therapy in HIV-infected patients allows to extend their life and to avoid AIDS. The one of the side effects of applied therapy is chronic kidney disease (CKD), which has become an important cause of such patients death. The aim of this study was retrospective analysis of kidney function of HIV positive patients, who are treated with antiretroviral therapy, on the basis of examination of urinary levels of some proteins, as biomarkers of kidney function. We measured concentration of Retinol-Binding Protein (RBP), Neutrophil Gelatinase-Associated Lipocalin (NGAL) and beta 2 microglobulin ($\beta_2$M) concentration in urine samples of these patients. The current results were compared with these conducted on the same patients but 7 years earlier.

**Materials and methods:** The morning urine samples were collected from 34 patients treated at Outpatient and Treatment Counseling Center HIV/AIDS in Wroclaw and 30 people, who are not suffering from HIV or any kidney diseases (comparable group). The urinary concentration of selected proteins were measured by ELISA tests. The value of Estimated Glomerular Filtration Rate (eGFR), plasma creatinine concentration and number of lymphocytes CD4(+) in blood were received from patients’ medical documentary.

**Results:** A reduction of the amount of all proteins in patients' urine, compared to year 2011 was observed. Has not been proven adverse effect of tenofovir (TNV) on renal function, but probably it was connected with introduce a new prodrug (tenofovir alafenamide – TAF instead TNV) to treatment, which is less toxic to most of the HIV-infected patients. Results also show a positive correlation between concentration of plasma creatinine and urinary $\beta_2$M level, and negative correlation between eGFR and concentration of RBP in urine. Additionally the influence of existing HCV infection and the number of lymphocytes CD4(+) on the examined biomarkers in urine has been shown.

**Conclusion:** This retrospective study confirm the importance of systematic monitoring kidney function in HIV-infected patients treated with antiretroviral therapy. Examined urine biomarkers are useful for the assessment of nefrotoxicity of applied therapy and others factors. This allows to take faster and more effective actions to prevent the loss of kidney function.

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The Attentional Boost Effect does not increase long-term memory in euthymic bipolar patients.

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**Background:** In the Attentional Boost Effect (ABE), stimuli encoded with to-be-responded targets are recognized more accurately than stimuli encoded with to-be-ignored distractors. To date, several studies have demonstrated that the ABE is robust in young adults, whereas it is eliminated in healthy older adults and in several clinical populations. Bipolar disorder is characterized by cognitive deficits in attention abilities, even in the euthymic phase of the disease. We investigated whether a significant ABE occurred in these patients.

**Materials and methods:** 28 euthymic bipolar outpatients and 30 healthy control subjects were recruited. To control for the effects of age on the ABE, we divided both samples in two sub-groups: young-adult (range: 18-35 years) and adult (range: 36-59 years). Participants encoded a sequence of pictures associated to either red (target) or green (distractor) squares. They were instructed to pay attention to the stimuli and simultaneously press the spacebar whenever a target square appeared on the screen. After a 15-minute interval, memory for the encoded stimuli was tested in an old-new recognition task.

**Results:** We found a worst performance in the detection task in bipolar patients, in both age groups. More importantly, young-adult and adult bipolar patients did not show the memory facilitation typically produced by the ABE. Regarding the control group, we found a robust ABE in young-adult participants, whereas the effect was eliminated in adult participants.

**Conclusions:** These data support the view that bipolar disorder is characterized by attentional deficits, even in the remission phase. We suggest that the increase in the attentional demands of the detection task subtracted attentional resources from the encoding of target-associated stimuli in bipolar patients, thus eliminating the ABE. In agreement with previous results, the lack of a significant ABE in older control participants confirms the hypothesis of a naturally-occurring age-related decline in the facilitatory mechanisms underlying the ABE.

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Selvita R&D portfolio overview

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Selvita is a clinical stage drug discovery company engaged in the research and development of novel cancer therapies, as well as provision of integrated drug discovery services. Selvita Early Discovery programs include: Immunooncology platform, Epigenetic platform, program targeting metabolic abnormalities in cancer, as well as an early discovery stage programs in the area of protein kinases. We are working on a number of novel oncology targets such as CDK8, SMARCA2/4, A2A/B, SHMT2 or STING. The company’s most advanced R&D program is SEL24, a dual PIM/FLT3 kinase inhibitor currently in Phase I/II clinical study, licensed to Menarini Group. The second most advanced program is SEL120, the first-in-class small molecule inhibitor of CDK8 with potential use in hematological malignancies, colorectal cancer and breast cancer, currently in IND-enabling studies. In August 2017 Selvita signed a partnering agreement with The Leukemia and Lymphoma Society for preclinical and Phase I clinical development of SEL120 in area of AML, within the Therapy Acceleration Program. The company has alliances and partnerships with more than fifty large and medium-sized pharmaceutical and biotechnology companies from USA and Europe, including R&D partnerships with Merck, H3 Biomedicine, Menarini Group and The Leukemia and Lymphoma Society.

Innovative Biologics and Biosimilars R&D and Manufacturing

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Polpharma Biologics is a division of Polpharma Group, an established pharmaceutical company licensed by the FDA to manufacture drugs for therapeutic use in humans. Polpharma Biologics’ core business focus of producing and selling Biosimilar drugs is now expanded by offering world class contract R&D services and cGMP production capability to external customers. Our mission is to provide our customers with world class development capabilities and a capacity for industrial-scale supply, enabling them to advance their medicinal product and help patients in need. Currently, Polpharma Biologics has 3 biotechnology products in full development and has further 3 in the early development stage for life threatening, chronic and inflammatory diseases. Our first product will be launched in 2020. We also expect to be in the first wave of submission in US. In early 2016 we began our first international clinical trial with our biological medicine, which has covered a group of almost 480 patients. The trial will support registration and is
being conducted in clinical centers around the world; 20% of patients will be recruited in Poland. Our world class team of scientists and engineers provides more than just their long-term experience and know-how but solutions that bring products to the market faster. Even more, our unique modular **one-stop-shop** concept allows a customized selection of the services you need. A unique team of young Polish scientists and international experts with global experience works on complex biopharmaceutical structures, monoclonal antibodies and other therapeutic proteins. We have also number of Polish expat scientists at post PhD level that have joined us from international companies. Many young scientists have found their place in the Polpharma Biologics Laboratories in Gdańsk, where as many as 12 nationalities are working in the team and future personnel is being educated through the introduction of paid internship programs.

Polpharma Biologics clinical manufacturing center located in Gdansk is one of the most modern biotech laboratories in Europe. Our highly skilled and motivated team operates state-of-the-art equipment for optimization and upscaling of analytical and production methods up to GMP-grade clinical material for biosimilar and innovative programs. For commercial production we can process in Gdansk 250/ 1000 or 2*10001 cell culture production. Customized formulations for both drug-substance and drug-product can also be developed in-house and subsequently tested for stability according to ICH standards. Intense cross-functional collaboration between analytical, process development, formulation and manufacturing departments and usage of comparable equipment drives fast and successful technological transfers.

Summarize Polpharma Biologics offers a **one-stop-shop** approach - fully integrated solutions along the biopharmaceutical development and production value chain to serve today’s and tomorrow’s global market needs.