



70<sup>TH</sup>  
ANNIVERSARY

***Neuropharmacology towards neuroplasticity:  
A journey from historical roots to future  
innovations***

scientific symposium celebrating  
the 70<sup>th</sup> Anniversary of the Maj Institute of Pharmacology  
Polish Academy of Sciences

December 2 - 3, 2024, Kraków, Poland

**Abstract book**

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## Welcome Address

*Dear Colleagues and Friends,*

We have the honor to invite you to the celebration of the 70<sup>th</sup> Anniversary of the Maj Institute of Pharmacology Polish Academy of Sciences (IP PAS) and the inauguration of the international scientific symposium, which will take place on December 2-3, 2024, in Kraków.

Over the past seven decades, IP PAS has established itself as a leading research institution in Poland, specializing in neuropsychopharmacology. Our scientific pursuits in 14 departments and state-of-the-art research facilities focus on understanding the mechanisms of central nervous system (CNS) disorders and developing novel bioactive substances for treating psychiatric and neurological conditions. The upcoming symposium is designed to highlight the institute's contributions to the field, both past and present, and to showcase the cutting-edge research currently being conducted. We are honored to host a distinguished lineup of plenary speakers which will offer deep insights into the key milestones in neuropharmacology, assess the current state of research, and share innovative strategies for developing effective and safe psychotropic medications. Among the notable talks, Dr. Phil Skolnick (USA) will discuss the development of glutamate-based antidepressants, while Professor Mart Saarma (Finland) will present groundbreaking research on novel mechanisms of neuroprotection. Additionally, Professor Micaela Morelli (Italy) will explore the profound impact of international collaborations on neuropharmacology, and Prof. Christian P. Müller (Germany) will shed light on the pharmacotherapy of substance use disorders (SUD).

In addition to plenary lectures, we are excited to provide a platform for the next generation of researchers at IP PAS. Our young scientists will present their findings, offering fresh perspectives on neurodegeneration, rapid-acting antidepressants, and the role of serotonin receptors in synaptic plasticity. The symposium will also feature poster sessions, offering a glimpse into the diverse research carried out across the institute and beyond. We are confident that this symposium will foster fruitful discussions, inspire new collaborations, and contribute to the ongoing advancement of neuropharmacology. We invite researchers, clinicians, and scholars from around the world to join us in celebrating this special occasion and to be part of the vibrant exchange of ideas that will shape the future of our field.

We view this occasion not only as an opportunity to present scientific advancements but also as a gathering of the IP PAS family, where we can collectively honor the legacy of those who came before us. Your presence will be an essential part of this celebration as we acknowledge and cherish the work of the scientific minds and staff whose dedication, over many decades, has helped us reach this important milestone.

We warmly invite you to join us in celebrating our rich history, engaging with the latest discoveries, and exploring the exciting future of our institute and the field of neuropharmacology.

Sincerely,

### **The Organizing Committee**

Prof. Irena Nalepa, Prof. Władysław Lasoń, Prof. Małgorzata Filip, Helena Domin, PhD, Małgorzata Frankowska, PhD, Anna Krzemińska, PhD, Kamila Piotrowska, M.Sc

## Program

Monday, December 2, 2024

10.00 a.m. - 12.00 p.m.	<b>A tour to the Maj Institute of Pharmacology Polish Academy of Sciences &amp; Center for Development of New Pharmacotherapies of Central Nervous System Disorders, CEPHARES</b>
12:30 - 1.00 p.m.	<b>Registration (Hotel Premier)</b>
1.00 p.m. - 1.15 p.m.	<b>Welcome address</b> Prof. Małgorzata Filip, PhD
1.15 - 1.45 p.m.	<b>The history &amp; scientific potential of the Maj Institute of Pharmacology Polish Academy of Sciences</b> Prof. Małgorzata Filip, PhD Prof. Jan Rodriguez-Parkitna, PhD
1.45 - 2.15 p.m.	<b>Speech by the Polish Academy of Sciences representatives and awarding state decorations</b>
2.15 - 2.30 p.m.	<b>Coffee break</b>
2.30 - 3.30 p.m.	<b>The research potential of the Maj Institute of Pharmacology Polish Academy of Sciences</b> <b>Piotr Chmielarz, PhD</b> Department of Brain Biochemistry, Maj Institute of Pharmacology, Polish Academy of Sciences, Krakow, Poland <i>Targeting alpha-synuclein aggregation in Parkinson disease</i> <b>Barbara Chruścicka-Smaga, PhD</b> Department of Neurobiology, Maj Institute of Pharmacology, Polish Academy of Sciences, Krakow, Poland <i>The role of the glutamatergic system in the activation of rapid-acting antidepressants</i> <b>Katarzyna Popiołek-Barczyk, PhD</b> Department of Neurochemistry, Maj Institute of Pharmacology, Polish Academy of Sciences, Krakow, Poland <i>Exploring the histamine H3 receptor: Implications for neuropathic pain relief</i> <b>Marcin Siwiec, PhD</b> Department of Physiology, Maj Institute of Pharmacology, Polish Academy of Sciences, Krakow, Poland <i>Serotonin receptors as region-dependent regulators of synaptic plasticity</i>
3.30 - 4.00 p.m.	<b>Coffee break</b>
3.30 - 4.00 p.m.	<b>Poster session 1</b>
4.00 - 5.00 p.m.	<b>The inaugural lecture</b> <b>Phil Skolnick, Ph.D., D.Sc. (hon.)</b> Indivior Inc., Midlothian Turnpike, N. Chesterfield, USA <i>Development of glutamate-based antidepressants: looking back and to the future</i>
5.00 - 7.00 p.m.	<b>Welcome party</b>

## Tuesday, December 3, 2024

9.45 - 10.00 a.m.	<b>Registration (Hotel Premier)</b>
10.00 - 11.00 a.m.	<b>Plenary lecture</b> <b>Prof. Mart Saarma, PhD</b> Institute of Biotechnology, HiLIFE; University of Helsinki, Finland <i>Cell stress regulating CDNF protein protects neurons cells by novel mechanism</i>
11.00 - 11.30 a.m.	<b>Coffee break</b>
11.30 a.m. - 12.30 p.m.	<b>Plenary lecture</b> <b>Prof. Micaela Morelli, PhD</b> University of Cagliari, CNR Institute of Neuroscience, Cagliari, Italy <i>How scientific and cultural international collaborations uplift knowledge on neuropharmacology research and impact on new generation: value and future perspectives</i>
12.30 - 1.30 p.m.	<b>Poster session 2</b>
1.30 - 2.15 p.m.	<b>Lunch break</b>
2.15 - 3.15 p.m.	<b>Plenary lecture</b> <b>Prof. Christian P. Müller, PhD</b> University Clinic, Friedrich-Alexander-University of Erlangen-Nuremberg, Erlangen, Germany <i>The pharmacotherapy of drug use disorders – past, present and future</i>
3.15 - 3.30 p.m.	<b>Closing ceremony</b> Prof. Małgorzata Filip, PhD; Prof. Irena Nalepa, PhD; Prof. Władysław Lasoń, PhD

## Organizer



Maj Institute of Pharmacology  
Polish Academy of Sciences



Ministry of Science and Higher Education  
Republic of Poland



Doskonała  
Nauka

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**INAUGURAL LECTURE****IL. 1.**

Skolnick P., PhD, DSc (hon.)

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**The development of glutamate-based antidepressants: look back and to the future**

The development of glutamate-based antidepressants has revolutionized the treatment of depression. A brief intravenous infusion of the NMDA antagonist ketamine at subanesthetic doses results in a profound reduction in depressive symptomatology within hours; this response can be maintained for a week or more. A decade elapsed between preclinical studies demonstrating the antidepressant-like actions of NMDA antagonists and clinical validation of this concept; almost 3 decades elapsed before the first NMDA antagonist was approved (by the FDA and EMA) for use in treatment-resistant depression. In this presentation, I will discuss a) the converging lines of preclinical evidence that led to the clinical testing of ketamine; b) the factors that contributed to the long 'incubation periods' for going from 'bench to bedside' and subsequent approval of an NMDA antagonist; and c) the prospects of improving the "scalability" of treating depression with NMDA antagonists. I will also discuss the potential for exploiting other glutamatergic mechanisms to develop antidepressants that retain both the speed of onset and efficacy produced by ketamine without the limiting side effects (dissociation) associated with NMDA receptor blockade.

**PLENARY LECTURES****PL. 1.**

Saarma M.

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**Cell stress regulating CDNF protein protects neurons by novel mechanism**

Parkinson's disease (PD) affects about 10 million people, and no treatment exists that can slow down or stop the disease progression. In PD, midbrain dopamine (DA) neurons degenerate and die, causing major motor and non-motor symptoms. We discovered an endoplasmic reticulum (ER) located protein with neurotrophic factor (NTF) activities - cerebral dopamine neurotrophic factors (CDNF). We have solved the three-dimensional structure of CDNF and shown that their structure and mode of action radically differ from other known NTFs. CDNF can protect and repair midbrain DA neurons in rodent and non-human primate neurotoxin PD models more efficiently than other NTFs. However, unlike other NTFs, CDNF is located in the ER, where they regulate ER stress and unfolded protein response (UPR) pathways. We have now discovered that UPR sensors PERK and IRE1 $\alpha$  are CDNF receptors. CDNF binding to PERK and IRE1 $\alpha$  is crucial for the survival of mouse and human stem cell-derived dopamine neurons in culture. We found that CDNF binds to BiP, but its binding to BiP was dispensable for the neuroprotective and neurorestorative activity of CDNF. Herantis Pharma Plc. has tested CDNF in phase I-II clinical trials in PD patients, and CDNF achieved its primary endpoints: safety and tolerability. Since CDNF cannot pass through the blood-brain barrier (BBB), it is delivered directly into the patient's brain via catheters installed during invasive surgery. We have recently discovered a fragment of CDNF (ngCDNF) that can pass through the BBB after subcutaneous administration. Using ngCDNF may allow peripheral delivery, avoiding intracranial surgery and facilitating the treatment of non-motor symptoms of PD.

**PLENARY LECTURES****PL. 2.**

Morelli M.

