Abstract Book

ISBN: 978-83-942760-8-9

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The project is financed by the Excellent Science Program

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Pavone F.

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Whole-cortex dynamics and interactions with the hippocampus

Battaglia F.

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Recent advances in anatomical and functional imaging techniques in humans and animals have led to a profound reappraisal of the principles of cortical mapping, emphasizing whole-brain networks that are organized according to functional and connectivity gradients.

Yet, how spontaneous activity propagates in the neocortex remains to be understood. Moreover, hippocampal-cortical interactions are thought to be crucial for memory processes, but are typically studied with focus on a handful of putative „hub" areas, for example the prefrontal cortex. Here, we investigate the fine-grained spatiotemporal dynamics of spontaneous activity in the entire dorsal cortex by making use of simultaneous recordings of wide-field voltage sensitive dye transients (VS) and cortical ECoG, complemented by hippocampal LFP in anesthetized mice.

Both VS and ECoG show cortical avalanches, large collective excitatory events, that we dub avalanches. Small avalanches are characterized by a limited number of co-activation modes involving a sub-set of cortical networks (related to the Default Mode Network), while larger avalanches tend to involve a substantial portion of the cortical surface and can be clustered into two broad families: one immediately preceded by Retrosplenial Cortex activation and mostly involving medial-posterior networks, the other initiated by Somatosensory Cortex and extending preferentially along the lateral-anterior region.

Rather than only differing in terms of size, these two set of events appear to be associated with markedly different brain-wide dynamical states: they are accompanied by a shift in the hippocampal LFP power spectrum, from the ripple band (smaller) to the gamma band (larger avalanches), and correspond to opposite directionality in the cortex-to-hippocampus causal relationship. These results provide a concrete description of global cortical dynamics, and shows how cortex in its entirety is involved in bi-directional communication in the hippocampus even in sleep-like states. This will likely have consequences for our theoretical understanding of memory processes, in ways that will have to be understood.

PL 3

Omics, metabolic health and obesity

Paczkowska-Abdulsalam M.

Medical University of Bialystok, Bialystok, Poland

Obesity, with its growing worldwide prevalence and a significant impact on the risk of cardiovascular disease and type 2 diabetes development, has emerged as a major public health concern. Interestingly, some obese individuals appear to be somehow protected from the detrimental effects of excessive adipose tissue accumulation and preserve a favorable metabolic health status. These individuals remain normoglycemic, insulin sensitive, and hypotensive with proper blood lipid levels, despite their high BMI. Multiple independent observations have led to the concept of the metabolically healthy obese (MHO) phenotype, yet no consensus has been reached to date regarding the main mechanism behind this phenomenon. This talk highlights recent advances regarding the use of omics technologies to investigate the MHO phenotype and identify pathways involved in the mechanisms underlying metabolic health deterioration.

PL 4

Psychedelic Research in the 21st Century

Nichols D.E.

University of North Carolina, Chapel Hill, North Carolina, United States

This talk will discuss the natural sources of psychedelics and their use in ancient times. Chemical structures of important psychedelics will be shown, but there will not be any discussion of their chemistry. The brain target for psychedelics will be illustrated, and a brief mention made of how psychedelics are tested in nonhuman species. There will be a brief discussion of recent structural biology studies, where the structure of LSD within the 5HT2A and 5HT2B receptors will be described. Finally, highlights of recent successful clinical studies will be covered, where psilocybin was combined with psychotherapy to treat anxiety, depression, and certain addictions.

S. 01-1

Towards antiviral strategies against SARS-CoV2

Neyts J.

KU Leuven, Leuven, Belgium

An automated platform to test multiple candidate antiviral drugs is used to meet time pressure and health professionals demands during the current pandemic. I allows to characterize already known RNA polymerase inhibitors (e.g. favipiravir) and viral protease inhibitors (e.g. indinavir) assay against the established and emerging strains of SARS-CoV-2 using a multi-well cytopathic test. Candidate drugs compositions are confirmed using a Syrian hamster model, prone to respiratory infection with SARS-CoV-2. Beneficial effects of combined therapy in other viral infections (e.g. HCV, HIV) and promising results of preclinical studies on the proposed drugs combinations, suggests that development of COVID-19 therapy based on already characterized direct antivirals can be introduced fast into clinical practice.

S. 01-2

Drug repurposing: can you teach an old dog new tricks?

Pyrć K.

Jagiellonian University, Krakow, Poland

Screening of small chemical compounds for their inhibitory potency on SARS-CoV-2 papain-like protease (PApro) revealed that chemical derivatives of acridine, a heterocyclic compound family used as antiseptics, can bind to the enzymatic pocket of PApro. This leads to failure in processing orf1ab encoded non-structural genes of the virus and inhibition of Vero cell infection in a concentration dependent manner. Acriflavine was tested also using human ACE2 transgenic mice susceptible to SARS-CoV-2 infection and found more effective than remdesivir used as comparator. The inhibitory concentration of acriflavine was in nanomolar range, below its genotoxic or cytotoxic levels. Molecular modelling suggests that PApro pocket is occupied by two acriflavine molecules in parallel, thus there is suggestion that a tandem acriflavine molecule should have even better antiviral properties.

S. 01-3

Chemically modified mRNA

Jemielity J.

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For several decades, scientists from all over the world have been trying to discover effective methods of combating diseases that are difficult to treat with traditional methods, such as cancer, genetic rare diseases, and the last year in every aspect of our lives has been dominated by the pandemic caused by the coronavirus SARS-CoV-2. The hope for improving this situation is the so-called gene therapy, in which a therapeutic is delivered in the form of a genetic recipe, which is then expressed in the cells of the patient. In recent years, messenger RNA (mRNA), which is the genetic recipe for a specific protein, has received a great deal of attention in this context. A kind of culmination of these efforts was the development of mRNA vaccines against coronavirus, which were the first to be approved for widespread use. On the way to effective mRNA-based therapies, there have been a number of problems that have been solved, but there is also room for improvement. During the lecture, the speaker will present the idea of gene therapies and their enormous potential beyond anticancer and antiviral therapies. He will talk about the main problems associated with the development of this novel therapy and ways to solve them using biological and chemical methods, including those developed at the University of Warsaw.

S.01-4

Beyond SARS-CoV-2 sequencing

Sanak M.

Jagiellonian University Medical College, Krakow, Poland

Popularity of new generation sequencing (NGS) during the current COVID-19 pandemic enabled a real-time monitoring of the pathogen variation. Emerging variants represent adaptation of the virus to a new human host, with increasing transmissibility and evasion of adaptive immunity. GISAD database, designed to monitor influenza, has almost 1,7 millions of full SARS-CoV-2 genomes as now. In this presentation a research idea is proposed on these sequencing data, because NGS provides also interesting data on the host response. RNA sequencing data of clinical oropharyngeal swabs contains reads of human mRNA derived from epithelial and inflammatory cells. These human mRNA fragments can be successfully aligned and quantified. An example of differentially expressed human genes in samples stratified by a high or low cellular response to the infection is presented. It highlights the role of mTORC signalling cascade during COVID-19 induced inflammation.

S. 02-1

Designing a truly unbiased dataset for machine learning and virtual screening

Tran-Nguyen V.K., Jacquemard C. and Rognan D.

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Comparative evaluation of virtual screening methods requires a rigorous benchmarking procedure on diverse, realistic, and unbiased datasets. Recent investigations from numerous research groups unambiguously demonstrate that artificially constructed ligand sets classically used by the community (e.g. DUD, DUD-E, MUV) are unfortunately biased by both obvious and hidden chemical biases, therefore overestimating the true accuracy of virtual screening methods. We herewith present a novel dataset (LIT-PCBA) specifically designed for virtual screening and machine learning [1]. LIT-PCBA relies on 149 dose-response PubChem bioassays that were additionally processed to remove false positives, assay artifacts, and keep active and inactive compounds within similar molecular property ranges. To ascertain that the dataset is suited to both ligand-based and structure-based virtual screening, target sets were restricted to single protein targets for which at least one X-ray structure is available in complex with ligands of the same phenotype (e.g. inhibitor, inverse agonist) as that of the PubChem active compounds. Preliminary virtual screening on the 21 remaining target sets with state-of-the-art orthogonal methods (2D fingerprint similarity, 3D shape similarity, molecular docking) enabled us to select 15 target sets for which at least one of the three screening methods is able to enrich the top 1%-ranked compounds in true actives by at least a factor of two. The corresponding ligand sets (training, validation) were finally unbiased by the recently described asymmetric validation embedding (AVE) procedure to afford the LIT-PCBA dataset, consisting in 15 targets, 7844 confirmed active and 407381 confirmed inactive compounds. The dataset mimics experimental screening decks in terms of hit rate (ratio of active to inactive compounds) and potency distribution. It is available online at http://drugdesign.unistra.fr/LIT-PCBA for download and for benchmarking novel virtual screening methods, notably those relying on machine learning [2].

Literature

1. Tran-Nguyen VK, Jacquemard C, Rognan D. LIT-PCBA: An Unbiased Data Set for Machine Learning and Virtual Screening. J Chem Inf Model, 2020, 60: 4263-4273

2. Tran-Nguyen VK, Bret G and Rognan D. True Accuracy of Fast Scoring Functions to Predict High-Throughput Screening Data from Docking Poses: The Simpler the Better. J Chem Inf Model, in press.

S. 02-2

Computational methods for flagging compounds likely to cause false outcomes in biological assays

Kirchmair J.

University of Vienna, Vienna, Austria

Background: Small molecules interfering with biological assays continue to pose significant challenges in compound screening. In recent years a number of computational approaches have been introduced for flagging potentially "badly behaving compounds", "bad actors", "nuisance compounds" or "frequent hitters". In this work we present Hit Dexter, an advanced web service for vetting compounds regarding their behavior in different types of biological assays.

Material and methods: Machine learning models for frequent hitter prediction, trained on large sets of chemical and biological data, form the core of Hit Dexter. These models are complemented by a number of rule-based and similarity-based approaches for the assessment of the risk of colloidal aggregation and other undesirable properties.

Results: On holdout data, the Hit Dexter machine learning models achieved high accuracy and good early enrichment. The models were also able to correctly characterize compounds with specific biological and physicochemical properties, such as compounds linked to dark chemical matter or colloidal aggregation. Among the most interesting findings of this study is that the Hit Dexter models identify large fractions of likely frequent hitters among approved drugs. Importantly, predictions of the individual Hit Dexter models are generally in good agreement and consistent with those of Badapple, an established statistical model for the prediction of frequent hitters.

Conclusions: The Hit Dexter platform, available at https://nerdd.univie.ac.at, provides a powerful and secure web service for the flagging of compounds for which extra caution should be exercised with positive assay readouts.

Acknowledgements: This research is supported by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation)—project number KI 2085/1-1, the Bergen Research Foundation (BFS)—grant no. BFS2017TMT01, the China Scholarship Council (201606010345), the Ministry of Education of the Czech Republic—project numbers NPU I-LO1220 and LM2015063.

S. 02-3

The role of the secondary binding pocket in GPCR pharmacology

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G-protein coupled receptors (GPCRs) are considered important therapeutic targets due to their pathophysiological significance and pharmacological relevance. Endogenous ligands bind to the orthosteric binding pocket (OBP) embedded in the intrahelical space of the receptor. During the last years, however, it has been turned out that in many receptors there is secondary binding pocket (SBP) located in the extracellular vestibule that is much less conserved. In some cases it serves as a stable allosteric site harbouring allosteric ligands that modulate the pharmacology of orthosteric binders. In other cases it is used by bitopic compounds occupying both the OBP and SBP. In these terms, SBP binding moieties might influence the pharmacology of the bitopic ligands. Together with others, our research group showed that SBP binders contribute significantly to the affinity, selectivity, functional activity, functional selectivity and binding kinetics of bitopic ligands.

S. 03-1

Whole exome and genome sequencing for discovery of novel human diseases

Płoski R.

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In 2012 Department of Medical Genetics (Warsaw Medical University) has acquired Illumina HiSeq 1500 which allowed to establish whole exome sequencing (WES) as method for both research and diagnostic purposes. Since then we have performed > 3,000 WES analyses, most of which aimed at finding diagnosis in patients suspected to suffer from rare disorders with a genetic basis. We also established whole genome sequencing (WGS) which we use to precisely map breakpoints in patients with symptomatic balanced chromosomal translocations. During the lecture selected findings will be presented illustrating how these approaches enable diagnosis as well as discovery of novel diseases (i.e. those caused by mutations in genes not yet associated with known human disorder), including those which are potentially treatable.

S. 03-2

New (epi)genetic diagnostic tools for cancer - liquid biopsy and beyond

Giefing M.[1], Kowal-Wiśniewska E.[1], Jaśkiewicz K.[1], Kiwerska K.[1], Bartochowska A.[2], Wierzbicka M.[2], Ustaszewski A.[1], Jarmuż-Szymczak M.[1]

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Background: Liquid biopsy is a novel, minimally invasive diagnostic procedure thought to revolutionize cancer diagnostics. It is based on a blood test and requires no more than a vial of freshly collected sample. This breakthrough is made possible by the unprecedenced power of high throughput sequencing that allows to identify and quantify small DNA fragments (usually 70-200 bp) released from cells, including cancer cells, to the bloodstream. These DNA fragments, so called cell-free (cf) DNA, were shown to be overrepresented in cancer and to have a cancer specific genetic and epigenetic profile. We have undertaken the challange to identify such cfDNA alterations in blood plasma of head and neck squamous cell carcinoma (HNSCC) patients with the aim to establish a panel of markers for early diagnosis, prognosis and early detection of reccurence. However, in contrast to most research conducted worldwide, we focused on cfDNA methylation changes as the putative marker.

Material and methods: We used the MethHC database (569 HNSCC samples) to identify differentially methylated DNA regions between tumors and control samples. Candidate regions (n=17) were analyzed by bisulfite DNA pyrosequencing in HNSCC tumor samples (n=64) and non-tumor tissue collected during laser-assisted uvulopalatoplasty (LAUP) (n=13). Bisulfite conversion was conducted using the EZ DNA Methylation-GoldTM Kit (Zymo Research) and samples were analyzed using the Pyromark Q48 Autoprep pyrosequencer (Qiagen). Mean methylation level in a sample was calculated from the analyzed CG dinucleotides in a given region. Samples with methylation level above the cut-off (the highest methylation value in a control sample plus two times standard deviation) were considered as hypermethylated (two sided MannWhitney test, α=95%, $p < 0.05$). The QiAamp MinElute ccfDNA Mini Kit (Qiagen) and the EpiTect Plus DNA Bisulfate Kit (Qiagen) were used for isolation and bisulfite conversion of cfDNA from patients plasma (n=111) and age adjusted control group (n=62). Best candidate regions were chosen based on statistical analysis using the Receiver Operating Curve (ROC).

Results: We have idetified 14 differentially methylated genes in the group of HNSCC tumor samples compared to the control group. So far, we have analyzed 4 regions for methylation level in cfDNA samples and controls in different time points. Importantly, we have observed decreased cfDNA methylation level after tumor resection and increased at the time of recurrence in several selected CpGs. Obviously, these CpGs are good candidates for a epigenetic diagnostic panel to be used in HNSCC.

Conclusions: In conclusion, we show that cfDNA methylation is a promising marker for an epigenetic diagnostic and prognostic panel in HNSCC. We expect that the combination of the candidate regions identified in our study will be the basis of a new panel that can be brought to the clinic in the next years.

Acknowledgements: Liquid biopsy – the tool for detection of “cancer fingerprints” in the blood of head- and neck- cancer patients. POIR.04.01.04-00-0003/17-00.

S. 03-3

Multigene panel testing in oncology clinical practice - pros and cons

Sąsiadek M.

Wroclaw Medical University, Wrocław, Poland

S. 03-4

Somatic pathogenic mutations in normal mammary tissue of breast cancer conservation surgery patients – incidental or causal?

Kostecka A.[1,2], Nowikiewicz T.[3,4], Olszewski P.[2], Koczkowska M.[1,2], Horbacz M.[2], Andreou M.[2], Salazaar R.[5], Mair T.[5], Heinzl M.[5], Madanecki P.[1], Gucwa M.[1], Davies H.[6], Skokowski J.[7], Pęksa R.[8], Śrutek E.[3], Szylberg Ł.[9], Jankowski M.[3], Zegarski W.[3], Tiemann-Boege I.[5], Dumański J.[2,6], Piotrowski A.[1,2]

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Post-zygotic genetic changes - DNA structural and sequence variants create a complex mosaic landscape of an individual that might underlie the pathogenesis of cancer. Breast cancer affects one in eight women worldwide and the majority of cases are sporadic tumors. The past two decades uncovered the molecular complexity of breast tumors, yet still little is known about the genetic landscape of normal mammary gland tissue. During life mammary gland remains mitotically active and, as a result of hormonal stimulation, undergoes cycles of proliferation, lactation and involution. Here we hypothesized that these factors increase the mutational burden in normal, glandular tissue that may explain recurrent disease in breast conservation surgery patients. Hence, we investigated the DNA sequence variants inpaired sets of normal mammary gland (UM) and primary tumor (PT) samples collected from 52 individuals diagnosed with sporadic breast cancer that underwent breast conservation surgery. We detected clearly pathogenic variants of breast cancer driver genes, including PIK3CA, TP53, AKT1, CDH1 and MAP3K1 in the uninvolved mammary gland tissue. To ensure higher sensitivity and confidence of variant detection we additionally employed the duplex sequencing technology. Ultradeep targeted sequencing of UM samples revealed low frequency pathogenic variants of PIK3CA and TP53 genes, the main drivers of breast tumors. Our results provide evidence for the presence of heterogenous landscape of somatic mosaic pathogenic genetic variants in the normal breast tissue sampled distant from the primary tumor site. These variants can lead to disease recurrence or affect the response to treatment. This raises a question about the oncogenic potential in nontumor mammary gland tissue of breast conservation surgery patients.

S. 04-1

Biomarkers of schizophrenia

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Background: Schizophrenia is a highly heritable, chronic, severe, disabling neurodevelopmental brain disorder with a heterogeneous genetic and neurobiological background, which is still poorly understood. To allow better diagnostic procedures and therapeutic strategies in schizophrenia patients, use of easily accessible biomarkers is suggested.

Material and methods: Biomarkers of schizophrenia might be divided into central, which are either studied by neuroimaging techniques or they are different analytes studied *postmortem*, and peripheral biomarkers which are more easily obtainable, mostly from body fluids, including cerebrospinal fluid, saliva, blood, and urine. Biomarkers' categorization criterion can be a method used to define those biomarkers (imaging, genomic, proteomic, epigenomic, metabolomic, transcriptomic biomarkers), or biological category to which biomarkers belong (DNA-, RNA- or protein-based biomarkers, metabolites or biomarkers belonging to different anatomical systems).

Results: The most frequently used biomarkers in schizophrenia are those associated with the neuroimmune and neuroendocrine system, metabolism, different neurotransmitter systems and neurotrophic factors. Schizophrenia represents altered homeostasis of immune/inflammatory, oxidative stress, endocrine and metabolic signaling processes that mediate and affect dopaminergic and serotonergic neurotransmission. Numerous molecular substrates, responsible for the imbalance in homeostatic signaling, were used to discover validated biomarkers in schizophrenia.

Conclusions: There are still no validated laboratory tests and biomarker(s) for schizophrenia diagnosis, prognosis, or prediction of the treatment response. Selected serum analytes were suggested to indicate a reproducible biological signature, but these studies need replication in larger population and in longitudinal studies which will be controlled for confounding variables such as sex, age, body mass index and medication. The future use of biomarkers should improve diagnosis, therapy monitoring and prediction of treatment outcome leading to the improvement of the quality of life in patients with schizophrenia and decrease of health costs worldwide.

Acknowledgements: All the efforts of the members of the Laboratory for molecular psychiatry from Ruder Boskovic Institute, as well as altruism of all the subjects who took part in our studies are extremely appreciated.

S. 04-2

What's the use of biomarkers for depression?

Strawbridge R.

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Background: Major depressive disorder (MDD) is a highly burdensome condition, partly due to its high prevalence, low rates of treatment and relative high rates of treatment-resistance, chronicity and recurrence of episodes.

Material and methods: I will review broadly the evidence of biomarkers that have been investigated in depression, with their variety of potential future uses spanning risk factors for the disorder, diagnostic markers, prognostic risk factors, personalised treatments, mechanisms for current and novel treatment targets. These primarily surround the biological systems of monoamine and other neurotransmitters, neuroanatomy, neuroendocrine, neuroplasticity, inflammation and the microbiome.

Results: While there is a wealth of evidence indicating hundreds of potential biomarkers that could be valuable for one or more of the above uses, the evidence is not currently reliable or extensive enough for their use at present. I provide some examples of promising avenues. A key reason for heterogeneity of evidence at present is indeed the heterogeneity with which depression presents, both in terms of symptom variability, illness course and comorbidities.

Conclusions: Despite the lack of consistent evidence of biomarkers at present there is the potential for current and future research to address these gaps but the research requires interpretation with scientific critique.

Acknowledgements: Centre for Affective Disorders (CfAD) at the Institute of Psychiatry, Psychology & Neuroscience, King's College London. Biomedical Research Centre at the Maudsley, London. Professors Anthony Cleare and Allan Young.

S. 04-3

Zinc deficiency and affected signaling networks as biomarkers and causative factors of Autism Spectrum Disorders

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Background: Genetic factors might be largely responsible for or facilitate the occurrence of Autism Spectrum Disorders (ASD). Still, in addition to a combination of autism-related genes, specific environmental factors may act as risk factors triggering the development of the disorders. Recently, zinc signaling in the brain was linked to the development of ASD. While for nutritionists, zinc is an essential trace element, for biochemists, a component of enzymes and other proteins, for neuroscientists, zinc is a crucial ionic signal and neurotransmitter at synapses.

Material and methods: Based on several studies conducted in the past using in vitro systems and animal models for zinc deficiency, we investigated the pathomechanisms of abnormal zinc homeostasis on synaptic function, brain morphology and connectivity, and behavioral outcomes.

Results: Prenatal zinc deficiency affects key processes at synapses in the Central Nervous system, alters the microbiome, increases inflammation, and through this, alters neuronal function, ultimately affecting brain connectivity and lateralization. We propose a mechanism, how low zinc status is linked to ASD pathology mechanistically, focusing on a dysregulation of synaptic SHANK protein networks.

Conclusions: Zinc as part of the metallome of the body may be an important prognostic marker and biomarker, and low zinc status linked to ASD mechanistically.

S. 05-1

Applied epigenomics: insights into the pathogenesis of Multiple Sclerosis

Jagodic M.

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Multiple Sclerosis (MS) is a leading cause of progressive disability in young adults. Although the cause remains unknown, vast epidemiological data establish MS as a complex disease influenced by genetic and environmental factors. Epigenetic mechanisms, such as DNA methylation, histone posttranslational modifications and non-coding RNAs, orchestrate activity of the genome in response to environmental cues and may provide understanding of molecular mechanisms underpinning disease development and progression. To understand MS pathogenesis, we are measuring DNA methylation patterns and transcription in discrete cell types from unique clinical cohorts in combination with functional studies in experimental models *in vitro* and *in vivo*. One of the main challenges with studying diseases such as MS is the limited access to the target tissue - *the brain*. Recent technical and analytical advances in methods to survey epigenetic modifications genome-wide and from them infer genome activity, opened up possibilities to study brain tissue and mechanisms that underlie neurodegeneration in MS patients.

S. 05-2

Imaging deep: Sensory and state coding in subcortical circuits

Gründemann J.

University of Basel, Basel, Switzerland

Our brain integrates inputs from an ever-changing sensory environment and aligns them with internal states to continuously form new memories and adjust behavioural output. In this lecture I present two recent studies where we used a longitudinal *in vivo* miniature microscope Ca^{2+} imaging approach to track the activity of large neuronal populations in the amygdala and auditory thalamus of mice across different behavioural paradigms. We identify changes in the activity levels of two major, non-overlapping populations of principal neurons in the basal amygdala that predict switches between exploratory and non-exploratory (defensive, anxiety-like) states. We find that sensory responses in the amygdala occur independently of behavioral state encoding during associative fear learning. In addition to amygdala coding, we monitored the neural activity of large populations of auditory thalamus (medial geniculate body, MGB) upon associative fear learning and found that MGB neurons exhibit functional cell classes similar to those previously identified in amygdala circuits (e.g. fear cells, extinction cells), while population level coding of associated stimuli was stable across days. Our data identifies MGB as a site for neuronal plasticity in associative fear learning upstream of the basolateral amygdala that might drive plasticity in downstream limbic brain areas. Furthermore, inhibiting activity in amygdala-projecting MGB neurons during fear learning leads to an imbalance of plasticity in auditory thalamus. Our data suggest that amygdala and thalamus are part of a distributed population code for fear learning.

S. 05-3

Central amygdala - ventral tegmental area - cortical circuits in motivation for social interaction and food reward

Knapska E.

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Background: Social dysfunctions in autism are often explained by a deficit in motivation and reward processing specific to social stimuli. However, the functional dissociation between the neural circuits processing social and non-social rewards is not known. Here, we compared involvement of the central amygdala (CeA) – ventral tegmental area (VTA) – cortical circuits in motivation for social interaction and for food.

Material and methods: We tested social motivation in familiar rats after a 3-week separation and motivation for pressing the lever for food in Progressive Ratio Test. Using opsins targeted to behaviorally activated neurons we tagged CeA cells implicated in social and food reward and inhibited or stimulated them to test whether the populations of social and food cells functionally overlap. Next, we tested the role of specific pathways in social motivation and motivation for food using chemogenetic constructs.

Results: Optogenetic manipulations revealed that the social and food circuits in the CeA overlap only partially. Through chemogenetic manipulations of specific projections we identified a crucial role of the CeA-VTA pathway, and the dopaminergic VTA-anterior cingulate (ACC) and VTA-orbitofrontal cortex (OFC) pathways in social motivation but not in motivation for food reward. In contrast, we found that the ACC-CeA and OFC-CeA inputs are involved in both social and non-social motivation.

Conclusions: Together, these findings establish the CeA-cortical pathways as a node for regulating social motivation, providing new insights into the social reward processing.

Acknowledgements: This work was supported by a European Research Council Starting Grant (H 415148).

S. 06-1

New developments in understanding mechanisms of drug resistance in the treatment of depression

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Treatment-resistant depression (TRD) in major depressive disorder (MDD) is challenging as many patients fail to achieve remission. The regulatory defines TRD as insufficient treatment response to any two different antidepressants (ADT) given at an adequate dose and duration in the current depressive episode in patients with MDD. A range of alternative TRD definitions results from the heterogeneity of illness stages and severity in treatment-resistant/refractory/chronic depression.

The TRD predictors include comorbid anxiety disorder, suicidality, depressive symptoms severity and previous number of episodes with some evidence for current depressive episode duration, psychotic symptoms, socioeconomical status, number of hospitalizations, early age of onset, and high relapse rates with treatment non-adherence commonly contributing to the TRD development.

The ADTs for TRD promote glutamate release and include serotonergic hallucinogens, muscarinic receptor antagonists, mGluR2 antagonists, and $GABA_A$ sub-type-selective negative allosteric modulators or inverse agonists. Alternatively, antidepressant effect is observed in molecules elevating BDNF levels, enhancing TrkB receptor signaling, or promoting mTORC1 activation. The boost of glutamate release and AMPA receptors stimulation results in enhanced BDNF release, TrkB stimulation, mTORC1 activation, with protein synthesis restoring neuronal plasticity and neurogenesis in MDD. AMPAkines mediate acute and long-term antidepressant effects and the activation of AMPA receptors in the mPFC appears to be a common pathway for the antidepressant effect.

The drug resistance in MDD hardly fits the models set for monoaminergic ADTs. The treatment response includes symptomatic remission and functional recovery with the reduction in exacerbations, relapses, quality of remission, number of concomitant medications. The TRD research shall adopt meaningful response and burden-reduction concept known as transformative treatment paradigm. The treatment effect associated with transformation is defined as rapid, marked and enduring psychological change, where 'psychological' refers to perception, cognition and action or behavior and is contextual to multi-level, biologically informed, context-dependent and process-based approach to the phenomenon.

This presentation reports the TRD clinical data as related to the research interpretation for novel non-monoaminergic ADTs with focus on clinically meaningful response/recovery definition.

S. 06-2

Somatic therapies for TRS depressions

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Background: Although patients with treatment-resistant depression (TRD) constitute a distinct minority, their treatment consumes a major portion of the clinician›s time. Somatic therapies can be used when a medication course (i.e. dose increase, switching antidepressants or adding medication) is found to be ineffective. The aim of this lecture is to review somatic therapies utilized in the treatment of TRD and present the results of personal research conducted at the Second Department of Psychiatry, at the Institute of Psychiatry and Neurology in Warsaw.

Materials and methods/methodologies: The first presented study involved assessing the mental state of patients before and after the implementation of subthalamic nucleus stimulators allowing the patients to be subject to deep brain stimulation (STN-DBS), with a particular focus on the presence of depression and anhedonia. The second study examined the efficacy and safety of morning bright light therapy (BLT) in treatments of patients suffering from bipolar and unipolar disorders, however not manifesting any seasonal patterns, who at the time of the study were also undergoing major depressive episodes (MDE). The third showcased study investigated whether depression-related insomnia can be modulated through repetitive transcranial magnetic stimulation (rTMS). The fourth study focused on the comparison between unilateral (ULECT) and bilateral (BLECT) eletrocnvulsive therapy (ECT) in treatments of recurrent affective disorders (i.e. assessing safety, efficacy and their impact on cognitive processes).

Results: The results showed an improvement in mental status within a month after beginning STN-DBS stimulation, taking form of improvements of moods and subsidence of sadness, apathy and anhedonia. In the second study BLT was more efficacious than a placebo substitute in a population of drug-resistant patients. The third study confirmed the beneficial effects of rTMS on moods of patients suffering from depression. The results of the fourth study showed that ULECT does not differ from BLECT in antidepressant efficacy and proved the former to be more safe over the latter.

Conclusions: Somatic therapies are well tolerated by patients and could be used as a good alternative for inneffective pharmacotherapy in treatment-resistant depression. The next step towards a broader understanding of somatic therapies for treatment affective disorders in our complex project is an upcoming study concerning the comparison between BLECT and pharmacotherapy in the treatment of bipolar disorders.

