4th International Seminar on Behavioral Methods

08.09.2016 KATOWICE, 09-10.09.2016 WISŁA
POLAND

Various species - similar behaviors

More information available on the website: **www.behavioral-methods.pl**

Venues:

WORKSHOP 08.09.2016
Center for Experimental Medicine, Medical University of Silesia in Katowice, 4 Medyków St, 40-752 Katowice

SEMINAR 09-10.09.2016
Hotel Stok, Jawornik 52a, 43-460 Wisla

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Members: Marta Głowacka, Marta Grabowska, Aniela Grajoszek, Marta Nowacka, Anna Sługocka

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Under the auspices of Rector of the Medical University of Silesia in Katowice

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September 8th 2016. Thursday

I. Morning session: Workshop
Center for Experimental Medicine, Medical University of Silesia in Katowice, 4 Medyków St, 40-752 Katowice

Good practice in behavioral experiments for biomedical research; experiment design and procedure preparation; most commonly used behavioral tests; how to properly conduct experiment

10:00 a.m.-1:00 p.m.	**Paweł Boguszewski,** *Department of Neurophysiology, Nencki Institute of Experimental Biology, Warsaw, Poland*
1:00-2:00 p.m.	**Lunch**

II. Afternoon session: Workshop
Center for Experimental Medicine, Medical University of Silesia in Katowice, 4 Medyków St, 40-752 Katowice

Data collection and data analysis in a nutshell; frequent pitfalls, mistakes and errors - and how to avoid them

2:00-5:00 p.m.	**Albert Willemsen,** *M.Sc. Noldus Information Technology bv, Wageningen, Netherlands*
5:30 p.m.	Shuttle bus for workshop participants *(to Hotel STOK, Wisła)*
8:00 p.m.	**Welcome dinner -** *Hotel STOK - Wisła, 52 Jawornik St.*

September 9th 2016. Friday

I. Morning session.Rodent models I

Sexual behavior as a model in behavioral research; abnormal development of monoaminergic neurons implicated in mood fluctuations and bipolar disorder; predatory aggression and violence in rats; a mouse model for Down syndrome

9:00-9:30 a.m.	**Maria del Mar Dierssen Sotos,** *Centre for Genomic Regulation, Barcelona, Spain:* The challenge of understanding intellectual disability in Down syndrome
9:30-10:00 a.m.	**Claude Brodsky,** *Ben Gurion University, Negev, Israel:* Abnormal development of monoaminergic neurons is implicated in mood fluctuations and bipolar disorder
10:00-10:30 a.m.	**Christopher Pryce,** *Preclinical Laboratory for Translational Research into Affective Disorders (PLaTRAD), Switzerland:* Mouse models for the study of behavioural states corresponding to RDoC psychopathologies
10:45-11:00 a.m.	**Coffee break**

11:00-11:30 a.m.	**Michał Biały,** *Department of Experimental and Clinical Physiology, Medical University of Warsaw. Poland:* The socio-sexual activity of male rat as a model of appetitive behavior
11:30-12:00 a.m.	**Dick Jaarsma**, *Department of Neuroscience, Netherlands, Rotterdam:* An automated horizontal ladder for locomotion analysis in mice

I. Morning session. Neuropharmacology

Behavioral methods in pharmacological research on transgenic mice in models of neuronal diseases such as schizophrenia, anxiety disorders, autism; interaction between brain, gut and microbiome and how it applies to stress and immune-related disorders, including irritable bowel syndrome, obesity and neurodevelopmental disorders; metastasis and BBB

9:00-9:30 a.m.	**Michał Toborek**, *Miller School of Medicine, University of Miami:* Running interference: physical activity prevents methamphetamine-induced aberrant neurogenesis
9:30-10:00 a.m.	**Natalia Pawlas,** *Institute of Occupational Medicine and Environmental Health:* New psychoactive substances Nowe substancje psychoaktywne
10:00-10:30 a.m.	**John Oberdick,** *Department of Neuroscience, The Ohio State University:* Developing a preventive pharb macotherapy for neonatal abstinence syndrome
10:45-11:00 a.m.	**Coffee break**
11:00-11:30 a.m.	**Elena Wenzler,** *TSE-System Inc:* New Insights in Behavioral Phenotyping: tools and instrumentations
12:30-1:30 p.m.	**Lunch**
1:30-2:30 p.m.	**Poster session**

II. Afternoon session. Behavioral research in human and other primates

Cognitive abilities and communication in primates; eye-tracking methods in human; impact of exercise during pregnancy on the next generation; amygdala-orbitofrontal interactions in primates: approach-avoidance decision making; bilingual learning vs. brain plasticity