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**How scientific and cultural international collaborations uplift knowledge on neuropharmacology research and impact on new generation: value and future perspectives**

Neuropharmacology is a cutting-edge scientific field because it includes psychiatric and neurodegenerative diseases that still do not have effective therapies for their treatment. Therefore, disciplines such as neuropharmacology need more important exchanges and relationships among different scientific groups than other disciplines. My lecture will face several aspects necessary to set up a fruitful collaboration among research groups, starting from a well-established field of research, original ideas, and forefront techniques, combined with the presence of translational research perspectives and young, talented researchers who can ensure the future of the collaboration. Research on Parkinson's disease (PD), basal ganglia, and the interaction between dopamine, the principal neurotransmitter involved in PD, and adenosine and dopamine receptors were the basis for a collaboration that started at the end of the 80th between my research group at the University of Cagliari (Italy) and several colleagues of the Polish Academy of Sciences of Krakow. The results of these collaborations were very fruitful. They brought several publications in international journals on the positive effect that blockade of adenosine A2A receptors could play on the stimulation of dopamine receptors in preclinical models of PD and on their neuroprotective effects. Moreover, based on the neurotoxic and neuroinflammatory effects of 3,4-methylenedioxymethamphetamine (MDMA) in the brain areas involved in PD, we have produced, through collaboration with research groups of the Polish Academy of Sciences, several publications on the potential mechanisms of these effects and the interaction of MDMA with caffeine. Efficient research is carried out preferentially at the international level since the exchange of information allows the select the best and most original ideas and produces innovation.



**PLENARY LECTURES****PL. 3.**

Müller C.P.

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**The pharmacotherapy of drug use disorders – past, present, and future**

Substance use disorder (SUD) is a widespread psychiatric disorder causing suffering for the affected individual and society. From the early days of recognizing this disorder, there was a wish for pharmacotherapy. An increasing understanding of the neurobiological mechanisms of drug use and abuse identified pharmacological targets in the brain for which compounds were developed. They were successfully validated in animal models, which can now model many symptoms of a SUD. As the neurochemical effects of distinct SUDs differ, so do the identified compounds for pharmacotherapy, as shown in the examples of alcohol and psychostimulants. Although preclinical research has provided numerous candidate substances, only very few were approved for alcohol use disorder (AUD) treatment and none for psychostimulant use disorder (PUD) in humans.

Furthermore, the approved substances are not widely used by AUD patients due to low efficacy and limited compliance. Here, we analyze the reasons for this failure and discuss potential ways to improve in the future. Recent neurobiological research has revealed that SUD core symptoms such as acute drug consumption, withdrawal, abstinence, and others – that occur in time-shifted phases - have distinct underlying neurobiological processes, each with a unique neuropharmacology. Preclinical research has identified compounds that improve single symptoms, but none of them improves all of them. Here, we propose a new type of SUD pharmacotherapy. As symptom sequence and suffering are highly individual, combining a pharmacological tool-box with an ecological momentary assessment (EMA), augmentation tool may be useful. An artificial intelligence coupled EMA device, which patients will receive, may serve as a near real-time diagnostic monitor, a phase-sensitive individualized treatment guide, and a therapeutic success monitor. It is suggested to use different drugs in precise phase-sensitive timing to treat the sequentially emerging single symptoms rather than 'one-pill-for-all.' This new level of technology-augmented pharmacotherapy may offer long-missed advancements in SUD therapy.

## PRESENTATIONS

### PR. 1.

Chmielarz P., Maziarz K., Alwani A., Burda G., Jankowska-Kieltyka M., Barut J., Nalepa I., Kreiner G.

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#### **Alpha-synuclein pathology and models in Parkinson's Disease research**

**Background:** Parkinson's Disease (PD) is a progressive neurodegenerative disorder characterized by motor dysfunction and gradual loss of dopaminergic neurons. A key pathological hallmark of PD is the presence of Lewy Bodies - intraneuronal inclusions containing aggregated and phosphorylated alpha-synuclein (ASN). Recent evidence suggests that misfolded ASN can spread through neuronal connections in a prion-like manner, potentially explaining the progressive nature of PD pathology. Growing evidence indicates that pathological processes in PD likely begin years before motor symptom onset, providing a potential window for therapeutic intervention. Understanding mechanisms governing ASN aggregation and spread is crucial for developing early therapeutic strategies.

**Material and methods:** Recent development of methods to reproducibly aggregate monomeric recombinant ASN into self-templating, infectious fibrils - alpha-synuclein preformed fibrils (PFFs) - has enabled new approaches to modeling PD pathology. We utilize these PFFs both *in vivo* and *in vitro* to investigate mechanisms of pathological ASN accumulation and potential protective treatments. *In vitro*, we use primary dopaminergic neuron cultures exposed to PFFs to study cellular mechanisms of ASN aggregation in disease-relevant cell type. *In vivo*, we employ targeted stereotactic injections of PFFs to induce the spreading of ASN pathology through anatomically connected brain regions.

**Results:** We employ established PFF models in several projects investigating mechanisms protecting against ASN aggregation. Building on our previous work showing the protective effects of neurotrophic signaling against PFF-induced pathology, we investigate the modulation of the endolysosomal pathway in protection against ASN aggregation. We have demonstrated that ghrelin receptor agonist MK-0677 reduces the accumulation of pathological ASN in dopaminergic neurons and attenuates PFF-induced motor deficits. We investigate how constitutively active Akt protects neurons through modulation of the endolysosomal pathway. Furthermore, we investigate novel approaches targeting ASN aggregation using functionalized graphene nanoparticles.

**Conclusions:** Current advances in modeling and understanding ASN pathology provide the foundation for developing novel therapeutic approaches to slow PD's progression.

**Acknowledgements:** This research was funded by National Science Centre, Poland, grants number 2019/35/D/NZ7/03200 (Sonata 15) and 2021/42/E/NZ7/00246 (Sonata BIS 11).

## PRESENTATIONS

### PR. 2.

Chruścicka-Smaga B., Pochwat B., Machaczka A., Szewczyk B., Pilc A.

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#### **The role of the glutamatergic system in the antidepressant-like effects of psilocybin**

For many years, research conducted in the Department of Neurobiology has focused on the role of glutamatergic (Glu) system modulation in the pathophysiology and treatment of depression. Depression remains the leading cause of disability worldwide. Despite advancements, the mechanisms involved in the pathogenesis of depression are still not fully understood, and despite the availability of monoaminergic antidepressants and psychotherapies, remission rates are unsatisfactory. Therefore, there is a considerable need to search for new therapies characterized by a rapid onset of action, long-lasting therapeutic effects, and fewer adverse effects. Psilocybin, which belongs to a group of serotonin hallucinogens, had been known to exert rapid and prolonged antidepressant (AD) effects in humans even before classical monoaminergic antidepressants were introduced into clinical practice. Notably, this was the first demonstration that rapid and long-lasting AD effect could be achieved through the direct modulation of serotonergic (5-HT) signaling. However, serotonin hallucinogens may also induce significant undesired effects, limiting their clinical applicability.

Although 5-HT receptors are the primary molecular targets of psilocybin, other neurotransmitter systems, including Glu neurotransmission, also play a crucial role in its therapeutic effects. Our previous data have shown that the modulation of metabotropic glutamate 2/3 receptors (mGluR2/3) affects AD-like activity of hallucinogens at the behavioral and molecular levels. Here, we demonstrate that a low, inactive dose of mGluR2/3 receptor antagonist, LY341495, significantly enhances the AD-like potential of a low, inactive dose of psilocybin in tail suspension and novelty-suppressed feeding tests in mice. These effects are rapid (within 1 hour) and long-lasting (up to 7 days). Co-administration of LY341495 also modulates psilocybin-induced head twitch responses that refer to hallucinogenic-like behavior. Moreover, the behavioral outcomes correlate with changes in synaptic protein levels in the mouse prefrontal cortex.

Serotonergic hallucinogens are undoubtedly the focus of extensive research as promising rapid-acting antidepressants. However, our co-administration approach better addresses the complexity of depression's symptoms and pathophysiology mechanisms, potentially reducing adverse effects while improving therapeutic outcomes.

**Acknowledgements:** This research is funded by the National Science Centre, grant number 2023/49/B/NZ7/02978, given to Andrzej Pilc.

## PRESENTATIONS

### PR. 3.

Popiolek-Barczyk K.

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#### **Exploring the histamine H<sub>3</sub> receptor: Implications for neuropathic pain relief.**

Besides its well-known role as a peripheral mediator of immune, vascular, and cellular responses, histamine plays a crucial role in the central nervous system (CNS), particularly in regulating sleep and wakefulness. Moreover, increasing evidence supports the involvement of histamine H<sub>3</sub> receptor (H<sub>3</sub>R) in the modulation of neuropathic pain, which remains challenging to manage. However, the precise mechanism of H<sub>3</sub>R action in pain remains unknown. Currently, there is one clinically used drug and the first marketed H<sub>3</sub>R antagonist/inverse agonist, pitolisant (Wakix<sup>®</sup>, Ozawade<sup>®</sup>), which is successfully used in human therapy for adults suffering from narcolepsy with or without cataplexy and Obstructive Sleep Apnoea (OSA). In February 2023, pitolisant received its first approval for treating narcolepsy in adolescents and children within the EU. Our study aimed to determine the analgesic potency of a pitolisant, and novel H<sub>3</sub>R antagonist/inverse agonist, E-98 (1-(7-(4-chlorophenoxy)heptyl)-3-methylpiperidine)), in a preclinical model of neuropathic pain (chronic constriction injury [CCI] of the sciatic nerve in mice). The impact of H<sub>3</sub>R antagonists/inverse agonists on mechanical (von Frey) and thermal (cold plate) stimuli was investigated. The compounds were injected intraperitoneally (intraperitoneally (i.p.) in a single dose (1, 5, 10, and 20 mg/kg). Additionally, to deepen our knowledge of the histaminergic system, we have also performed immunohistochemical staining to examine H<sub>3</sub>R presence within the spinal cord of control and neuropathic animals. Moreover, we assessed the influence of E-98 on glial cells (microglia and astrocytes) activation within the lumbar spinal cord. The anti-inflammatory properties of E-98 (10 μM) were screened in primary microglial and astroglial cell cultures. Here, we performed a set of ADME tests, together with screening for compound toxicity, to build a pharmacological profile of the new H<sub>3</sub>R antagonist/inverse agonist. The H<sub>3</sub>R antagonist E-98 attenuated nerve injury-induced hypersensitivity in a dose- and time-dependent manner. Chronic E-98 treatment demonstrated a time-dependent analgesic effect, correlated with reduced microglial and increased astroglial cell activation in the lumbar spinal cord. *In vitro* studies showed a decreased pro-inflammatory IL-6 level in both cell cultures. Furthermore, our preliminary data showed profound, dose-dependent analgesia following pitolisant treatment. We observed co-localization of H<sub>3</sub>R with spinal neurons, microglia, and astrocytes and in primary glial cell cultures. These findings help elucidate the mechanisms of H<sub>3</sub>R antagonists/inverse agonists in pain modulation, highlighting glial cells as potential targets alongside neurons. Additionally, this research underscores the histaminergic system as a promising target for pain management strategies in humans.