S. 06-3

Mechanism of antidepressant failure in an animal model of treatment-resistant depression

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A substantial proportion of depressed patients fail to benefit from antidepressant drug treatment. Until very recently there have been no validated animal models for treatment-resistant depression (TRD), reflecting the requirement that a valid animal model of depression should respond to antidepressant treatment. This paradox was resolved by the recent discovery of novel treatments, such as deep brain stimulation (DBS) and ketamine, which are effective in TRD. We propose that an animal model for TRD should be based on (i) a validated animal model of depression which (ii) does not respond to conventional antidepressants but (iii) does respond DBS and ketamine; and we present evidence that the chronic mild stress (CMS) model implemented in Wistar-Kyoto (WKY) rats meets these three criteria. Recovery from CMS following chronic treatment with both the antidepressant drug venlafaxine (VFX) in Wistar rats, and either DBS or optogenetic stimulation (OGS) of the prelimbic region of medial prefrontal cortex (mPFC) in WKY, was blocked by injection of the AMPA receptor antagonist NBQX at the same site in prelimbic cortex. This common mechanism in mPFC for a conventional antidepressant in Wistar and DBS/OGS in WKY suggests that antidepressant resistance in WKY may result from the failure of a critical input to mPFC. In a test of this hypothesis, VFX was rendered effective in WKY rats by weekly OGS of ventral hippocampal (vHPC) afferents to mPFC. This effect was not seen with OGS of dorsal hippocampal afferents to mPFC. VFX was also rendered effective in WKY rats when combined with weekly OGS of prelimbic afferents to nucleus accumbens core (NAcC), but not vHPC afferents to NAcC. We conclude that antidepressant treatment resistance results from an inability to activate a vHPC-mPFC-NAcC pathway in the treatment-resistant brain.

S. 06-4

Cortical serotonin 5-HT_{1A} receptor biased agonism - a novel mechanism for effective therapy of treatment-resistant depression

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Background: Treatment of depression is undergoing a profound rethink. Unlike classical antidepressants, ketamine possesses rapid-acting antidepressant (RAAD) activity and is efficacious in profoundly depressed and treatment-resistant patients. However, ketamine also elicits side-effects which limit its use. Interstingly, recent studies point to an important role of cortical serotonin 5-HT_{1A} receptors in ketamine's mechanism of action. Indeed, ketamine's antidepressant-like effects in rodents are blocked by local pre-frontal cortex (mPFC) microinjection of 5-HT_{1A} receptor antagonists. Moreover, ketamine elicits robust phosphorylation of extracellular regulated kinase (pERK) in cortex (a response typical of 5-HT_{1A} activation) and inhibition of pERK prevents the antidepressant-like action of ketamine.

Materials and methods: A novel 'biased agonist', NLX-101, which preferentially targets post-synaptic cortical 5-HT_{1A} heteroreceptors was identified and tested in a range of *in vitro*, *ex vivo* and *in vivo* tests, covering cellular signaling, electrophysiology, neurochemistry, behavioral pharmacology and brain imaging.

Results: NLX-101 has high affinity and exceptional selectivity for 5-HT_{1A} receptors. It exhibits higher potency for pERK1/2 signaling vs other responses. *Ex vivo*, it preferentially activates mPFC 5-HT_{1A} receptors in c-Fos and pERK1/2 experiments. NLX-101 also preferentially stimulates pyramidal neurons and elicits dopamine release in mPFC. In fMRI imaging, NLX-101 preferentially activated cortical regions whereas other agonists elicited wide effects. NLX-101 exhibits strong antidepressant-like activity in the rat forced swim test (FST), completely reversing depressive-like behavior upon acute administration – unlike currently-used clinical antidepressants. NLX-101 also exhibits strikingly rapid activity in the rat Chronic Mild Stress (CMS) model of depression: after a single day, NLX-101 completely rescued the deficit in sucrose consumption induced by CMS, a behavioral marker of anhedonia, whereas other antidepressants require weeks of treatment. The antianhedonic properties of NLX-101 persist for up to 4 weeks after cessation of treatment, suggesting that a long-lasting remodeling of neuronal function has taken place. This is supported by observations that NLX-101 increases BDNF levels and augments synaptic plasticity in hippocampal regions.

Conclusions: The data suggest that the preferential cortical pERK1/2 activation by NLX-101 seen in tests of receptor signaling translates to neuronal plasticity and enhanced antidepressant-like activity in models of behavior. The mechanism of action of NLX-101 in the mPFC appears to mimick that of ketamine and suggests that 'biased agonist' activation of 5-HT_{1A} receptors in this brain region may therefore constitute a promising strategy to achieve a robust and safer RAAD profile.

Acknowledgements: Studies were funded by collaborative academic research grants and by Neurolixis Inc.

S. 07-1

MRI-based in vivo histology - myth or reality

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I am presenting the novel concept of in vivo histology using magnetic resonance imaging (MRI) of the brain. After two decades of developing and validating computational anatomy approaches for investigation of brain's structure, we now take on the challenge quantifying the underlying MRI measurable tissue properties – myelin, iron and tissue water. To this end, we use relaxometry-based MRI that delivers high-resolution (1mm) quantitative maps of MT saturation, transverse and longitudinal relaxation rate as well as proton density. I describe the cumulated evidence that links the biophysical models behind this technique with empirical data in large-scale cohorts. Additionally, I embark on novel ways to obtain tissue property information from diffusion-weighted data beyond the well-established diffusion tensor model. I wrap up my presentation with an outlook for the added value of these techniques for clinical neuroscience.

S. 07-2

Time-Frequency Characterization of Resting Brain in Bipolar Disorder during Euthymia—A Preliminary Study

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The aim of our study was to investigate the baseline brain activity in bipolar disorder (BD) patients during euthymia state, with the use of a variety of resting state functional magnetic resonance imaging (rs-fMRI) analyses, such as regional homogeneity (ReHo), amplitude of low frequency fluctuations (ALFF), fractional ALFF (f/ALFF), ALFF-based functional connectivity (FC). According to our hypothesis, above-mentioned methods will differentiate BD from healthy controls (HC) indicating dissimilarities between the groups within different brain structures. 42 individuals divided into two groups of euthymic BD patients (n = 21) and HC (n = 21) were enrolled to rsfMRI study. Typical band ALFF, f/ALFF, as well as ReHo indexes were analyzed. Regions with altered ALFF were chosen as region of interest for seed-to-voxel analysis of FC. As opposed to HC, BD patients revealed: increased ALFF in left insula; increased f/ALFF in left superior frontal gyrus, left superior temporal gyrus, left middle occipital gyrus, right putamen, and bilateral thalamus. Compared to HC, the BD group presented higher ReHo values in the left superior medial frontal gyrus and lower ReHo values in the right supplementary motor area. FC analysis showed significant hyper-connectivity within the BD group between left insula and bilateral middle frontal gyrus, right superior parietal gyrus, right supramarginal gyrus, left inferior parietal gyrus, left cerebellum, and left supplementary motor area. This is the first rs-fMRI study combining ReHo, ALFF, f/ALFF in euthymic BD patients. ALFF, f/ALFF as well as REHO analysis revealed significant differences between two studied groups. Although results obtained with the above methods enable to identify group-specific brain structures, no overlap between the brain regions was detected. Our study shows that combination of rs-fMRI methods may complement each other, revealing the bigger picture of the complex resting state disturbances in BD.

S. 07-3

Reduced regional homogenity after intraocular lens (IOL) implantation - resting-state fMRI study

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Background: Cataract is a disease characterized by clouding of the lens, which leads to vision disturbances. It is widespread mainly among elderly people and currently, the only effective treatment is the surgery to replace the disturbed lens with a new one. The current study aimed at determining the differences in regional homogeneity (one of the resting-state fMRI measures) before and after the interocular blue light blocking lens implantation in cataract patients.

Material and methods: In the study took part fifteen elderly participants (10 women; mean age: 61,8 ± 8,6 y.o.). They performed two imaging sessions with a resting-state procedure.

Results: The results indicated differences in regional homogeneity in several brain regions, specifically reduced values after the implantation of blue light blocking lens.

Conclusions: The altered regional homogeneity values after the surgery could be explained by adaptation to the modified vision of the cataract patients.

Acknowledgments: The study was funded by the Polish National Science Centre and partly supported by the Foundation for Polish Science.

S. 07-4

Altered Functional Connectivity Differences in Salience Network as a Neuromarker of Suicide Risk in Euthymic Bipolar Disorder Patients

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Background: The occurrence of death by suicide in patients diagnosed with bipolar disorder is as much as 60 times greater than in the general population. Even during the state of euthymia patients are characterized by suicide risk. The aim of the study is to investigate the baseline brain activity in euthymic bipolar disorder patients in regard to suicide risk. We hypothesized that patients compared to healthy control group will demonstrate altered functional connectivity among resting state networks which will be directly related to current suicide risk.

Methods: 41 subjects were enrolled in the study consisting control group (n = 21) and euthymic bipolar disorder patients group (n = 20). Functional magnetic resonance imaging was used to evaluate resting state brain activity and ROI-ROI functional connectivity analysis was performed. Suicidal risk was estimated using The Suicide Behaviors Questionnaire-Revised.

Results: A two sample t-test revealed decreased functional connectivity between regions involved in the salience network in patients compared to the control group. This decrease was negatively correlated with current suicide risk.

Conclusion: Obtained results suggest the association between risk of suicide and activity of regions responsible for functions such as learning from mistakes, prospective thinking, and sensory integration.

S. 08-1

The role of serotonin in brain development and behaviour: the SSRI case

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Background: Serotonin plays a key role in brain development. Two main factors increasing serotonin levels in the developing brain are inherited down-regulation of the serotonin transporter (SERT) and maternal SSRI (selective serotonin reuptake inhibitor) use to treatment maternal depression. In case of the latter condition, SSRIs are able to cross the placenta and thereby reach the fetus brain to act at the serotonin transporter expressed in the fetal brain. Here we aimed to investigate and compare the developmental effects of genetic and pharmacological serotonin transporter inhibition and to unravel the putative underlying mechanisms.

Materials and methods: Perinatally fluoxetine exposed and SERT knockout rats were subjected to a behavioural test battery throughout early life, adolescence and adulthood, to genome-wide transcriptomics analyses, computational modeling of sensory task performance, and functional magnetic resonance imaging (fMRI).

Results: We found that both perinatally fluoxetine exposed and SERT knockout rats displayed a delay in motor and reflex development, decreased socio-affective behaviour, and increased repetitive behaviour. Transcriptomics analyses revealed differential expression of myelin-related genes in the hippocampus and prefrontal cortex. Furthermore, structural changes were found in the somatosensory barrel cortex. Using the gap crossing task, in which animals have to use their whiskers to cross a gap between platforms in the dark, it was found that an early life increase in serotonin levels was associated the need of less whisker touches to cross the gap. Subsequent computational modeling of the gap crossing data revealed a lack of adaptive motor control, such that the animals were exploring their environment in a less targeted but a more 'information inclusive' manner. Lastly, whisker stimulation during fMRI revealed that perinatally SSRI exposed rats exhibit increased activity of memory and sensory information processing areas, suggesting that compensatory changes take place influencing sensory information processing in later life.

Conclusions: Early life inhibition of the serotonin transporter, either by knockout of the serotonin transporter or by its pharmacological inhibition, leads to autism-like behavioural changes, which may be related to developmental changes in myelination and sensory systems.

Acknowledgements: Dutch council for scientific research.

S. 08-2

Stress exposure as a risk factor for the development of psychiatric disorders: a role for the serotoninergic system

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The term “psychiatric disorders” refers to an umbrella term that summarizes a plethora of pathologies of the CNS that may also be named mental illness or mental disorders.

The etiology of psychiatric disorders is still not fully known, but it is broadly accepted that the interaction between the genetic makeup and the exposure to environmental factors plays a pivotal role.

Among the environmental factors, stress is widely recognized as one of the main precipitating factors for psychopathologies.

On these bases, the strategy we employed in the last years was to combine the two aspects (gene and environment) by exposing mutant rats (with alteration of serotonergic genes: SERT KO, TPH1, and TPH2 KO rats) to different environmental paradigms.

In conclusion, the data presented confirm that stress effects depend on the time and length of exposure, and that alterations of the serotoninergic system influence the response to an environmental challenge.

In particular, we found that SERT KO rats are characterized by alterations in basal conditions, increased response to positive environments, and altered response to negative situations.

Similarly, lack of serotonin (in Tph 1 and 2) leads to differences in the expression of plastic markers in basal conditions and an impaired response to an acute challenge.

By showing this data, we would like to convince you that these models are good tools to study psychiatric disorders from different aims such as:

Identification of the molecular mechanism involved in the development of these pathologies,

Identification of new diagnostic and predictive biomarkers

Identification of novel therapeutic target

And to test the efficacy of new pharmacological treatment.

S. 08-3

Maternal and central serotonin: contribution to the early development in rodents

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Serotonin, is a monoamine working as an autacoid in the periphery and as a neurotransmitter in the central nervous system. Tryptophan hydroxylase (TPH) is a rate limiting enzyme of serotonin synthesis. It converts tryptophan (Trp) to 5-hydroxytryptophan (5-HTP) and belongs to the family of pterin-dependent hydroxylases, that also comprises tyrosine and phenylalanine hydroxylases (PAH). In mammals TPH has 2 isoforms: TPH1, responsible for serotonin synthesis in periphery, and TPH2, which is restricted to serotonergic neurons in the raphe nuclei in the brain and in the enteric nervous system. Since in adult mammals serotonin cannot cross the blood-brain barrier, these two enzymes define two serotonin systems with independent regulation and different functions. During development, besides its own production by TPH1 starting embryonic day E14, and TPH2 starting E12, there are other sources of serotonin, including maternal 5-HT, which is actively transported through the placenta via the serotonin transporter (SERT). In the early phases of embryonic and postnatal life, 5-HT is a trophic factor that modulates not only cell proliferation, migration and differentiation in the brain and in peripheral tissues but also cell survival and synaptogenesis, through its role in the connective organization of the CNS.

This talk will give an overview of possible sources of serotonin during prenatal development and behavioral and physiological consequences of central 5-HT deficiency in rodents.

S. 08-4

Affective communication in rodents: serotonin and its modulating role in ultrasonic vocalizations

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Mice and rats are highly social animals. Their rich social behavior repertoire includes the emission of ultrasonic vocalizations (USV), of which many different call types exist. Such call types were repeatedly associated with distinct affective states. For instance, rats emit long, low-frequency 22-kHz USV in aversive situations, such as predator exposure or aggressive encounters, while short, high-frequency 50-kHz USV occur in appetitive situations, such as rough-and-tumble play and sexual interactions, or in response to amphetamine treatment. Moreover, converging results obtained in selective breeding, devocalization, and playback studies suggest that 22-kHz and 50-kHz USV serve as socio-affective signals with distinct communicative functions. There is robust evidence indicating that 22-kHz USV act as alarm calls, while 50-kHz USV function as social contact calls. In my talk, I will provide examples from work in both mice and rats that reveal how ultrasonic calling is modulated by serotonin (5-hydroxytryptamine, 5-HT). In mice, for example, lack of central 5-HT due to Tph2 deficiency is associated with reduced emission of pup calls known to stimulate maternal caregiving behavior. In rats, deficiency of the serotonin transporter leads to a decrease in the production of alarm 22-kHz USV, despite high levels of anxiety-related behavior. Moreover, the amphetamine-induced increase in the production of 50-kHz USV can be blocked by the 5-HT2c receptor agonist CP 809,101, while the 5-HT2c receptor antagonist SB 242084 potentiates amphetamine-induced 50-kHz USV emission. Together, this shows that manipulations targeting the 5-HT system alter affective ultrasonic communication in rodents throughout life. Modulatory effects are probably associated with the important role that 5-HT plays in regulating mood and social behavior.

S. 09-1

Chronobiology, social rythms and bipolar disorder

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Circadian rythms are the fundamental property of all living organisms. Virtually every physiological and mental function in human beings varies as a function of time of day. Contemporary lifestyle and technological progress imply a growing disturbance of biological rythms. Chronobiology is a new area of grip for the treatment of affective disorders, including bipolar disorder. Disturbances in circadian rhythms have been documented in: depression, bipolar disorder, seasonal depression, premenstrual syndrome. Biological rhythm disturbances can be a marker of affective diseases (especially bipolar disorder). They are also found in healthy relatives of patients. It is unclear whether the altered biological rhythms are the cause or the result of affective disorders. Core symptoms of affectie disorders, i.e., disturbed mood and sleep disturbances, follow circadian variations and are experienced by patients. Sleep disturbances are core symptoms assessed in major depression rating scales (HAMD, MADRS) and mania rating scales (Young scale, Bech-Rafaelsen scale). Disruption of circadian rhythm was identified, even in drug-naïve BD patients. Actigraphy confirmed this finding, independently of mood status. Studies showing alterations in daily profiles of melatonin levels and cortisol reinforced circadian dysregulations in BD.

Chronotype is the behavioral manifestation of underlying circadian rhythms of myriad physical processes. A person's chronotype is the propensity for the individual to sleep at a particular time during a 24-hour period. Eveningness (delayed sleep period) and morningness (advanced sleep period) are the two extremes with most individuals having some flexibility in the timing of their sleep period. Most people are neither evening nor morning types but lie somewhere in between. In general population eveningness is associated with higher rates of bipolar spectrum symptoms and unfavourable affective temperaments traits.

Social factors such as the timing of meals, work schedules, the schedules of other family members, and even, to some extent, the timing of television programs can all have a substantial influence on an individual's social rhythms and, in turn, on their circadian rhythms. There is a particular connection between specific kinds of instability and the recurrence of illness episodes. Psychosocial stressors and life that alter the patterning of daily life may have destabilizing effects on the body's natural rhythms. Life events which may appear harmless (or even beneficial) from a psychological perspective may still be linked with considerable changes in daily routines. These disruptions can, in turn, place substantial stress on the body's capacity to maintain stable biological rhythms (particularly sleep-wake, energy, alertness, and appetite rhythms) that are usually synchronized in the absence of an affective episode. Stressful events have the capacity to impact the circadian system via increases in autonomic arousal that can, in turn, alter sleep-wake cycles, timing (and amount) of food consumption, and normal circadian patterns of release of other hormones. Events of any size or severity can lead to significant changes in daily routines (like a child going to school, or a new dog that requires morning walks). Major life stressors such as moving house or getting a divorce can not only have a negative psychological impact on the individual, but may also disrupt social rhythms.

If we increase the regularity of patients' daily routines (specifically, their often erratic sleep/wake cycles, meal times, and times of rest versus activity) we thereby help strengthen their otherwise vulnerable circadian systems. Interpersonal and social rythms therapy (IPSRT) directly incorporates social rhythm theories into the framework of interpersonal psychotherapy, initially developed by Klerman for the treatment of unipolar depression. IPSRT is geared toward stabilizing patients' routines while simultaneously improving the quality of their interpersonal relationships and their performance of key social roles.

S. 09-2

Lithium – mechanism of action in association with bipolar disorder's neurobiology

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Background: Lithium has been the first mood-stabilizing drug and is now regarded as the drug of the first choice for prophylaxis of bipolar disorder (BD). Besides mood-stabilizing activity, lithium exerts anti-suicidal, antiviral, immunomodulatory, and neuroprotective effects. The mechanisms of these effects can be associated with lithium's therapeutic activity in BD and related to the pathogenesis of this disorder.

Material and methods: This is a narrative review of the biological mechanisms of lithium in relation to the pathogenesis of BD.

Results: The effects of lithium on intracellular signalling has been known for several decades. Lithium influences the phosphatidylinositol system, mostly by inhibiting inositol 1-monophosphatase. This was a basis for the inositol hypothesis of bipolar disorder and lithium action formulated by Berridge in 1989. Lithium also inhibits protein kinase C (PKC), similar to another mood stabilizer, valproate what prompted using tamoxifen, a PCK inhibitor, in the treatment of mania. Another action on intracellular signalling is inhibition by lithium of adenyl cyclase. Since the mid-1990s, the inhibiting effect of lithium on the enzyme glycogen synthase-3 beta has been advocated as the most important mechanism, responsible for a variety of neurobiological effects of lithium, including adverse effects in bipolar disorder. Lithium stimulates the brain-derived neurotrophic factor (BDNF) system. The BDNF gene polymorphism has been shown in the pathogenesis of bipolar disorder and in the prophylactic effects of lithium. Biological rhythm disturbances have been important in the pathogenesis of the bipolar disorder. An association of the polymorphisms of clock genes with lithium prophylactic response was demonstrated and in clinical conditions, a positive effect of lithium administration on chronotype. The candidate genes associated with lithium prophylaxis are mostly related to a predisposition to BD. Antiviral effect of lithium on herpes viruses may be connected with pro-cognitive action in BD. The immunomodulatory effect of lithium can attenuate the "low-grade inflammation" in BD. Finally, a neuroprotective effect of lithium can improve neuroplasticity in BD and make this ion a possible candidate for therapeutic application in neurodegenerative disorders.

Conclusions: Many mechanisms of the biological effects of lithium are related to the pathogenetic processes of bipolar disorder.

Acknowledgements: None.

S. 09-3

Biomarkers of staging in BD

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Bipolar disorder (BD) is one of the severe mental disorders characterized by early onset, chronic course, high relapse rate, impaired social, professional and cognitive functioning (also in periods of remission) and high mortality due to both suicide and frequent comorbidity of somatic diseases resulting in various consequences and complications. For many years clinical trials have been conducted aiming to recognize and understand the pathophysiological processes underlying bipolar disorder. A considerable part of these studies relies on determining the activity of biomarkers defined as biochemical parameters, whose presence or intensity is either a constant, process- or disease specific, phase-independent feature (trait marker), or sensitive to changes, that may be a reflection of certain stages or phases of a disease and its severity (state marker).

According to many researchers, a large part of the changes and biochemical phenomena detected in patients with bipolar disorder can be considered as so called allostatic markers. The term 'allostasis' means the ability of the body to restore and maintain the new balance in response to adverse environmental and pathogenetic factors. It is a condition in which organism stays alive, but suffers the negative consequences that cumulate over time. An example of this phenomenon may be a development of bipolar disorder along with its subsequent episodes. In 2009 Kapczinski et al. proposed an integrated model trying to detail the subsequent phases of clinical and biological advancement of BD. This model suggests that in the course of BD a gradual progression towards heavier, more treatment-resistant and more impairing form of the disease can be observed. It is a result of accumulation of intertwined and mutually influencing pathophysiological processes that compose the allostatic load. These processes include oxidative and nitrosative stress, inflammatory processes, neuroregeneration and neuroplasticity disturbances and the phenomenon of excitotoxicity stemming from the excessive activation of glutamate transmission leading to neuronal damage.

Kapczinski distinguished four stages of advancement of bipolar disorder characterized by different clinical picture, prognosis, treatment response and the necessity to use specific strategies. Stage 1 is characterized by full symptomatic remission after episodes with a return to premorbid functioning. In Stage 2 between episodes, symptoms of comorbid psychiatric disorders (dependence or abuse of alcohol or othersubstances, anxiety, etc.) are present and have impairing influence on functioning. There also occurs apossibility of rapid-cycling course of the disease in stage 2. Cognitive impairment is recognized inneuropsychological tests, but not disclosed in examination and functioning of a patient. Stage 3 istyped by subsyndromal affective symptoms between episodes, overt clinical impairment of cognitivefunctioning, gradual shortening of the duration of periods of euthymia, increasing number ofexacerbations, evident impairment of family and work functioning. Stage 4 is an intensification of the stage 3 characteristics with a progressive invalidation and deterioration of a patient.

We conducted our own research project to investigate markers of allostasis in bipolar disorder. The study included a total of 133 participants with a diagnosis of bipolar disorder (type I, type II or NOS - on the basis of DSM-IV-TR). 61 of them were in the current episode of depression,23 persons in the current manic or hypomanic state and 49 persons in remission. The control group of healthy volunteers consisted of 50 people.

Based on our results some hypothesis, requiring further examination, can be formulated:1) Increased TBARS levels in serum may be a state marker reflecting the presence of acute episode of the disease and a trait marker of a more advanced stage of bipolar disorder (stage 3 and 4); 2) Reduced zinc concentration in serum may be a marker of depression as a state - particularly in patients with BD type I and more advanced stages of BD (stage 3 and 4); 3)sTNFR-60 kDa and sTNFR-80 may be a marker of melancholic syndrome and depression exacerbation in bipolar disorder; 4) reduced level of sIL-2R and increased level of sTNFR-80 may be biomarkers of advanced form of bipolar disorder (stage 3 + 4); 5)elevated level of sIL-1RA can be a trait marker of bipolar disorder and elevated sTNFR-80 level –a state marker reflecting the presence of depressive episode; 6) Elevated concentration of copper may be a marker for an early stage of BD (Stage 1). Our analyses are the part of current, extensive studies that provide insight into the biological substrate of the disease. Their future integration may open an opportunity to create a battery of clinically useful laboratory markers of bipolar disorder.

S. 09-4

Neurocognitive and motor deficits in BD – neuroimaging and clinical studies

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Increasing number of studies show motor functions deficits in psychiatric disorders. The largest number of those has been devoted to patients with schizophrenia (SZ). They present neurological soft signs (NSS) and cerebellar soft signs (CSS) as well as neurocognitive deficits in form of implicit motor learning impairments. It has been suggested that NSS may represent one of the overlapping intermediate phenotype, reflecting a common genetic and neurodevelopmental impairment between SZ and BD patients. Despite the growing number of studies evaluating neurological impairments in SZ, only few studies showed them in BD patients group. The aim of the study is to evaluate the severity of motor dysfunctions in the form of NSS, CSS and implicit motor learning impairments in BD as compared to the group of healthy controls (HC) and the group of SZ patients and to evaluate their neurobiological underpinnings with the use of functional magnetic resonance imaging (fMRI) methods. According to my hypothesis, BD patients will show significant disturbances of motor functions in the form of NSS, CSS and implicit motor learning impairements of intensity comparable to patients with SZ and significantly greater than in HC group.

We have examined 33 euthymic BD patients, 33 SZ patients during functional remission and 31 HC. NSS were assessed using the Neurological Evaluation Scale (NES). CSS were evaluated with the use of the International Cooperative Ataxia Rating Scale (ICARS) scale. Implicit motor learning was measured using Serial Reaction Time Task (SRTT) procedure. fMRI data were acquired using a 3T Siemens Skyra MR System.

Our results showed increased severity of NSS and CSS in BD patients compared with the healthy control group. For the first time, we have shown that NES and ICARS scores and subscores do not differentiate BD and SZ patients. SRTT analysis revealed no indices of learning in BD patients. For the first time, we have shown the presence of inverted learning curve features in this clinical group. In addition, BD patients showed a comparable severity of implicit motor learning as patients with SZ. Neuroimaging studies has show deficits within fronto-thalamo-cerebellar network in BD patients group. There is a need to conduct further neuroimaging studies to assess neurocorrelates of movement functions deficits in BD.

S. 10-1

Sano Centre: Towards Holistic Computational Medicine

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Background: The future of medical treatment is the development of computational medicine, a new branch of science, a combination of medicine and computer science. Nowadays, we know that every patient is different, so treatment should be tailored to each patient.

Material and methods: Since 2000 DICE Team (http://dice.cyfronet.pl/) has been involved in the research focused on the elaboration of problem-solving environments and decision support systems for medicine on top of distributed computing infrastructures. The main aim was to provide *repeatability*, *replicability* and *reproducibility* in the context of extreme-scale computing methodology. This research is financed mainly by projects of the European Commission and the main partners are the University of Sheffield, University of Amsterdam, Juelich Supercomputing Centre, LMU and Leibniz Supercomputing Centre in Munich.

Results: As a result of this successful scientific collaboration a new scientific The main aim was: *Sano Centre for Computational Personalized Medicine - International Research Foundation* was established in 2019, in Krakow (https://sano.science/). Six Sano research teams cover such in-silico medicine areas as: modelling and simulation, data science, artificial intelligence and machine learning methods, image processing, IT methods in medicine, large-scale computing, and decision-making support systems. Researchers at Sano use the computing and storage resources of PL-Grid, a.o. the Prometheus computer at Cyfronet AGH.

Conclusions: Scientists working at Sano Centre develop advanced algorithms, modelling methods, computer simulations and artificial intelligence tools that will support doctors in the diagnostic and treatment process. It is extremely valuable from the point of view of an individual patient but equally important is the fact that thanks to personalized diagnostics and therapy, the social costs associated with treatment are reduced. Moreover, modern computer technologies developed in Sano can also be used in research laboratories of pharmaceutical and biotechnology companies.

Acknowledgements: Sano Centre is financed by the European Union's Horizon 2020 Research and Innovation Programme Teaming (grant 857533, 15 M€), the International Research Agendas Programme of the Foundation for Polish Science grant MAB PLUS/2019/13 co-funded by the European Union in the scope of the European Regional Development Fund (10 M €), and Polish Ministry of Education and Science (after 2023, 5 M €).

S. 10-2

Sequence-defined polymers – a next generation data storage materials

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Background: Since ancient times, people had to store information in order to pass gained knowledge to the next generations. Even though the current technology is very advanced it cannot keep up with growing numbers of bits due to the short life time of memory devices. An interesting data storage medium is natural DNA that carries our genetic code and all instructions necessary for reproduction and proliferation of all known living organisms. It was demonstrated that DNA can be also used to store a binary code that can represent text or computer processor instructions using a two-symbol system 0 and 1.

Material and methods: Polymers for data storage have been obtained according to protocol of oligonucleotides synthesis and characterized by mass spectrometry and ion-exchange chromatography. Encoded data was read by MS/MS or nanopore sequencing. Digital materials were fabricated using layer-by-layer assembly of alternatively charged polyelectrolytes/digital polymers. The film growth was followed by ellipsometry and characterized by AFM.

Results: The sequence-defined synthetic polymers (SDPs) are an interesting media for data storage. The information is written into monomer sequence by iterative synthesis and can be edited by light. These synthetic macromolecules can be decrypted by tandem mass spectrometry or nanopore. However, the data capacity in these materials is determined by the macromolecules length. This limitation can be overcome by the spatial organization of digital polymers using non-covalent synthesis e.g. Layer-by-layer assembly of polyelectrolyte.