2:30-3:00 p.m.	**Bert Timmermans,** *School of Psychology, King's College Aberdeen. UK:* Dual interactive eye tracking with virtual avatars: Interaction dynamics predicting behavioral outcomes
3:00-3:30 p.m.	**Manuel Carreiras,** *Basque Center on Cognition, Brain and Language, Bilbao, Spain:* The bilingual brain
3:30-4:00 p.m.	**Alicja Niedźwiecka,** *Neurocognitive Development Lab, Faculty of Psychology, University of Warsaw, Poland:* Parent-infant interactions and infant cognitive development

4:15-4:30 p.m.	**Coffee break**
4:30-5:00 p.m.	**Przemyslaw Tomalski,** *Neurocognitive Development Lab and Developmental Psychology Unit, Faculty of Psychology, University of Warsaw, Poland:* Measuring and training attention control in infancy with eye-tracking
5:00-5:30 p.m.	**Hayley Ash,** *University of Stirling, UK*, The long-term impact of infant rearing background on the affective state of common marmosets (Callithrix jacchus)
5:30-6:30 p.m.	**Poster session**
7:00-8:00 p.m.	Special lecture: **Chris de Zeeuw,** *Erasmus University Rotterdam, Erasmus Medical Center (MC), Rotterdam, Netherlands:* Cerebellar Modules and Learning Rules
8:00 p.m.	**Barbecue evening**

September 10th 2016. Saturday

I. Morning session. Rodent models II

Vasopressin signaling and stress hormones in rats, pathophysiology of autism; neuroanatomical and neurochemical correlates of cognitive processing bias (such as optimism/pessimism) in animal models

9:00-9:30 a.m.	**Gernot Riedel,** *University of Aberdeen, Aberdeen:* Inter- versus intra-laboratory comparison of home cage behaviour
9:30-10:00 a.m.	**John Oberdick,** *Department of Neuroscience, The Ohio State University:* The L7 gene and control of emotional behaviors by the cerebellum
10:00-10:30 a.m.	**Karl Schilling,** I*nstitute of Anatomy, Rheinische Friedrich-Wilhelms-Universität, Bonn, Germany:* Quantifying and interpreting behavior: making sensible use of basic statistics
10:45-11:00 a.m.	**Coffee break**
11:00-11:30 a.m.	**Paweł Boguszewski,** *Department of Neurophysiology, Nencki Institute of Experimental Biology, Warsaw, Poland:* Contemporary social interaction paradigms in behavioral neuroscience
11:30-12:00 a.m.	**Ewelina Knapska,** *Nencki Institute of Experimental Biology, Warsaw, Poland:* How to study neuronal correlates of emotional contagion in rats?

I. Morning session. Neurorehabilitation

Drugs supporting rehabilitation after stroke; new therapy methods of hand disability after stroke; virtual reality in treatment of neurological diseases; OUN diseases: Alzheimer disease, Parkinson, multiple sclerosis

9:00-9:30 a.m.	**Jacek Jurkojć,** *Faculty of Biomedical Engineering, Silesian University of Technology, Poland:* Virtual reality as future of modern rehabilitation
9:30-10:00 a.m.	**Andrzej Małecki, I. Garbowska,** *The Academy of Physical Education in Katowice:* Rahabilitation of patients with central nervous system dysfunctions with multimedia and virtual reality
10:00-10:30 a.m.	**Iwona Sarzyńska-Długosz,** *2nd Department of Neurology, Neurorehabilitation Ward, Institute of Psychiatry and Neurology, Warsaw, Poland:* Multimodal sensory stimulation in severe disorders of consciousness
10:45-11:00 a.m.	**Coffee break**
11:00-11:30 a.m.	**Marek Łos,** *Dept. Clinical & Experimental Medicine, Integrative Regenerative Med. Center (IGEN), Linköping University Sweden:* Modern approaches of regenerative medicine - production of corneal limbal cells as an example
11:30-12:00 a.m.	**Tomasz Wolny,** *Katedra Kinezyterapii i Metod Specjalnych Fizjoterapii, Akademia Wychowania Fizycznego im. Jerzego Kukuczki w Katowicach:* Wpływ długotrwałego spożywania środków psychoaktywnych na rozwój czuciowej neuropatii obwodowej – doniesienie wstępne.
12:30-1:30 p.m.	**Lunch**
1:30-2:30 p.m.	**Poster session**

II. Afternoon session. Noah's Ark

Cognitive abilities and affective states in flock; personality of horses; the origin of cognitive patterns in bears; spatial behavior and learning in reptiles; communication abilities and self-awareness in elephants and dolphins; cognitive abilities and intelligence in fish; vision in Drosophila

2:30-3:00 p.m.	**Anna Wilkinson,** *Univeristy of Lincoln, UK:* Cold-blooded cognition comparisons across classes.
3:00-3:30 p.m.	**Jennifer Vonk,** *Oakland Univeristy, USA:* Bear-ly scratching the surface of Bear Cognition.
3:30-4:00 p.m.	**Alex Mauss,** *Max Planck Institute of Neurobiology, Martinsried, Germany:* Neural mechanisms underlying flow-field selectivity and visually guided course control in "Drosophila"
4:15-5:00 p.m.	**Poster session**
5:00 p.m.	**Good-bye meeting with snacks**