**Acknowledgments:** Work was financed by a grant from the National Science National Center, Poland (SONATA 2019/35/D/NZ7/01042).

## PRESENTATIONS

### PR. 4.

Siwiec M.

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#### **Serotonin receptors as region-dependent regulators of synaptic plasticity**

Despite decades of research, our knowledge about the mammalian serotonin system remains limited, partly because of the ubiquitous nature of this evolutionarily ancient neurotransmitter. This short presentation aims to outline some outstanding problems in serotonin research that have been investigated in the Department of Physiology of the Maj Institute of Pharmacology. Studies into the outputs of the dorsal raphe serotonin system and its receptors will be briefly summarized, with a particular focus on the 5-HT<sub>7</sub> receptor as an example of the complicated nature of GPCR signaling in neuronal plasticity. Then, the talk will shift focus to the current scientific goals of the Physiology group, namely studying the plasticity of the dorsal raphe network itself and its regulation by various anatomically defined synaptic inputs as well as local circuits. This short introduction will hopefully offer the audience a glimpse into the challenges of serotonin neurophysiology. It will also present some of the research methods employed at the Department of Physiology to help answer longstanding questions about the systems neuroscience of serotonin signaling.

**Acknowledgments:** National Science Centre grants 2016/23/N/NZ4/03224; 2016/21/B/NZ4/03618; 2023/49/B/NZ4/03592

## POSTER SESSIONS

## PRECLINICAL RESEARCH

## P. 1.

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### Effects of $\beta$ -caryophyllene administration in rat schizophrenia model – potential involvement of the endocannabinoid system

**Background:** Schizophrenia is a complex mental disorder affecting approximately 1% of the population. Characterized by disturbances in thought, perception, and behavior, it often leads to significant disability and a reduced quality of life. Genetic, environmental, and neurobiological factors—such as disturbances in neurotransmission and neuroinflammation—are believed to contribute to its onset and progression. Recent studies suggest that the endocannabinoid system (ECS) is involved in the pathophysiology of schizophrenia, highlighting the potential beneficial effects of activating cannabinoid receptor type 2 (CB2R) in models of the disease. The natural CB2R agonist is  $\beta$ -caryophyllene (BCP), which is found in plants such as *Cannabis sativa*. The aim of this study is to evaluate the effects of BCP on schizophrenia-related changes in an animal model of the disease.

**Materials and Methods:** *In vitro* assays (NO release and MTT assays), were conducted on LPS-stimulated astrocytic cells. In behavioral studies, Wistar rats were used to evaluate locomotor activity and anxiety level in open field test (OFT). The animals were administered MK-801 (0.3 mg/kg, *i.p.*) for 5 days, while BCP (25 mg/kg, *i.p.*) or vehicle was administered once daily for 14 days. After OFT, brain tissue was collected, and dopamine (DA), serotonin (5-HT), and their metabolites (DOPAC, 3-MT, HVA, 5-HIAA) levels were assessed in the frontal cortex (FCX) using the HPLC method. Inflammatory markers interleukin-6 (IL-6) and interleukin-10 (IL-10) were analyzed in the FCX and hippocampus (HIP) using ELISA kits.

**Results:** In *in vitro* studies, several BCP concentrations significantly decreased NO release in astrocytic cells ( $p < 0.0001$ ). In the OFT, BCP administration counteracted MK-801-induced hyperlocomotion ( $p < 0.05$ ). MK-801 increased the DA level in the FCX ( $p < 0.05$ ) and decreased the DA/HVA metabolism ratio, whereas BCP reversed this effect. Moreover, BCP significantly elevated 5-HT and 5-HIAA levels in the FCX. MK-801 caused an increase in IL-10 levels in the FCX; however, BCP restored its level to the control condition.

**Conclusions:** BCP counteracted several disturbances observed after MK-801 administration, which represent characteristic changes in an animal model of schizophrenia. Further studies are necessary to evaluate the potential beneficial effects of CB2R activation in this disease.

**Acknowledgements:** The study was financed from NSC Preludium Grant no. 2022/45/N/NZ7/04059 and statutory funds.

## P. 2.

Bryk A.<sup>1</sup>, Witek K.<sup>1</sup>, Wydra K.<sup>1</sup>, Suder A.<sup>1</sup>, Pieniążek R.<sup>1</sup>, Jastrzębska J.<sup>1</sup>, Piprek R.P.<sup>2</sup>, Filip M.<sup>1</sup>

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<sup>2</sup>*Department of Comparative Anatomy, Institute of Zoology and Biomedical Research, Jagiellonian University, Kraków, Poland*

### **Obesity-inducing diet alters behavioral and biochemical status in rat obesity phenotypes.**

**Background:** Obesity is a global epidemic affecting more than 2.5 billion people worldwide. Although increased intake of energy-dense foods rich in fat causes weight gain and obesity, some individuals are resistant. Our study aimed to evaluate behavioral and biochemical changes in rats after a chronic obesity-inducing diet (OID).

**Material and methods:** Wistar male and female rats were divided into three equal groups after 12 weeks of feeding with OID (45% energy from fat) or standard diet (SD; 10% energy from fat). Rats were shared according to their change in body weight; those with the lowest weight gain were classified as obesity-resistant (OR), whereas those with the greatest weight gain were classified as obesity-prone (OP). Rats were investigated in several behavioral screening tests (locomotor activity (LAM), novel object recognition (NOR), forced swim test (FST), elevated zero maze (EZM)) and serum analysis (triglycerides, cholesterol fractions, alanine aminotransferase, and glucose levels). The measure of their body and internal organs weight was conducted. Tissue samples (liver and fat) were stained in hematoxylin and eosin, Masson's trichrome, and Periodic acid–Schiff stain.

**Results:** Our findings showed that an OID changes body weight individually, and OP rats have higher body weight and body weight gain index vs OR by 35-40%. At the behavioral level, male OP rats had more open-space entries in the EZM than OR and shorter swimming times in the FST compared to SD males. After one hour in the NOR test, a lower recognition index was observed only in females. However, no effect of OID was observed on the LAM in either males or females. At the biochemical level, OP and OR rats had higher total cholesterol and HDL levels than SD females. Histological analysis showed that adipocytes from OP rats (both male and female) were larger than those from SD and OR rats, which correlates with increased body weight. OP males had less liver weight than OR males, and less glycogen storage than SD and OR males and females. OP males and females had much higher numbers of fatty hepatocytes than SD and OR.

**Conclusions:** Our results indicate that postnatal exposure to an OID predisposes to biochemical changes in OP and OR rats. Performed behavioral analyses do not explain differences in obesity in rats.

**Acknowledgments:** Funds by the Polish National Science Centre (Kraków, Poland) research grant no. 2021/43/B/NZ5/03340 and the statutory funds of the Maj Institute of Pharmacology PAS.



**P. 3.**

Ciechanowska A., Rojewska E., Piotrowska A., Pawlik K., Ciapała K., Mika J.

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**Blockade of XCL1 and its receptors - XCR1 and ITGA9 reduces symptoms of hypersensitivity to mechanical and thermal stimuli in a neuropathic mice.**

**Background:** Even though the progress of science and medicine is huge, neuropathy is still an unsolved problem for clinicians. Opioid drugs, which are an important remedy in other painful conditions, turn out to be ineffective in about 50% of neuropathic pain patients. What is more, there is an urgent need for combined therapies that may offer a lower risk of side effects. After damage to the nervous system, there is an increase in the level of immune and glial cells markers, and the concomitant growth in the level of inflammatory markers such as interleukins and chemokines is observed. Our study focused on XCL1 and its two receptors – the classical chemokine receptor XCR1 and ITGA9, which affinity to was discovered relatively recently.

**Material and methods:** We investigated the effect of XCL1, XCR, and ITGA9 blockade on mechanical (von Frey) and thermal (cold plate) hypersensitivity in mice model of neuropathic pain (Chronic Constriction Injury to the sciatic nerve – CCI model) and naive animals. Single intrathecal injections were performed at day 7 in naive mice and after CCI. Chronic intraperitoneal injections of minocycline were performed for 7 days. Behavioral tests were assessed 1, 4, 24, 48 and/or 98 h after drug administration.

**Results:** We have shown that together with increasing hypersensitivity to mechanical and thermal stimuli, there is an increase in XCL1 level in the spinal cord up to day 35 after injury. After the intrathecal administration of XCL1 to naive mice, we have observed the development of mechanical and thermal hypersensitivity. This hypersensitivity was then diminished by blockade of both XCL1 receptors – XCR1 and ITGA9, showing they are both involved in nociceptive transmission. Moreover, the combined administration of XCL1-neutralizing antibodies with two opioids enhanced morphine, not buprenorphine analgesic effects in the CCI model. We have also demonstrated that repeated administration of minocycline, which relieves pain symptoms, reduces the mRNA and protein level of pronociceptive XCL1 after 7 days of chronic intraperitoneal treatment in the CCI model.

**Conclusions:** Our results suggest that XCL1/XCR1 and XCL1/ITGA9 signaling contribute to developing pain hypersensitivity. Importantly, the blockade of both XCL1 and the two receptors studied seems to be an interesting approach for further studies.