Conclusions: Information encoded in sequences of synthetic polymers can be edited by light. It is possible to erase, reveal, or edit data using light trigger. To increase data storage capacity Layer-by-Layer assembly of polyelectrolytes can be applied. The sequence-defined synthetic polymers might be relevant materials to the development of archives for long-term data storage. However, the efficient data reading methodology has to be developed.

Acknowledgements: R. Szweda thanks J.F. Lutz for mentoring during her stay in ICS CNRS Strasbourg, France (funding: ERC *680897*, MSCA ITN *642083)* and Polish National Science Centre (2018/31/D/ST5/01365) for received funding to carry on research on SDPs.

S. 10-3

Using markers in targeted diagnostics. Development of stable isotope labeled ionization enhancers for the sensitive analysis of biomarkers

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Background: Although modern medical diagnostics uses hundreds of different molecular biomarkers to examine the patient's condition, it must be assumed that plenty of them remain undiscovered, because their abundance is too low to be detected by modern analytical methods. The aim of our research is to develop a sensitive method of molecular biomarker analysis and to lower their limit of detection as much as possible.

Material and methods: We have proposed an efficient and straightforward methods of peptide–quaternary ammonium salt (QA) conjugate synthesis. QA were used by us as ionization enhancers for the analysis of trace amounts of peptides. We have proposed linearand bicyclicQA as ionization enhancers for analysis of peptides at the attomole level. We designed two new classes of quaternary ammonium isobaric tags for relative and absolute quantitation (QA-iTRAQ 2-plex), combining the advantages of isobaric markers with increasing sensitivity mass spectrometric detection caused by incorporation to the peptide permanent charge. We tested the QA-iTRAQ 2-plex reagents on various peptides as well as protein tryptic digests and podocyte cells.

Results: The incorporation of the developed ionization markers into a peptide molecule increases the sensitivity of detection by mass spectrometry by three orders of magnitude as compared to the parent peptide. We have proposed linearand bicyclicQA as ionization enhancers for analysis of peptides at the attomole level. Obtained results suggest usefulness of the isobaric ionization tags for relative and absolute quantification of trace amounts of peptides.

Conclusions: The application of such new ionization tags may revolutionize the comprehensive proteomics and result in development and quantitative analysis of new biomarkers based on proteins of low abundance.

Acknowledgements: This work was supported by a grant No. UMO-2016/23/B/ST4/01036 from the NSC, Poland. We would like to thank Andrzej Reszka (Shim-Pol, Poland) for providing the access to Shimadzu instruments.

S. 10-4

New ways to record and replay medical operations - Holographic MedAssistant

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Background: Today options to view/record/replay operations are very limited. Every operation require dedicated stuff performing actions, devices needed to perform expected actions and additional tools in case if something will go wrong and unplanned action will have to be done. All of this require a lot of space in operating room. This is way all need to be very well planned and prepared to prevent pushing around between people, devices and cables. Besides helping people each operation gives opportunity to educate future doctors to allow them be better prepared for their own performed operations. Because of very limited number of people who could be educated during operations there were implemented cameras. Each camera allow recording different part of operating space and later such movies could be replayed to learn from it. Such solution allowed to review operations unlimited times and train unlimited number of people with it. The biggest disadvantage of such solution is that in most of cases recording is limited to 2-3 perspectives (2-3 cameras were recording operations).

Material and methods: The concept of a holographic medical assistant (Holographic MedAssistant) using advanced technology devices - MS Azure Kinect DK spatial cameras and MS HoloLens 2 glasses allows to record movies as 3D space. It records 2D movie and besides that record depth data which allows to generate thanks to those data 3D movies. This technology has of course some disadvantages. One camera can create 3D movie only from data which is in front of it, for example space behind visible person will not be recorder. Workaround for this is to use two cameras where second one record space potentially not visible be first one. Thanks to that, two cameras can build 3D movie space with almost no blind spaces. Only requirement is to merge data from 2 cameras to create one full recording.

Results: Final result of this technology is full recording in 3D. Such movie allow to view recorded operation from nay different positions and angles. That is a huge advantage which adds value missing in normal 2D movie, where viewer is restricted to angle how camera was placed and setup.

Conclusions: A new visualization tool for advanced personalized medical education has been proposed.

Acknowledgements: Financed by NCBR. Grant No. LIDER/17/0064/L-11/19/NCBR/2020.

S. 11-1

Effects of cocaine self-administration on neuroplastic mechanisms: evidence from rats lacking the serotonin transporter

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Background: Previous studies have clearly shown that reduced expression and function of the plasmalemmal serotonin transporter is closely associated with an anxious and pro-depressive phenotype and promotes higher cocaine **intake in rats**. Rats lacking the serotonin transporter (SERT-/-) show increased sensitivity to environmental stimuli, in line with the personality trait sensory processing sensitivity (SPS), but little is known about the underlying mechanisms in a condition of co-presence of addictive and pro-depressive states.

Thus, given the pivotal role of glutamate in environmental sensitivity and in cocaine seeking behavior, the aim of our study was to evaluate whether changes in the glutamate synapse in reward-related brain areas of naïve and SERT$^{-/-}$ exposed to repeated short-access (ShA, 1h/day) or long-access (LgA, 6h/day) cocaine (COC) or amphetamine (AMPH) self-administration (SA) could contribute, at least in part, to higher drugs intake.

Material and methods: Male SERT$^{-/-}$ rats were allowed to self-administer COC (0.5 mg/kg/infusion) or AMPH (0.03 mg/kg/infusion) during daily ShA or LgA session, for a total of 17 days.24 hours following the last SA session, we analysed the expression of glutamate system components in the nucleus accumbens shell (sNAc) and core (cNAc).

Results: SERT$^{-/-}$ rats self-administer more cocaine under both ShA and LgA conditions, while they display increased AMPH intake underLgA, but not ShA, conditions. SERT gene deletion increases the motivational and psychomotor effects of COC and AMPH, respectively.

Deletion of SERT determined an overall reduction of NMDA and AMPA receptor subunits and their scaffolding proteins in the cNAc, mimicking the COC and AMPH-induced changes in wild-type animals. LgA, but not ShA, in SERT$^{-/-}$ rats increased glutamatergic signaling in cNAc, but not in sNAc, suggesting that SERT removal reorganizes the glutamate synapse, contributing to the escalation of cocaine seeking.

Conclusions: These results suggest that the liability of SERT$^{-/-}$ rats to compulsive COC and AMPH intake may, at least in part, depend upon lack of SERT. Hypersensitivity of the glutamatergic synapse in the cNAc, a subregion involved in the incubation of drug seeking, may contribute to the increased vulnerability to addiction observed in SERT$^{-/-}$ rats and may contribute to the negative emotional state observed in drug users after drug discontinuation.

Acknowledgements: Sponsored by ERANID Grant 'STANDUP' project to FF and JH

S. 11-2

The neural mechanisms driving the transition from regular to compulsive cocaine intake in rats lacking the serotonin transporter

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Background: Drug addiction is characterized by compulsive drug intake. It is thought that the transition from controlled regular to uncontrolled compulsive drug intake is associated with a neural shift from brain areas implicated in functions such as arousal to areas exerting more complex functions such as emotion regulation. In humans and experimental animals, inherited down-regulation of the serotonin transporter is associated with anxiety and increased risk for drug addiction. We aimed to investigate the neural circuitry involved in the transition to compulsive cocaine intake as a function of inherited serotonin transporter down-regulation.

Materials and methods: Serotonin transporter (SERT) knockout and virally-mediated SERT down-regulation rats were subjected to either short (1 hr) or long access (6 hr) intravenous cocaine self-administration to measure regular and compulsive cocaine intake, respectively. 24 Hrs into withdrawal emotion was assessed using the elevated plus maze test. Cocaine self-administration thereafter commenced. 24 Hrs after the last self-administration session the animals were sacrificed to measure through ex vivo immunohistochemistry CRF (corticotrophin releasing factor) levels in the hypothalamus (mediating arousal) and the amygdala (mediating emotion). In a second experiment we subjected rats with a history of long access cocaine or sucrose self-administration to structural magnetic resonance imaging (MRI).

Results: We found that SERT knockout rats display increased cocaine intake under both short and long access cocaine self-administration conditions. 24 Hours into withdrawal from short and long access cocaine self-administration SERT knockout rats displayed increased anxiety. We could mimic the increased cocaine intake under short access conditions by down-regulating SERT in the median raphe, and the increase cocaine intake under long access conditions by down-regulating SERT in the dorsal raphe. Furthermore, we found that SERT down-regulation in the median raphe affected CRF levels in the hypothalamus, and SERT down-regulation in the dorsal raphe affected CRF in the amygdala. Using MRI we found volume changes in the amygdala and dorsal raphe in SERT knockout rats with a history of compulsive cocaine intake.

Conclusions: The transition to compulsivity in drug addiction could relate to a neural shift from the median raphe to the dorsal raphe projecting to the amygdala.

Acknowledgements: Dutch council for scientific research.

S. 11-3

Sphingolipids in depression-induced alcoholism

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Background: Major depression and alcohol addiction are characterized by high co-morbidity (Lalanne et al., 2015). Ceramide/acid sphingomyelinase (ASM) system is proposed as a mechanism for depression-induced alcoholism. Clinical and preclinical studies showed the association between increased ASM activity in tissues and depression as well as alcohol addiction (Kornhuber et al., 2005; Gulbins et al., 2013; Müller et al., 2017). We investigated interactions between depression and alcohol dependence in animals with ASM overexpression (ASMtg).

Material and methods: The depression/anxiety-like behavior of ASMtg mice treated with alcohol on the model of free choice drinking or forced administration was assessed in a battery of behavioral tests. Alcohol effects on brain tissue were evaluated by high-performance liquid chromatography. The changed in extracellular monoamine levels as a response to alcohol were evaluated by in-vivo microdialysis. Immunohistochemical analysis of the morphology of monoaminergic systems in the dorsal hippocampus (DH) was performed.

Results: Free choice alcohol consumption reversed increased depression level and normalized ASM activity in the DH of ASMtg, but not wild type (wt) mice. Decreased tissue monoamine levels were selectively reversed by alcohol drinking in several brain structures of ASMtg mice. ASM overexpression potentiated the dopamine response to alcohol in the DH and nucleus accumbens, but reduced norepinephrine response. Dopaminergic and serotonergic innervation was preserved in ASMtg and wt mice. In contrast, forced alcohol treatment had depressogenic in ASMtg and wt mice and did not affect ASM activity.

Conclusions: Free-choice alcohol drinking, but not forced alcohol treatment reduces depression-like behavior selectively in depressed animals through the normalizing brain ASM activity and monoamine homeostasis. This mechanism can be considered as a new treatment target specifically for depression-induced alcoholism.

Acknowledgements: This work was supported by the IZKF Erlangen (project E13).

S. 11-4

Paraventricular thalamic control of opioid withdrawal and relapse

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Background: The paraventricular thalamus (PVT) to the nucleus accumbens (NAc) pathway has been shown to drive aversion in naïve animals and more recently has been implicated in the aversive states experienced during opioid withdrawal. However, the role of the PVT→NAc pathway in opioid relapse is unclear.

Materials and methods: Here we used optogenetics and chemogenetics to investigate the contribution of the PVT→NAc pathway in heroin relapse in two different heroin self-administration models in rats. In one model, rats underwent home-cage abstinence prior a cued relapse test, and in the other, they underwent extinction training, a procedure that has been likened to cognitive behavioral therapy.

Results: We found that the PVT→NAc pathway is both sufficient and necessary to drive aversion in a real-time conditioned place aversion (rtCPA) test and heroin seeking after abstinence but not after extinction training. The ability of extinction to reduce the contribution of the PVT→NAc pathway to drive heroin seeking was paralleled by a loss of synaptic plasticity in PVT inputs onto a specific subset of NAc neurons, the D1 medium spiny neurons (MSNs). Thus, the extinction training procedure may exert therapeutic reductions of opioid seeking by altering synaptic plasticity within the PVT→NAc pathway, ultimately resulting in reduced aversion and drug seeking.

Conclusions: Overall, these results point to the PVT→NAc pathway as a key component of the neural circuitry driving aversion and heroin seeking after forced home-cage abstinence and identify this pathway as a potential therapeutic substrate by which the extinction procedure can reduces relapse.

S. 12-1

Defining and Mining Therapeutic Targets in Psychostimulant Use Disorder

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Background: Cocaine misuse and cocaine use disorder (**CUD**), an acquired brain disorder, continue to be major public health challenges. There remains a critical unmet need to discover novel therapeutic strategies to normalize mechanistic drivers of CUD and treat CUD effectively. Dampened signaling through the central serotonin (**5-HT**) 5-HT_{2C} receptor (**5-HT_{2C}R**), a member of the 5-HT_2R receptor family, is a key component of the mechanisms of action underlying the cognitive and behavioral dimensions of CUD. The anti-obesity medication and 5-HT_{2C}R agonist lorcaserin (APD-356, Belviq[â]) suppresses intake of cocaine, nicotine and opioids (e.g., oxycodone) in preclinical self-administration studies, and has demonstrated efficacy to improve smoking cession rates in a phase II study.

Methods: The orthosteric site of the 5-HT_{2C}R binds 5-HT and has been the traditional target for ligand discovery, and recent studies have identified positive allosteric modulators (**PAMs**) that increase the efficacy of 5-HT.

Results: Several analogues of our new molecule series potentiated 5-HT_{2C}R-, but not 5-HT_{2A}R, mediated signaling in cultured cells and did not appreciably displace binding to 5-HT_{2C}R, 5-HT_{2A}R or 5-HT_{2B}R. A selected PAM exhibited a favorable overall pharmacokinetic and behavioral profile in rats, and inhibited cocaine-seeking behaviors in rats.

Conclusions: Importantly, the advances in novel molecule discovery and expansion of knowledge of allosteric modulation of the 5-HT_{2C}R could have a profound impactin improving the course and treatment of CUD.

Acknowledgements: NIH NIDA DA038446; VidaLibreBio, Inc. UTMB Center for Addiction Research.

S. 12-2

The novel psychoactive substance methoxetamine induces persistent behavioral abnormalities and neurotoxicity in rats

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Background: Methoxetamine (MXE) is a novel psychoactive substance that can induce several short-term effects on emotional states and behavior. However, little is known about the persistent emotional and behavioral effects of MXE. Moreover, neurotoxic effects of MXE have been hypothesized, but never demonstrated in vivo.

Materials and Methods: To clarify these issues, rats received repeated treatment with MXE every other day (0.1-0.5 mg/kg, i.p., × 5), and 7 days later they were challenged with MXE (0.1-0.5 mg/kg, i.p.). Behavioral effects of MXE were first evaluated by measuring emission of ultrasonic vocalizations and locomotor activity after each administration. Thereafter, persistent behavioral effects of MXE were evaluated, starting 8 days after challenge, through elevated plus maze, spontaneous alternation, novel object recognition, and marble burying tests. After completion of behavioral analysis, neurotoxic effects of MXE were evaluated by measuring densities of dopamine transporter, tyrosine hydroxylase, and serotonin transporter in various brain regions.

Results: Repeated treatment and challenge with MXE affected neither calling behavior nor locomotor activity of rats. Conversely, rats previously treated with MXE exhibited behavioral alterations in the elevated plus maze, marble burying and novel object recognition tests, suggestive of increased anxiety and impaired non-spatial memory. Noteworthy, the same rats displayed dopaminergic damage in the medial prefrontal cortex, nucleus accumbens, caudate-putamen, substantia nigra pars compacta, and ventral tegmental area, along with accumbal serotonergic damage.

Conclusions: Our findings show for the first time that repeated administration of MXE induces persistent behavioral abnormalities and neurotoxicity in rats, which can help elucidating the risks associated with human MXE consumption.

Acknowledgements: None.

S. 12-3

Dopamine system dysregulation, glial cells alteration, and behavioral correlates after repeated exposure to the synthetic cannabinoid receptor agonist JWH-018

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Background: Since 2004, herbal mixtures broadly known as Spice/K2 drugs, containing synthetic cannabinoids such as JWH-018, have been marketed as a legal marijuana surrogate. Previous studies of our group showed that JWH-018 has CB1-receptor dependent reinforcing properties and increases dopamine (DA) transmission selectively in the shell of the NAc at the dose of 0.25 mg/kg i.p. Despite the widespread and growing use of Spice/K2 drugs, limited information is available on the effects induced by repeated exposure to synthetic cannabinoids on mesolimbic and mesocortical DA transmission and on glial cells.

Material and methods: In order to test whether repeated administration of JWH-018 is able to (i) modulates behaviour and emotional state, (ii) affect dopamine transmission and its responsiveness to motivational stimuli, and (iii) is associated with CB1 receptors and glial cell alterations, adult male rats were administered with JWH-018 JWH-018 (0.25 mg/kg i.p.) once a day for 14 consecutive days. At multiple time points after drug discontinuation (1 hours, 24 hours, 7 days), we performed behavioural, electrophysiological, neurochemical, and molecular evaluation.

Results: Repeated JWH-018 exposure (i) induces anxious and aversive behaviours and withdrawal signs, (ii) decreases spontaneous activity and number of dopamine neurons in the VTA. Moreover, (iii) we observed a decreased dopamine sensitivity in the NAc shell and core, but not in the mPFC, to the first salient taste stimulus (chocolate); conversely, after the second exposure, dialysate dopamine fully increased in the NAc shell and core but not in the mPFC. Finally, (iv) astrogliosis (mPFC, NAc shell and core, VTA), microgliosis (NAc shell and core), and downregulation of CB1Rs (mPFC, NAc shell and core) were found in selected dopamine brain areas.

Conclusions: Taken together these results suggest that repeated JWH-018 exposure may reflect a useful model to clarify the detrimental effects of recurring use of Spice/K2 drugs.

S. 12-4

Mechanisms and neurotransmitter systems involved in the neurotoxic effects of 3,4-methylenedioxymethamphetamine (MDMA) administration during adolescence

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Background: The amphetamine-related drug 3,4-methylenedioxymethamphetamine (MDMA, "ecstasy") is widely used as a recreational drug by young people. However, previous studies have consistently demonstrated that MDMA has neurotoxic potential, and evidence in mice shows that MDMA may induce dopaminergic damage, particularly at the level of the nigrostriatal system. In order to characterize how the number of administrations influenced the severity of MDMA-induced dopaminergic damage and to describe the localization and persistence of this damage, we evaluated the changes in tyrosine hydroxylase (TH) and dopamine transporter (DAT) in different regions of the mouse brain. Moreover, we investigated whether dopaminergic damage was associated with noradrenergic, GABAergic, and serotonergic damage, by evaluating the changes in noradrenaline transporter (NET), glutamic acid decarboxylase-67 (GAD-67), and serotonin transporter (SERT). Finally, we evaluated the gender-dependence of the oxidative stress processes induced by acute MDMA administration.

Material and Methods: Male and/or female mice received acute (4 ˣ20 mg/kg i.p., 2-h interval) or chronic (14/28/36 ˣ10 mg/kg i.p.) MDMA treatments depending on the marker evaluated, and were sacrificed at different time points after drug discontinuation.

Results: After acute MDMA, the isoform 1 of SOD (SOD1), which catalyzes the dismutation of the superoxide radical (O2−) produced by mitochondria into molecular oxygen (O2) and hydrogen peroxide (H2O2), increased in striatal TH-positive terminals, but not in nigral neurons, of males and females, while the isoform 2 of SOD (SOD2) increased in striatal TH-positive terminals and nigral neurons of males only.

Male mice receiving chronic MDMA administration showed reduced levels of DAT-positive fibers in caudate–putamen (CPu) and medial prefrontal cortex (mPFC) and reduced levels of TH-positive nigral neurons. These mice also displayed increased NET-positive hippocampal fibers, reduced GAD67-positive neurons in CPu and hippocampus and reduced GAD67-positive fibers in mPFC. The reductions in dopaminergic markers and GAD-67 persisted at 3 months after MDMA discontinuation. Finally, MDMA never modified the levels of SERT.

Conclusions: These results provide further insight into the localization and persistence of MDMA-induced dopaminergic damage, and show that this effect may associate with GABAergic, but not noradrenergic or serotonergic, damage. Moreover, these results suggest that MDMA-induced neurotoxic effects are gender-dependent and dopaminergic neurons of males could be more sensitive to SOD2-mediated toxic effects.

S. 13-1

Bidirectional interaction between the gut microbiome and the endocannabinoidome

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For several decades, and starting some 25 years from its discovery, the only plant cannabinoid (phytocannabinoid) with an established mechanism for its pharmacological actions has been D^9-tetrahydrocannabinol (THC). To THC are ascribed the most important euphoric and psychotropic effects of recreational preparations (e.g. marijuana, hashish) obtained from those varieties of *Cannabis sativa* that are rich in this compound. These effects on the central nervous system are now known to be due to THC capability of activating endogenous G-protein-coupled receptors (GPCRs) that are among the most abundant such proteins in the mammalian brain: the type-1 cannabinoid (CB1) receptors. THC also activates another GPCR, the type-2 cannabinoid (CB2) receptors, through which it produces instead anti-inflammatory and immune-modulatory actions. The discovery of CB1 and CB2 receptors led to the finding of their endogenous agonists, later named endocannabinoids: *N*-arachidonoyl-ethanolamine (anandamide) and 2-arachidonoyl-glycerol (2-AG). The chemical signaling system composed of CB1 and CB2 receptors, the two endocannabinoids and the anabolic and catabolic enzymes regulating endocannabinoid levels, became known as *the endocannabinoid system*.

Later, an *expanded* endocannabinoid system, including several non-endocannabinoid long chain fatty acid amides and esters, among which: a) the congeners of anandamide and 2-AG, b) the *N*-acyl-aminoacids, c) the *N*-acyl-neurotransmitters and d) the primary fatty acid amides, has been discovered. These lipid mediators often share with the two endocannabinoids biosynthetic and/or inactivating enzymes, but not necessarily their receptors, which instead include orphan GPCRs, ligand-activated ion channels and peroxisome proliferator-activated nuclear receptors (PPARs). These small molecules, therefore, should not be considered endocannabinoids *sensu stricto*, but instead as endocannabinoid-like mediators, and this expanded endocannabinoid system is becoming known as the *endocannabinoidome*.

The endocannabinoidome is involved in almost all aspects of mammalian physiology and pathology, and recent work from my and other laboratories have highlighted how this complex signalling system is modulated by, and in turn modulates, another fundamental player in several physiopathological conditions: the gut microbiome. I will present data on the endocannabinoidome-gut microbiome axis and its emerging importance in obesity and related neuropsychiatric disorders.

S. 13-2

Targeting FABP5 to Treat Pain and Inflammation

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Chronic pain is highly prevalent in the population and is a major reason for seeking medical care. Currently available analgesics exhibit limited efficacy, pronounced side-effect profiles, and/or addiction liability, highlighting the need to identify novel targets for the development of next-generation analgesics. Fatty acid binding protein 5 (FABP5) is a lipid transport protein that our group previously identified as an intracellular carrier for endocannabinoids. Inhibition of FABP5 reduces endocannabinoid transport to catabolic enzymes, elevates tissue endocannabinoid levels, and produces cannabinoid receptor-mediated antinociceptive effects. In parallel, FABP5 modulates the production of pro-inflammatory mediators via cannabinoid receptor-independent mechanisms at sites of tissue injury. Here, I will discuss our ongoing work aimed at developing small molecule FABP5 inhibitors as novel analgesics, describe mechanisms in sensory neurons through which FABP5 modulates pain, and present new preliminary data focusing on potential mechanisms underlying the antinociceptive effects of FABP5 inhibitors in a model of osteoarthritis pain.

S. 13-3

Lipid signaling and the recovery from traumatic events

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Background and purpose: PTSD is a heterogeneous disorder induced by trauma and results in severe long-term impairments of an individual's mental health and daily quality of life. Interestingly, PTSD does not develop in every individual exposed to trauma; thus, some individuals are more resilient than others. However, the molecular differences underlying trauma-induced dysregulation versus resilience to trauma are poorly understood. In this study, we aimed at shedding light on these processes of vulnerability and resilience.

Experimental approach: We used a single-trauma PTSD model in mice to induce long-term maladaptive behaviours. These maladaptations were analysed four weeks post-trauma in a longitudinal behavioural design in order to profile the individual animals into resilient, i.e. those that behave consistently like the unexposed group, or susceptible, i.e. those that developed PTSD-like behavioural phenotypes. The phenotype's classification was based on their individual responses in different behavioural experiments. We analysed the microbiome and circulating endocannabinoid levels of these mice during the development of PTSD, as well as long-term changes in brain phospholipid and transcript levels.

Key results: We found a plethora of molecular differences between resilient and susceptible individuals across multiple molecular domains, including lipidome, transcriptome, and the gut microbiome. Some of these differences were stable even several weeks after the trauma, indicating the long-term impact of traumatic stimuli on the organism's physiology. Furthermore, the integration of these multi-layered molecular data revealed that resilient and susceptible individuals have very distinct molecular signatures across various physiological systems.

Conclusions and implications: We showed that trauma induces individual-specific behavioural responses that, in combination with a longitudinal characterization of mice, can be used to identify distinct sub-phenotypes within the trauma-exposed group. These groups differ significantly not only in their behaviour but also in specific molecular aspects across a variety of tissues and brain regions. This powerful approach may reveal new targets and predictive biomarkers for the pharmacological treatment and prognosis of stress-related disorders.

S. 14-1

The association between FTO rs9930506 polymorphism, vitamin D levels and depression

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Background: In a genome-wide association study, the FTO gene was found to be linked to obesity. However, no data are available on the relation between FTO rs9930506 gene single nucleotide polymorphism and serum 25(OH)D levels. In addition, obesity and the serum vitamin D level are proposed to be involved in pathophysiology of depression.

Material and methods: Two hundred and eighteen morbidly obese women were recruited in this cross-sectional study. Real-time polymerase chain reaction was used to detect the A/G alleles of the rs9939506 polymorphism in the FTO gene. For all subjects, anthropometric measurements (body weight and height) were taken and the body mass index (BMI) was calculated. Blood tests were also performed, including total cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides, and fasting glucose levels. Serum 25(OH)D concentrations were measured using electrochemiluminescence immunoassay. The severity of depressive symptoms was assessed with the Beck's Depression Inventory (BDI-II).

Results: Studied women with morbid obesity were 42.2 ± 11.1 years old with mean BMI 45.9 ± 5.8 kg/m^2. It was noticed that the rs9930506 GG homozygotes had significantly higher body mass as well as lover serum 25(OH)D levels (14.8±6.9 ng/ml, n=64) than A-allele carriers (17.1±7.9 ng/ml, n=154, $p<0.05$). BMI were significant inversely associated with serum 25(OH)D levels in all genotypes. There was no association between FTO genotype, serum vitamin D level and BDI-II total scores in studied population.

Conclusions: GG genotype of FTO rs9930506 polymorphism might predispose to the lover serum vitamin D levels in morbidly obese women. Further longitudinal studies are needed to confirm these results.

S. 14-2

3,3'-diindolylmethane protects rat brain from perinatal asphyxia

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Background: According to the World Health Organization perinatal asphyxia represents the third most common cause of neonatal death. Although most of infants recover from a hypoxic-ischemic (HI) episode, many of them suffer from ischemic encephalopathy resulting in motor disability and cognitive disorder. The main methods of treatment of perinatal asphyxia are oxygen therapy and hypothermia. However, the possibility of side effects and short application time make it still necessary to search for new therapies.

Material and methods: In the present study, the *in vivo* model of rat perinatal asphyxia has been used. Rats underwent the intraperitoneal injection with *3,3'*-diindolylmethane (DIM) at 30 min, 24 h, 48 and 72 h after HI episode at a dose of 10 mg/kg of body weight. Sham operated and HI control rats were injected with corn oil. After 3 days, we investigated the levels of apoptotic and anti-oxidant factors. The qPCR analysis of the mRNA levels encoding *Bax*, *Bcl2*, *Gpx-3* and *Sod1* has been used. To check the protein expression levels of BAX, BCL2, FAS, CASP-3, GPx-3 and SOD-1 we applied the western blot analyses. Caspase-9 activity was determined by the enzyme-linked immunosorbent assay (ELISA).

Results: HI injury decreased the mRNA expression of the anti-apoptotic gene *Bcl2* and increased the mRNA expression of the antioxidative enzyme *GPx3*. Treatment with DIM partially reversed these effects. However, there were no changes in *Bax* or *Sod1* mRNA expression levels. Western blot analyses showed that HI insult increased the protein levels of FAS and SOD-1 and decreased BCL2 protein level. DIM reduced the protein levels of FAS, CASP-3, GPx3 and SOD-1, but it did not change the levels of BAX and BCL2. Moreover, DIM reduced hypoxia/ischemia-evoked caspase-9 activity.

Conclusions: We demonstrated for the first time the neuroprotective capacity of DIM in an *in vivo* model of rat perinatal asphyxia which corresponds to hypoxic/ischemic episodes in human newborns. The neuroprotective effect of the compound was mediated *via* inhibition of HI-induced apoptosis as well as normalization of expression of oxidative stress parameters. Therefore, we postulate that DIM is a promising therapeutic tool to prevent HI-induced brain injury.

Acknowledgements: This study was financially supported by grant no. 2018/31/B/NZ7/01815 from the National Science Centre of Poland and the statutory fund of the Maj Institute of Pharmacology of the Polish Academy of Sciences in Krakow, Poland.

S. 14-3

Coarse-grained simulations for identification and analysis of transport pathways in proteins: encouraging speed-accuracy trade-off

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Background: Biochemical reactions occur in the active sites of enzymes, secured by several essential catalytic residues, which are mostly buried inside the protein core & connected to the bulk solvent through molecular transport pathways called tunnels. These tunnels were proven to be important targets for protein engineering & drug discovery. Since most tunnels are transient in their nature, molecular dynamics (MD) simulations are the method of choice for their study. However, given the rare opening of tunnels, their identification and analysis often require extensive and time-demanding simulations. Coarse-grained (CG-MD) simulations are, in general, capable of overcoming sampling limitations of classical molecular dynamics (cMD). Therefore, we investigated to what extent CG models are suitable for tunnel analysis given their reduced resolution.

Material and methods: ReplicatedcMD & CG simulations (5x 5μs for each model) were carried out for model system haloalkane dehalogenase (DEHAL) enzyme using AMBER18 package following recommended standards in the field and using SIRAH CG model. Tunnels were analyzed with the CAVER3 package.