LECTURE SESSION

L1. THE LONG-TERM IMPACT OF INFANT REARING BACKGROUND ON THE AFFECTIVE STATE OF COMMON MARMOSETS (CALLITHRIX JACCHUS)

Ash H

Behaviour and Evolution Research Group, University of Stirling, Stirling, UK

Early life environment, including family separation, can have a major influence on cognition and behaviour, increasing the risk of anxiety or depression-like symptoms. We compared the performance of family reared and supplementary-fed common marmosets (*Callithrix jacchus*) in a battery of measures, including a novel cognitive bias task for use in non-human primates. No significant differences between rearing conditions were found in responses to human or novel object tests, suggesting no effect on neophobia. There were no differences in cognitive bias task acquisition time, and only minor differences in response to the probes, with supplementary-fed marmosets demonstrating a mildly reduced expectation of reward. Similarly, in a 2 bottle preference test for anhedonia, no difference was found between rearing conditions in consumption of appetitive milkshake, although supplementary-fed triplets showed a very small reduction of interest in reward at the lowest concentration. This consistent pattern of results suggests that the supplementary feeding at this facility did not have a major effect on learning and affective state in adulthood. Therefore, while family separation is not recommended, this particular practice should be used if it is necessary, to promote the welfare of the animals.

L2. THE SOCIO-SEXUAL ACTIVITY OF MALE RAT AS A MODEL OF APPETITIVE BEHAVIOR

Bialy M

Department of Experimental and Clinical Physiology, The Medical University of Warsaw, Warsaw, Poland

The sexual behavior of male rats is appetitive behavior strongly related to social interaction and cues from other rats. At least five independent factors regulate male sexual activity: anticipatory, initiation copulatory efficiency, number of intromission and hit (intromission) ratio. Even such processes like penis erections are regulate by different hormonal and neuronal networks at different behavioral context (copulation, noncontact erections or ex copula erections). These factors and sexual activity were analyzed in relation to neuronal networks, as well as hormonal and pharmacological background of arousal, motivation, learning and memory of emotional state, social interaction, and expression of emotion, activation of rewarding system at the physiology and pathophysiology (depression, autism, addiction, cardiovascular diseases or diabetes). Also sexual interactions give new windows to understand of the role of ultrasonic vocalizations emitted by rats. Our new results show, that postejaculatory 22-kHz vocalizations reflect expression of positive emotion during relaxation state.

L3. CONTEMPORARY SOCIAL INTERACTION PARADIGMS IN BEHAVIORAL NEUROSCIENCE

Boguszewski PM

Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland

Analysis of animal behavior is one of the fundamental tools used by behavioral neuroscientists. There are many experimental approaches based on observation of single animal, usually exploring artificial environment or stimuli and performing forced tasks with limited set of responses measured. The alternative approach implies using the social interaction paradigms. There are numerous advantages of this concept. A major implies taking benefit of natural stimulus, i.e., presence of another conspecific animal(s), in opposite to artificial objects or elaborated tasks. Also, natural repertoire of animals reactions can be measured as dependent variable. Various paradigms of social interaction in laboratory rodents have been successfully developed and contributed strongly to investigation and identification of neuronal mechanisms of aggression, learning and memory, social defeat, stress, anxiety, individual differences in emotional reactivity and effects of psychoactive drugs. This unique ecological relevance and flexibility contributed to growing popularity of social interaction paradigms in past 10 years. It must be mentioned however, that such studies, due to its involvement of multiple animals, complexity and multidimensionality, present the researcher with challenges in experiment design, measurement techniques and data analysis. In my lecture I would review the latest achievements, newest methods and difficulties to be faced with behavioral tests based on social interactions.

L4. ABNORMAL DEVELOPMENT OF MONOAMINERGIC NEURONS IS IMPLICATED IN MOOD FLUCTUATIONS AND BIPOLAR DISORDER

Brodsky C

Department of Physiology and Cell Biology, Ben Gurion University, Negev, Israel

Subtle mood fluctuations are normal emotional experiences, whereas drastic mood swings can be a manifestation of bipolar disorder (BPD). Despite their