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**P. 4.**

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**Evaluating 5-HT<sub>2A</sub> and 5-HT<sub>5A</sub> Antagonists on DOI-Induced Head-Twitch in Rats via Markerless Deep Learning Analysis**

**Background:** Serotonergic psychedelics, with high affinity and specificity for 5-HT<sub>2A</sub> receptors like 2,5-dimethoxy-4-iodoamphetamine (DOI), reliably induce a head-twitch response in rodents – a behavior characterized by paroxysmal, high-frequency head rotations. Traditionally, this response is manually observed and counted, which is time-consuming and subjective. Although automation could simplify and facilitate data collection, existing methods require the surgical implantation of magnetic markers into the rodent's skull or ear.

**Material and methods:** This study evaluates the potential of a digital marker-less workflow for detecting head-twitch responses using deep learning algorithms. High-speed video data were analyzed using the DeepLabCut neural network to track head movements, and the Simple Behavioral Analysis (SimBA) toolkit was employed to classify specific head-twitch events.

**Results:** In studying DOI (0.3125, 0.625, 1.25, and 2.5 mg/kg) effects, the deep learning algorithm workflow demonstrated significant correlation with the human observations. As expected, the preferential 5-HT<sub>2A</sub> receptor antagonist ketanserin (0.625 mg/kg) reduced head-twitch responses induced by DOI (1.25 mg/kg). In contrast, the 5-HT<sub>5A</sub> receptor antagonists SB 699551 (3 and 10 mg/kg) and ASP 5736 (0.01 and 0.03 mg/kg) did not attenuate these responses.

Previous drug discrimination studies demonstrated that these 5-HT<sub>5A</sub> receptor antagonists attenuated the interoceptive cue of a potent hallucinogen LSD, suggesting their anti-hallucinatory effects. However, our findings support the head-twitch response as selective for 5-HT<sub>2A</sub> and not 5-HT<sub>5A</sub> receptor inhibition.

**Conclusions:** We conclude that the DeepLabCut and SimBA toolkits offer high objectivity and can accurately and efficiently identify compounds that induce or inhibit head-twitch responses, making them valuable tools for high-throughput research.

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**P. 5.**

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**The novel third-generation antipsychotic drugs, brexpiprazole, and lumateperone, modulate CYP enzymes in the liver. A pharmacological significance**

**Background:** Antipsychotic drugs are used to treat schizophrenia and other psychotic-related symptoms. They are administered to patients for a long time, often in combination with other drugs that are substrates of cytochrome P450 (CYP) enzymes. Our present work aimed to investigate the effects of prolonged administration of the third-generation antipsychotic drugs brexpiprazole and lumateperone on the expression and activity of CYP drug-metabolizing enzymes in rat liver and on the expression of transcription factors regulating CYP enzymes.

**Material and Methods:** Male Wistar rats received lumateperone or brexpiprazole (1 mg/kg ip) once daily for two weeks. 24 h after the last dose, their livers were excised, and the activities (HPLC), protein levels (Western blotting), and mRNAs (qRT-PCR) of CYP enzymes were measured. In parallel, the expression of transcription factors (Western blotting, qRT-PCR) was assessed.

**Results:** The investigated antipsychotics produced a broad-spectrum effect on cytochrome P450 expression and activity. Brexpiprazole increased the expression and activity of CYP1A, CYP2A, CYP3A1/2, and the activity of CYP2B and CYP2C11 but decreased the expression/activity of CYP2Ds and CYP2E1 enzymes. Lumateperone produced similar changes in the investigated CYPs but did not affect CYP1A1/2 and CYP2E1. The observed changes in CYP enzymes were accompanied by adequate alterations in transcription factors: both drugs increased the expression of PXR and decreased that of PPAR $\gamma$ . Moreover, brexpiprazole increased the expression of AhR.

**Conclusion:** In conclusion, brexpiprazole and lumateperone affect the regulation of CYP-drug metabolizing enzymes at a transcription level, which may lead to drug-drug metabolic interactions of pharmacological and clinical importance during long-term therapy with these antipsychotics.

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**P. 6.**

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**Psilocybin effects on brain neurotransmission and rat behavior.**

**Background:** Psilocybin, belonging to the tryptamine class of psychedelics, exerts its effects in the brain by dual action on 5-HT<sub>2A</sub> and 5-HT<sub>1A</sub> receptors located mainly on pyramidal cells and GABAergic interneurons in the frontal cortex. Psilocybin has various therapeutic effects, among others, in depression and mood disorders. The aim of this project was to investigate psilocybin effects on brain neurotransmission and animal behavior in naive and stressed rats.

**Material and Methods:** Psilocybin effect on neurotransmission was studied using microdialysis in the rat. A wet dog shake (WDS) test was applied to monitor hallucinogenic activity, an acoustic startle response test was used to study the efficacy of sensorimotor gating, the light-dark box (LDB) test was used to measure animal anxiety, an alkaline comet assay was performed to show DNA damage. Chronic mild stress (CMS) was applied as a model of anhedonia observed in depression.

**Results:** Psilocybin at doses 0.1, 0.3, and 0.6 mg/kg increased the release of NA, DA, 5-HT, ACh, glutamate, and GABA in the frontal cortex of naive rats. In contrast to 25I-NBOMe, a selective agonist of 5-HT<sub>2A</sub> receptors, psilocybin did not produce hallucinogenic activity and did not disrupt sensorimotor gating. Furthermore, psilocybin showed an anxiolytic effect. Psilocybin and 25I-NBOMe did not produce oxidative damage of DNA in the frontal cortex and hippocampus of naive rats, whereas ketamine and MDMA increased oxidative stress. The stressed animals showed depressive-like behavior expressed as anhedonia. Psilocybin (0.6 mg/kg) increased the KCl-evoked release of NA, DA, 5-HT, and glutamate extracellular levels in non-stressed animals, while no increase in NA, DA, and glutamate was found in the stressed rats. The GABA extracellular level decreased in both groups, but a stronger effect was seen in stressed animals. Administration of psilocybin resulted in oxidative DNA damage in the nuclear fraction obtained from the frontal cortex and hippocampus of both non-stressed and stressed rats.

**Conclusion:** The use of single low doses of psilocybin may have some beneficial properties in naive rats and fewer harmful effects. The lack of psilocybin effect in stressed animals may result from losing 5-HT<sub>2A</sub> receptor functionality.

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**P. 7.**

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**Functional Selectivity and *In Vivo* Efficacy of Trisubstituted 1,3,5-Triazines as Histamine H<sub>4</sub> Receptor Modulators in Rodent Models of Asthma**

**Background:** Histamine receptors play critical roles in immune responses, with the histamine H<sub>4</sub> receptor (H<sub>4</sub>R) being pivotal in modulating inflammation and immune cell recruitment. H<sub>4</sub>R's involvement in asthma is significant, as it influences mast cell, eosinophil, and T-cell activation, contributing to airway inflammation and hyperreactivity. Targeting H<sub>4</sub>R offers potential for novel therapeutic strategies.

**Material and Methods:** A series of 16 6-methylpiperazinetriazine-2-amine derivatives containing five-membered heterocyclic rings at the fourth position of triazine core were synthesized and tested for their affinity towards H<sub>4</sub>R. Comprehensive functional assays were employed to characterize the intrinsic activity of the compounds in G-protein-coupled and  $\beta$ -arrestin-mediated pathways. Functional selectivity in the  $\beta$ -arrestin pathway was assessed using two cellular models: split-luciferase complementation and reporter gene approach. Selected compounds were further characterized using an ERK phosphorylation assay. Finally, the most active compounds were tested in a protease-induced asthma model in C57BL/6J mice.

**Results:** Binding experiments revealed a good affinity for the H<sub>4</sub>R, with most compounds exhibiting  $K_i$  values in the submicromolar range. All ligands were found to be moderate antagonists/inverse agonists in the G-protein-coupled pathway, as indicated by their effects on cAMP production in CHO cells expressing H<sub>4</sub>R. Investigation of the  $\beta$ -arrestin pathway revealed a reproducible substitution pattern in the heterocyclic thiophene ring that favored either moderate agonistic or potent antagonistic activity. Introducing methyl, chloride, or bromide at position 5 of the thiophene ring resulted in agonistic activity toward  $\beta$ -arrestin, while placing these substituents at positions 3 and 4 generated  $\beta$ -arrestin pathway antagonists. *In vivo*, treatments with three selected, most active positional isomers in the protease-induced asthma model reduced allergic airway inflammation, confirming their potential to alleviate asthmatic symptoms.

**Conclusions:** We identified a series of positional isomers as potent H<sub>4</sub>R ligands where the substitution pattern was responsible for the occurrence of functional selectivity in the  $\beta$ -arrestin pathway. This functional selectivity did not correlate with efficacy *in vivo*, suggesting that while  $\beta$ -arrestin signaling may influence various receptor responses, other factors likely contribute to the compounds' *in vivo* effects in animal model of asthma.

## P. 8.

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### **Psilocybin-induced miRNA and proteomic changes in the prefrontal cortex in the context of treatment-resistant depression**

**Background:** Psilocybin has gained significant attention as a novel antidepressant, particularly for its efficacy in treatment-resistant depression (TRD). Psilocybin is widely recognized for exerting its effects particularly in the prefrontal cortex. However, the precise molecular mechanisms underlying its action remain unclear. This study aimed to investigate the expression levels of miRNAs and protein profiles after psilocybin administration to elucidate its molecular effects.

**Materials and methods:** Two rat strains were used: Wistar Kyoto (WKY, an animal model of TRD) and Wistar Han (WIS, a normotypic control). Psilocybin was administered at a dose of 0.3 mg/kg. Two time points were examined: 4 hours (for miRNA analysis) and 7 days (for proteomic analysis). Small RNA sequencing was employed to assess miRNA expression in the prefrontal cortex 4 hours after psilocybin administration. Mass spectrometry was used to quantify protein levels, with proteomic analysis performed separately for neuronal cell populations, which were isolated from tissue using fluorescence-activated cell sorting (FACS).

**Results:** More than 50 miRNAs showed differential expression between WKY and WIS rat strains. Psilocybin treatment influenced a smaller subset of miRNAs, with distinct patterns of alterations observed between WIS and WKY rats. Several miRNAs were selected for further analysis, including rno-miR-212-3p, rno-miR-223-3p, rno-miR-34a-5p, rno-miR-3064-5p, and rno-miR-383-3p. Proteomic analysis revealed significant differences between WIS and WKY rats, particularly in metabolic processes. Psilocybin treatment affected the expression of proteins involved in RNA metabolism and mRNA processing, including biosynthesis and splicing.