Results: Since we have initially observed the compromised secondary structure of DHEAL in CG simulations, we have developed a protocol for restraining α-helices and β-sheets. This protocol reduced secondary structure loss to around 5% in α-helices and effectively prevented disruption of β-sheets. Importantly, using the optimized protocol the protein RMSD was maintained at an acceptable range (< 5Å), comparable to other extensively used CG models. Regarding their application for tunnel analysis, CG simulations managed to identify all experimentally confirmed tunnels in agreement with cMD. The geometry of CG tunnels is similar to cMD, but there is a tendency for tunnels being somewhat narrower and longer. Additionally, we have systematically identified several tunnels not observed in cMD previously, suggesting improved sampling of tunnels in significantly reduced run-times.

Conclusions: CG models constitute a promising approach to investigate tunnels in proteins with significantly reduced computational demands compared to traditional methods, opening new possibilities for the identification of tunnels in large protein systems & massive datasets.

Acknowledgments: This research is supported by National Science Centre, Poland (2017/26/E/NZ1/00548), and the calculations were performed at the Poznan Supercomputing and Networking Center.

S. 14-4

Monitoring concentration profiles of anthracycline antibiotics in pediatric oncology patients

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Background: The effectiveness of chemotherapy in pediatric cancer patients depends on the correct dosage. One of the most frequently used groups of cytostatics in various chemotherapeutic regimens are anthracycline antibiotics. The narrow therapeutic index these group of drugs combine with the inter- and intra-individual pharmacokinetic differences can results in high risk of side effects as well as ineffective therapy. The best approach to determining the correct dosage is implementation of personalized therapy. The essence of this process is to monitor the concentration of cytostatic drugs in the patient's biological fluids. The aim of the research was to develop quick, simple and sensitive analytical methods allowing the determination of the concentration of epirubicin hydrochloride, doxorubicin hydrochloride and idarubicin hydrochloride in plasma and urine samples collected from the patients treated in the Department of Pediatrics, Hematology and Oncology, University Clinical Center in Gdansk.

Material and methods: Based on the optimized analytical methods the profile of doxorubicin, epirubicin and idarubicin was determine in plasma and urine samples of 15-years old patient with Hodgkin's Lymphoma, who undergoing the oncological therapy conducted according to the EuroNet-PHL-C1 protocol for childhood HL, 17-years old patient with RMS of the head and neck area, treated according to the CWS Guidance protocol and 19-years old patient with metastatic alveolar rhabdomyosarcoma (RMA), respectively. Doxorubicin was administered in the form of a 4-h and 3-h intravenous infusion at a dose of 40 and 20 mg/m^2, respectively. The samples of plasma and urine for epirubicin profiles were collected from the end of 6-h intravenous, in turn idarubicin received orally at a dose of 10 mg once a day.

Results: The obtained results showed that the maximum concentration of doxorubicin in plasma and urine occurs already 4 hours after the end of the infusion, for epirubicin the highest concentration in the tested biological fluids was achieved immediately after the end of the infusion, while for idarubicin the highest concentration in plasma was achieved after 4 hours, and in urine 20 h after oral administration.

Conclusion: The obtained results indicate that urinary anthracycline levels were significantly higher than those calculated in the plasma samples. It can suggest that exposure of hospital personnel to the anthracycline in urine samples occurs long after the end of their received by patients.

Acknowledgements: The study was supported by the project, POWR.03.02.00-00-I014/17-00, which was co-financed by the European Union through the European Social Fund under the Operational Programme Knowledge Education Development 2014–2020.

S. 14-5

Comparison of social behaviour and ultrasonic vocalisation in male and female rats with life-long genetic depletion of brain serotonin

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Background: Tryptophan hydroxylase 2 (TPH2) is a rate-limiting enzyme of brain serotonin synthesis. Therefore, TPH2 - deficient (Tph2-KO) rats represent a valuable model to study the consequences of central serotonin depletion. The Tph2-KO animals are completely lacking serotonin in the brain from birth throughout the whole life. Brain serotonin manipulations are thought to be associated with central nervous system processes, including social behavior, aggression and communication.

The goal of the current study was to investigate genotype and sex differences in social behaviour and communication. Therefore, Tph2-KO and Tph2-WT male and female rats were examined in a social interaction test (SIT).

Material and methods: Two unfamiliar rats of matched genotype, sex and body weight (±5 g) were placed in the open field arena, and their social behaviour and ultrasonic vocalisation were recorded. The following active social behaviours were scored: sniffing, anogenital sniffing, social grooming, following behaviour, climbing, as well as sexual activity and fighting with the conspecific. Additionally, ultrasonic vocalisations (USVs) were measured and classified into several categories. In adult laboratory rats, two main types ofUSVs have been described: the low (22-kHz) and high (50-kHz) frequency calls. The low, termed an “alarm” vocalization, has been associated with negative social experiences. The high may be detected in appetitive contexts, including social interactions.

Results: We report that both male and female TPH2-KO rats demonstrated disturbed patterns of social behaviour and communication. Male but not female TPH2-KO rats, manifested fighting episodes and copulatory-like behaviour directed toward their male partners. Moreover, male TPH2-KO rats emitted significantly more 50-kHz USVs, characterised by wider bandwidth compared to controls. In contrast, female TPH2-KO rats spent significantly less time on social interactions and demonstrated much less 50-kHz USVs as compared to the WT control group.

Conclusions: The present study confirms the role of central serotonin in the regulation of social behaviour and points to increased aggression due to life-long serotonin depletion.

Acknowledgments: This study was supported by the grant ERA-NET Neuron II JTC 2015 Respond and by the Statutory Activity of the Maj Institute of Pharmacology, Polish Academy of Sciences, Krakow, Poland.

S. 14-6

Characteristics of CD4+ T cell *N*-glycans in Hashimoto's thyroiditis

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Background: Hashimoto's thyroiditis (HT) is an autoimmune disease characterized by chronic inflammation of the thyroid gland. This disease causes the loss of immune tolerance to the thyroid's antigens, which results in the destruction of thyrocytes by activated T cells and hypothyroidism. The most diverse group of T cells expressing CD4 co-receptor are T helper cells (Th), also referred to CD4+ T lymphocytes. Most of T cell receptors undergo *N*-glycosylation, post-translational modification of proteins consisting in the enzymatic attachment of sugar residues. *N*-glycosylation was found to be altered in inflammation and autoimmunity. **The aim of the research:** is was to analyze *N*-glycosylation of CD4+ T cells in HT in comparison to healthy donors.

Material and methods: The blood samples collected from patients with HT before treatment (HT1; n=14), and after initiation of L-thyroxine (HT2; n=26) and from healthy subjects (CTR; n=18) were used in this study. CD4+ T cells were isolated from peripheral blood mononuclear cells (PBMCs) using an automatic magnetic sorter (autoMACS Pro Separator). *N*-glycans were released from CD4+ proteins by *N*-glycosidase F digestion and analyzed by normal-phase high-performance liquid chromatography (NP-HPLC) after fluorescent labeling. NP-HPLC chromatograms were integrated into 30 *N*-glycan peaks (GP). The annotation of *N*-glycan peaks was performed based on the glucose unit values compared to GlycoStore database. The Kruskal-Wallis test was performed to assess the statistical significance of the obtained results ($p<0,05$).

Results: The results showed the statistically significant differences in *N*-glycan content on CD4+ cells between HT and control samples. The amount of the diantennary complex-type structures, most with core fucose and some of them with bisecting *N*-acetylglucosamine was decreased in HT1 group (GPs: 8, 10, 11, 12, 14, 17, and 19) and HT2 donors (GP 11). The level of sialylated diantennary complex-type *N*-glycan (GP 22) was found to be elevated in HT1 group in comparison to healthy control CD4+ cells.

Conclusions: The detected changes in *N*-glycosylation of T lymphocytes may be important to chronic inflammation mediated by these cells in HT. Further analyzes are needed to confirm this hypothesis.

Acknowledgments This study was supported by the National Science Center (grant No. UMO-2015/18/E/NZ6/00602).

S. 14-7

Tolerance development and neurotoxicity induction after repeated treatment with the hallucinogenic compound 25I-NBOMe

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Background: NBOMes are highly toxic serotonin 5-HT2A receptor agonists. 4-Iodo-2,5-dimethoxy-N-(2-methoxybenzyl)phenethylamine (25I-NBOMe) is one over three most popular representatives of NBOMe family. Our earlier studies on animal model confirmed the strong contribution of 5-HT2A receptor in the effect of 25I-NBOMe on neurotransmission and rat's behavior. In this study we aimed to investigated the effect of 25I-NBOMe-repeated doses on neurotransmission as well as on neurotoxicity induction in the rat brain.

Material and methods: Wistar Han rats received seven subcutaneous injections of 0.3 mg/kg 25I-NBOMe, once daily. The neurotransmitter release was measured using microdialysis in freely moving animals and the hallucinogenic activity was assessed in the wet dog shake test. We used comet and TUNEL assays to investigate DNA damage and apoptosis, respectively. The distribution of the drug was studied using electrospray ion trap mass spectrometry. Moreover, immunohistochemical assessment of the cells number was performed.

Results: Repeated administration of 25I-NBOMe decreased the cortical release of neurotransmitters in response to a challenge dose and reduced hallucinogenic activity. Toxicity assays indicated DNA damage but not apoptotic signal after acute and chronic administration. A high concentration of 25I-NBOMe was detected in several brain regions after chronic treatment and changes in the number of cells were observed.

Conclusions: The attenuated release of neurotransmitters and reduced hallucinogenic activity indicated on tolerance development. 25I-NBOMe-caused DNA damage and alteration in cells number may result from the induction of oxidative stress. However, absence of apoptosis signal suggests lack of permanent cell death. 25I-NBOMe accumulation in the brain tissue after chronic administration may lead to metabolism impairments of this drug.

Acknowledgements: This study was supported by the National Science Centre, Kraków, Poland (grant no. 2016/21/B/NZ7/01131) and statutory fund of the Maj Institute of Pharmacology Polish Academy of Sciences. MH acknowledges the support of InterDokMed project no. POWR.03.02.00-00-I013/16.

S. 14-8

Molecular patterns of XCL1 and its receptors after central and peripheral nervous system damages

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Background: We compared how direct and indirect damage influence on immunological changes at the level of the central nervous system. Traumatic brain injury affects cells death and is causing secondary inflammatory cascade. Similarly, peripheral nerves injury leads to alterations of immune factors at the spinal cord level. Recently literature suggested, that inflammatory reactions initiated and regulated by chemokines play a crucial role in central nerve system disorders. The goal was to study changes in the XCL1/XCR1 and XCL1/ITGA9 axes in the brain and the spinal cord after central or peripheral nervous system damages, respectively.

Materials and methods: We compared the pattern of expression of XCL1, XCR1, ITGA9 at different time points (24 hours - 5 weeks), in mouse model of traumatic brain injury (TBI) caused by controlled cortical impact (using a pneumatically driven piston to induce well-controlled injury) in the cortex and after peripheral nerve damage (caused by chronic constriction injury of the sciatic nerve) in the spinal cord. Changes in mRNA level were assessed by RT-qPCR and changes in protein level by ELISA/ Western Blot.

Results: After TBI, we observed strong up-regulation of mRNA expression of both *XCL1* and *XCR1* at all tested time points. The strongest expression of the ligand was observed 2 weeks after the injury, while that of the *XCR1* receptor, at the 4th day. The up-regulation of *ITGA9* was also significant, especially on the 4th day following injury. In the spinal cord, after CCI, we have also observed strong up-regulation of *XCL1* at all tested time points, but no changes were observed in the subjected receptors. The strongest expression of the *XCL1* was observed 4 days after nerve injury. We also observed changes of the XCL1 and its receptors protein levels in selected time points following TBI and/or CCI.

Conclusions: We reported a concomitant and time-dependent expression of XCL1 as a consequence of the central and peripheral nervous system damages. Noticeable differences may be correlated with different populations of cells participating in these injuries and the direct or indirect violation of the CNS. The XCL1 can be considered as one of the triggers responsible for neuroimmunological disturbances, therefore XCR1 and ITGA9 may be important targets for pharmacological intervention after CNS and/or PNS injury.

Acknowledgments: Supported by the NCBiR grant ERA-NET NEURONCOFUND/1/LEAP/15/17 and NCN grant OPUS11 2016/21/B/N24/00128

S. 14-9

The effects of ACEA 1021, NMDA receptor antagonist, on neurotoxicity of dexamethasone – the preliminary histopathological and immunohistochemical study

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It has been noted that chronic exposure to elevated levels of glucocorticoids (GCs) results in neurodegenerative changes in the central nervous system, particularly in the limbic system and hippocampus, structures regulating behaviour, memory and other cognitive functions. Those changes include hippocampal subfield CA1 and CA3, prefrontal cortex and striatum volume reduction, dendritic atrophy and decreased neurogenesis. GCs potentiate stress or ischemia-induced accumulation of excitatory amino acids (EAA) in the extracellular space of hippocampus and engender hippocampal neurons damage by facilitating the glutamate/Ca^{2+} cascade. ACEA 1021 (licostinel), a selective antagonist glycine site associated with the NMDA receptor complex, has been reported to prevent the excitotoxic action of high extracellular glutamate levels. The aim of the histopathological and immunohistochemical study in the CA3 subfield of hippocampus was to evaluate the neuroprotective effect of ACEA 1021. It was performed in the animal model of neurotoxicity induced by administration of dexamethasone (DEX) to Albino mice at the dose of 16 mg/kg/day for 14 days.

ACEA 1021, at the doses: 1.25, 2.5, 5.0 mg/kg/day, ip, was administered 15 min before DEX each day. 48 h after the last injection of drugs mice were anesthetised and the brain of mice were subjected to histopathological examination by hematoxylin-eosin staining. Intracellular oedema with formation of vacuoles and shrinking of pericarions in the CA3 subfield of hippocampus, indicative of neuronal dysfunction, was assessed using light microscope. Moreover, immunohistochemical staining using Proliferating Cell Nuclear Factor (PCNA) antibody was also performer. The ratio of immunopositive cells to the total number of cells in the hippocampal CA3 subfield was determined.

Administration of DEX at the dose of 16 mg/kg/day for 14 days showed significant increase in damaged pyramidal neurons and decrease of the proliferative activity in the CA3 subfield of the hippocampus. The number of damaged neurons in this region of hippocampus was reduced in the group receiving ACEA 1021 at the dose of 5 mg/kg/day together with DEX. The immunohistochemical study showed increase in the proliferative activity in this group of mice.

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

Supported by the grand from Medical University of Lublin, No DS 449.

S. 14-10

Mesenchymal stem cells and extracellular vesicles cultured on graphene-based substrates diminish osteoarthritis-related pain in a rat model

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Background: Osteoarthritis (OA) is a joint disease leading to chronic pain and movement limitations. Current therapeutic possibilities are limited to symptomatic control, therefore novel methods of treatment are greatly required. Mesenchymal stem cells (MSCs) and their bioactive derivatives such as extracellular vehicles (EVs) have been demonstrated to have regenerative potential for cartilage, which may attenuate OA development in patients. In the present study we investigate the efficacy of MSCs cultured on graphene (GO)-based composites and MSC-derived EVs in treating OA symptoms.

Materials and methods: OA was induced in rats by intra articular (i.a.) injection of 1 mg of sodium monoiodoacetate (MIA). After 14-days, 10^6 of human adipose tissue-derived MSCs (AT-MSCs) or EVs harvested from MSCs conditioned medium (AT-MSC-EVs) were administrated i.a. AT-MSCs were cultured on standard cell culture plates or surfaces coated with layer of GO-based substrates (native GO or Au-modified GO). Pain behaviour was measured every 7 days up to 42 days post-MIA injection, when animals were sacrificed and tissue was collected for further analysis.

Results: OA animals showed a gradual decrease in pain threshold, which was not observed in naïve rats. Native AT-MSCs and AT-MSC-EVs diminished MIA-induced allodynia at the end of the experiment, but failed to restore paws weight bearing difference during walking. AT-MSCs cultured on GO-based surfaces did not exhibit analgesic properties, however AT-MSC-EVs harvested from MSCs cultured on GO-based scaffold diminished allodynia, but had no effect on paws weight bearing disturbance. In turn, AT-MSC-EVs harvested from MSCs cultured on Au modified-GO surface diminished MIA-induced allodynia and tended to restore normal paws weight bearing pattern. Microtomographic and histological pictures showed less severe cartilage deficits in AT-MSCs/EVs-treated groups.

Conclusions: The innovative feature of our research is the use of novel biocompatible GO-based materials to improve MSCs and EVs efficacy in cartilage regeneration in OA. Our data indicate that AT-MSCs and AT-MSC-EVs possess analgesic and anti-degenerative properties and might be a promising tool for OA treatment.

Acknowledgements: Research funded by the National Center for Research and Development, Poland (BioMiStem grant no. STRATEGMED3/303570/7/NCBR/2017) and Maj Institute of Pharmacology, Polish Academy of Sciences statutory funds.

Proteomic analysis of undifferentiated and retinoic acid-differentiated human neuroblastoma SH-SY5Y cells – implications for neuroprotection studies

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Background: Human neuroblastoma SH-SY5Y cell line is a commonly accepted cellular model of Parkinson's disease and widely used as a first-line screening platform for neuroprotective drugs. Since there is still ongoing discussion whether undifferentiated or neuronally-differentiated SH-SY5Y cells are better for studies on neurodegeneration/neuroprotection, we performed proteomic analysis of both cell phenotypes.

Material and methods: Undifferentiated (UN) and 7-day retinoic acid (RA)-differentiated SH-SY5Y cells were subjected to proteomic analysis by nano-LC/MALDI-TOF/TOF methods (Bruker Daltonics). Data were analyzed by FlexAnalysis software (Bruker Daltonics) and further processed using Mascot algorithm (Matrix Science) against the Swiss-Prot database.

Results: 511 proteins were identified with Mascot score higher than 30, of which 339 were common for both analytical groups, while 172 proteins showed different abundance in both cell phenotypes. 83 and 89 proteins were characteristic for undifferentiated and differentiated cells, respectively. Based on Panther Go-Slim Molecular Function analysis, 67 of the regulated proteins belong to the binding category (the percent of gene hits against total number of genes equals 39.6%) and 44 proteins belong to the catalytic activity category (26.0%), the other categories contain less than 10 proteins each. Additional ontology Pathway analysis of cell phenotype-regulated proteins revealed the substantial changes in categories directly or indirectly associated with neurodegenerative processes, such as: inflammation mediated by chemokine and cytokine; integrin signaling; Wnt signaling; cytoskeletal regulation by Rho GTPase; ubiquitin-proteasome pathway; Parkinson disease and Huntington disease.

Conclusion: The optimal choice of neuronal-like cell model for studies on neurodegeneration/neuroprotection should also be based on the proteomic data.

Acknowledgements: The study was supported by statutory funds of the Maj Institute of Pharmacology of the Polish Academy of Sciences.

Effect of airborne particulate matter (PM) from Kraków on the course of EAE in mice and thymic and peripheral lymphocyte subpopulations, Th17 and Treg

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Background: Exposure to airborne particulate matter (PM) is believed to adversely affect the incidence and course of autoimmune diseases, including multiple sclerosis (MS). The Kraków city is one of the areas with the highest air pollution by PM in Poland. The aim of the study was to evaluate the effect of inhalation exposure to PM collected in Kraków (PM Krk) on the course of the MS model, autoimmune encephalomyelitis (EAE) in mice, and on the subpopulations of T lymphocytes - Th17 and Treg - in the thymus and lymph nodes.

Material and methods: Mice were exposed to PM Krk (1 g/m^3) or to distilled water for 6 hours daily, 6 days a week for the entire duration of the experiment. PM Krk was collected in Krakow and provided by the Faculty of Chemistry of the Jagiellonian University. On day 10th of PM exposure the EAE was induced with the MOG_{35-55} peptide. Mice were sacrificed at the peak of symptoms' intensity. Th17 and Treg lymphocytes were determined among thymocytes and lymph node cells with flow cytometry.

Results: The applied protocol has generated EAE of a very mild intensity only in a part of the animals (60 and 67%), and exposure to PM Krk had no effect on the incidence and course of the disease. PM Krk in control mice lowered the percentage of both Th17 and Treg lymphocytes in the thymus and increased the intracellular expression of IL-17 without affecting these subpopulations in lymph nodes. EAE lowered the percentage of Treg cells in the lymph nodes. Exposure of EAE mice to PM Krk increased only the expression of Treg subpopulation marker, Foxp3, in the thymus.

Conclusions: The exposure to PM Krk did not affect the course of EAE. However, it caused some changes in the differentiation of lymphocytes in the thymus, which could indicate an immunosuppressive effect of PM Krk, possibly related to its specific chemical composition.

Acknowledgements: The study was supported by the APARIC grant No.2015/16/W/ST5/00005 funded by National Science Centre, Poland, and statutory funds of Maj Institute of Pharmacology, Polish Academy of Sciences, Kraków, Poland.

MicroRNAs as regulators of signaling pathways in the brain of three strains of mice displaying different sensitivity to restraint stress

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Background: Our previous studies have shown that three strains of mice, i.e. norepinephrine transporter deficient (NET-KO), SWR/J and C57BL/6J (used as WT) display different behavioral response to restraint stress (RS): NET-KO and SWR/J are regarded as resilient while C57BL/6J – susceptible to RS, as measured by their response in the forced swim test. We also characterized serum microRNAs (miRNAs) as molecular markers of this response.

In our present study we show that certain miRNAs are altered upon RS in various regions of the mouse brain, and these alterations might be related with the strain-specific response to RS.

Materials and Methods: The brain regions (prefrontal cortex; PFCx and ventral tegmental area; VTA) were obtained from experimental animals (males, ca. 4.5 m old) under control conditions as well as immediately after RS. The miRNAs were purified, and Custom TaqMan Array MicroRNA Cards (with 192 miRNAs) were run. The results were analyzed by QbasePlus Software (Biogazelle), followed by two-way ANOVA, and DIANA miRpath v.3.0 was used for miRNA pathway analysis. Links between miRNAs and their targets were also determined.

Results: The groups of miRNAs detected in the PFCx and VTA and differentiating reaction of NET-KO and SWR/J mice form WT to RS were found to regulate steroid biosynthesis, and the following signaling pathways: MAPK, FoxO, AMPK, as well as mTOR signaling pathway. The mRNAs regulated by more than one miRNA and connected with the most of pathways were further analyzed.

Conclusion: Bioinformatics analyses of miRNAs which were altered significantly by RS in PFCx and VTA of three strains of mice indicate pathways which might be important for stress response. However, the type of regulation depends on reaction to stress – susceptibility or resilience.

Acknowledgement: This work was supported by the National Science Centre Poland (grant number 2016/23/B/NZ4/01086).

The impact of MK-801 and 1MeTIQ on the activity of antioxidant enzymes in the rat's hippocampus

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Background: NMDA antagonists such as MK-801 are used as animal model of schizophrenia. Induction of schizophrenia results in a significant oxidative stress by increasing the levels of antioxidant enzymes. Because 1-methyl-1,2,3,4-tetrahydroisoquinoline (1MeTIQ) is an endogenous amine with neuroprotective and antipsychotic properties, in this study we tested its effect on the activity of antioxidant enzymes: catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx) and glutathione reductase (GR) disturbed by MK-801 injection.

Methods: Male Sprague Dawley rats received chronic injections (by 7 consequtives days) of MK-801 (0.1 mg/kg, *i.p.)* or 1MeTIQ (25 or 50 mg/kg, *i.p.*). The combined groups received MK- 801 20 minutes after 1MeTIQ administration. The control group received saline injections. 2 hours after last dose of 1MeTIQ rat's hippocampus were dissected and the activity of CAT, SOD, GPx and GR were measured by ELISA kits.

Results: Received data shows that chronic MK-801 administration produced increase the activity of SOD, GPx and GR. Similar effect was observed after chronic treatment with 1MeTIQ in a dose 50 mg/kg. Interestingly, combined treatment with MK-801 and 1MeTIQ completely reversed this effect.

Conclusion: Obtained results indicated that MK-801 administration induces oxidative stress by increasing the activity of antioxidant enzymes. 1MeTIQ antagonizes the biochemical effects induced by MK-801.

Acknowledgements: This study was financially supported through a grant from the National Science Centre Grant No. 2017/25/B/NZ7/01096.

Changes in monoaminergic neurotransmission in the striatum of a rat model of pain after administration of 1MeTIQ measured in microdialysis study

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Background: Osteoarthritis (OA) is the most common joint disease, affecting millions of people worldwide. A symptoms usually develop slowly and worsen over time, with characteristics that involve progressive degradation of articular cartilage and resulting chronic pain. The population of people suffering from OA is characterized by a frequent coexistence of mental disorders. The monoiodoacetate (MIA) model has become a standard for animal modelling symptoms of OA. 1MeTIQ (1-methyl-1,2,3,4-tetrahydroisoquinoline) is a neuroprotective compound synthesized in the mammalian brain. The aim of the study was to investigate striatal neurotransmission in an osteoarthritis pain model and to evaluate the effect of 1MeTIQ on this neurotransmission.

Material and methods: Male Wistar rats received a single injection of MIA into the knee joint to induce pain-like responses and progressive degeneration of the joint. 1MeTIQ at a dose of 50 mg (per kilogram body weight) was administered intraperitoneally at three time points - the day before, 1 and 14 days after the administration of MIA. Striatal microdialysis was performed 28 days after MIA administration. Biochemical analysis with HPLC methodology was carried out to evaluate monoamines levels in rats striatum microdialysates after this procedures.

Results: Result obtained from experiment showed that DA levels were similar in all groups. In turn, the levels of its metabolites DOPAC and HVA were elevated in the pain model group. This effect was reversed by the administration of 1MeTIQ. Moreover, in the combined group (MIA with 1MeTIQ) the highest decrease in levels of 5HT and its metabolite 5-HIAA was recorded. The elevated levels of NA and its metabolite NMN were observed in the pain model group, this effect also was reversed after receiving 1MeTIQ doses.

Conclusions: The study showed that elevation of DOPAC, HVA, NA and NMN levels was characteristic in the MIA pain model group, these effects were reversed by administration of 1MeTIQ. An increase in the HVA level and a decrease in the level of 3MT suggest an increased activity of the MAO enzyme (monoamine oxidase) in pain model group. More preclinical studies are needed to investigate the association of pain with neurotransmission.

Acknowledgements: This study was financially supported through statutory funds from the Maj Institute of PAS.

PRO-RESOLVING FPR2 AGONISTS REGULATE NECROTIC AND APOPTOTIC CELL DEATH IN THE LPS STIMULATED PRIMARY MICROGLIA CULTURES: LINK TO NEUROPROTECTION

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Background: Many evidences suggest that Formyl-Peptide Receptor 2 (FPR2) play various functions in brain. Binding to FPRs agonists triggers intracellular cascades involved in the regulation of inflammatory response and neuroendocrine functions. Recent findings have pointed a central role of FPR2 also in the regulation of cell proliferation, apoptosis and necrotic damage. Therefore, here our study was to determine, whether specialized pro-resolving mediators (SPMs) such as lipoxin A4 (LXA4), its analog - aspirin triggered lipoxin (AT-LXA4) as well as new non-peptidic agonist with ureidopropanamide scaffold (MR-39) are able to modulate the death processes evoked by lipopolysaccharide (LPS) treatment in primary microglial cells.

Material and Methods: Primary microglia cultures were prepared from cortices of 1-2 days old Sprague-Dawley rats offspring. Microglia cells were pre-treated with WRW4 (FPR2 antagonist) and/or lipoxin A4 (LXA4), aspirin triggered lipoxin A4 (AT-LXA4) as well as compound MR-39. After 1 h of incubation the nonspecific immune system activator – lipopolysaccharide (LPS; 100 ng/ml) was added. Time-dependent (3-24h) cell death/viability was determined by lactate dehydrogenase release (LDH test), caspase-3 and mitochondrial potential JC-1 assay.

Results: Treatment of microglia cells with LXA4 and AT-LXA4 (0,01-0,1 mM) suppressed the LPS-evoked LDH release after 3 h of incubation, while protective effect of MR-39 (1-5 mM) was demonstrated after 24 h of incubation. Importantly, the neuroprotective effect of all FPR2 agonists was attenuated by FPR2 receptor antagonist (WRW4) pre-treatment.

In subsequent studies, the influence of FPR2 agonists on the potential of the mitochondrial membrane and the activity of caspase-3 was assessed. It was shown that the tested ligands significantly inhibited the apoptotic damage of microglial cells in the experimental model used. AT-LXA4 (0,1 mM) and MR-39 (1mM) attenuated the LPS-induced caspase-3 activity, while MR-39 (1 mM) inhibited also the LPS-induced decrease in the mitochondrial membrane potential (JC-1).

Conclusions: Our novel discoveries showed the usefulness of the new ureidopropanamide formyl-peptide receptors agonist in the neuroprotection and demonstrated FPR2 receptor in microglia cells as an attractive molecular target for the development of pro-resolving strategies.

Acknowledgements: Supported by the grant no. 2017/26/M/NZ7/01048, National Science Centre, Poland.

Candidate Alzheimer's disease biomarker miR-483-5p lowers TAU phosphorylation

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Background: MicroRNAs have been demonstrated as key regulators of gene expression in the aetiology of a range of diseases including Alzheimer's disease (AD). Recently, we identified miR-483-5p as the most upregulated miRNA amongst a panel of miRNAs in blood plasma specific to prodromal, early-stage Alzheimer's disease patients. Here we investigated the functions of miR-483-5p in AD pathology.

Materials and Methods: The interaction between miR-483-5p and its predicted target mRNAs was probed using a luciferase assay, with outputs measured by RT-qPCR or immunoblotting techniques. Knockout of miR-483 was accomplished using the CRISPR/Cas9 system. We demonstrated the effects of miR-483-5p on protein expression and phosphorylation using SDS-PAGE, immunoblotting and densitometry.