**Conclusions:** Biochemical analyses revealed substantial differences between TRD-like WKY rats and control WIS rats at both the miRNA and protein levels. Similarly, psilocybin treatment induced strain-specific changes in miRNA and protein expression, with limited overlap between the two strains. These findings highlight the molecular complexity of psilocybin's action and its potential strain-dependent effects.

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**P. 9.**

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**The cytochrome P450 2D6 (CYP2D6)-catalyzed synthesis of dopamine in human neuronal cells**

**Background:** Dysfunction of the brain dopaminergic system may be implicated in different neuropsychiatric diseases. The polymorphic cytochrome P450 2D6 (CYP2D6) is engaged in the metabolism of psychotropic drugs and endogenous neuroactive substrates, so its activity may affect the prevalence of those diseases and therapy outcomes. *In vitro* studies using liver/brain microsomes and cDNA-expressed CYP2Ds revealed that the efficiency of human CYP2D6 in synthesizing dopamine was much higher than that of rat CYP2Ds. The CYP2D-catalyzed dopamine formation was also shown *in vivo* in the rat brain microdialysis model. In the present study, we have focused on investigating tyramine hydroxylation to dopamine in human neuronal cells.

**Material and methods:** A human neuroblastoma cell line (SH-SY5Y) of wild-type (WT) and genetically modified *via* overexpression of the *CYP2D6* gene (*CYP2D6-OX*) was used. The overexpression of *CYP2D6* was verified by assessing *CYP2D6* mRNA (qRT-PCR), CYP2D6 protein level (Western blotting), and CYP2D6 activity measured as a rate of bufuralol 1'-hydroxylation (HPLC with fluorescence detection). Tyramine was added to the incubation medium, and the formation of dopamine from tyramine in neuronal cells was measured using HPLC with coulometric detection.

**Results:** The investigated *CYP2D6-WT* and *CYP2D6-OX* cell lines displayed a basal level of dopamine. Dopamine was synthesized from tyramine by CYP2D6 in both neuronal cell lines. The kinetic parameters were calculated based on dopamine formation's dependence on the time and substrate (tyramine) concentration in the incubation medium ( $K_m$  and  $V_{max}$ ). The rate of tyramine hydroxylation to dopamine was significantly higher in the transgenic cell line with the overexpression of the *CYP2D6* gene compared to the WT cell line.

**Conclusions:** We have demonstrated that human neuronal cell line can synthesize dopamine from tyramine. Assuming that dopamine is produced from endogenous tyramine in the human brain *in vivo* and considering *CYP2D6* gene polymorphism, the amount of dopamine formed *via* this alternative pathway may differ in individual subjects. Thus, interindividual differences in CYP2D6 activity in the brain may affect the predisposition to neurological or psychiatric disorders.

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**P. 10.**

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**New evidence for selective activation of nonnuclear ER signaling by PaPE-1 in A $\beta$ -challenged mammalian brain neurons**

**Background:** Alzheimer's disease (AD) is the predominant cause of dementia, accounting for 60-80% of reported cases. Current AD therapies provide moderate symptom relief but do not alter disease progression. In recent years, nonnuclear estrogen receptor (ER) signaling has emerged as a potential therapeutic target to induce neuroprotection, avoiding the deleterious effects elicited by the activation of classical nuclear ERs. This inspired us to test the neuroprotective capacity of the Pathway Preferential Estrogen 1 (PaPE-1) in experimental models of AD. We recently demonstrated that PaPE-1 confers neuroprotection against A $\beta$ -induced toxicity, as evidenced by reduced level of amyloid-beta (A $\beta$ )-induced neurodegeneration, restored neurite outgrowth, and inhibited expression of AD-related genes. The aim of the present study was to examine the selective activation of nonnuclear ER signaling by PaPE-1 in A $\beta$ -challenged mammalian brain neurons.

**Material and methods:** In this study, we verified the selective targeting of PaPE-1 (10  $\mu$ M) to membrane fraction of ERs and provided evidence for its beneficial role in a cellular model of AD. Experiments were performed on mouse primary neurons in *in vitro* cultures that were pretreated with 10  $\mu$ M A $\beta$ . Molecular analyses such as qPCR, ELISA, and assessment of DNA methylation of specific genes such as *Esr1*, *Esr2*, and *Gper1* were then performed. These analyses were complemented by immunofluorescence labeling and confocal microscopy.

**Results:** A $\beta$  reduced the protein levels of ERs in cytosolic and membrane fractions, thus confirming the impairment of ER signaling typical of AD. PaPE-1 reversed A $\beta$ -induced effects only in the membrane fraction of receptors that supports nongenomic signaling-oriented effects. PaPE-1-evoked increases in the protein levels of membrane fraction receptors ESR1/ER $\alpha$  and ESR2/ER $\beta$  corresponded to DNA hypomethylation of *Esr1* and *Esr2*. MAP2-specific labeling confirmed the neuroprotective potential of PaPE-1, as the compound counteracted the decrease in MAP2 immunofluorescence staining in response to A $\beta$ .

**Conclusions:** In this study, we demonstrated for the first time that the mechanism of action of PaPE-1 on ER signaling in A $\beta$ -challenged mammalian brain neurons involves membrane-localized ESR1/ER $\alpha$  and ESR2/ER $\beta$ , which are upregulated through DNA hypomethylation.

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**P. 11.**

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**The role of the immune system in the development of hypersensitivity in leptin-deficient Lep<sup>ob/ob</sup> and streptozotocin-induced diabetic models in mice**

**Background:** Diabetic neuropathy is a chronic disease resulting from nerve damage and affects as many as 50% of people with diabetes. In turn, one of the most progressive diseases of civilization is obesity; at least 39% of the world's population is overweight, and 13% is obese. Obesity is also strongly associated with the occurrence of chronic pain, including neuropathic pain. Unfortunately, neuropathic pain treatment is unsatisfactory; therefore, it is so important to understand the mechanisms that determine the development of hypersensitivity to develop effective pharmacotherapy with particular consideration of its etiology and gender. According to numerous reports, immune factors such as interleukins and chemokines are associated with the pathophysiology of neuropathic pain development, but their role in diabetes and obesity remains unclear. Therefore, this study aimed to determine the participation and role of cytokines and their receptors in the model of diabetes and obesity in male and female mice.

**Material and Methods:** The experiments were performed on male and female C57BL6/J mice in a model of diabetic neuropathy induced by streptozotocin (STZ, 200 mg/kg, i.p.) and leptin-deficient ob/ob (LepKO) mice in a model of genetic obesity. To determine the development of mechanical and thermal hypersensitivity, two behavioral tests were performed, respectively, the von Frey test and the cold plate test. In addition, the level of cytokines and their receptors in the spinal cord collected from the studied mice was determined using RT-qPCR and/or ELISA.

**Results:** The development of neuropathic pain symptoms was observed in both the obesity and diabetes models. Interestingly, increased expression of genes encoding CCL2, CCL5, and IL-18 was observed in the spinal cord during diabetes-induced neuropathic pain, whereas obese mice showed an increase in IL-1 $\beta$  levels. Additionally, these changes were also sex-dependent.

**Conclusions:** The presented results indicate that cytokines are involved in developing hypersensitivity in both diabetes and obesity and suggest the need for further research in this area to develop effective, cause- and gender-specific treatments for chronic pain.

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**P. 12.**

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**Western diet and neurodevelopment: a maternal influence on offspring's behavior**

**Background:** The rising incidence of neurodevelopmental disorders, including autism spectrum disorders (ASD) and attention deficit hyperactivity disorder (ADHD), is linked to prenatal, perinatal, and postnatal genetic and environmental factors. Recent animal studies suggest that a Western diet (WD) during pre-pregnancy or lactation may negatively impact brain development. This study aimed to investigate how maternal WD affects offspring behavioral phenotypes on postnatal days (PND).

**Material and Methods:** Wistar Han dams were fed either a control diet (CD) or a WD during gestation and lactation for 14 weeks. Post-weaning, offspring of both sexes were switched to a CD. Behavioral analyses were conducted using locomotor activity (LA), novel object recognition test (NOR), marble burying (MB), open field (OF), and self-grooming (SG) tests on PND 30, 60, and 90. On PND 90, offspring were sacrificed for molecular analysis, and blood, brain, adipose tissue, brown fat, heart, liver, spleen, and kidneys were collected for further examination.

**Results:** Male offspring exposed to a WD had significantly higher body weights compared to those on a CD, with a trend toward increased adipose tissue in both sexes. Glucose levels remained stable regardless of diet or sex. Surprisingly, brain weight was higher in the WD group for both sexes. In the OF test, WD male offspring exhibited significantly more line crossings on PND 30 and 60 but not on PND 90, with no significant differences in center time across diets or sexes. In the SG test, WD males groomed significantly longer on PND 30 than PND 60 and 90, with decreasing latency across testing days; no such differences were noted in females. No significant differences were observed in LA, NOR, or MB tests between CD and WD groups for either sex.

**Conclusions:** To sum up, exposure to a maternal WD induces behavioral changes, which are evident in OF and SG tests, particularly in male offspring during early life. Therefore, our research supports the idea that maternal nutrition during pregnancy and lactation has a significant impact on neurodevelopmental disorders such as ASD and ADHD, which may serve as valuable indicators for assessing the long-term effects of maternal diet in adults.

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**P. 13.**

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### **Side effects of fluoxetine treatment on the morpho-functional state of male rats' gonads with PTSD developed in peripubertal age**

**Background:** Among the pharmacological treatments available for childhood PTSD, fluoxetine (FLX) remains one of the most commonly prescribed antidepressants. The challenge can arise from the dual burden imposed by PTSD and FLX on the developing male reproductive system. This study aimed to investigate the combined impact of PTSD developed in peripubertal age and FLX treatment on male gonads, utilizing a rodent model.

**Material and methods:** The experimental design included three groups: control, PTSD-only, and PTSD+FLX. Male rats, 40 days old, were exposed to combined stress followed by repeated stress, then treated with FLX: 10 mg/kg for 21 days (the control group and PTSD group obtained the corresponding volumes of the vehicle). After the last FLX administration, behavioral assessments were conducted using the Elevated Plus Maze test to prove the presence of PTSD signs. Morpho-functional and histological analyses of the gonads and hormones investigations were performed.

**Results:** PTSD simulation resulted in increased anxiety-like behaviors noticed by an increase in the time spent in closed arms and an increase in anxiety index. Both PTSD and fluoxetine groups showed a decrease in serum total testosterone and follicle-stimulating hormone, alterations in testicular histology, characterized by pericellular edema and apoptosis of sustentocytes. FLX administration also led to thickening of blood vessel walls and degeneration of spermatogenic cells, suggesting hypospermatogenesis. In rats with PTSD and FLX administration, the frequency of intraepithelial vacuole detection was significantly higher than in rats with only PTSD. Epididymal histology showed changes that characterize blood circulation disorders, accompanied by a disturbance in the blood vessel wall permeability and the development of foci of peritubular edema in certain parts of the organs in the PTSD and PTSD+FLX groups. Notably, in the FLX-treated group, we observed the gonadosomatic index elevation, a decrease in the epididymis mass, and the number of sperm from the cauda of epididymis by 25%. **Conclusions.** The findings highlight the dual burden of PTSD and FLX on male reproductive health, emphasizing the need for further research into therapeutic strategies that minimize delayed adverse reproductive outcomes in pediatric patients undergoing SSRI treatment.

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**P. 14.**

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**Omega-3 fatty acid-enriched diet reverses maternal high-fat diet-induced depression-like behavior and myelin-related changes in rat offspring.**

**Background:** Maternal high-fat diet (HFD) during pregnancy and lactation induces a depression-like phenotype and myelin-related changes in rat offspring, which persist into adulthood. Considering the plasticity of the developing brain, we aimed to investigate whether these adverse effects could be reversed by a diet enriched with omega-3 fatty acids (Ω3D).

**Material and methods:** The effects of Ω3D administered from the post-weaning period until adulthood (63rd day of life) were examined in offspring exposed to maternal HFD (60% energy from fat) during gestation and lactation. Behavioral assessments included the forced swimming test and locomotor activity. Molecular analyses were performed to evaluate myelin-related changes in the prefrontal cortex (PFCTX) of adult offspring using RT-qPCR, ELISA, and immunofluorescence staining.

**Results:** Ω3D reversed increased immobility time and decreased sucrose preference in adult offspring induced by maternal HFD without affecting the animals' locomotor activity. Molecularly, Ω3D normalized the reduced expression levels of myelin-oligodendrocyte glycoprotein (MOG) and myelin and lymphocyte protein (MAL) in males and MOG in females in the PFCTX, changes initially induced by maternal HFD. Additionally, Ω3D normalized the number of oligodendrocytes (precursor and mature cells) in the prelimbic, infralimbic, and cingulate cortex, mainly in males, which were reduced following maternal HFD exposure.

**Conclusions:** These findings suggest that Ω3D supplementation may play a pivotal role in mitigating behavioral and neurobiological changes caused by adverse prenatal conditions, highlighting its potential therapeutic value.

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**P. 15.**

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**Exploring Myelination and the Endocannabinoid System in Fetal Alcohol Spectrum Disorders: Insights into Pathogenesis and Therapeutic Potential**

**Background:** Fetal Alcohol Spectrum Disorders (FASD) result from prenatal alcohol exposure (PAE), leading to cognitive, behavioral, and motor impairments. Disrupted myelination, a crucial process for neural signal transmission regulated by the endocannabinoid (eCB) system, may underlie these deficits. The eCB system comprises cannabinoid receptors (CB1/CB2), endocannabinoids (anandamide [AEA] and 2-arachidonoylglycerol [2-AG]), and associated enzymes. We hypothesize that FASD disrupts this system, impairing glial cells and myelin formation. This study aimed to investigate the impact of neonatal ethanol exposure (a model of FASD) on eCB levels and myelin-related changes in the hippocampus of rats.

**Material and Method:** Neonatal rats were administered ethanol (5 g/kg, 22.66% v/v, i.g.) from postnatal days (PND) 4 to 9 to model FASD. Pups were weaned on PND21, sex-separated, and euthanized on PND22. Brains were rapidly extracted, and hippocampi were dissected. Endocannabinoid levels were quantified using liquid chromatography-mass spectrometry (LC-MS), and myelin-related changes were assessed via ELISA.

**Results:** In the hippocampus of ethanol-exposed female rats, AEA levels increased, while 2-AG levels decreased. In contrast, male ethanol-exposed rats exhibited reduced 2-AG levels without changes in AEA. Furthermore, PAE led to a reduction in the expression of myelin basic protein (MBP), myelin oligodendrocyte glycoprotein (MOG), myelin and lymphocyte protein (MAL), and 2',3'-cyclic nucleotide 3'-phosphodiesterase (CNPase) in male rats.

**Conclusions:** Ethanol exposure disrupts the eCB system and myelination in neonatal rats, with sex-specific effects. Future studies will explore how eCB modulation influences myelination and cognitive outcomes in FASD models. These findings underscore the eCB system as a potential therapeutic target for neurodevelopmental deficits in FASD.

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**P. 16.**

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**Dual-Targeting Histamine H<sub>3</sub> and Sigma-1 Receptor Ligands as Candidates for the Treatment of Neuropathic Pain**

**Background:** The treatment of complex, multifactorial diseases using single-target therapies often fails to achieve satisfactory efficacy. Consequently, the pharmaceutical industry and academia have turned their attention to strategies that modulate multiple biological targets simultaneously. Considering the well-documented interplay between histamine H<sub>3</sub> (H<sub>3</sub>R) and sigma-1 (σ<sub>1</sub>R) receptors, there is significant potential for developing dual-acting ligands to manage various pain conditions.

**Material and methods:** In this study, we synthesized a series of 11 novel compounds, including derivatives of piperidine, morpholine, azepane, benzoxazole, benzothiazole, and chalcones. These compounds were evaluated for their affinities at H<sub>3</sub>R, σ<sub>1</sub>R, sigma-2 (σ<sub>2</sub>R), and μ-opioid (MOR) receptors using *in vitro* assays.

**Results:** Among the tested compounds, several demonstrated strong antagonistic activity at H<sub>3</sub>R, with selectivity for the intended targets. Notably, the compound KSK107 emerged as a lead candidate due to its promising pharmacological profile. Subsequent *in vivo* studies confirmed its pain-relieving properties, validating its potential as a multi-target analgesic agent.

**Conclusions:** This research supports the growing evidence that multi-target ligands can offer a more integrative and effective approach to pain management by modulating multiple pathways. Such compounds have the potential to deliver improved efficacy and safety profiles compared to current monotherapies.

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**P. 17.**

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**Effect of 1MeTIQ on monoamine release disorders induced by MK-801 in the rat hippocampus**

**Background:** Dysregulation of monoaminergic systems can cause both positive and negative symptoms of schizophrenia. MK-801 acts as an antagonist of NMDA receptors and may induce schizophrenia-like symptoms. 1-Methyl-1,2,3,4-tetrahydroisoquinoline (1MeTIQ) is a reversible MAO inhibitor with neuroprotective, antidepressant- and anxiolytic-like effects.

The aim of the present study was to investigate the impact of acute 1MeTIQ administration on the disturbances in the release of monoamines (dopamine [DA], serotonin [5-HT], noradrenaline [NA]) in the rat's hippocampus (HIP) caused by MK-801; 1MeTIQ was administered 20 minutes before MK-801 injection using *in vivo* microdialysis study.

**Material and methods:** The release of DA, 5-HT, and NA were measured in the HIP in freely moving rats. After the microdialysis experiment, the dialysate samples were analyzed using an HPLC apparatus for electrochemical detection.

**Results:** *In vivo* microdialysis study showed that in the HIP, 1MeTIQ given alone and combined with MK-801 increased the release of DA (100% and 60%, respectively). The same analysis indicated that in the HIP MK-801 significantly decreased the release of 5-HT (up to 50%). In the combined group, 1MeTIQ completely reversed this effect and increased the release of 5-HT (approx. 180%). *In vivo* microdialysis study showed that 1MeTIQ given alone and combined with MK-801 increased the release of NA (approx. 50%) in the HIP.

**Conclusions:** Our study indicated the ability of 1MeTIQ to reverse NMDA receptor antagonist-induced disturbances in the activity of the serotonergic system. At the same time, 1MeTIQ administered alone or in combination with MK-801 increased the release of the remaining monoamines, DA, and NA in rat's HIP.

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**P. 18.**

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**Maternal diet enhances offspring's vulnerability to cocaine-seeking**

**Background:** Cocaine use disorder is the compulsive use of cocaine that causes medical, psychological, and behavioral consequences. It is well-documented that the maternal diet significantly influences the proper development of offspring. However, there is no information on the development and maintenance of cocaine use disorders in offspring exposed to maternal high-sucrose (HSD) or fructose (FRU) diet consumption during pregnancy and lactation.

**Material and methods:** Male offspring rats after maternal HSD or FRU diet exposure during pregnancy and lactation were used in the intravenous cocaine self-administration procedures in an increasing fixed ratio (from FR1 to FR5) reinforcement schedule with a stable dose of cocaine (0.5 mg/kg/infusion) or increasing doses of cocaine (from 0.25 to 1 mg/kg/infusion) under a stable FR1. Later, we evaluated the rat's motivation in a progressive ratio test, abstinence in extinction training, and reinstatement of cocaine-seeking induced by conditional or unconditional stimuli. Finally, we evaluated the expression of melanocortin type 4 receptors (MC4Rs) and pharmacological intervention of MC4Rs antagonist (ML 00253764) in cocaine-seeking behavior.

**Results:** In comparison to the maternal HSD, the maternal FRU diet is sufficient to evoke either alternation in the acquisition and maintenance of cocaine abuse or produce vulnerability to low cocaine doses in male offspring. Similarly, both perinatal HSD and FRU diet leads to an increased reinstatement of cocaine-seeking behavior. Interestingly, maternal HSD overconsumption evokes differences in the expression of MC4Rs in reward-related rat brain regions. At the same time, intervention with MC4Rs antagonist reduces the reinstatement of cocaine-seeking behavior.

**Conclusions:** Our findings underlie the important role of maternal diet during pregnancy and lactation in the offspring's development. Excessive maternal FRU overconsumption can change offspring's vulnerability and sensitivity to cocaine. Here, offspring exposed to maternal HSD or FRU intake leads to increased reinstatement of cocaine-seeking behavior. Furthermore, based on neurochemical and behavioral analyses, MC4Rs can be a potential target for new drugs to prevent cocaine relapse in human addicts.