Results: Using TargetScan and miRTarBase we identified 3 candidate targets of miR-483-5p: mRNA for TAU, one of the key proteins in AD, and for ERK1/ERK2, two kinases known to mediate toxic phosphorylation of TAU. We showed that TAU mRNA is not a direct target of miR4835p, however, ERK1 and ERK2 showed reductions in both mRNA and protein levels in cells transfected withmiR-483-5p. Consistently, Knockout of miR-483 with CRISPR/Cas9 resulted in an increase in ERK1 mRNA. The decrease in ERK1 mRNA translated to a reduction in ERK1 and pERK1 proteins and a resultant fall in phosphorylation of TAU and pTAU. The same pattern was then emulated in the neuronal SK-N-MC cells. We used U0126 as a known inhibitor of MEK1/2 (of which ERK1 is a substrate) as a positive control, demonstrating that reducing ERK1 activity outside the miR-483-5p system also impacts TAU and pTAU levels.

Conclusions: We describe here the functional activity of miR-483-5p in the context of TAU pathology, a constituent part of the AD aetiology. This validates our earlier finding of this molecule in the plasma of prodromal AD patients, showcasing the viability of targeting the miR-483-5p pathway in potential future AD therapies.

Acknowledgements: This work has been supported by the Polish National Science Centre grant OPUS 2018/29/B/NZ7/02757, by the EU Horizon2020 FETOPEN grant, agreement no 737390 (ArrestAD), and by the funding from the Polish Ministry of Science and Higher Education within 2016-2020 funds for the implementation of international projects (agreement no 3548/H2020/COFUND/2016/2).

The effect of chronic treatment with MK-801 and 1-methyl-1,2,3,4-tetrahydroisoquinoline on activity of antioxidative enzymes in rats' frontal cortex

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Background: Oxidative stress (OS) generates reactive oxygen species (ROS) what cause a damage to lipids, proteins and nucleic acids in many tissues, including the brain. OS and is highly neurotoxic and may result in cellular dysfunction leading to death of a cell. The physiological first line of defense against ROS are antioxidative enzymes: glutathione peroxidase (GPx), glutathione reductase (GR), catalase (CAT) and superoxide dismutase (SOD). Schizophrenia is a severe mental illness, which may be modeled in animals by administration MK-801, an NMDA receptor antagonist. Bulk of evidence suggest, that pathophysiology of schizophrenia is related to interconnection of NMDA receptor hypofunction and OS. Understanding the mechanism of this association may result in developing new promising therapies. 1-methyl-1,2,3,4-tetrahydroisoquinoline (1MeTIQ) is an endogenous compound, found in mammalian brain, with neuroprotective, pro-cognitive and anxiolytic properties. The aim of the present study was to investigate the impact of 1MeTIQ on the activity of antioxidative enzymes in rats' frontal cortex after treatment with MK-801.

Material and methods: Male Sprague Dawley rats were injected for 7 consecutive days with saline, MK-801 (0.1 mg/kg), 1MeTIQ (25 or 50mg/kg) or MK-801 combined with both doses of 1MeTIQ (6 treatment groups). 2 hours after last injection, rats were decapitated and FCX was dissected for analysis of enzymes' activity (GPx, GR, CAT and SOD) with use of applicable assay kits.

Results: In MK-801-treated animals higher activity of all antioxidative enzymes was found. 1MeTIQ (25 mg) given alone did not affect enzymes' activity. 1MeTIQ (50 mg) given alone caused increased activity ($p<0.05$) of GPx and SOD. 1MeTIQ (25mg) reversed the effect of MK-801 and decreased activity of GR ($p<0.05$) and CAT ($p<0.01$). 1MeTIQ (50 mg) lowered GR ($p<0.01$), CAT ($p<0.01$) and SOD ($p<0.05$) activity and reversed MK-801 action.

Conclusions: Higher activity of antioxidative enzymes suggest enhanced oxidative stress in frontal cortex after MK-801 administration. 1MeTIQ, especially in dose of 50 mg, reverses the effect of MK-801 and restores enzymatic activity. This results suggest that pharmacological model of schizophrenia leads to enhanced oxidative stress and 1MeTIQ play an antioxidative action in model of the illness.

Acknowledgements: The study was financed from NSC Grant No. 2017/25/B/NZ7/01096.

Priming in response to pro-inflammatory stimulation in healthy and osteoarthritic synoviocytes in an in vitro model

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Background: Osteoarthritis (OA) is a disease of the musculoskeletal system. It manifests itself with the gradual joint damage and to the deterioration of the OA patient's mobility, but the direct cause of OA development is still unknown. Nowadays, OA is seen by clinicists as disorder demonstrating complex and multi-tissue pathologies involving cartilage, subchondral bone and increasingly recognized inflammation of the synovium. The soft tissue lining the spaces of joints consists mainly of fibroblast-like synoviocytes (FLS), which release hyaluronic acid and lubricin to synovial fluid, but also secretes molecules promoting the development of inflammation, such as chemo- and cytokines.

Material and methods: The present study investigates the response to pro-inflammatory stimulation of FLS isolated from OA (FLS-OA) and control patients in *in vitro* conditions. This approach aims to determine the cellular responses to synovium inflammation which is observed in OA development and can help to propose new potential therapeutic strategies. For this purpose, we stimulated cells with LPS and IFNγ then we used RNAseq method for analysis of cells transcriptome and ELISA to check changes in the level of proteins secretion to the medium.

Results: Transcriptome of FLS-OA and control cells showed significant differences in response to pro-inflammatory stimulation with both compounds. Genes grouped with gene ontology tool were mainly responsible for inflammatory response, apoptosis and homeostasis. We observed that expression of genes coding pro-inflammatory proteins was higher in FLS-OA. Proteins secreted into the medium highly related to OA (such as chemokines or cathepsins) were verified and changes in their expression between control and FLS-OA were confirmed with ELISA.

Conclusions: Our study showed that FLS-OA are capable of more potent reaction on inflammation then control cells. FLS-OA may not only attract immune cells more effectively causing a vicious circle, but also increase cartilage degeneration by upregulation of proteases activity. Understanding the mechanism(s) of the hyperactivity of these cells could help the better understanding of the role of synoviocytes in OA progression.

Acknowledgements: Supported by the National Science Centre, Poland, grant OPUS no. UMO-2016/23/B/NZ7/01143, 2017-2020.

Fisetin, a plant-derived flavonoid as a new promising tool in neuropathic pain management – evidences from behavioral studies in mice

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Background: Neuropathic pain impairs daily activities, quality of life and social functioning of patients all over the world and its conventional management results in poor clinical outcomes. Analgesics that are used to treat this pathology produce remarkable side effects, and, moreover, the great majority of people obtain only partial relief. Therefore, new therapeutic approach is necessary. Fisetin, a plant-derived flavonoid, displays a variety of pharmacological activities, including antioxidant, anti-allergic, anti-depressive and neuroprotective properties, however, its usefulness in relieving neuropathic pain needs to be explored. Mechanisms underlying the actions of this interesting substance involve modulation of intracellular pathways such as PI3K/AKT/mTOR, ERK and MAPK as well as inhibition of several pro-inflammatory cytokines. The following study was aimed to evaluate how fisetin influences pain-related behavior in mice model of neuropathic pain.

Materials and methods: According to Bennet and Xie, mice were exposed to chronic constriction injury of the sciatic nerve (CCI model). On the day 7 after surgery, single intraperitoneal and intrathecal administrations of fisetin were performed. To assess mechanical and thermal hypersensitivity, the von Frey and cold plate test were used, respectively. Additionally, the intrathecal co-administration of fisetin with morphine and buprenorphine were conducted.

Results: Fisetin dose-dependently attenuated mechanical hypersensitivity after both, intraperitoneal and intrathecal injections on the day 7 in neuropathic mice. Thermal hypersensitivity was also significantly reduced after intrathecal administration. Moreover, single intrathecal administration of fisetin enhanced morphine-induced analgesia.

Conclusions: The present study extends the knowledge of the effects of fisetin on neuropathy, although further studies should be undertaken to fully elucidate the precise mechanisms for fisetin actions and its influence on other opioids. Obtained results emphasise that flavonoids, which display wide spectre of biological activities, might be promising tool for polytheraphy of neuropathic pain.

Acknowledgments: Supported by National Science Centre grant OPUS11 2016/21/B/N24/00128 and statutory founds of the Maj Institute of Pharmacology, Polish Academy of Sciences.

Methodical analysis of Morris water maze task: impact of strain and tool compounds on variety of parameters measured with ANY-maze

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Background: Morris water maze (MWM) is widely used to investigate spatial learning and memory in rodents, primarily rats and mice. It is used to measure the effects of neurocognitive disorders on spatial learning, potential treatments or to study how age influences cognitive function and spatial learning.

Over the years, different versions of this test have been performed with large amount of variables (e.g. number of trials, training duration, etc.). The results vary significantly, so the variables should be kept constant in the laboratory to draw correct conclusions.

At present C57BL/6J mice are used in the majority of MWM studies due to consistent results and low count of non-performers among inbred strains. Therefore there is a discussion if learning and memory abilities of CD-1 mice (outbred strain), which are commonly used across different laboratories, are comparable with C57BL/6J mice.

Material and methods: Present studies were undertaken to investigate similarities and differences between C57BL/6J and CD-1 mice in MWM. Tool compounds, MK-801 (0.3 mg/kg) and scopolamine (1 mg/kg), were administered 30 min before the first trial to establish their potency to impair learning and memory of mice. Additionally, memantine or rivastigmine were administered 30 min before tool compounds in order to reverse induced deficits. Mice were trained for 5 subsequent days (4 trials/day) and tested on 6th day. The animals were video-recorded and with the use of ANY-maze program several variables were measured, including parameters regarding animal's movement in relation to the NE zone or platform zone.

Results: Both C57BL/6J and CD-1 animals memorized platform's localization. 8 (of 15) parameters concerning NE zone, and 7 (of 9) concerning platform zone differed significantly between the two strains ($p<0.05$), revealing slightly better performance of the C57BL/6J strain in MWM. Administration of MK-801 or scopolamine significantly disrupted mice's performance in all measured parameters, which were partially reversed by chosen compounds.

Conclusions: The results obtained for both strains were comparable i.e. both strains learned to find the platform with equal effectiveness and the tool compounds impaired their learning abilities in a similar manner. However, MK-801 was much potent amnestic comparing to scopolamine.

Acknowledgements: This study was supported by Statutory Funds from the Maj Institute of Pharmacology, Polish Academy of Sciences.

The problem of evaluating ligand as a drug candidate in the process of drug design – analysis with the use of big data

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Background: Drug design is a complex process that is still burdened with high risk and uncertainty. Generally, drug design can be interpreted as a mapping within two groups of variables representing chemical compounds, the first group of which are molecular descriptors related to the molecular representation of a chemical compound. The second group of variables is related to the properties of a chemical compound. Descriptors and properties are two types of chemical compound representation. While the descriptors' formalism has been exhaustively described, the typology of properties has been dealt with much less. The research dealt with the problem of ligand evaluation as a potential drug, in particular the analysis of the ligand efficiency estimator as one of the types of representations of substance properties used in drug design.

Material and methods: The scope of the research included in silico analysis of the large molecular databases of ChEMBL (www.ebi.ac.uk) and PubChem (pubchem.ncbi.nlm.nih.gov). As part of the research, a database of smaller literature data was also created, which was subjected to comparative analyzes: Binding Database, Psychoactive Drug Screening Program PDSP and data from Mortenson 2018, Johnson 2016, Johnson 2019, Hopkins 2014, Schultes 2010 and Gharagheizi 2013.

Results: The biological activities expressed in the pAC50 scale after transformation to LE analyzed as a function of 1 / HAC in the domains of the largest available data always show the typical hyperbolic effect. Since LE is an interaction of pAC50 and factor 1 / HAC, the presence of a hyperbolic effect proves that the value of LE is largely determined by 1 / HAC, which is represented by the hyperbola as a function of HAC. The conducted analyzes allow to describe the full systematics of properties as a representation of a chemical compound, which can be related to a molar scale, weight scale or the scale of a single molecule. It has also been shown that the so-called binning, in particular high resolution binning, can be an effective method of searching for structure-activity relationships within big data.

Conclusions: The problems analyzed are particularly important due to the growing interest in fragment-based molecular design, single molecule biophysics and single molecule biology, which are currently under development and will need to be correctly interpreted in chemoinformatics and QSAR modeling.

Acknowledgements: This work was supported by the University of Silesia in Katowice.

Impact of two-week cannabidiol administration on the n-6/n-3 PUFA ratio in the white skeletal muscle in a rat model of high fat diet-induced obesity

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Background: Nowadays, a global obesity epidemic continues to grow worldwide. This complex disorder results in excessive accumulation of lipids in several tissues, such as skeletal muscle, leading to the development of oxidative stress and inflammation, which are the background of insulin resistance (IR). The latest data indicate that cannabidiol (CBD), a component of medical marijuana (*Cannabis*), may be a potentially useful factor in the treatment of obesity. Therefore, our study aimed to investigate the influence of two-week CBD administration on the n-6:n-3 polyunsaturated fatty acids (PUFAs) ratio in different lipid fractions (FFA, DAG, TAG and PL), oxidative stress parameters and inflammatory pathway in the white gastrocnemius muscle in a rat model of high fat diet-induced obesity.

Materials and methods: All designed experiments were performed on Wistar rats fed high-fat diet (HFD) or standard rodent chow for 7 weeks, and subsequently injected with CBD (10 mg/kg for the last 2 weeks of a diet regime).Lipid content and oxidative stress parameters were assessed using gas-liquid chromatography (GLC), immunoenzymatic and/or colorimetric methods, respectively. The total expression of proteins was measured by Western blotting.

Results: Our results showed that, feeding rats a HFD for 7 weeks influences the fatty acids (FAs) composition in different lipid fractions, especially n-6 PUFAs, in the skeletal muscle tissue with predominant glycolytic metabolism. Concomitantly, we observed an increased oxidative stress parameters and local inflammation development.Importantly, we reported for the first time, that two-week CBD administration significantly improved the n-6:n-3 PUFA ratio, and shifted the equilibrium towards anti-inflammatory n-3 PUFAs (in the FFA, DAG and PL fractions) in rats fed HFD. The above changes were accompanied by a reduced lipid peroxidation products generation as well as attenuation of inflammatory response in the white gastrocnemius muscle after CBD treatment in obese rats.

Conclusions: The conducted research showed that CBD under the conditions of increased supply of FAs in the diet affects PUFA profile, thereby exhibiting a beneficial effects in the treatment of obesity and related disturbances.

Acknowledgements: Publication financed under the project No. POWR.03.02.00-00-I051/16 from European Union funds, PO WER 2014-2020 (Grant No. 01/IMSD/G/2019) and supported by the National Science Centre of Poland (Grant No. 2017/26/D/NZ3/01119).

Behavioural and molecular changes in poly (I:C) treated male rats

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Background: Autism spectrum disorder (ASD) is a common neurological disease with a higher prevalence in males than females (3:1 ratio). One of the most common hypotheses to explain ASD occurrence postulates that immune, environmental and/or genetic defects during neurodevelopment, may altere excitatory and inhibitory circuits. This excitation/inhibition imbalance can be caused by the impaired density of interneurons, reduced levels of parvalbumin (PV) or glutamic acid decarboxylase (GAD), compromised GABA-ergic neurotransmission and many more. There is strong evidence suggesting that maternal infection during pregnancy correlates with an increased risk of developing ASD in the child. One of the most frequently applied experimental MIA protocols is based on prenatal exposure of pregnant dams topolyinosinic:polycytidylic acid (poly (I:C)). Therefore, we used poly (I:C) model to asses brain levels of GAD and PV. Additionally, we measured repetitive patterns of behaviours that represent a key symptom of ASD.

Material and methods: Sprague-Dawley rat dams received a single intraperitoneal injection of poly (I:C) (5 mg/kg) or vehicle at gestational day 15. Cognitive flexibility of adult male offspring was then measured using the attentional set-shifting task (ASST). After the ASST procedure, locomotor activity and repetitive beahoviours were automatically scored using actometers. Additionally, levels of PV and GAD in the cerebellum, cortex, striatum and hippocampi were measured using an appropriate ELISA kit.

Results: We observed a higher number of repetitive behaviours and increased activity in poly (I:C) treated rats.

Rats with modelled ASD had also compromised cognitive flexibility reflected by impaired reversal learning. In addition, poly (I:C) rats have altered set formation. Moreover, autistic-like rats have diminished levels of PV and GAD in the cerebellum.

Conclusions: Here we were able to show that maternal infection during pregnancy may influence the levels of the inhibitory neurotransmitters, which may, in fact, result in destabilization of neuronal homeostasis and consequently in stereotyped patterns of behaviour and cognitive rigidity.

Acknowledgement: Supported by NSC grant: 2016/23/B/NZ7/01131.

Identification of molecular markers in animal model of treatment resistant depression

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Background: Wistar Kyoto rat strain (WKY) is a genetic model of drug resistant depression, often used in preclinical studies. Using a well-validated model of chronic mild stress (CMS), it has been demonstrated that WKY do not respond to antidepressant drugs (ADs) administration Comparison between rat Wistar Han and WKY strains allows to study possible biochemical or molecular differences in the rat brain, for example in habenular nuclei (Hb). Our previous studies indicate that Hb can play the potential role in the pharmacotherapy of depression. Since the role of micro RNAs (miRNAs) as biomarkers of response to pharmacotherapy is increasingly emphasized, in the present studies, we performed an analysis of miRNA expression in the rat Hb.

Materials and methods: Experiment was carried out on two strains of rats: Wistar Han and WKY. The brains were isolated from decapitated animals and rapidly frozen on dry ice. Brain sections (20 µm) containing the region of interest – habenula – were obtained using a Jung CM 3000 cryostat microtome. The slices were then attached on PEN-membrane 2,0 µm microscope slides and stained with Cresyl Violet from LCM Staining Kit according to the manufacturer's instructions, to expose the medial and lateral habenular nuclei, which were then obtained using Laser Capture Microdissection. miRNAs were isolated using miRNeasy Micro Kit according to the manufacturer's instructions. The expression of miRNAs was measured using TaqMan Array Rodent MicroRNA A + B Cards Set v3.0. The results were analyzed using the Biogazelle qbase program.

Results: Our preliminary study with the use of Custom TaqMan Array MicroRNA Card, containing 191 primers for individual miRNAs, showed that five of them significantly differentiated the WKY strain from Wistar Han. Especially interesting is the increased expression of miRNA-674 and miRNA-221-3p observed in WKY, what confirms the association of pro-depressive behavior and ADs resistance.

Conclusion: The obtained results, although preliminary, strongly point not only to the significance of habenular nuclei as a brain region important for studies on depression but also, they indicate the significance of microRNAs as an important level of epigenetic regulation which might help to clarify the mechanisms underlying the pro-depressive behavior of WKY rats.

Acknowledgments: Statutory Activity of Maj Institute of Pharmacology Polish Academy of Sciences.

The evaluation of tremorolytic properties of zolpidem in animal models of parkinsonian and essential tremor

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Background: Tremor is a very common symptom of neurological diseases such as Parkinson's disease (PD) or essential tremor (ET) and negatively affects the patient's quality of life. Current pharmacotherapy for tremor is not effective. Deep brain stimulation of the internal globus pallidus and the subthalamic nucleus for PD or the ventral thalamus for ET is used to attenuate its advanced drug-resistant forms. Interestingly, in all of these structures, $GABA_A$ receptors with α1 subunits are present. Moreover, mice lacking the $GABA_A$α1 subunit protein gene show severe tremor and are one of the models of ET. In turn, zolpidem, which acts mainly on $GABA_A$α1 receptors, almost immediately reduced motor symptoms in a report on patients with advanced PD. It is suggested that PD tremor arises from abnormal activity of both the basal ganglia with key dopamine and cerebello-thalamo-cortical circuits regulated by GABA. The pathological changes in cerebellar GABAergic transmission appear to be a contributing factor of ET.

Materials and methods: This study aimed to evaluate the tremorolytic properties of zolpidem, a positive allosteric modulator of $GABA_A$α1 receptor, in models of ET and PD tremor in rats. To model ET, acute administration of harmaline (15 mg/kg ip) was used and the intensity of harmaline-induced tremor and locomotor activity were measured using Force Plate Actimeters. To induce tremulous jaw movements (TJMs), which are a model of PD-like tremor, two different compounds were used – pimozide (PIM, 1 mg/kg ip, for 7 days) or tetrabenazine (TBZ, 2 mg/kg ip). TJMs were recorded and counted by an observer.

Results: Harmaline induced tremor of the whole body with a peak frequency between 9-12 Hz, and both TBZ and PIM induced clear TJMs. Unfortunately, zolpidem in none of the tested doses (0.5, 1.0, 1.5 mg/kg ip) did not inhibit either harmaline-induced tremor or TJMs. Zolpidem in a dose-dependent manner reduced only the locomotor activity in control rats.

Conclusions: The present results suggest that zolpidem in the tested doses has no tremorolytic activity in the harmaline model of ET and PIM/TBZ model of PD-like tremor but further studies are needed to investigate if stimulation of other $GABA_A$ receptor subunits could be effective in tremor inhibition.

Acknowledgements: Supported by the statutory funds of Maj Institute of Pharmacology PAS, Department of Neuropsychopharmacology, Kraków, Poland and Jagiellonian University Medical College, Kraków, Poland: N42/DBS/000176.

Aripiprazole and antidepressants affect the BDNF mRNA expression and Akt signalling pathway in the neurodevelopment model of schizophrenia in rats

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Background: Recent studies suggest that impaired glutathione synthesis and dopaminergic transmission are important factors in the pathophysiology of schizophrenia. Moreover, some studies have suggested that antidepressants (ADs) are able to increase in the activity of atypical antipsychotics which may efficiently improve the treatment of negative and some cognitive symptoms of schizophrenia. In addition, it is know that Akt, serine/threanine protein kinase is involved in many cellular processes and neuronal plasticity. While, Akt-1 is involved in cellular survival, cell proliferation and protein synthesis pathway. In the present study, we investigated the influence of repeated co-treated with escitalopram (ESC) or mirtazapine (MIR) and aripiprazole on the BDNF mRNA expression andp-Akt/Akt-1 signalling pathway in the neurodevelopment model of schizophrenia in adult rats induced by glutathione deficit during early postnatal development.

Materials and Methods: Between the postnatal days (p5-p16), male pups were treated with the inhibitor of glutathione synthesis, L-buthionine-(S,R)-sulfoximine (BSO 3.8 mmol/kg, sc., daily) and the dopamine uptake inhibitor (GBR 12909, 5 mg/kg, sc., every second day) alone or in combination. ESC or MIR and aripiprazole were given repeatedly, once daily for 21 days before the tests. The tissue (hippocampus and prefrontal cortex) for biochemical assays was dissected on p92.

Results: The present study indicated that the inhibition of glutathione synthesis inearly postnatal development induced schizophrenia-like behaviour and decreased the BDNF mRNA expression in adult rats, and these behavioural and biochemical deficits were reversed by repeated treatment with a higher dose of aripiprazole and also by co-treatment with aripiprazole and ineffective doses of ADs. In addition, p-Akt/Akt-1signalling pathway in this neurodevelopment model of schizophrenia was decreased and co-treatment with ADs and aripiprazole reversed this effect, especially in the frontal cortex. Further studies are need to explain the mechanism of this action.

Conclusions: The above data suggests that this neurodevelopment rat model of schizophrenia induced by glutathione deficit by repeated treatment with BSO alone and together with GBR 12909inearly postnatal life may be usefulness for studies on the pathomechanism of schizophrenia.

Acknowledgement: This study was financially supported by grant from the National Science Center 2016/23//B/NZ7/01280, Poland.

NETWORK ANALYSIS TO DISENTANGLE CANNABIDIOL PHARMACOLOGY IN COMPLEX DISEASES: FOCUS ON NEUROPATHIC COMPONENT OF OSTEOARTHRITIS

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Background: In recent years, the paradigm of the 'single target–single drug' shifted towards 'multi-target drugs'. Evaluating drug effects as the result of multiple interactions in a complex network, has yielded unprecedented opportunities to understand the functioning of biological systems and design therapeutic strategies aimed at modifying disease processes rather than controlling symptoms. Cannabidiol (CBD) is non-psychoactive constituent of cannabis interacting with variety of molecular targets, while osteoarthritis (OA) is a chronic joint disease with a complex etiology, resulting in chronic pain that includes neuropathic component. Hereby presented results evaluate tools for computational analysis of CBD's mechanism of action in various complex diseases, such as OA.

Materials and methods: Molecular targets of CBD were established through literature search. Diseases associated genes were obtained through Open Targets Platform. Venn analysis was performed to find common targets between CBD, neuropathic pain and OA. Subsequent analysis of protein-protein interactions was performed with STRING-DB. Most influential node was assessed by centrality degree. Analgesic potential of CBD was assessed by Hargreaves test and Kinetic Weight Bearing (KWB) in MIA-induced model of OA in rats.

Results: Literature search allowed us to find 65 molecular targets of CBD, whereas Open Targets Platform provided 970 and 2472 genes associated with neuropathic pain and OA, respectively. CBD interacted with only 30 targets at concentrations below 2mM, while 10 of them were associated with both OA and neuropathic pain. We were able to cluster the targets as either ionotropic receptors or genes related to endocannabinoid system. PPARγ was the most influential CBD target in the OA-associated target network. CBD was able to restore impaired weight bearing in KWB, however produced thermal hyperalgesia in Hargreaves test.

Conclusions: Our analysis was based on publicly available data and allowed us to evaluate number of potential CBD targets involved in the neuropathic component of OA. These results imply complex mechanism of CBD action that could be both pro- and anti-nociceptive. Initial experimental findings confirm this hypothesis, however further validation of our results is essential. If successful, it may prove bioinformatic approach as the meaningful tool for prediction of mechanism of action in the disease of interest.

Acknowledgements: supported by statutory funds.

CCR1 and CCR3 antagonism brings beneficial effects in neuropathic pain by affecting microglia and neutrophils

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Background: Millions of people worldwide suffer from neuropathic pain, however there is still no efficient treatment. Recent studies indicate that blockade of chemokine receptors by their antagonists may bring beneficial properties on neuropathic pain pathophysiology through the influence on various cell types.The aim of the study was to investigate the impact of CCR1 and CCR3 antagonists on pain related behaviors and protein level of microglia and neutrophils markers and moreover checked whether targeting microglia and neutrophils using their inhibitors may diminish pain related behaviors.

Materials and methods: To evoke neuropathic pain-like symptoms, chronic constriction injury (CCI) of the sciatic nerve were performed in the rodents. Next, CCR1 (J113863) and CCR3 (SB328437) antagonists were administrated intrathecally. Moreover, intraperitoneally were administrated minocycline and 4-Aminobenzoic acid hydrazide (4-ABAH), which are able to inhibit microglia and neutrophils activation, respectively. After drugs administration the von Frey test were provided to measure mechanical hypersensitivity. The western blot method was used to measure the protein level of Ionized calcium binding adaptor molecule 1 (IBA-1, microglia marker) and myeloperoxidase (MPO, neutrophil marker) in the spinal cord after CCI of rats sciatic nerve.

Results: J113863 and SB328437 diminished pain related behaviors as measured by von Frey test, both after single and repeated intrathecal administration. Additionally, inhibition of activity using minocycline and 4-ABAH has also brought beneficial effects on mechanical hypersensitivity. Western blot analysis, shows that J113863 lowers the level of IBA-1, having no impact on MPO, while SB328437 reduces MPO level without influence IBA-1.

Conclusion: Our results prove that both agonists have strong antinociceptive properties however their mechanism of action is different. Moreover, it seems, that under neuropathic pain conditions targeting microglia and neutrophils may bring beneficial properties. Our data provides new evidence that CCR1 and CCR3 may indeed play a significant role in the neuropathic pain development by modulating spinal neuroimmune interactions, suggesting that its blockade may have potential therapeutic utility.

Acknowledgments: Supported by National Science Center, Poland, OPUS-11-2016/21/B/NZ4/00128.

The nonpeptide OXTR agonist reverses memory deficits in Novel Object Recognition Test in MAM model of schizophrenia

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Background: Schizophrenia is a chronic, debilitating disease affecting about 1% of the global population. Its symptoms can be divided into three clusters: positive (psychotic; hallucinations), negative (social withdrawal, lowered motivation) and cognitive (deficits in learning and memory). Methylazoxymethanol acetate (MAM) is a mitotoxin that disrupts brain development when administered to a pregnant rat dam. Behavioral and anatomical changes in the offspring resemble those present in schizophrenia patients.

Oxytocin (OXT) is a neuropeptide involved in social functions, including social memory and deficits in oxytocin's production or secretion are present, among others, in depression and schizophrenia. Previous studies on exogenous OXT administration to schizophrenia patients have provided inconsistent results. This may be due to the low tissue penetration of peptides and the short half-life of OXT. Newly synthesized, nonpeptide OXTR agonist, LIT-001 may bring a breakthrough in the oxytocin studies.

Materials and methods: The neurodevelopmental model of schizophrenia was obtained by MAM intraperitoneal administration on GD 17 to pregnant rat dams. Male offspring were tested at 60-70 PND. Novel Object Recognition Test (NORT) was used to evaluate the pro-cognitive effect of agonist LIT-001 (1 mg/kg) in the MAM model of schizophrenia. Intraperitoneal injection of either LIT-001 or vehicle was done 30 min prior to the first phase of the NORT.

Results: MAM animals presented memory impairments and increased activity.LIT-001 reversed memory deficits but did notinfluence exploration time or overall mobility of animals.

Conclusions: Results suggest that LIT-001 can reverse cognitive deficits present in the MAM model of schizophrenia, improving animal performance in NORT. These preliminary results require further testing on a larger cohort of animals and with other doses of LIT-001. As oxytocin is mainly involved in social activities, the efficacy of the OXTR agonist against MAM-induced social dysfunctions warrants further studies.

Acknowledgments: This study was funded with Diamentowy Grant 0049/DIA/2019/48 to D.P.

Improvement of analytical results quality in neuroscience based on ACh determination - Good Methodology Practice (GMP)

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Background: Acetylcholine (ACh) is one of the most important neurotransmitters in the peripheral and central nervous systems. It is responsible for activating muscles in the neuromuscular junction and is involved in processes such as arousal, attention, and motivation. It is very important to use appropriate methods to accurately detect and determine this neurotransmitter in biological samples with a complex matrix from the clinical perspective. The limitation is also a very low analyte concentration and a small volume of tested samples. The research aim was to assess the accuracy of the obtained results using various approaches to analytical calibration.

Materials and methods: The determination of ACh in mice brains was carried out using microdialysis in freely moving animals. The assay was performed using high-performance liquid chromatography with electrochemical detection (HPLC-ED) - Alexys Antec neurotransmitter analyzer. The ACh analysis was based on the initial separation of HPLC in ionic vapours, online enzymatic conversion of ACh to hydrogen peroxide, and detection of the analyte on the platinum electrode. Four calibration methods: External Calibration (EC), Standard Addition Method (SAM), Integrated Calibration Method in the version of Complementary Dilution Method (ICM/CDM), and the basic variant of Integrated Calibration Method (ICM) were tested.

Results: The obtained limit of detection (LOD) and limit of quantification (LOQ) of the method were 0.09 and 0.27 nM, respectively. The influence of non-linear fitting to the measurement points and the number of points for calibration lines was examined. The accurate values were obtained only in a linear fitting (RE~2%) for the SAM approach. While for the traditional EC method, relative errors were -5.28% and -12.40% for six and two experimental points. ICM and ICM/CDM methods enabled the most accurate detection and determination of this neurotransmitter in biological samples with RE below 1% for extrapolative estimations. The obtained results were characterized by very good precision (RSD ~ 2%).

Conclusions: The use of the HPLC-ED system enabled fast and sensitive ACh determination in the biological samples. The proposed strategy (GMP) allowed obtaining accurate results in samples with such a small analyte concentration. The comparison of obtained results calculated in various ways allowed an effective assessment and compensation of systematic errors due to different interference effects.

Acknowledgments: The research was financed by Polish National Science Centre (projects no 2016/21/B/NZ7/01131; 2014/13/B/NZ7/02237) and partly by statutory funds of the Maj Institute of Pharmacology, PAS, Poland.

Effect of coumarin on kynurenic acid production in rat brain cortex in vitro

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Background: Coumarin (1,2-benzopyrone) is a natural secondary metabolite widely occurring in plants. It was isolated for the first time from *Dipteryx odorata* (Aubl.) Willd. (tonka bean) but also has been found in *Melilotus officinalis* (L.) Pall. (*Fabaceae*), *Galium odoratatum* (L.) Scop. (*Rubiaceae*) as well as *Hierochloe odorata* (L.) P. Beauv. (*Poaceae*) and many others. Modification of coumarin core leads to a diversity of natural or synthetic derivatives, which were shown to display potential pharmacological properties including anti-inflammatory, anticancer or antioxidant.

Kynurenic acid (KYNA), a metabolite of kynurenine, is endogenous antagonist of ionotropic glutamate receptors. It exhibits the strongest affinity towards the glycine binding site of NMDA receptor and weaker activity to AMPA and kainate ones. Brain synthesis of KYNA is primarily catalyzed by kynurenine aminotransferases (KAT I and KAT II). KYNA has a potent neuroprotective, anti-inflammatory, and anticonvulsive properties and disturbing of its production can be involved in the pathogenesis of neurodegenerative disorders such as Parkinson`s, Alzheimer`s and Huntington`s diseases, epilepsy, as well as schizophrenia.

The aim of this study was to evaluate the effect of coumarin on the brain formation of KYNA in rat brain cortex *in vitro*.

Material and methods: The research was carried out on Male Wistar rats. The effect of coumarin on KYNA production was examined in cortical slices. KATs activity was measured in rat brain homogenates incubated with L- KYN and different concentrations of coumarin. The quantity of produced KYNA was determined by HPLC method with fluorescence detection. Statistical evaluation was done using one-way ANOVA with the post-hoc Bonferroni test, assuming $P < 0.05$ as significant.

Results: Coumarin at the concentration of 0.01; 0.1 and 1 mM decreased KYNA production in the brain cortical slices to 52 % ($P<0.01$);44 % ($P<0.01$) and 55 % ($P<0.01$) of control, respectively. Coumarin used at the concentration of 0.01 -1 mM did not alter neither KAT I nor KAT II activity.

Conclusion: The data obtained here suggest that coumarin might decrease KYNA production in rat brain cortex.

Acknowledgments: Supported by the grant from Medical University of Lublin, No DS 457

Effects of Tat-NR2B9c on cocaine-seeking behavior in rat model

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Background: The cocaine use disorder (CUD) is a global problem, which affects people of all ages and gender, causing behavioral, neuronal and molecular changes. Cocaine, as an extremely powerful stimulant, changes the expression and the composition of subunits in the ionotropic glutamatergic N-methyl-D-aspartate receptors (NMDAR; Smaga et al., 2019). In previous research, we found that in the rat cocaine self-administration (SA) model the levels of the gene (Grin2B) and protein (GluN2B) in the ventral hippocampus were increased (Smaga et al., 2021). Currently, the role of NMDAR subunits in CUD is not well-understood.

Material and methods: We have studied the effects of Tat-NR2B9c (NA-1) – the postsynaptic density-95 protein (PSD-95) inhibitor, after it's intravenous injection on cocaine-seeking behavior, in extinction/reinstatement procedure, in male rats.

Results: Our findings demonstrated that in animal with history of cocaine SA (0.5 mg/kg/infusion) and during drug-free period with extinction training, NA-1 in a dose of 7.8 mg/kg/day decreased cocaine-seeking behavior. Injection of NA-1 given 3 times (at 48, 24 and 1 h) before the test reduced cocaine- and cue-induced relapse shown by decreases in the number of active, but not inactive, lever presses. At the same time, the NA-1 attenuated the level of GluN2B/PSD-95 complexes in brain areas.

Conclusions: The GluN2B subunit of the NMDA receptor has a role to control cocaine-seeking and reinstatement behavior, while the NA-1 may be considered as a valid treatment to prolong cocaine abstinence.

Acknowledgements: This research was supported by the National Science Center (Kraków, Poland) grant no. UMO-2015/17/B/NZ7/02935. The authors declare no conflict of interest.

4’-methoxy-1-naphthylfenoterol as adjuvants for doxorubicin-based anticancer therapy

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Background: Doxorubicin (DOX) is well established chemotherapeutic agent currently investigated as immunotherapy booster. In addition to its direct tumoricidal activity, DOX stimulates cell surface exposure of damage-associated molecular patterns (DAMPs) as calreticulin to induce immunogenic cell death and enhances the permeability of tumor cells to granzyme B produced by cytotoxic T lymphocytes. However, many tumors have intrinsic or acquired resistance to DOX. Here, we targeted the G protein-coupled receptor 55 (GPR55) and β2-adrenergic receptor (β_2AR) to enhance DOX accumulation and its anticancer activity in CT26 murine colon carcinoma cells. We utilized 4’-methoxy-1-naphthylfenoterol (MNF), that was previously demonstrated to inhibit growth of cancer cells *in vitro* and *in vivo* through simultaneous β_2AR activation and GPR55 inhibition.

Material and methods: DOX levels were measured fluorometrically in DOX-loaded CT26 cells pretreated with MNF, fenoterol (MNF’s parent compound), salmeterol (β_2AR agonist) or ML193 (GPR55 antagonist). Phosphorylation of key signaling nodes was studied by western blotting. Apoptosis and necrosis were assessed in real-time using RealTime-Glo kit from Promega. Cell viability was measured colorimetrically in MTT assays based on the reduction of tetrazolium dye by metabolically active cells.

Results: MNF activity significantly increased accumulation of DOX in CT26 cells in a dose- and time-dependent manner. Fenoterol and salmeterol displayed little to no effect on DOX incorporation, whereas ML193 efficiently increased intracellular levels of DOX. These results imply that GPR55 inhibition is crucial for DOX accumulation.

MNF displayed anti-apoptotic effect on CT26 cells during the first 6 h of treatment, but increased necrosis at 18 to 24 h timepoint. Early antiapoptotic action of MNF coincided with increase in phosphorylation of pro-apoptotic Bad at inhibitory Ser112. In parallel, MNF suppressed pro-proliferative c-Raf/MEK/ERK signaling cascade, as evidenced by significant drop in phosphoactive levels of cRaf, MEK1/2, and ERK1/2. These events were accompanied by significant decrease in phosphorylation of several ERK1/2 substrates, namely: cPLA, p90RSK, and Mnk1.

Conclusions: Taken together, these results identified MNF as efficient inhibitor of pro-proliferative signaling and enhancer of DOX incorporation in CT26 colon cancer cells.

Acknowledgements: This work was supported National Science Center (SONATA 2018/31/D/NZ7/01350).

Effect of chronic treatment with 25B-NBOMe on neurotransmitter release, behaviour and genotoxicity in rats

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Background: 25B-NBOMe (2-(4-bromo-2,5-dimethoxyphenyl)-N-[(2-methoxyphenyl) methyl]ethanamine) is a novel psychoactive compound belonging to phenethylamine class of serotonergic hallucinogens. It is characterized with subnanomolar affinity to 5-HT2A receptor and demonstrates potent hallucinogenic activity. Up to date there is no data concerning the effect of 25B-NBOMe chronic administration in rats. The aim of this study was to find out the effect of repeated administration of 25B-NBOMe on brain neurotransmission, anxiety and possible genotoxic damage, in comparison to an acute administration.

Materials and methods: The study was conducted on male Wistar-Han rats (280-300 g). The animals were treated with single or repeated (0.3mg/kg for 7 consecutive days) doses of 25B-NBOMe. The cortical, striatal and accumbal extracellular DA, 5-HT and glutamate levels were assessed using microdialysis in freely moving animals. The hallucinogenic effect was measured using the wet-dog shake (WDS) test. Genotoxicity was evaluated with the use of Comet Assay, while anxiety level was monitored using light/dark box test.

Results: Repeated treatment with 25B-NBOME decreased the release of DA, 5-HT and glutamate in response to a challenge dose of 0.3 mg/kg in all studied brain regions in comparison to acute treatment. Chronic administration of 25B-NBOMe reduced the number of WDS episodes, starting from day two. Results obtained with Comet Assay showed that repeated administration of 25B-NBOMe leads to DNA damage in the rat frontal cortex and hippocampus and induced anxiety after acute and chronic administration as measured in the light/dark box test.

Conclusions: Repeated treatment with 25B-NBOMe leads to a rapid development of tolerance which can be observed in the reduction of WDS episodes and decrease in DA, 5-HT and glutamate release. The level of anxiety after single and repeated doses was comparable between treatmentswhile still significantly stronger in comparison to control group. The observed genotoxic effect may result from production of free radicals after chronic administration of 25B-NBOMe.

Acknowledgements: The study was supported by National Science Center, Poland grant no. 2016/21/B/NZ7/01131

Psychedelics as Microglia Immunomodulators

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Background: Psilocybin and N, N-dimethyltryptamine (DMT) belong to a family of psychoactive compounds traditionally termed psychedelics. The compounds are emerging as the novel, and more effective anti-depressant drug candidates in psychiatry. Interestingly, psychedelics can induce brain plasticity and possess immunomodulatory and neuroprotective properties, but the molecular and cellular mechanisms responsible for their effects on neural tissue are poorly understood. Here we demonstrate, how DMT and psilocin influence microglia biology. Microglia are brain residing, specialized immune cells, which protect the brain from pathogens penetrating the blood-brain barrier. Moreover, microglia are significant players in the process of neuronal plasticity and brain tissue regeneration. Unfortunately, as a result of the disease, microgliamay become overactivated and cause neural tissue injury, brain damage, or neurodegeneration.

Materials and Methods: Primary microglial cells were isolated from DBA1 mouse brains and treated with DMT or psilocin in the presence of LPS to mimic brain inflammation and microglial activation. The hallmarks of activated microglia, their morphological and immunological phenotypes were tested by immunostaining and FACS. Microglial activity and neuronal damage with and without DMT and psilocin treatment were measured using neuronal-microglial co-culture by real-time 24h observations.

Results: DMT and psilocin downregulated levels of TLR4, Nf-κβ p65, and CD80, which are microglial proteins upregulated upon inflammation. Psilocin also upregulated TREM2, a receptor involved in regulating microglial phagocytosis during infection, toxic protein clearance, and synaptic pruning. Notably, abnormalities in TREM2 occur in various neuropsychiatric conditions. Finally, we also observed that psilocin but not DMT attenuated healthy neuron phagocytosis by LPS-activated microglia *in vitro*.

Conclusions: We have demonstrated that DMT and psilocin potently influence microglia to prevent their inflammatory properties and may activate regenerative processes in neuronal cells. Therefore psychedelics should be considered as therapeutic candidates in brain disorders, where pathogenesis involves excessive inflammation and abnormal microglia activity.

Pharmacological assessment of zebrafish-based cardiovascular models

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Background: Cardiovascular diseases are the leading cause of mortality and morbidity worldwide. Although heart medications have been proven to be effective for the prevention or treatment of cardiac dysfunction, the search for safer therapies is intensively conducted.

In a related effort, zebrafish has become a powerful tool in cardiovascular research. Small size, optical transparency, and rapid cardiovascular system development allow for the *in vivo* high-throughput screening of substances that modulate heart parameters.

Up until now, a few cardiotoxic drugs have been proposed as zebrafish-based heart failure models. Doxorubicin, β-adrenergic agonists, and terfenadine are characterised by well-documented cardiotoxicity. Therefore, the study aimed to reveal how zebrafish heart responds to cardiotoxic treatments and then to determine whether human medications are able to manage heart dysfunction in zebrafish.

Material and methods: Zebrafish were exposed to both cardiotoxic and cardioprotective drugs to 96 hours post-fertilisation. The compounds were compared regarding the elicited changes in cardiovascular parameters and morphological alterations using ImageJ and DanioScope (Noldus).

Results: Importantly, zebrafish mimicked the response of the human heart to cardiovascular drugs. The greater part of impaired cardiovascular parameters and cardiac abnormalities were alleviated by β-adrenergic and angiotensin receptor ligands. It confirms the functionality of human cardiovascular agents in the zebrafish model.

Conclusions: Here, we developed the zebrafish-based bioassay for a faster and more accurate diagnosis of cardiotoxicity and to monitor the response of the treatment with cardioprotective drugs. In our view, the results emphasise a value of a zebrafish model in preclinical research and particularly to screen drug candidates. Zebrafish may thereby serve for testing of compounds with or without known pharmacological utility.

Acknowledgements: The authors acknowledge financial support from the National Science Centre, Poland (Grant 2017/25/B/NZ7/02654).

Chronic antidepressants treatment reverses depressive-like changes induced by olfactory bulbectomy and zinc deficiency animal model of depression

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Background: Depression is serious health problem but it's pathophysiology is still unknown. Currently used antidepressants do not always provide the desired results and many patients suffer from treatment-resistant depression. Results from clinical studies suggest that zinc deficiency (ZnD) may be an important risk factor for depressive disorder as well as a factor decreasing the effect of antidepressants.

Aims & Objectives: The main aim of this project was to examine whether ZnD might be an essential variable in inducing resistance to antidepressants. To elucidate that, male Spraque-Dawley rats were subjected to olfactory bulbectomy (OB) model of depression and to ZnD. The effectiveness of standard antidepressants in this model were evaluated. Moreover, the level of monoamines (serotonin, dopamine and noradrenalin) in the PFC and Hp were analysed.

Method: The bilateral removal of olfactory bulbs was performed as described previously. In control rats (Sham) the bulbs were left intact. 7 days following surgery, rats were fed zinc deficient diet (3mg Zn/kg) or zinc adequate diet (50mg Zn/kg) for 3 weeks. Then, escitalopram (ESC), venlafaxine (VEN) 10 mg/kg, *i.p.* or combined ESC/VEN (inactive dose 1 mg/kg, *i.p.*) and zinc (5 mg/kg) treatment begun.Following 3 weeks of drug administration the behaviour of rats was examined in open field (OFT), forced swim (FST) and sucrose intake (SIT) tests. Monoamine levels were measured byhigh-performance liquid chromatography (HPLC).

Results: Chronic treatment with ESC reduced hyperactivity of rats in OFT and immobility time in FST in OB+ZnD model. Such effect was not observed after VEN or join ESC/VEN and zinc treatment. Moreover, treatment with ESC/VEN and zinc increased sucrose consumption in rats subjected to ZnD+OB model. Chronic treatment with ESC or join ESC/VEN and zinc elevated the level of dopamine and its metabolite (HVA) in the Hp while, level of serotonin and noradrenalin remain unchanged.

Conclusions: The OB+ZnD model induces more pro-depressive effects than either model alone. SSRIs seems to be more effective and can be used as reference drugs in this model. Obtained results indicated that the OB+ZnD model may induce a drug-resistance in rats. Alterations in the dopamine system seems to underlay changes induced by OB+ZnD and antidepressants effects.

Acknowledgements: This study was supported by NCN grant 2017/25/N/NZ5/02714

Structural modifications in the distal, regulatory region of H3R antagonists leading to the identification of a potent antiobesity agent

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Background: Recent pharmacological and (pre)clinical studies confirmed that histamine H_3 receptor (H_3R) ligands are effective in the treatment of sleep disorders (narcolepsy), cognitive impairment, pain/itch, stroke, depression, schizophrenia, Alzheimer's disease, attention deficit hyperactivity disorder, dementia, obesity and neurodegenerative disorders. Beyond that, several investigations reflect the paramount role of neuronal histamine in feeding behavior mediated by central H_3 and H_1 receptors. So far, numerous H_3R antagonists inhibited food intake and caused profound weight loss in various rodent obesity models.

Material and methods: The synthesis of desired final compounds was achieved through the two-step synthetic route. The phenoxy alkyl bromides were obtained mainly by one-step alkylation of commercially available phenols with 1,3-dibromopropane in propan-1-ol under reflux conditions. Obtained precursor bromides were then coupled with proper amine in a mixture of ethanol/water with powdered potassium carbonate and a catalytic amount of potassium iodide.

Results: A series of 4-pyridylpiperazine derivatives with varying regulatory region substituents proved to be potent histamine H_3R ligands in the nanomolar concentration range. The most influential modification that affected the affinity toward the H_3R appeared by introducing electron-withdrawing moieties into the distal aromatic ring. The putative protein-ligand interactions responsible for their high affinity were demonstrated using molecular modeling techniques. Furthermore, selectivity, intrinsic activity at the H_3R, as well as drug-like properties of ligands were evaluated using *in vitro* methods. Moreover, pharmacological *in vivo* test results of compound **KSK94** (structural analogue of Abbott's A-331440) clearly indicate that it may affect the amount of calories consumed, thus act as an anorectic compound.

Conclusions: Both, the high affinity and significant *in vivo* activity of compound **KSK94** gives hope for the discovery of a unique compound among a vast variety of H_3R ligands. This compound has been chosen as a new lead structure for the development of anti-obeseH_3R ligands and the mechanism of its action would be a subject for further studies.

Acknowledgements: We are pleased to acknowledge the generous support of the National Science Center, Poland granted on the basis of decision No. 2020/36/C/NZ7/00284 and 2016/23/B/NZ7/01063.

Development of a multiplexed protein panel using a targeted proteomics approach for the study of CDK4/6 inhibitors resistance in HR+ breast cancer

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Recurrent and metastatic disease limit the survival of patients with breast cancer. Despite the improved disease control with CDK4/6Inhibitors (CDK4/6I), not all patients respond to these therapy. Our aim is to perform a quantitative evaluation of marker proteins with a developed multiplexed panel using targeted-mass-spectrometry-based proteomics for 25 proteins central to CDK4/6I resistance.

We developed Multiple Reaction Monitoring(MRM) MS methods for the 25 target proteins from the CDK/RB/E2F-pathway using synthetic heavy-isotope-labeled standards with the aim of creating MRM assays to enable specific, sensitive and precise quantitation of these proteins in small amounts samples. Moreover, we developed a high resolution peptide fractionation system using high-pH micro-flow liquid chromatography(LC) which is required to overcome the problem of small samples amounts while improving analytical assay sensitivity in the analysis of complex biological matrices such as breast cancer biopsies. Human breast cancer cell lines were used as model during method development. Nuclear proteins from cell lysates were isolated, reduced, alkylated and digested with trypsin. The resulting tryptic peptides were micro-flow fractionated into 60 fractions, concatenated in 24 fraction and were used for peptide detection and quantification.

Our developed micro-flow fractionation method allowed us to work on limited amounts of samples (60 ug) and increased the possibility to detecting low abundance proteins such as cell cycle components. Using the cell models, we are able to identify and quantify 19 proteins from our panel: CDK1, CDK2, CDK4, Cyclin B1, Cyclin D1, Cyclin D3, Cyclin E1, RB1, E1F-1, E2F-3, E2F-4, E2F-5, ESR1, TOP2A, TYMS, EZH2, MKI67, BIRC5, FAT1.

We have developed a highly specific MS-based multiplexed assay with peptide standards targeting 25 proteins relevant to CDK4/6I breast cancer treatment. Our micro-flow fractionation method increased assay sensitivity and allows for the analysis of small sample amounts (<100 ug). We will apply this workflow to samples such as different cell lines, Patient Derived Xenografts models, breast cancer tissues and FFPE samples in order to identify the predictive value of these potential biomarkers for responsiveness to CDK4/6I.

The presented project is carried out within the First Team programme of the Foundation for Polish Science co-financed by the European Union under the European Regional Development Fund (First TEAM/2017-4/33).

Design and synthesis of squaramide derivatives of PZM21: a new opioid receptor molecular probes in the study of the structure-selectivity relationship

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Background: Opioid receptors (OR) have been key drug targets of medicinal chemistry due to their role in the modulation of pain perception. The opioid receptor ligands are effective pain therapeutics.Although many μ, κ, and δ ORs ligands are known to date, there is still no clear understanding of the ligand-receptor interactions and structure-function relationships underlying the distinct biological effects upon receptor activation or inhibition. Thus, studying structurally similar ligands, but showing different biological effects could help in better understanding the fundamental interactions responsible for the observed differences.

Material and methods: A series of squaramide derivatives of the opioid biased agonist PZM21 was synthesized, and their intrinsic activity of the μ, κ, and δ ORs were established. The computational methods (i.e., molecular docking and molecular dynamics simulations) have been used for investigating ligand-receptor interactions.

Results: As a part of our study of opioid receptors, the structure of well-known G-protein-biased μOR agonist PZM21 has been modified. The bioisosteric replacement of molecule core led to a change in intrinsic activity, giving new squaramide derivative as potent μOR/κOR antagonist. Moreover, further systematic modifications of its terminal aromatic moiety have been carried out. The emerged SAR studies showed that μOR/κOR/δOR selectivity could be affected by simple structural changes. Computational analysis indicated the possible binding modes and ligand-receptor interactions.

Conclusions: The resulting group of squaramide derivatives of PZM21 is useful as molecular probes in recognizing key ligand-receptor interactions and thus provides molecular clues for designing new ligands with increased OR selectivity.

Acknowledgments: The study was supported by the grant OPUS 16 nr 2018/31/B/NZ7/03954 financed by the Polish National Science Centre and Statutory Fund of the Maj Institute of Pharmacology PAS.

The effect of ageing and cerebral serotonin deficit on the activity and protein level of brain and liver cytochrome P450 2D (CYP2D) in female rats

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Background: Brain cytochrome P450 (CYP) is involved in the local metabolism of endogenous substrates (steroids, neurotransmitters), drugs and toxins. The aim of the study was to assess the changes in CYP2D activity during the aging process and as a result of serotonin deficiency in the brain.

Material and methods: The experiment was carried out on female wild-type Dark Agouti rats (mature 3.5-months-old and aged 21-months-old) and in tryptophan hydroxylase 2 (TPH2)-deficient animals. The activity of CYP2D was studied by measurement of the rate of bufuralol 1'-hydroxylation in microsomes derived from the liver and selected brain structures (HPLC). Besides, the protein levels (Western blotting) of brain and liver CYP2D were estimated. The data were statistically analyzed using a Student's t-test and One-way ANOVA followed by *post-hoc* Tuckey's test.

Results: The CYP2D activity and protein levels were decreased in the cerebral cortex, hippocampus, cerebellum and liver, but increased in the brain stem in aging females. In the other examined structures (frontal cortex, hypothalamus, thalamus, striatum) the enzyme activity did not change. In aging TPH2-deficient females, The CYP2D activity and protein levels were decreased in the frontal cortex, hypothalamus and brain stem, remaining unchanged in other brain structures, relative to aging wild-type females.

Conclusions: The results indicate thatthe aging process and TPH2-deficit affect the CYP2D activity and protein level in female rats. This may have a negative impact on the compensatory capacity of CYP2D in the synthesis of serotonin and dopamine in the female brain structures involved in cognitive and emotional functions, and on drug metabolism in the liver.

Acknowledgements: This study was supported by the grant ERA-NET Neuron II JTC 2015 Respond and by the Statutory Activity of the Maj Institute of Pharmacology, Polish Academy of Sciences, Krakow, Poland.

RP-18 Thin Layer Chromatographic Investigations of Skin Permeability of Steroids

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Background: Steroids are an important class of pharmacologically active drugs which are administered by different routes including transdermal delivery. Understanding of steroids' ability to cross the skin barrier is therefore important. Transdermal permeability of drugs has been studied by many techniques, including *in vitro* permeation experiments on excised human skin, animal skin, cultured human skin cells or synthetic membranes. It is also known that skin permeation is linked to some easily obtained physicochemical parameters of a molecule, including its partition coefficient between aqueous and organic layers log P_{ow}. Thin layer chromatographic retention parameters are connected with log P_{ow}, so TLC chromatographic descriptors may be used as predictors of skin permeability.

Material and Method: Retention factors R_f were obtained for 16 steroids (cortisol, hydrocortisone acetate, methyltestosterone, progesterone, testosterone propionate, testosterone heptanoate, cortisone acetate, prednisolone, estrone, estradiol benzoate, desoxycorticosterone acetate, tibolone, spironolactone, eplerenone, digoxin, dexamethasone) by RP-18 thin-layer chromatography using pH 7.4 phosphate-buffered saline - acetonitrile 30:70 (v/v) mixture as a mobile phase. $R_M$parameters calculated according to an equation: R_M = log (1/R_f - 1) were correlated with skin permeability coefficients (log K_d), calculated according to the Potts model (using the non-commercial EpiSuite software), preADMET software and obtained from literature sources.

Results: Correlations between the calculated log K_p values and R_M were linear (R^2 = 0.89 for EpiSuite and 0.77 for preADMET, respectively). The results were confirmed using 5 experimental K_p values available in the literature.

Conclusions: Analysis of correlations of R_M values with log K_d proved that the single chromatographic run approach used in this study gives sufficiently good results and using R_M^0 values extrapolated to zero concentration of the organic modifier is not necessary.

Acknowledgements: This research was supported by an internal grant of the Medical University of Lodz no. 503/3-016-03/503-31-001.

Proteomic analysis of ectosomes derived from normal thyroid follicular epithelium and anaplastic thyroid carcinoma

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Background: An increasing number of studies confirm that ectosomes, which are a subpopulation of extracellular vesicles, play a functional role at various stages of cancer progression. They have also potential applications in cancer management as diagnostic and prognostic biomarkers. The aim of this study was to identify proteins present in ectosomes released by normal and cancer thyroid cells.

Material and methods: Two cell lines were used in these research: anaplastic thyroid carcinoma (8305C) cells and normal thyroid follicular (Nthy-ori 3-1) cells. Ectosomes were isolated from conditioned media concentrated by low-vacuum filtration by differential centrifugation. Liquid chromatography coupled to tandem mass spectrometry was used to investigate the protein content of the ectosome proteome. Next, Gene Ontology (GO) analysis was performed using UniProt Database to classify identified proteins according to the biological processes, molecular functions and their cellular origin.

Results: In cargo of Nthy-ori 3-1-derived ectosomes a total of 561 proteins was identified, while 8305C-derived ectosomes carried only 462 proteins. Among the identified ectosomal proteins, 314 were common.Ninety four percent of identified proteins were also identified in other EVs studies. According to GO the most abundant groups of proteins for both types of ectosomes were those connected with cytosolic or membrane origin. In 8305C-derived ectosomes, several cancer-associated proteins were found, which suggests their possible role in cancer promotion.

Conclusions: The present study provides first, comprehensive proteomic analysis of anaplastic thyroid carcinoma-derived ectosomes. Identification of biologically relevant proteins in ectosomes holds a promise for a deeper understanding of disease pathogenesis and evaluating ectosomes as novel therapeutic and biomarker targets.

Acknowledgements: This work was supported by grants from POB BioS (U1U/P03/DO/13.17 and U1U/P03/DO/14.56), the Jagiellonian University in Kraków (N18/DBS/000007) and from the Polish National Science Center (UMO-2017/25/B/NZ4/01403).

Driver's oncogenes molecular programs in human neoplasia: a functional transcriptomics analysis

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Background: Cancer is the primary cause of maximum death in western countries. Tendencies of improving diagnosis and treatment in breast and prostate cancer in the European countries allow predicting that in the upcoming decade the cancers of the lung, colon/rectum, and pancreas will be the primary causes of deaths in the European Union.

The main objective of this study to build a systematic, transcriptomics and proteomics map of molecular pathways to determine the leading oncogenes such as cellular proteasome machinery, mutated TP53, KRAS, and hyperactive CMYC in human neoplasia. Large-scale transcriptomics and proteomics analysis were performed in a panel of cancer cell lines with CRISPR-Cas9 knockouts of the oncogenes.

Materials and Methods: Bioinformatics approaches used to obtain the results which included pre-processing and mapping of reads, expression counts, and statistical analysis. We had analysed three different cancer types (lung, colon, & pancreatic) with three different oncogenes (mutantTP53, KRAS, & CMYC). Data quantification had been done by FastQC and trimmomatic tool then processed for the mapping with HISAT2 tool. Differentially regulated genes were predicted by the DEseq2 method and separately submitted to Cytoscape plugin ClueGO for pathway analysis.

Results: After the removal of insignificant reads, data quantification resulted in 96.47% good quality reads. Mapping of the obtained significant reads with the reference dataset leads to a significant score of ~97-98%. The obtained score value shows the successful mapping of the reads with the reference genome. To find common pathways between up and down differentially regulated genes predicted by the Deseq2 method observed a total of 204 up and 4 down common pathways. Finally, with the above approach total of 63 signatures with the broadest potential meaning for cancer progression were found, including 42 up and 21 down-regulated genes common in all cell lines. From the observed genes; targets were selected for *in vitro* validation to choose possible therapeutic targets to confirm the effect of oncogene editing on transcript and protein levels first in the studied cancer cell lines.