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**P. 19.**

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**Research directions and main scientific achievements of the Department of Brain Biochemistry of Maj Institute of Pharmacology in the years 2007-2023**

The analysis of research of the Department of Brain Biochemistry of the Maj Institute of Pharmacology published in 2007-2023 indicates three main scientific trends: searching for intracellular signaling alterations involved in symptoms of depression, drug addiction, and antidepressant drug action mechanisms; the study of inflammatory components of psychiatric disorders; and the development of new transgenic models to study the mechanism of neurodegenerative diseases. To achievements in signaling alterations belong: describing the pattern of changes of the expression of  $\alpha$  subunits of all G protein families in the prefrontal cortex, hippocampus and amygdala in models of morphine addiction and fear-impaired memory; the discovery that the blocking of GABA transporter (GAT1) by tiagabine prevents cocaine evoked increase of cortical responsiveness of  $\alpha$ 1AR; pretreatment with paroxetine does not influence the reduction of cAMP in the  $\beta$ 1AR pathway of the rat cortex connected to the antidepressive activity of ECS, rTMS; anhedonia is associated with decreased  $\alpha$ 1AR density and the modulation of Hsp72, Hsp90 level in cortico-limbic neuronal circuits; antidepressants may differentially regulate intracellular signaling from  $\alpha$ 1AR subtypes ( $\alpha$ 1A-,  $\alpha$ 1B- and  $\alpha$ 1D-AR). Immunological research includes discoveries: therapeutic impact of antidepressant therapies in animal models is related to the reduced pro-inflammatory activity of macrophages; selective ablation of glucocorticoid receptors in noradrenergic neurons enhances the pro-inflammatory activity of macrophages in response to selected stimuli in vitro; the exposure to particulate matter (a component of air pollution) increases oxidative stress and inflammatory processes in mice. Lastly, the department developed and characterized several transgenic mouse models. Key findings from neuropsychiatric models include sex-dependent behavioral effects of GR deletion in noradrenergic neurons (anxiety/depression in females, stress resilience in males) and demonstrating CREM involvement in antidepressant responses. Utilizing a unique selective neurodegeneration model based on TIF1A deletion in specific target populations revealed neuroprotective roles of autophagy in Huntington's disease. Recent achievements utilizing TIF1A/flox mice focus on Parkinson's disease, including demonstrating neuroprotective properties of noradrenergic transmission and developing a novel CRISPR/Cas9-based model for noradrenergic neuron degeneration.



**P. 20.**

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**Effect of lysine acetylsalicylate on the levels of oxidative stress markers in rat brain cortex after traumatic brain injury**

**Background:** Traumatic brain injury (TBI) is considered a major global health concern, with no current approved drug for its treatment. Excess intracellular Ca<sup>2+</sup> and excitotoxicity in TBI lead to excessive production of reactive oxygen species (ROS) and ultimately to oxidative stress. Cyclooxygenases – COX-1 and COX-2 are the main enzymes responsible for the conversion of arachidonic acid (AA) into prostaglandins (PGs) and reactive oxygen species (ROS), both of which take an active part in neuroinflammation. The use of acetylsalicylic acid (ASA)-like drugs in modern medicine is extensive. The key mechanism of action for ASA is the irreversible inhibition of COX-1 and COX-2 enzymes. ASA penetrates well through the blood-brain barrier, which can affect the exchange of prostaglandins in the CNS in both normal and pathological conditions.

**Material and methods:** The study was conducted on 28 male Wistar rats weighing 200-250 g. TBI was caused by mechanical damage from a metal weight (450 g) free-falling in a vertical pipe from a height of 170 cm onto the rat head (*Marmarou C.R. et al., 2009*). All animals were randomized into 3 groups: intact rats (n=10), control – rats with TBI (n=8), rats with TBI + lysine acetylsalicylate (LASA), 30 mg/kg, intraperitoneally (n=10). Administration of LASA was performed 1 hour, 24 hours, and 48 hours after TBI. Oxidative stress biomarkers (advanced oxidation protein products (AOPP), thiobarbituric acid reactive substances (TBARS), carbonylated proteins (PC370/PC430) were studied using spectrophotometry in fraction S1 of the brain cortex.

**Results.** The levels of TBARS, PC370, and AOPP in the S1 fraction of cortex in rats with TBI were increased compared to the intact animals by 39.7% (P<0.05), 34.2% (P<0.05) and 27% (P<0.01), respectively. Simultaneously, there were no significant changes in levels of PC430. After administration of LASA, a significant decrease in the levels of AOPP by 33.9% (P<0.05) was noted compared to the group of rats with TBI.

**Conclusions.** The directed changes in biomarkers of carbonyl-oxidative stress in the brain cortex indicate the complexity of oxidative damage caused by TBI. In our study, LASA demonstrated effectiveness in reducing the levels of AOPP solely in rats with TBI. These results provide insight into some potential therapeutic effects of LASA, but further studies are needed to investigate the therapeutic efficacy of this drug in TBI.

**P. 21.**

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**Spatial analysis of risperidone-induced gene expression in the murine forebrain**

**Background:** Profiling treatment-induced gene expression reveals molecular mechanisms underlying drug action. The effects of risperidone on gene expression were examined in the mouse forebrain using an approach that combines RNA sequencing and in situ hybridization.

**Material and methods:** Male C57BL/6N mice (9-10 weeks old) received risperidone (*i.p.*, 0.5 mg/kg) or saline injection (n = 6 per group). Brains were extracted 2 hours after drug administration, frozen, sectioned, and used for spatial transcription profiling. A customized method for sequencing read alignment was used to avoid mismatched reads in 3' transcript regions. Clustering was performed with the Seurat package using a shared nearest neighbor algorithm and visualized as a UMAP projection of the whole dataset. Cluster-wise statistical comparisons were used to extract region-specific effects of drug treatment.

**Results:** Unsupervised clustering showed gene expression patterns reflecting neuroanatomy of the forebrain. Brain area-specific changes in the regulation of 95 transcripts were found in 12 clusters (p < 0.01, log<sub>2</sub>ratio = 0.8, minimum expression threshold = 0.2). Enrichment analysis of the risperidone-induced genes revealed 18 that were previously connected to the etiology of schizophrenia. Further exploratory analysis indicated that this subset of risperidone-regulated transcripts (e.g., *Cacna1i*, *Caln1*, *Smpd3*, *Phactr3*) was also associated with schizophrenia in human genome-wide studies (GWAS).

**Conclusions:** Spatial analysis of risperidone-induced gene expression reveals complex expression patterns, confirming its known effects and identifying novel gene candidates affected by drug and disease. Most importantly, it also localizes transcriptional alterations of genes previously linked to human schizophrenia. These genes may serve as potential molecular switches between physiological brain plasticity disease-related and treatment-induced changes.

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## POSTER SESSIONS

## OTHER TOPIC

## P. 22.

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**Immune Response Disturbances: A Key Factor in Brain Pathology**

**Background:** Prolonged inflammation is central to neurodegenerative diseases like stroke and Alzheimer's disease (AD). Current therapies remain limited, highlighting the need to unravel the molecular mechanisms of these conditions. Therefore, new challenges arise for understanding the molecular basis of civilization diseases, defining innovative therapeutic strategies and novel model systems, multidisciplinary application of knowledge in the field of physics, chemistry, and experimental biology, and conducting preclinical research considering new model tools that can lead to more effective basic and alternative therapies.

**Material and methods:** The Department of Experimental Neuroendocrinology at IP PAS conducts research to test the hypothesis that brain immune regulation, neuroinflammation reduction, and enhancement of endogenous resolution mechanisms (RoI) could represent innovative therapeutic strategies for neurodegenerative diseases. This proof-of-concept study integrates advanced research tools: *in vitro* microglial cell models, *ex vivo* organotypic hippocampal cultures simulating ischemia (via Oxygen Glucose Deprivation), and *in vivo* APPNL-F/NL-F transgenic mice as a model of late-onset Alzheimer's disease. Furthermore, the study includes the development of novel drug-like molecules and theranostic nanocarriers aimed at mitigating brain pathology.

**Results:** We have identified age-dependent cognitive learning and memory deficits in APP<sup>NL-F/NL-F</sup> mice and disturbances in the RoI, which are determined by the animal's age, genetic background, and brain structure. Moreover, our studies showed that new ligands of the FPR2 receptor actively suppress the inflammatory reaction induced by a non-specific activator of the immune system. Their protective and anti-inflammatory effects were observed in cultures of microglial cells (*in vitro*). We also provide new evidence for the neuroprotective potential of CsA in ischemia-induced brain injury (*ex vivo* 3D organotypic culture systems).

**Conclusions:** We believe that our innovative approach to modulating brain inflammation with new drug-like molecules and targeted immunosuppressive therapies could lead to new strategies for managing Alzheimer's disease and stroke, addressing significant economic and social challenges.

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**P. 23.**

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**Activation of non-nuclear estrogen receptors with PaPE-1 as a novel therapeutic approach in post-treatment of hypoxic and ischemic injuries**

**Background:** Pathway Preferential Estrogen-1 (PaPE-1) is an innovative compound specifically designed to target a subpopulation of estrogen receptors (ERs), i.e., non-nuclear ERs. This substance poses a promising direction in development of therapies since it is devoid of hormonal adverse side effects associated with non-specific activation of these receptors. Our study explores the complex mechanisms underlying action of PaPE-1 and provides evidence of its efficacy in mitigating damage caused by hypoxic and ischemic events, particularly in a post-treatment paradigm.

**Material and methods:** In our research, we utilized an *in vitro* model of hypoxic/ischemic injury, established by exposing primary neocortical cell cultures to hypoxic or ischemic conditions for 6 hours. This was followed by an 18-hour reoxygenation phase with the administration of PaPE-1, mimicking the reperfusion phase observed in patients. To assess the neuroprotective potential of PaPE-1, we used a variety of both biochemical and molecular analyses.

**Results:** Since the beginning of this project, we studied a plethora of parameters that PaPE-1 was able to regulate, such as apoptosis, excitotoxicity and mitochondrial damage. In hereby work, we focus on more indirect effects of its neuroprotective actions associated with autophagy, oxidative stress, and neuroinflammation. All of these processes have been observed to play pivotal roles in the emergence of hypoxia- and ischemia-induced injury. In our study, administering PaPE-1 six hours after the initial injury proved effective in normalizing parameters dysregulated due to hypoxia and ischemia. This included formation of autophagy-related vesicles, as well as expression of both genes and proteins involved in autophagy. Moreover, PaPE-1 influenced expression of factors associated with neuroinflammation and the markers of stroke severity, such as metalloproteinases, arginase or hypoxia-inducible factor 1-alpha.

**Conclusions:** Taking our findings into consideration, we postulate that activation of non-nuclear estrogen receptors with PaPE-1 is a promising therapeutic direction in combating neurodegenerative diseases associated with hypoxia and ischemia. Notably, in our research, we focused on extending the therapeutic window, addressing the most dire limitation of currently available stroke therapies.

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**P. 24.**

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**Over 30 years of research on wild lettuces and chicory in the Department of Phytochemistry IP PAS**

**Background:** The tribe Cichorieae of the Asteraceae comprises about 150 lettuce (*Lactuca* L.) species and seven species of chicory (*Cichorium* L.). Of these, *L. sativa* L., a popular leafy vegetable, is of major economic importance. Another member of the *Lactuca* genus, *L. virosa* L., as well as some other closely related wild lettuces, is a source of "lactucarium," an old remedy for pain, cough, anxiety, and insomnia. Its medicinal properties have been attributed to sesquiterpene lactones of lactucin-type. The same compounds are synthesized by chicory, a plant utilized both as a vegetable and traditional medicine. Roots of *C. intybus* L., the most popular species of the genus, are valued as a traditional herbal remedy used to relieve symptoms of mild digestive disorders and temporary loss of appetite.

**Material and methods:** The investigated plants were grown in the Garden of Medicinal Plants, Maj Institute of Pharmacology, PAS, Kraków, where voucher specimens were deposited. Tissue cultures of different types were obtained and maintained in the Department of Phytochemistry IP PAS. The structures of isolated compounds were elucidated by spectroscopic methods (NMR, HRMS). The hot plate, tail-flick, and locomotor activity tests on mice were used to assess the pharmacological activity of the plant preparations and isolated compounds.

**Results:** Chemical composition of 20 wild lettuces and 5 species of chicory were studied: (I) 11 lettuce species were investigated for the first time; (II) eighteen new, previously not described natural products were characterized in lettuces and chicories (one from a hairy root culture); (III) several dozen of specialized metabolites were described for the first time as metabolites of lettuce or chicory (including different types of tissue cultures) and structures of some previously known natural products were corrected; (IV) hairy roots of *C. intybus* and *L. virosa* turned out to be a rich source of polyphenolic antioxidants; (V) analgesic and sedative properties of lactucin and its derivatives, which are characteristic metabolites of *Lactuca* and *Cichorium* spp., have been proven.

**Conclusions:** Our reports confirm the possibility of using wild lettuce and chicory as a source of biologically active metabolites and as a functional food due to the presence of compounds with potential health-promoting properties. The metabolomic profile of wild lettuces often reflects their taxonomic position within the genus *Lactuca*.

## P. 25.

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### **In silico SwissADME Evaluation of Antibacterial NHC-silver acetates and halides complexes**

**Background:** NHC-silver(I) complexes have garnered interest in medicinal chemistry for their antibacterial and anticancer potential. Their effectiveness hinges on the NHC core structure and nitrogen substituents, which can be adjusted to optimize lipophilicity and control silver ion release. Their antibacterial action mainly stems from the gradual release of silver ions, and this effect is enhanced by the complexes' lipophilicity, which aids cell penetration. The link between lipophilicity and biological activity is a central focus in ongoing research on these compounds.

**Material and methods:** SwissADME, developed by the Swiss Institute of Bioinformatics (SIB), is a web-based tool used to predict drug-likeness, pharmacokinetics, and medicinal chemistry suitability of small molecules—key properties in drug discovery. While widely used in medicinal chemistry, SIB does not guarantee its results. This work characterizes antibacterial NHC-silver complexes to identify factors influencing biological activity, specifically assessing if more active complexes differ in SwissADME parameters. Since the computational methods utilized are based on molecular mechanics and various topological and atom-based models, we have explored the electronic structures of representative NHC-silver complexes using DFT calculations and compared the results with the reported experimental stabilities.

**Results:** We have demonstrated that the easily accessible pharmacokinetic parameters provided by the SwissADME platform cannot be directly used to explain the biological activity of a given silver complex. Since the biological activity of Ag-NHC complexes is primarily determined by the presence of silver ions, the theory proposed by some authors that lipophilicity enhances antibacterial activity is not supported by our findings.

**Conclusions:** The bulky and lipophilic within any NHC system influence the strength of the carbon-silver bond and its susceptibility to hydrolysis. Consequently, lipophilicity and solubility and etc. parameters for entire complexes provided by theoretical models like SwissADME are secondary factors and should not be used in isolation to compare different NHC systems. Stability and susceptibility to hydrolysis of NHC-silver complexes can be linked to the LUMO absolute values mapped on the electron isodensity surface, as evidenced by DFT calculations. The optimal lipophilicity value for antibacterial Ag-NHC complexes should be considered unknown.

**P. 26.**

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**Preparation of hierarchical scaffolds for the regeneration of osteochondral defects**

Due to increasing life expectancy and lifestyle, there is a constant demand for more innovative, functional materials for medical applications. Particularly in demand is the manufacturing of implants, where the focus is on personalizing the properties of the materials obtained so that they can regenerate the tissues of a specific patient. Additionally, the use of commercially available implants is associated with a risk of rejection. Therefore, researchers are focusing on developing novel biomaterials. The scaffolds constructed for the regeneration of osteochondral tissue defects should be able to reproduce a three-dimensional microenvironment that mimics the native tissue. An interconnected, porous architecture ideal for cell adhesion, proliferation, and differentiation should characterize them.

Furthermore, the scaffolds should exhibit mechanical properties comparable to native cartilage or bone tissue. Using suitable polymers with precise geometry and micro/macromolecular organization is a promising approach to obtaining hierarchical and gradient scaffolds for osteochondral tissue regeneration. For this purpose, materials engineering techniques include solvent casting/porogen leaching or freeze-drying techniques. This study presents the possibility of combining solvent casting, porogen leaching, and freeze-drying methods to produce tissue engineering scaffolds based on calcium phosphates, polyesters, and polysaccharides. The obtained materials may find application in the treatment of osteochondral tissue defects.

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**Transfersomes: an innovative strategy for targeted skin cancer therapy**

**Background:** Skin cancer poses a growing global health concern, necessitating the development of targeted and non-invasive therapeutic approaches. Transfersomes, ultradeformable lipid vesicles capable of overcoming the skin barrier, have garnered attention for their ability to deliver drugs directly to the affected site. This study explores the formulation and application of transfersomes loaded with anticancer agents to enhance the efficacy of skin cancer treatment while minimizing systemic side effects.

**Material and Methods:** Transfersomes were prepared using a thin-film hydration technique with subsequent sonication. The formulation was optimized by varying lipid composition, surfactant ratio, and drug loading efficiency. Physicochemical characterization was performed to assess vesicle size, zeta potential, and stability. *In vitro* release studies and *ex vivo* skin permeation tests were conducted using Franz diffusion cells. Additionally, antioxidant compounds were incorporated into transfersomes to evaluate their synergistic effect on therapeutic outcomes.

**Results:** The optimized transfersomes exhibited an average size of 120 nm, high encapsulation efficiency (>90%), and excellent stability over four weeks. *In vitro* release profiles demonstrated a sustained release of the encapsulated drug, with over 75% release within 24 hours. Skin permeation studies confirmed efficient penetration into deeper skin layers while maintaining localized drug concentration. The addition of antioxidants significantly enhanced the oxidative stress reduction, providing a dual therapeutic effect.

**Conclusions:** Transfersomes represent a promising delivery system for targeted skin cancer therapy. Their ability to encapsulate and deliver anticancer agents efficiently, coupled with their synergistic antioxidant properties, offers a novel approach to improving treatment outcomes. Future *in vivo* studies are planned to validate these findings and assess the safety and clinical applicability of transfersome-based therapies.



**P. 28.**

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**Exotic QSAR**

**Background:** Drug design and development face complex technological and economic challenges, with evidence indicating a long-term decline in R&D productivity as fewer drugs gain approval relative to the rising research costs. Drug market success is influenced by compound quality, marketing, and unmet medical needs, with bestsellers viewed as the "winners" among FDA approvals. Economics is often considered a "soft science," making structure-economic QSARs (Quantitative Structure-Activity Relationships) challenging to model. Despite its unconventional nature, emerging trends suggest that molecular simplicity could help reduce drug attrition rates. However, a direct correlation between molecular complexity and market performance remains largely unexplored, highlighting an important area for further research.

**Methods:** The FDA data was obtained from the FDA Compilation of CDER NME and the New Biologic Approvals database. Alternatively, we extracted the data from Drugs.com, ClinCalc, and ChEMBL. The drug-likeness data was obtained from the DrugBank database. The ZINC's FDA-approved drugs dataset was transformed into the 2D map using the t-distributed stochastic neighbor embedding dimensionality reduction method. The reduced chemical space was divided into clusters based on their similarity to molecular structure.

**Results:** In the context of pharmacoconomics, the mean sales corresponding to the most densely populated MW frequencies within TOP populations remained high. At the same time, the increase of the maximal frequency MWs from 300-400 Da to 400-500 Da in the Lipinski region and the increasing importance of biologics in a recent decade vs. the 2000-2009 TOP population are the two most essential effects when probing the TOP populations. In this context, comparing the TOPs and FDA trends revealed parallel behavior; however, the TOP drugs are older than the combined FDA approvals population.

**Conclusions:** The TOP drug rankings reflect pharmaceutical development shaped by pharmaco-economic market trends. A shift toward lower logP values, common among TOP-listed drugs, is not observed in FDA-approved drugs. Additionally, key molecular fragments, such as benzene and diazobenzene, are characteristic of TOP drugs, supporting more efficient drug design. Together, these trends make TOP-listed drugs valuable benchmarks for guiding pharmaceutical R&D.

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