Conclusion: Collectively this study revealed that upon selecting the therapeutically promising protein and transcript signatures can be used to understand the mechanisms within the studied pathways and allowing for more informative functional validation.

Acknowledgement: Financial support -2017/26/E/NZ5/00663

Significance of CCL17/CCL22/CCL2 signalling in nociceptive transmission and morphine analgesic potency

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Background: Recent studies indicates that chemokines signaling pathways are crucial in neuropathy development, however the involvement of CC chemokine receptor 4 (CCR4) has not been completely elucidated. Therefore, we examined the role of CCR4 and its ligands (CCL17, CCL22, CCL2) on the development of hypersensitivity in naive mice as well as in morphine-induced tolerance in mice exposed to chronic constriction injury (CCI) of the sciatic nerve.

Material and methods: Single intrathecal injections of C021 (CCR4 antagonist) and its ligands CCL17/CCL22/CCL2 were performed on naive mice. Moreover the repeated intraperitoneal injections of C021 with morphine were performed on mice after Chronic Constriction Injury (CCI model) of the sciatic nerve. Hypersensitivity were evaluated by von Frey/cold plate tests. Spinal changes in mRNA levels were analyzed by RT-qPCR at day 14th after CCI.

Results: The results of our research demonstrated that after sciatic nerve injury the expression of CCL17 and CCL22 are spinally unchanged, in contrast to CCL2, which remains significantly upregulated till day 14th after CCI. Importantly, our results give evidence that in naive mice CCL2, CCL17 and CCL22 may evoke pain-related behaviors through CCR4, since its pronociceptive effects are diminished by C021. In CCI-exposed mice the pharmacological blockade of CCR4 enhances the analgesic properties of morphine and delay development of morphine-induced tolerance.

Conclusions: Our results suggest that the modulation of the activity of the chemokine signaling pathways could be beneficial for the potentiation of an analgesic effect of available drugs in neuropathic pain therapy.

Acknowledgments: Supported by National Science Centre, Poland (OPUS 2016/21/B/NZ4/00128) and by statutory funds of the Department of Pain Pharmacology, Institute of Pharmacology PAS. Joanna Bogacka acknowledges the support of InterDokMed project no.POWR.03.02.00-00-I013/16.

A method of obtaining highly active hyaluronidase on an industrial scale

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Background: Hyaluronidases refer to a group of enzymes that catalyse the hydrolysis of certain complex carbohydrates such as hyaluronic acid and chondroitin sulphates. In medicine it has been used for its approved indications in general to increase the absorption of drugs into tissue in such fields of as orthopaedics, surgery, ophthalmology, oncology, dermatology. Presently, there is market feedback regarding reported cases of shortage of the medicinal products based on high quality hyaluronidase. This article presents an economical method of obtaining hyaluronidase on an industrial scale leading to a product with high specific activity which does not require the use of dangerous substances or complicated and expensive techniques.

Materials and methods: Tissue extraction was performed in aqueous solution of acetic acid with continuous cooling.Precipitation of ballast compounds was achieved using CaCl2.The extract was filtered on a sieve separator and the filtrate was directed to a disc centrifuge. The supernatant was placed in a tank with a cooling jacket and filtered using ceramic filters. The extract was concentrated using a membrane with a cut-off of 30 kDa.The ballasts were discharged with ammonium sulphate.The solution was centrifuged, and the supernatant was adsorbed onto the chromatographic column - strong cation exchanger.Hyaluronidase was eluted with a linear gradient of ammonium acetate. In subsequent stages, the enzyme was precipitated with ammonium sulphate and subjected to chromatographic separation on Sephacryl S200. The fractions containing hyaluronidase were concentrated and microfiltered, and drying of the final solution was carried out in a freeze dryer. Hyaluronidase activity was determined using the pharmacopoeia method.

Results: Finally, after lyophilization 43.5 g of hyaluronidase with a specific activity of 14,341 U/mg was obtained from 250 kg of waste meat raw material.

Conclusions: In accordance to the method presented, it is possible to obtain a product with a high specific activity of 14,000-15,000 U/mg with a total yield of approx. 20%. The present preparation method does not involve complicated and expensive techniques, and results in a product compliant with Ph. Eur.

Acknowledgements: This work was financially supported by EU from the European Regional Development Fund. Project number: RPDS.01.02.01-02-0149/17. Project is being realized as a part of Regional Operational Programme of the Lower Silesian Voivodeship for years 2014 – 2020.

Analysis of the prevalence of mood disorders in type 2 diabetic patients – a pilot study

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Background: The concurrence of depression and diabetes mellitus (DM) has been increasingly noticed during recent years. So far, many factors and mechanisms are discussed to be involved in the pathogenesis of coexistence of these disorders. Therefore, the aim of this study was to estimate the frequency of depression in patients with DM type 2 and to detect the possible associations between antidiabetic drugs and mood disorders. The influence of long-term and short-term metabolic control on the risk of the development of depression was also measured.

Materials and methods: The study group consisted of 59 patients diagnosed with DM type 2 – 32 women and 27 men. The age of participants ranged from 50 to 89 years. Depression was measured using the Patient Health Questionnaire-9 (PHQ-9), and sociodemographic factors were gathered with the self-prepared survey. The metabolic control indicators were assessed via the Enzymatic Method for Glucose Determination (fasting glycemia) and High-performance liquid chromatography (HPLC) (HbA1C) in blood samples. The participants were treated with the following antidiabetic therapies: metformin (n=33), the combination of insulin and metformin (n=12), sulfonylureas (n=9), and diet (n=5).

Results: The frequency of depression in the studied group was estimated at 33,9%. The most common disorder found with PHQ-9 was Mild Depressive Disorder. The analysis of the impact of sociodemographic risk factors showed no gender effect on depression in the study group. However, it was observed that lower education significantly increased the risk of developing mood disorders. Analyzing the indicators of metabolic control, including fasting glycemia ($r=0,25$, $p<0,05$) and HbA1C ($r=0,31$, $p<0,01$), a positive correlation between these parameters and PHQ-9 score was found. The analysis of variance (ANOVA) showed no influence of antidiabetic therapy on depressive symptoms.

Conclusions: The study demonstrates a similar frequency of depressive disorders in the observed population as in data from world literature. Level of education and impaired short and long-term compensation might be linked with an increased risk of developing depressive disorders in the studied group of patients with DM type 2. The results demonstrated no indication that the form of antidiabetic therapy may exert an anti-depressive effect.

Acknowledgements: This research was funded by Poznan University of Medical Sciences.

Behavioral effects of two new generation synthetic cathinones: 4-MeO-PVP and 4-CMC

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Background: Synthetic cathinones, belonging to new psychoactive substances, have been present on the market since 2008. They produce effects similar to those of old drugs of abuse, such as methamphetamine or MDMA, by enhancing dopaminergic (DA), noradrenergic (NE) or serotoninergic (5-HT) neurotransmission in the central nervous system. Cathinones with high selectivity for DA system are considered to act as psychostimulants and to have strong addictive potential, while cathinones with similar affinity for DA/5-HT systems produce empathogenic effects and are endowed with lower abuse potential. Here we present behavioral effects in mice of two new generation synthetic cathinones: 4-MeO-PVP (more DA-specific) and 4-CMC (equipotent at DA and 5-HT).

Materials and methods:

- All experiments were performed using adult male C57BL/6J or DBA/2J mice.
- Spontaneous locomotor activity was measured using open field chambers equipped with infrared beams.
- Behavioral sensitization was assessed by measuring changes of spontaneous locomotor activity induced by drugs on 1st and 7th days of administration and after 10-day abstinence.
- Rewarding properties were measured using biased conditioned place preference (CPP) paradigm.
- Motor coordination of mice was assessed using accelerating rotarod.

Results:

- 4-MeO-PVP and 4-CMC after acute administration increased spontaneous locomotor activity of C57BL/6J and DBA/2J mice with distinct profiles. Both drugs increased horizontal activity, while only 4-MeO-PVP consistently increased vertical activity.
- Both 4-MeO-PVP and 4-CMC produced behavioral sensitization in DBA/2J mice.
- 4-MeO-PVP but not 4-CMC produced CPP.
- Neither 4-MeO-PVP or 4-CMC disturbed motor coordination of mice after acute treatment. 4-MeO-PVP at the high dose (20 mg/kg) improved performance of mice on the rotarod.

Conclusions: Both 4-MeO-PVP and 4-CMC produced psychostimulant effects in mice as they stimulated spontaneous locomotor activity. Effects of more selective for DA system 4-MeO-PVP were more pronounced, since it increased spontaneous vertical and forced (rotarod) activities of mice. Both drugs are endowed with abuse liability as they produced behavioral sensitization after intermittent treatment. As expected, abuse potential of 4-MeO-PVP is stronger what was confirmed by CPP induction in mice.

Acknowledgements: Supported by the Medical University of Lodz, Poland.

All procedures were approved by the Local Ethical Commission for Experimentations on Animals in Łódź.

Genetic landscape of meningiomas and its implication to lipidome composition

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Background: Meningiomas are the most common benign brain tumors but sometimes they can evolve to higher grade. Therefore, basic research, which enables to understand the relation between genetic mutations and metabolic alterations is essential. Thus, the aim of this study was to assess genetic profile of meningiomas as well as to analyze the influence of genetic changes on lipid composition.

Material and methods: 82 brain tumors were obtained during neurosurgical procedures. Directly after lesions excision chemical biopsy using solid-phase microexraction fibers was performed. Then lipidomic analysis was carried out using liquid chromatography coupled with high resolution mass spectrometry, Q Exactive Focus. The remaining part of the lesion was stored as a paraffin tissue blocks. Then, genetic testing towards the presence of mutations in the following genes: NF2, TRAF7, AKT1, KLF4, PIK3CA were performed.

Results: Genetic profiling of mengingiomas revealed that majority of lesions had mutation in NF gene (71% of samples). Mutation in AKT1 and TRAF7 genes were present only in the absence of NF mutation. The percentage of samples with these changes was 22% and 9%, respectively. No mutations in PIK3A or KLF4 gene were observed.

Lipidomic analysis revealed homogenous structure of meningiomas and the data did not clearly reflected the histological type of the collected samples. On the other hand, lipidomic phenotype showed some relation with the NF status.

Conclusions: Genetic landscape of meningiomas in Polish patients is similar to general population. The NF mutation was found in the majority of samples. Moreover, the presence of NF do not accompany AKT1, TRAF7, PIK3A and KLF4 mutants. Lipidomic analysis, on the other hand, confirmed homogenous nature of benign brain tumors, with some observation suggesting potential influence of genetic alterations on tumor lipidome.

Acknowledgements: Genotyping of meningiomas was funded by the National Science Center Poland, within the research grant No 2019/33/N/ST4/00286. Lipidomic analysis of meningioma was funded by the National Science Center Poland, within the research grant No 2015/18/M/ST4/00059. The authors would like to acknowledge Supelco/ MilliporeSigma for kindly supplying the SPME probes, Thermo Fisher Scientific for granting the access to a Q-Exactive Focus mass spectrometer, and Anchem for the technical support.

THE EFFECT OF ALFA LIPOIC ACID ON CARDIAC SPHINGOLIPID METABOLISM IN RATS ON A HIGH-FAT DIET REGIME

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Background: High-fat diet (HFD) causes an enhanced supply of fatty acids (FAs) in the circulation, which results in lipid metabolism imbalance. This disturbance leads to excessive lipid accumulation in cell as triacylglycerols (TAGs) contributing to development of lipotoxicity. Furthermore, TAGs may be esterified to sphingolipids, which increased concentration in the heart contributes to pathologic changes in cardiac structure and function. There is no data describing influence of alfa lipoic acid (ALA) on sphingolipid metabolism in the left ventricle. The purpose of this study was to establish the impact of ALA, which is known for its antioxidant properties on sphingolipid pathway.

Material and methods: The experiment was carried out on male Wistar rats implementing standard chow or high-fat diet for 8 weeks. Animals were divided into four groups: control, HFD, ALA, HFD+ALA. Intragastric ALA solution (500 mg/kg body weight; at dose of 2 ml/kg body weight/once daily) was administered for the last two groups during the whole experiment. The left ventricle was excised and immediately frozen in liquid nitrogen and stored at -80°C. The expression of ceramide *de novo* synthesis enzymes (SPTLC1, SPTLC2) was performed using Western Blott technique. Sphingolipid fractions were determined by high performance liquid chromatography. Data were analyzed by one-way ANOVA followed by a respective post-hoc test. The statistical significance was defined as $p< 0.05$.

Results: In our study, high-fat feeding resulted in a significant increase in sphinganine and ceramide concentration, which was consistent with increased expression of SPTLC1 and SPTLC2 compared to the control group. Moreover, as a result elevated availability of FAs in the diet with ALA treatment caused decrease in ceramide accumulation in left ventricle in comparison with the HFD group. In the same group we also observed decreased expression of ceramide *de novo* synthesis enzymes in relation to the HFD group.

Conclusions: The obtained data disclosed that ALA relevantly reduced ceramide content in cardiac muscle under high-fat diet condition. The observed changes in *de novo* ceramide synthesis pathway pointed up a potential beneficial influence of ALA on excessive sphingolipid accumulation in the heart.

Acknowledgement: This work was supported by the Medical University of Bialystok, Poland (Grant No. SUB/1/DN/20/006/1118; SUB/1/DN/20/004/1118).

Characterization of human gastrointestinal cells exposed to vitamins: Raman spectroscopy and imaging studies

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Background: Cancer of gastrointestinal tract, such as gastric cancer and colorectal cancer, is a common type of cancer worldwide. Natural antioxidants, such as vitamins, are widely considered as potential anti-cancer agents. In this study, vitamin C and vitamin E, were tested in terms on their effect on molecular composition of human normal colon cells (CCD-18Co) and human gastric cancer cells (HTB-135).

Material and methods: CCD-18Co cells, treated with different concentrations of vitamin C, and HTB-135 cells, exposed to different concentrations of vitamin E, were biochemically and structurally characterized by means of Raman spectroscopy and imaging.

Results: Results of this study show that cellular composition of CCD-18Co and HTB-135 cells can alter depending on vitamins concentrations tested. Specific Raman spectroscopy bands, related to amino-acids, proteins, nucleic acids and lipids, can serve as biomarkers describing chemical changes in CCD-18Co and HTB-135 cells exposed to vitamins.

Conclusions: Raman spectroscopy and imaging can be applied as valuable techniques used to monitor chemical alterations in human colon and gastric cells subjected to different vitamin C and E concentrations exposure.

Acknowledgements: This work was supported by the National Science Centre of Poland (Narodowe Centrum Nauki) UMO-2017/25/B/ST4/01788.

Toxicity assessment and visualization of cell structures using new fluorophores from the group of coumarin derivatives

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Fluorescent probes based on small organic molecules have become indispensable tools in modern biology because they provide dynamic information concerning the localization and quantity of the molecules of interest, without the need of genetic engineering of the sample. Moreover, fluorescent probes offer great potential to identify and treat surgical tumors by clinicians. For any biomedical applications, it is mandatory to investigate the cytotoxicity of fluorescent probes prior to their utilization into biological applications. This study evaluated the cytotoxicity of novel coumarin-derived fluorophores using the MTT (3- (4,5-dimethylthiazolyl-2) -2,5-diphenyltetrazolium bromide) cell viability assay on a non-small cell lung cancer line (A549). The test measures the metabolic activity of cells incubated in the presence of test compounds. The test used the ability of the enzyme mitochondrial dehydrogenase to reduce the tetrazolium salt to the colored formazan. The amount of formazan formed is directly proportional to the number of viable, actively proliferating cells in culture. Cytotoxicity of the tested compounds was compared with the doxorubicin compound, which has proven toxicity to cancer cells. After the MTT test, it was shown that the coumarin derivatives tested are safe for cells and do not affect their physiological properties after both 3 and 24 hours of incubation. The aim of this study was also to evaluate the ability to penetrate biological membranes and visualize cellular structures using novel compounds based on coumarin scaffolds. Analysis performed with the Leica DMiL LED Fluo inverted microscope. These compounds have been shown to be effective as fluorescent dyes. Colocalization studies of new coumarin derivatives were also performed using commercial probes LysoTracker and MitoTracker Red. The partial localization of new coumarin derivatives in lysosomes was confirmed.

Acknowledgements: The authors are grateful to the Foundation for Polish Science (Warsaw, Poland) - Project POWROTY (Contract No. POIR.04.04.00-00-1E42/16-00 - POWROTY/2016-1/4 – „Synthesis and photochemistry/photophysics studies of the intelligent luminescent molecular sensors for selective detection in biochemistry and chemistry").

BENEFICIAL EFFECT OF N-ACETYLCYSTEINE ON SPHINGOLIPID METABOLISM IN THE LEFT VENTRICLE OF OBESE RATS

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Background: Obesity as a crucial aspect of the metabolic syndrome is an important risk factor for further development of diabetes mellitus and cardiovascular diseases. It disrupts fatty acid (FA) metabolism and increases lipid deposition as triacylglycerols in nonadipose tissues such as the heart muscle. This lipid fraction may be a precursor for more lipotoxic intermediates from sphingolipid pathway such as ceramide (CER). Its excessive accumulation during overnutrition contributes to obesity-related cardiovascular dysfunction. Despite numerous studies about lipid metabolism in the left ventricle, there is a lack of data describing the influence of N-acetylcysteine (NAC) on sphingolipid pathway. In this research we assessed the effect of this antioxidant on sphingolipid synthesis and storage in cardiac muscle.

Material and methods: The experiment was conducted on male Wistar rats fed standard chow or high-fat diet (HFD) for 8 weeks. A half of rats from both groups were chronically receiving intragastric NAC solution (500 mg/kg body mass; at volume of 2 ml/kg body mass/once daily) during the whole feeding period. After rat anesthesia the left ventricle was excised, promptly frozen in liquid nitrogen and stored at -80°C. Sphingolipids content was measured by high-performance liquid chromatography. Immunoblotting was used to determine the expression of ceramide *de novo* synthesis pathway proteins: SPTLC1, SPTLC2 and proteins common for *de novo* synthesis and salvage pathway: LASS4, LASS5, LASS6. The data were analyzed by one-way ANOVA followed by an appropriate post-hoc test (statistically significant at $p < 0.05$).

Results: Our study showed that HFD administration caused a crucial rise in CER content and expression of heart-specific SPTLC2 from that in control group. Treatment with NAC significantly decreased CER content in lipid overload condition, which was in accordance with diminished SPTLC2 expression in the same group compared to the HFD group. In addition, SFA content was markedly reduced after NAC administration in the left ventricle in comparison with the control group.

Conclusions: This data clearly showed that decrease in SFA and CER content caused by NAC was consistent with decreased expression of ceramide *de novo* synthesis proteins. Hence, we may suspect that NAC may exert protective effect on heart metabolism and function.

Acknowledgement: Funded by the Medical University of Bialystok (Grant No. SUB/1/DN/20/004/1118).

DNAmoreDB, a database of DNAzymes

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Background: Deoxyribozymes, DNA enzymes, or simply DNAzymes are singlestranded DNA molecules that, like proteins and ribozymes, possess the ability to perform catalysis. Although DNAzymes have not yet been found in living organisms, they have been isolated in the laboratory through in vitro selection. Since their discovery in 1994, DNAzymes capable of catalyzing an ever-growing array of chemical transformations have found applications in diverse fields, such as biochemistry, nanotechnology, and therapeutics.

Results: DNAmoreDB is a comprehensive database resource for DNAzymes that collects and organizes the following types of information: sequences, conditions of the selection procedure, catalyzed reactions, kinetic parameters, substrates, cofactors, structural information whenever available, and literature references. Currently, DNAmoreDB contains more than 1000 entries with information about DNAzymes that catalyze 20 different reactions. We included a submission form for new data, a RESTbased API system that allows users to retrieve the database contents in a machinereadable format, and keyword and BLASTN search features. The database is publicly available at https://www.genesilico.pl/DNAmoreDB/.

Conclusions: DNAmoreDB is a database dedicated to DNAzymes that offers a single entry point for data that so far could be obtained only by meticulously analyzing many different sources, often difficult to browse, such as supplementary materials of published papers. Our database offers a user-friendly interface with different options to browse from lists of DNAzymes and publications, apply search filters, and sort the results depending on the user's preferences. Besides, advanced search options are available through keyword search, and by sequence.

Acknowledgements: We would like to thank our funding agency, the National Science Centre, Poland [2018/31/D/NZ2/01883 to A.P.S.] and all members of the Bujnicki laboratory for fruitful discussions.

Dendritic spines elongation in hippocampal CA1 subregion

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Background: Depression is one of the most widespread illnesses in the world. Among the many symptoms we can observe e.g. anhedonia, chronic fatigue, lack of motivation, low self-esteem or suicidal thoughts. There are various medicaments for this illness. Unfortunately their therapeutic effects appear after dozen of days of treatment. For that reason, currently many researches focus on the process of the development of depression. In order to develop new therapies, a better understanding of the mechanisms regulating this disorder is needed. Recently, the 5-HT7 receptor (5-HT7R) has been a subject of particular interest to scientists. It was shown that inhibition of 5-HT7R has antidepressant effects. Previously, we described a new 5-HT7R/MMP-9 signaling pathway that is associated with the reorganization of the dendritic spines in hippocampal neurons *in vitro*. However, the existence *in vivo* and the role of this pathway in physiology has not yet been deeply studied.

Material and methods: Using a combination of behavioral, biochemical and imaging methods, we analyzed 5HT7R/MMP-9 signaling and dendritic spine plasticity in the hippocampus of mice treated with the selective 5-HT7R agonist.

Results: We have found that the stimulation of 5-HT7R leads to the increase of the activity of MMP-9 in hippocampus. Biochemical analysis of the individual subregions of the hippocampus (CA1, CA3 and dentate gyrus (DG)) revealed, that this increase was mainly localized in the CA1 and DG subregions. Moreover, treatment with inverse agonist of 5-HT7R leads to decrease in MMP-9 activity in the hippocampus.

The morphometric analysis has showed, that 5-HT7R stimulation leads to the elongation of dendritic spines in the CA1 subregion of hippocampus - where 5-HT7R is highly expressed. Whereas, in DG subregion of hippocampus, the maturation of dendritic spines was observed upon activation of 5-HT7R. Moreover, the decrease of dendritic spines density was observed in all hippocampal subregions (CA1, CA3, DG).

Interestingly, we have found that 5-HT7R activation induces depressive-like behavior in mice in a MMP-9-dependent manner.

Conclusions: The 5-HT7R/MMP 9 pathway is specifically activated in the CA1 and DG subregions of the hippocampus and leads to dendritic spines remodeling. These processes are associated with the depression-like behavior.

Acknowledgements: This work was supported by the Foundation for Polish Science (POIR.04.04.00-00-43BC/17-00) and National Science Centre (2019/35/D/NZ4/02042).

The impact of LIG3 single-nucleotide polymorphisms on the risk of nonalcoholic fatty liver disease occurrence

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Background: Nonalcoholic fatty liver disease (NAFLD) is a common hepatic disorder. The pathophysiology of NAFLD is based on the interplay between the accumulation of free fatty acids, inflammation and oxidative stress. It may result in DNA damage, which should be repaired to protect hepatocytes. Probably, the impairment of DNA repair pathways might lead to the excess of reactive oxygen species and intensification of the problem. Since this situation can trigger a progression of the disease, we have decided to investigate the associations between NAFLD and singe-nucleotide polymorphisms (SNPs) in ligase 3 gene (LIG3), involved in DNA base excision repair (BER).

Material and methods: 359 participants were enrolled in the study and divided into two groups, i.e. 48 NAFLD patients and 311 healthy volunteers as a control group. We selected two SNPs: c.*83A>C (rs4796030) and c.*50C>T (rs1052536) of LIG3. The DNA was isolated from the whole blood of the patients and genotyping was performed using TaqMan probes. Statistical analysis was accomplished by multiple logistic regression. The results were calculated as odds ratios (ORs) with 95 % confidence intervals and considered statistically significant with a p-value < 0,05.

Results: The obtained findings showed that the C/C genotype, as well as the C allele of c.-468T>G of the c.*83A>C increased the risk of NAFLD occurrence, whereas the A allele of mentioned SNP decreased the risk. In the case of the second SNP in LIG3, c.*50C>T, there were no differences between the groups.

Conclusions: The results suggest that one of the selected variants in the LIG3 modulates the risk of NAFLD. We may suspect that the other genes related to BER may have an impact on the development of the fatty liver disease.

Acknowledgments: This study was supported by financing from a scientific research grant from the National Science Centre of Poland (No. 2019/35/O/NZ5/02502).

Evaluation of evolutionary patterning as a pipeline for identification of amino acid positions involved in the generation of drug-resistance

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Background: Evolutionary patterning (EP) is a strategy to identify amino acid positions under the influence of natural selection. EP uses the ratio of non-synonymous (dN) to synonymous substitutions (dS) of codons to assess the level of selective pressure. Here, we evaluated EP as a method to indicate amino acids involved in the generation of drug resistance in bacteria. We searched for amino acids under positive selective pressure in nine drug-resistance-associated proteins of *Mycobacterium tuberculosis*.

Material and Methods: We used a bioinformatic database consisting of genomic sequences of 3798 strains of *M. tuberculosis* to quantify the dN/dS ratio in nine proteins previously associated with drug resistance. The significant departure of codon-specific nucleotide substitution rates was tested with four different methods: Single Likelihood Ancestor Counting (SLAC), Fixed Effects Likelihood (FEL), MEME (Mixed Effects Model of Evolution), and Fast Unconstrained Bayesian AppRoximation (FUBAR). We used $p \leq 0.08$ significance level to infer selection in FEL, SLAC, and MEME analyses, while in FUBAR the cutoff limit to infer selection was set to 0.9 posterior probability. We considered the position confirmed when it was identified by at least three out of four methods.

Results: We identified 16 amino acid positions under positive selective pressure in nine drug-resistance-associated genes. Ten positions were previously recognized in the literature as associated with drug resistance. Six positions were not previously identified. It remains to be established in laboratory studies whether these newly identified positions influence *M. tuberculosis* drug resistance.

Conclusions: EP is an effective alternative method to genome-wide association studies to identify clinically important aminoacid positions involved in the generation of drug resistance.

Acknowledgments: This work is part of the research project financed by the National Science Center of Poland, grant number 2019/34/E/NZ6/00221.

Expression of clinically relevant drug metabolizing enzymes in liver pathology

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Background: Drug metabolizing enzymes expressed in the liver play a very important role in drug biotransformation, and together with enzymes in the gastrointestinal tract are major determinants of bioavailability of orally administered pharmaceuticals and drug-drug interactions. The available pharmacokinetic information suggest deficient function of the enzymes in liver dysfunction states.

Material and methods: The CYPs (CYP1A1,CYP1A2, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, CYP3A4, CYP3A5) and UGTs (UGT1A1, UGT1A3, UGT2B7, UGT2B15) enzymes in patients with various forms of liver damage (hepatitis C, primary biliary cholangitis, primary sclerosing cholangitis, alcoholic liver disease and autoimmune hepatitis) were measured using quantitative reverse transcription polymerase chain reaction (rt-PCR) and liquid chromatography-tandem mass spectrometry (LC-MS/MS) methods.

Results: CYP2C9 and CYP2C19 remained stable in all liver pathological states, and in comparable levels to the controls. As for the UGTs, protein levels of UGT1A1, UGT1A3 and UGT2B15 were also not changed. Alcoholic liver disease and primary biliary cholangitis were involved in the most prominent changes in protein abundances, with downregulation of 6 (CYP1A2, CYP2C8, CYP2D6, CYP2E1, CYP3A4, UGT2B7) and 5 (CYP1A1, CYP2B6, CYP2C8, CYP2E1, CYP3A4) significantly downregulated enzymes, respectively.

Conclusions: Drug metabolizing enzymes protein abundance is affected by the type of liver pathology.

Acknowledgements: The study was funded a grant by the National Science Centre, Cracow, Poland UMO-2020/37/B/NZ7/01466.

Spectrophotometric characteristics of organic functionalized luminescent sensors and their biological application for selective detection of bio-particles

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Albumin is the most abundant protein of the blood serum playing the role of a transport protein, transporting many important compounds such as hormones, fatty acids, metal ions, amino acids and drugs. The ability of albumin to bind toxins in order to expel them from the body shows its weight in the process of keeping organs and tissues in good condition. Therefore, the search for simple and convenient methods of determining the content of albumins, especially with the use of fluorescent probes, is important in medical diagnostics.

The selection of an appropriate fluorescence technique for biosensor testing depends on the nature of the biosensor and its spectral properties. To characterize information signaled by a biosensor or an optical sensor based on the fluorescence phenomenon, several techniques are available: change of fluorescence intensity, FRET (Förster resonance energy transfer), FLIM (Fluorescence life time imaging), FCS (Fluorescence correlation spectroscopy). The present research focuses on the technique of changing the fluorescence intensity.

As part of this work, research was carried out on the non-covalent interaction of new fluorescent sensors that are derivatives of the 2-amino-4,6-diphenylpyridine-3-carbonitrile with biomolecules, which is closely related to searches focused on the development of efficient spectroscopic probes dedicated to biochemical systems. These studies included, among other, determining the effect of bovine serum albumin (BSA) concentration on the fluorescence of the test compounds, as well as the association of the pyridine sensor with BSA, and the changes in fluorescence over time. Due to the significant changes in the spectroscopic properties of the pyridine derivatives tested under the influence of the combination with the substance analyzed - BSA and calculated binding parameters, it was shown that the analyzed compounds may find practical use as fluorescent markers for the determination of biomacromolecules.

The present work was funded by the Ministry of Science and Higher Education (MNiSW) under the Diamond Grant project, contract number 0072/DIA/2020/49.

Stress and ketamine, bimodal impact on cognitive function

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Background: The glutamate NmethylDaspartate receptor (NMDAR) non-selective antagonist ketamine was recently repurposed as a rapidly acting antidepressant, catalysing the vigorous investigation of glutamatesignalling modulators as novel therapeutic agents for depressive disorders. The long-term effects of ketamine use have not been known, including the cognitive sphere. It is well known that prolonged exposure to stress induces depression and cognitive impairment.

In this study we tested this working hypothesis, is it possible that ketamine, used in prolonged-regimen in rats could alleviate some aspects of stress-evoked memory deficits?

Material and methods: Stressed (immobilization - 2 hours daily for 21 days) and non-stressed rats were treated with ketamine (4 mg kg-1, *s.c.*) for 21 days and next followed by a battery of behavioural tests: Morris water maze (MWM) and Barnes maze (BM)), stereotypy (stereotypy test - ST), locomotor functions (Open field - OF) and anxiety behaviour (Elevated plus maze - EPM).

Results: Stressed rats (6-weeks-old) displayed significant decline in the spatial working and reference memory. The effect of chronic ketamine administration depended on the type of test and differed between control rats and animals simultaneously exposed to chronic stress. While in MWM the impact is quite unequivocal, because we have observed an improvement in spatial memory in stressed animals, and a deterioration in animals not stressed after ketamine administration. In the BM, the effect of ketamine changes in successive attempts, from favourable in the initial period to the negative at the end of the test (72 hours later) in the group of animals stressed and without significant impact on the control animals. The data obtained from the auxiliary tests did not show the effect of administration (ketamine or solvent) or used procedures on loco-motor efficiency (OF) or the level of anxiety (EPM) or stereotypy behaviour (ST) in tested rats. The applied dose of ketamine proved to be effective in both groups of animals, exposed and not exposed to stress, as assessed in the stereotypy test.

Conclusion: Taken together, these findings demonstrate that ketamine potently abolishes or prevent some kinds of stress-induced memory impairments and cognitive decline in rats, but in some circumstances, it could even increase damage of memory, especially in unstressed animals.

Effects of genetic impairment of the noradrenergic system on the expression of inflammatory markers in the SN/VTA neurons of mice.

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Background: Parkinson's disease PD is characterized by the loss of dopaminergic (DA) neurons in the substantia nigra and ventral tegmental area (SN/VTA), however, the disorder affects also noradrenergic (NA) neurons as observed in the locus coeruleus (LC) of PD patients. Moreover, impaired NA input exacerbates the DA cells loss in rodent PD models. Therefore the aim of our study was to evaluate whether progressive degeneration of NA cells has impact on the expression of inflammatory cytokines in the SN/VTA as inflammation is one of the hallmarks of the neurodegenerative process.

Material and methods: Mutant animals were created by crossing DbhCre with TIF-IA$^{flox/flox}$ mice, which resulted in knock out of TIF-IA gene in all noradrenergic cells and their subsequent death. Mice were sacrificed at the age of 12 weeks, when most of the LC neurons where lost. Excised SN/VTA region was rapidly frozen at -80°C. Next we examined mRNA and protein expression of IL-1β, IL-6 and IL-10 using qPCR with TaqMan probes and Western Blot method, respectively. Additionally, we evaluated the level of inflammatory cytokines using Mouse Inflammation Antibody Array (RayBiotech) in the homogenates of SN/VTA tissue.

Results: qPCR studies revealed a 2-fold increase in the expression of IL10 mRNA in the mutant animals. Immunoblotting did not show any significant changes in the protein level of IL10 or IL1β; however, the IL6 level was decreased by 32%. To see how the mutation affects other cytokines we performed semi-quantitative protein array for 40 cytokines, which revealed increased levels of pro-inflammatory cytokines and chemokines, like: GM-CSF, IFNγ, IL-13, I-TAC, KC, LIX, MCP1, MIP1α or TECK. On the other hand, increased levels of neuroprotective proteins TIMP1 and TIMP2 in mutant vs control animals were found.

Conclusions: Lack of induction of IL1β or IL6 may suggest that the degeneration of NA neurons in TIF-IA^{DbhCre} mice evokes only weak inflammatory state in the SN/VTA, as shown by the increased level of other proinflammatory cytokines. Moreover, neuroprotective events such as increased expression of IL10 or increased level of tissue inhibitors of metalloproteinases (TIMP1, TIMP2) were also observed. More than 12 weeks of observation would be required to understand the influence of these cytokines on the survival of DA neurons of SN/VTA in TIF-IA^{DbhCre} mice, however, it was impossible due to short lifespan of these animals.

Acknowledgements: Supported by NCN2017/25/B/NZ7/02406.

CPL500036 – a novel PDE10A inhibitor induces molecular changes during L-DOPA induced dyskinesia in a 6-OHDA rat model of Parkinson's disease

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Background: Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by the loss of dopaminergic neurotransmission, mainly in the striatum. Unfortunately, following years of exposure to dopamine replacement therapy, levodopa causes motor complications such as levodopa-induced dyskinesias (LIDs). Phosphodiesterase 10A regulates cAMP/cGMP downstream signaling (e.g. cAMP/PKA/DARPP-32) thus having a key role in the regulation of dopaminergic signaling in the direct and indirect striatal pathways. We aimed to verify the molecular response to a novel PDE10A inhibitor, CPL500036.

Methods: Male Wistar rats (n=9) were stereotactically injected into the left SNc with solvent or 6-OHDA. Four weeks after lesion, all groups of rats were treated daily for 16 days with L-DOPA (6mg/kg, i.p.) and a peripheral DOPA decarboxylase inhibitor (6mg/kg, i.p.) and tested compound CPL500036 in doses of: 0,03, 0,1 and 0,3 mg/kg i.p. Two weeks after the last treatment with CPL500036, rats were decapitated and brain structures were dissected, separately for the intact and lesioned side. cAMP and cGMP, were assayed in striatal homogenates by HPLC. Primary antibodies against striatal proteins used were: phospho-ERK 1/2$^{(Thr202/Tyr204)}$, ERK 1/2, phospho-DARPP-32$^{(Thr34)}$, DARPP-32, phospho-Glur$^{(Ser845)}$, GluR1, phospho-MSK$^{(Ser376)}$, MSK, TH, PDE-10. Stain-Free technology was used for normalization. Data were expressed as mean ± SD. The significance of differences among groups was calculated by two-way ANOVA with a post-hoc Bonferroni test. A value of $p \leq 0.05$ was considered significant.

Results: No differences in striatal PDE10A expression in CPL500036 treated rat in both sides of brain were observed. The level of cAMP and cGMP increased significantly in animals treated with the highest dose of CPL500036 in the intact side of the brain compared to lesioned side. The changes of phosphorylation of GluR1$^{(Ser\ 845)}$ were observed in both hemispheres of the animals treated with the dose of 0.3 mg/kg of CPL500036. No changes were observed in protein phosphorylation of DARPP-32$^{(Thr34)}$ and MSK$^{(Ser376)}$ in the lesioned side compared to intact side. pERK$^{(Thr202/Tyr204)}$ remained unchanged in both sides.

Conclusion: The presented results suggest that the innovative PDE10A inhibitor – CPL500036 – may constitute a novel therapy in the treatment of LIDs in Parkinson's disease. Further studies are required to unveil its molecular mechanism of action in the treatment of dyskinesias.

Acknowledgements: The present study was co-financed by Celon Pharma SA and the National Centreof Research and Development (No. POIR.01.01.01-00-0617/20)

The problem of polypharmacy and evaluation of celecoxib stability in multi-drug mixtures

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Background: Polypharmacy is a big challenge for the people conducting the therapy. On the one hand it brings many benefits, but on the other hand - the risk of adverse interactions that may worsen the patient's condition. Medicinal agents are tested for changes in pharmacokinetics, metabolism or biotransformation under the influence of various factors, including other medicinal substances. During each therapy, one should also remember about possible modifications of the structure of the molecule under the influence of substances contained in another drug or dietary supplement. These chemical interactions may result in increased drug degradation resulting in decreased drug efficacy. Pain is a complex symptom accompanying many diseases, especially chronic ones. The selection of appropriate medications should take into account these diseases to avoid interactions with other medications taken by the patient.

Celecoxib was the first coxib to be introduced into human use. This group of selective COX-2 inhibitors shows much lower gastrotoxic activity compared to other NSAIDs. Coxibs are mainly used to treat rheumatoid arthritis and ankylosing spondylitis, and to treat post-operative pain.

Materials and method: The presented work covers the stability analysis of celecoxib in mixtures with other drugs, i.e. antibiotics, other analgesics and anti-inflammatory drugs, antihistamines, purine alkaloids. The analyzed substances in the form of prepared three-drug mixtures were exposed to various temperatures. Samples taken at intervals specified in the test plan were analyzed using the thin layer chromatography technique with densitometric detection. The analysis was carried out on TLC F254 plates, using the mixture with the following composition: chloroform + acetone + toluene (12: 5: 2, v / v / v) as mobile phase. Optimized separation conditions made it possible to obtain well-formed peaks originating from the tested substances, next to the peaks originating from possibly arising degradation products.

Results: Analyzing the obtained results, it can be noticed that celecoxib is degraded most quickly in the presence of: diclofenac and loratadine at 70 ° C and doxycycline and loratadine at 25 ° C. This process is usually faster at elevated temperatures. The above observations were additionally confirmed by calculating the values of the basic kinetic parameters (k, t0.5, t0.1), determined for each mixture.

Conclusions: Celecoxib is a fairly persistent drug, however its durability may depend on the presence of other drugs. The obtained results indicate the necessity of further research on this problem and the analysis of possibly formed reaction products.

Cerebral ischemia influences the expression of neuroprotective and pro-inflammatory microglia and apoptosis markers in the brain – preclinical study.

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Background: Stroke is the third leading cause of death and disability worldwide. The concept that stroke is a disorder solely of blood vessels has been expanded to include the effects of a detrimental interaction between microglia and neurons. Specifically, manipulating the glia response to stroke is under intense investigation. Therefore, we aimed to investigate the expression of the neuroprotective M2 and pro-inflammatory M1 microglia markers as well as caspase-3 level (apoptosis marker) in the frontal cortex, dorsal striatum (DSTR) and hippocampus in order to characterize the neuromolecular changes that can contribute to pathological changes in brain areas affected by ischemic stroke.

Materials and methods: In our experiment, we used Sprague-Dawley rats (280-320 g). The animals were randomly assigned to the following groups: control, sham surgery (SHAM), and 90-minute middle cerebral artery occlusion (MCAO). Three days after the stroke onset, all animals were decapitated, and their frontal cortex, dorsal striatum, and hippocampus were isolated. The level of the CD-206 - a neuroprotective microglia marker and CD-86 - a pro-inflammatory microglia marker as well as caspase-3 level was determined by the Western Blot analysis.

Results: The expression of the CD-86 increased in the dorsal striatum ($p<0.05$) of animals that underwent 90-minute middle cerebral artery occlusion. Contrary, CD-206 decreased in the dorsal striatum and hippocampus in the same group ($p<0.05$). No changes in caspase-3 level were observed.

Conclusions: To sum up, the observed changes suggest that three days after transient stroke onset, microglia are polarized to pro-inflammatory M1 and not to M2 phenotype in the DSTR. This fact indicates an increase of neuroinflammation and a decreased neuroprotection from M2 microglia. Surprisingly, the level of caspase-3 did not change in the MCAO group. The reason for this may be the lack of apoptosis at this time-point after stroke or - which seems more likely - the use of an antibody against the inactivated form of caspase-3. In the future, we plan to determine the level of cleaved caspase-3 and the panel of pro-inflammatory cytokines in order to characterize the inflammation occurring after MCAO. Moreover, we aim to test the anti-inflammatory activity of the new-synthesized compounds, which may help in recovery after ischemic stroke.

Acknowledgements: This research was funded by National Science Centre, Poland grant: 2018/30/E/NZ7/00247.

Maternal modified diets during pregnancy and lactation change the expression of ASD-related genes in the prefrontal cortex of adult offspring rats

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Background: Maternal nutrition plays a critical role in fetal brain development and function. Autism spectrum disorder (ASD) is a highly heritable and heterogeneous neurodevelopmental disorder, characterized by significant social, communication, emotional and behavioral challenges, often not allowing for normal life in society and reduced quality of life. In the 21st century, the number of people diagnosed with ASD is still growing. Therefore, searching for pathogenesis mechanisms and risk factors of this complex disease is very important. The present study aimed to determine the effects of maternal modified diets during pregnancy and lactation on young adult rat offspring by considering the alteration in the expression profile of ASD-related genes in the prefrontal cortex.

Material and methods: Wistar rat dams were fed either a standard diet (SD) or modified diets: high-fat – (HFD) high-carbohydrate – (rich in sucrose; HCD) or mixed – (MD) during the pregnancy and lactation periods. At weaning postnatal day (PND) 22, offspring were separated according to sex, and fed a SD. For molecular analysis, at PND 63, male and female offspring rats were sacrificed, and the prefrontal cortex was dissected according to the rat brain atlas. Next, the expression of 23 ASD-related genes was assessed using TaqMan Gene Expression Custom Array Cards.

Results: In young adult offspring (PND 63) exposure to maternal modified diets, we observed a different pattern of expression of the ASD-related genes compared to the control group. In males, a maternal HFD significantly increased the expression of *Mecp2* and *Setd1b*. In turn, exposure to HCD maternal induced a decrease in *Setd1b* expression, and a similar effect was observed in male MD offspring. Additionally, maternal MD reduced *Shank2* levels. In the case of females, all studied diets reduced the expression of *Pten* and *Fmr1*. The maternal HCD additionally decreased the level of *Itgb3* and *Setd1b*, while the maternal MD decreased *Taok2* expression.

Conclusions: Inconclusion,we demonstrated that maternal modified diets may disrupt the normal expression of ASD-related genes in the young adult offspring's prefrontal cortex.

Acknowledgements: Supported by research grant 2018/29/N/NZ7/02703 from the National Science Centre, Poland. K.G. is a recipient of the doctoral scholarship ETIUDA (2020/36/T/NZ7/00540) from the National Science Centre, Poland.

The effect of chronic treatment with atypical neuroleptic asenapine on the neuroendocrine regulation of liver cytochrome P450

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Background: Cytochrome P450 (CYP) is a major component of the mixed-function oxidase system that catalyzes the metabolism of endogenous (steroid hormones, neurosteroids, monoaminergic neurotransmitters) and exogenous substances (drugs, toxins, mutagens). Genes coding for cytochrome P450 are regulated by endogenous hormones such as growth hormone, corticosteroids, thyroid hormones and sex hormones. Asenapine is a novel atypical neuroleptic approved for the treatment of schizophrenia and bipolar disorders, which exerts its therapeutic effects *via* the dopaminergic and serotonergic systems.

Materials and methods: The experiment was carried out on male Wistar rats. Asenapine was administered subcutaneously (s.c) at a dose of 0.3 mg/kg for a period of two weeks. Afterwards, livers were removed and microsomes were prepared. The activity of CYP enzymes in liver microsomes was estimated by measurement of the rates of CYP enzyme-specific reaction by the HPLC method with UV detection. The protein level was assessed by Western Blot. The mRNA levels was calculated by a quantitative real-time PCR. The pituitary growth hormone-releasing hormone (GHRH) level and serum hormone levels were measured by ELISA. The obtained results were elaborated statistically using Student's *t*-test.

Results: The obtained results show that chronic treatment with asenapine exerts a significant effect on different CYP enzyme activity in the rat liver. Asenapine significantly decreased the activity of CYP2B, CYP2C11 and CYP3A (testosterone hydroxylation at positions 16β; 2α and 16α; 2β and 6β, respectively). The CYP2C11, CYP3A1 and CYP2B1 protein and respective mRNA levels were decreased. The protein and mRNA levels of CYP2B2, and CYP3A2 were not significantly changed. The ELISA test revealed a significant decrease in the pituitary GHRH level and serum concentration of corticosterone and growth hormone, and an increase in the thyroid hormone triiodothyronine (T_3). The concentrations of thyroxine (T_4) and interleukins (IL-2 and IL-6) were not significantly changed after chronic asenapine treatment

Conclusions: The presented results indicate that chronic treatment with asenapine down-regulates cytochrome P450 and may slow the metabolism of CYP2B, CYP2C11 and CYP3A substrates (steroids and/or drugs). The observed effects of asenapine on cytochrome P450 may be related to its pharmacological action on dopaminergic, adrenergic and serotonergic receptors (in particular on D_2, 5-HT_{1A}, 5-HT_{2C} and α_2 receptors), which affect the endocrine regulation of liver CYP enzymes and signaling pathways mediating enzyme expression in the liver. The obtained results also indicate the necessity of testing new neuroactive drugs for their interactions with cytochrome P450 not only *in vitro*, but also *in vivo*, which enables the observation of full spectrum of their mechanisms of action on cytochrome P450 expression and activity, taking place in the brain and peripheral organs (e.g., the liver), including neuroendocrine regulation of the enzyme.

Acknowledgements: Supported by grant Preludium 16 no. 2018/31/N/NZ7/01857 from the National Science Centre, Kraków, Poland and by statutory funds from the Maj Institute of Pharmacology, PAS, Kraków, Poland. Przemysław Danek acknowledges the support of InterDokMed project no. POWR. 03.02.00-00-I013/16.

Mephedrone pre-exposure during adolescence affects contextual fear conditioning: The impact of minocycline, a matrix metalloproteinase-9 inhibitor

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Background: Mephedrone, a cathinone derivative, is a psychoactive substance mostly abused in adolescence, and may have a negative impact on cognitive processes in young adults and adults. Matrix metalloproteinase-9 (MMP-9) is an enzyme that plays an important role in both the physiology and pathology of the central nervous system (CNS), including learning and memory. The aim of the study was to evaluate the effect of repeated administration of mephedrone in adolescence on fear learning and memory processes in young adult and adult rats. Furthermore, the role of MMP-9 in fear conditioning and memory retrieval was evaluated.

Material and Methods: Theexperiments were carried out in male Wistar rats. Mephedrone (5 mg/kg or 10 mg/kg, ip) was administrated three times a day for 7 consecutive days, between the 30th and 36th postnatal days (PND). Minocycline (45 mg/kg, ip) was given once daily before mephedrone administration. Afterwards, the animals were assigned to experimental groups. One was subjected to contextual fear conditioning (training) after 4 (PND 40) and the other after 19 (PND 55) days following drug administration. Next, rats from both groups were subjected to contextual fear memory tests: 1day (PND 41 or 56) and 7 (PND 47 or 62) days after training.

Results: Both young adult and adult mephedrone-treated animals show a lower freezing in response to conditioned context in a dose-dependent manner. However, adults were more disrupted by mephedrone in context conditioning than young adults. Minocycline pre-treatment reversed deleterious effects of mephedrone on fear learning and memory.

Conclusions: Mephedrone pre-exposure differently impacts the contextual (hippocampal dependent) fear conditioning procedure in young adult and adult rats. The effect of mephedrone is more pronounced in adult animals due to their lower adaptive phenotype plasticity. Minocycline, the MMP-9 inhibitor given before mephedrone administration prevents impairment of fear learning and memory in both young adult and adult animals. These data suggest the participation of this enzyme in the deleterious effects of mephedrone on fear learning and memory.

Acknowledgements: This work was supported by Grant No. 2017/25/B/NZ7/01845 from the National Science Centre, Poland.

The influence of benzophenone-2 on the process of rats spermatogenesis

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Background: For many years it has been believed that the compounds used as chemical UV filters may only have adverse effects in the skin, i.e. cause irritation or allergic contact dermatitis. Recently, studies have indicated a structural similarity of benzophenone derivatives to natural estrogen. Thus, these compounds can interfere with the endocrine system, by binding to estrogen receptors and by interfering with the synthesis and metabolism of natural estrogens. Estrogenic action is currently the main focus of research, concerning benzophenone derivatives.

Results: Hormonal disturbances of sex hormones (17β-estradiol, progesterone and testosterone) and thyroid hormones as well as the prolactin overproduction are the most common causes of reduced sperm production and quality. The impact of BP-2 on the sperm cells, obtained from the epididymis, was demonstrated by the assessment of their number, mobility and morphology. It was demonstrate decrease number of sperm cells and increase the number of abnormalities in morphology. It was also shown changes in mobility of sperm: an decrease number of sperm with progressive movement, but increase sperm mobile but without progressive movement or immobile. Because testosterone is essential in the process of spermatogenesis and the male fertility, it was determined in both the blood and in the testes. It was shown decrease level of testesterone (total and free fraction) measured in blood and testes. Since the normal production of male reproductive cells are also affected by other hormones such as: the 17β-estradiol, progesterone, the thyroid hormones (fT3 and fT4) and the prolactin, the concentration of these hormones was also determined in blood. It was shown increased level of 17β-estradiol, luteinizing hormone and thyroid hormones but level of others hormones wasn't changed.

Conclusions: Environmental pollution xenobiotics, including agents that disrupt the functions of sex hormones, may be an important reason for the increased risk of infertility. Exclusion or providing evidence of adverse effects of BP-2 will allow to assess whether there should be restrictions on the use of this compound.

Acknowledgements: Funding Grant from National Science Centre 2016/21/N/NZ7/03284.

Aversive learning deficits and depressive-like behaviors are accompanied by an increase in oxidative stress in a rat model of fetal alcohol spectrum disorders: Protective effect of rapamycin

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Background: Ethanol is well known for its teratogenic effects during fetal development. Prenatal ethanol exposure (PAE) leads to many physical, behavioral and cognitive abnormalities in children, known as Fetal Alcohol Syndrome Disorders (FASD). In adulthood, these disorders can be manifested by learning and memory deficits and depressive-like behavior Ethanol-induced oxidative stress may be one of the factors that induces FASD development. Recent reports have shown that the mammalian target of rapamycin (mTOR), signaling pathway that acts via two distinct multiprotein complexes, mTORC1 and mTORC2, can affect oxidative stress and participate in neurotoxic effects of ethanol. The aim of the present study was to investigate which of mechanism: mTORC1 and/or mTORC2 or the mTOR-independent process is involved in this phenomenon and may attenuate memory deficits and affect anxiety-like behavior in rats with FASD.

Material and Methods: The experiment was performed with male Wistar rats. Rats model of FASD were intragastrically intubated with ethanol (5g/kg, 22.66% v/v) over postnatal day (PND) 4 to 9 (an equivalent to the third trimester of human pregnancy). Rapamycin (selective inhibitor of mTORC1), Torin-2 (non-selective mTORC1/mTORC2 inhibitor) and FK-506 (inhibitor of oxidative stress in an mTOR-independent manner) were given intraperitoneally at the dose of 3mg/kg, 30 min before ethanol treatment. The passive avoidance (PA) task (aversive learning and memory) and forced swimming test (FST) (depressive-like behaviors) were conducted in adult (PND60-65) rats. Furthermore, the biochemical parameters of oxidative stress, such as lipid peroxidation (LPO), as well as AP-sites were determined in the hippocampus and prefrontal cortex in adult (PND65) rats.

Results: Our results show that neonatal ethanol exposure leads to deficits in fear learning and memory, depressive-like behavior and increased oxidative stress parameters in the hippocampus and prefrontal cortex in adult rats. The ethanol effects were normalized only by rapamycin pre-treatment.

Conclusions: Our results clearly show that mTORC1 inhibitors may be useful as a preventive therapy in the disorders connected with prenatal ethanol exposure.

Acknowledgements: This work was supported by the Statutory Funds of Medical University of Lublin (DS 22/19).

Analog series-based scaffolds: design and exploration towards mGlu7receptor putative activity

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[3] *SELVITA, Krakow, Poland. Introduction Analog series-based scaffolds: design and exploration towards mGlu7receptor putative activity*

Introduction: Recent literature evidence showed that the mGlu7 receptor gained attention as an attractive therapeutic target for a number of CNS disorders, including schizophrenia, depression, anxiety, post-traumatic stress disorders, Rett syndrome, epilepsy, autism, ADHD and addiction. However, the research on mGlu7 receptor biology was hindered, mainly due to the poor accessibility of ligands with good selectivity and drug-like properties. So far, only a few compounds which influence the mGlu7 receptor are known (Fig 1). Most of the described compounds do not demonstrate high potency and/or subtype selectivity, brain penetration, or oral bioavailability. Most recently discovered ligands VU6012962 and VU6012962 enhance the ability to perform key studies on mGlu7 receptor, however, there is still an urgent need to search for novel mGlu7 ligands that enable the development of the first drug candidate in this class of target. The development of a novel chemical scaffold possessing activity towards the mGlu7 receptor is the aim of the present study. So far, a variety of chemotypes were synthesized and examined in vitro.

Scaffold design: In our search for a new scaffold generation, the structures of the tool compounds available at that moment (MMPIP and ADX71743) were applied. The bioisosteric concept has become a key strategy in our campaign (chemotypes: A1-A12 and (M1-M5)), additionally, the Cresset field methodology was applied as computer-aided drug design (chemotypes: A13-A17) (Fig 2 and Fig 3). In the first approach of our study, various new chemical entities were synthesized having terminal group arrangements similar to that of the reference compounds (Fig 4).

Results: Among all synthesized library of the compound containing selected key substituents (Fig 4), only 3-methyl-2,6-diphenylquinazolin-4(3H)-one (ALX-063) and 2-(2-chlorophenyl)-3-methyl-6-phenyl quinazolin-4(3H)-one (ALX-065) belonging to chemotype A-8 exhibited week NAM activity towards mGlu7 receptor (IC50 = 6.5 and 4.65 μM, respectively). The compounds were assessed in vitro in a forskolin-stimulated cAMP assay in T-Rex-293 cells expressing human mGlu7 receptors. Compounds were tested in the PAM, NAM as well as agonist modes. The preliminary ADME properties are shown in Table below (KS –kinetic solubility in HHB buffer, MS- metabolic stability liver microsomes mouse (% remaining after 60 min)).

Summary: The designing of 22 new chemotypes and synthesis of various derivatives led to the identification of two hit compounds. The ALX-065 was slightly more active than unsubstituted analogue ALX-063. The presence of chlorine atom increased lipophilicity and decreased both solubility and stability. Although the new compounds showed weak activity and poor solubility, the metabolic stability of ALX-063 and ALX-065 was slightly higher than ADX71743. Further optimization of the hit structures are underway.

Acknowledgments: This study was partially supported by the project PBS1/B7/8/2012 financed by the National Centre for Research and Development (NCBiR).

5-HT6/D2 receptor ligands based on N-skatyltryptamine scaffold with procognitive and antipsychotic potential

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Background: Starting from the unsubstituted scaffold of N-skatyltryptamine, we put efforts to rationalize the ring substitution in order to increase the affinity for 5-HT6R, which was our primary target of interest, while also searching for derivatives with mixed receptor profiles such as 5-HT2A/5-HT6/D2R. Selective 5-HT6R antagonists have been shown previously to produce procognitive and promnesic effects in several rodent models. Administration of 5-HT6R agonists was more ambiguous – in naive animals they did not alter memory or produced slight amnesic effects, while in rodent models of memory impairment, they ameliorated the condition just like antagonists.

Material and methods: Halogen substitution was of particular interest, so monohalogenated derivatives were synthesized and their affinity for serotonin and dopamine receptors was determined. Intrisic function of compounds which exhibited the highest affinity for 5-HT6R and D2R were evaluated. Substitution patterns resulting in affinity/activity switches were studied using homology modeling. Chosen hits were screened to determine their metabolism, permeability and CYP inhibition. Two the most promising compounds 15 and 18, were tested in Novel Object Recognition test (NORT) and in their ability to influence hyperactivity induced by MK-801.

Results: Several D2 receptor antagonists with additional 5-HT6R antagonist and agonist properties were identified. Using the identified hit compounds 15 and 18, we tried to sort out the difference between ligands exhibiting D2R antagonist function combined with 5-HT6R agonism, and mixed D2/5-HT6R antagonists in murine models of psychosis. In NORT both compounds reversed memory impairment. None of the tested compounds at all the tested doses inhibited hyperactivity induced by MK-801.

Conclusions: The series of potent dual 5-HT6R/D2R ligands were synthesized. Due to the fact that lead compounds exhibited good permeability and reversed memory impairment at low doses they could serve as molecular probes for CNS disorders models.

Acknowledgements: This study was supported by the National Science Centre within grants SONATA 2014/15/D/NZ7/01782, OPUS13 2017/25/B/NZ7/02929 and by statutory funds from the Maj Institute of Pharmacology Polish Academy of Sciences.

4-Fluoro-5-iodo-3-(1-methyl-1H-imidazol-5-yl)-1H-indole (AGH-194): orally bioavailable, highly selective, low-basicity 5-HT$_7$R receptor agonist

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Background: The poor quality (low selectivity and/or metabolic stability and/or) of the existing agonist molecular probes has hampered the research on the physiological and patophysiological roles of the 5-HT$_7$ receptor. The low selectivity of the classical i.e. highly basic aminergic receptor agonists can be attributed to the common mechanism underlying their binding to the receptor. The discovery of low-basicity full agonists of the 5-HT$_7$R receptor enabled finding of the first truly selective, functionally potent brain penetrant agonist molecular probe.

Materials and methods: Techniques used: radioligand displacement for affinity determination, CAMP fluorescence assays, Western blot for downstream effects, LC-MS in pharmacokinetic study, perfussion for histopathology, acute oral toxicity in female rats, microsome incubation for metabolic stability, PAMPA, CACO-2,

Results: AGH-194 is a strong, water soluble 5-HT$_7$R agonist (Ki = 2nM). It was found unusually selective (PDSP panel). Compound was well tolerated in numerous behavioural studies up to 20 mg/kg, well tolerated after repeated dosage in rats (1mg/kg for ~180 days), did not block PGP, exhibited moderate metabolic stability (mouse, rat & human microsomes), weak hERG inhibition IC50 = 5µM (automated patch clamp), was a strong CYP3A4 binder. High permeability was found in CACO-2 and PAMPA models, which was confirmed in PK study. AGH-194 was found to produce potent analgesic effect in mouse models of neuropathic pain (CCI and STZ). Oral acute toxicity study revealed toxic effects at 300 and 2000 mg/kg

Conclusions: Athorough pharmacodynamic, functional, ADME and pharmacokinetic characterization of 4-fluoro-5-iodo-3-(1-methyl-1*H*-imidazol-5-yl)-1*H*-indole (AGH-194) has shown its superiority over the classical 5-HT$_7$R agonists. The preclinical development of AGH-194 was halted due to insufficient safety, however this compound can be regarded an optimal tool compound for in vitro and in vivo 5-HT$_7$R activation.

Acknowledgements: This study was supported by the National Science Centre within grant OPUS13 2017/25/B/NZ7/02929, The National Centre for Research and Development grant Serotonergic analgesic therapy based on 5-HT7 receptor agonist (STEP7), Foundation for Polish Science START programme and by statutory funds from the Maj Institute of Pharmacology Polish Academy of Sciences.

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