importance for normal and pathological behavior, the mechanisms underlying endogenous mood instability are largely unknown. During embryogenesis, the transcription factor Otx2 orchestrates the genetic networks directing the specification of dopaminergic (DA) and serotonergic (5-HT) neurons. Here we behaviorally phenotyped mouse mutants overexpressing Otx2 in the hindbrain, resulting in an increased number of DA neurons and a decreased number of 5-HT neurons in both developing and mature animals. Over the course of 1 month, control animals exhibited stable locomotor activity in their home cages, whereas mutants showed extended periods of elevated or decreased activity relative to their individual average. Additional behavioral paradigms, testing for manic- and depressive-like behavior, demonstrated that mutants showed an increase in intra-individual fluctuations in locomotor activity, habituation, risk-taking behavioral parameters, social interaction, and hedonic-like behavior. Olanzapine, lithium, and carbamazepine ameliorated the behavioral alterations of the mutants, as did the mixed serotonin receptor agonist quipazine and the specific 5-HT2C receptor agonist CP-809101. Testing the relevance of the genetic networks specifying monoaminergic neurons for BPD in humans, we applied an interval-based enrichment analysis tool for genome-wide association studies. We observed that the genes specifying DA and 5-HT neurons exhibit a significant level of aggregated association with BPD but not with schizophrenia or major depressive disorder. The results of our translational study suggest that aberrant development of monoaminergic neurons leads to mood fluctuations and may be associated with BPD.

L5. CEREBELLAR MODULES AND LEARNING RULES

De Zeeuw CI

Departament of Neuroscience, Erasmus MC, Rotterdam, Netherlands; Netherlands Institute for Neuroscience, Royal Dutch Academy of Arts & Sciences, Amsterdam, Netherlands

The olivocerebellar system can be divided into modules that control a particular domain in motor and/or cognitive processing. Each module integrates the activity of Purkinje cells in the cerebellar cortex, cerebellar nuclei neurons and neurons in the inferior olive. Interestingly, the modules can be divided in at least two main types, characterized by the presence or absence of zebrin. Those that do and do not express zebrin show an intrinsic average firing frequency of approximately 60 and 90 Hz, respectively. In this lecture I will highlight the impact of this organization on the rules that guide cerebellar learning. Whereas the zebrin-positive zones, such as those controlling adaptation of the vestibulo-ocular reflex, appear to engage plasticity mechanisms that increase simple spike firing frequency during learning, the zebrin-negative zones, such as those controlling Pavlovian eyeblink conditioning, seem to trigger mechanisms that suppress simple spike activity. These different cerebellar cortical mechanisms will also induce different processes downstream in the vestibular and cerebellar nuclei, including slower tonic changes and faster rebound activity, respectively. Finally, the different intrinsic activity within the olivocerebellar modules will also affect the pathogenesis of cerebellar diseases, with Purkinje cell death occurring most prominently in zebrin-negative zones presumably reflecting enhanced cytotoxicity.

L6. THE CHALLENGE OF UNDERSTANDING INTELLECTUAL DISABILITY IN DOWN SYNDROME

del Mar Dierssen Sotos M

Cellular & Systems Neurobiology, Center for Genomic Regulation, Barcelona, Spain

Down syndrome (DS) is the most common genetic form of intellectual disability, with an estimated incidence of more than 200,000 cases per year worldwide. In DS brain, suboptimal network architecture and altered synaptic communication arising from neurodevelopmental impairment are key determinants of cognitive defects. Abnormal number, size or shape of dendrites and dendritic spines has been described in Down syndrome (DS) that correlate with learning and memory impairment. While many labs, including ours, have studied the brain structural alterations of different intellectual disability disorders, almost no information exists on how those lead to intellectual disability. Interestingly, either increasing or decreasing the number of dendritic spines (that act as neuronal connectors) will have detrimental effects on cognition. Regardless of their molecular cause, most intellectual disabilities are characterized by neural plasticity disruption, and therefore this is a natural target for therapeutic purposes. Our group has demonstrated that epigallocatechin-3-gallate (EGCG), the most abundant catechin of green tea, promotes learning and memory recovery, and produces extensive dendritic remodeling in DS mouse models and significantly improves memory, executive functions and adaptive behavior along with increased functional connectivity in specific brain regions of DS adults (Phase I and Phase II clinical trials). This has been a crucial step in treating intellectual disability that has opened new important questions.

L7. AN AUTOMATED HORIZONTAL LADDER FOR LOCOMOTION ANALYSIS IN MICE

Jaarsma D

Department of Neuroscience, Erasmus MC, Rotterdam, Netherlands

A variety of tests have been designed to examine motor function in mouse models of neurological and psychiatric disorder. Quantitative analysis of locomotion with automated gait analysis devices provide new opportunities to measure disease progression and evaluate the effect of interventional approaches. A recently developed automated horizontal ladder allows the study of several gait parameters, including step time, step length, slips and interlimb coordination, as well as aspects of motivation and avoidance behavior. The apparatus has been used in cross-sectional studies as a 'phenotyper' of different types of cerebellar mutants. We have found that the ladder is a powerful tool for monitoring disease course in mouse models of progressive adult-onset cerebellar disease. In addition, the apparatus uncovered unique locomotor patterns in non-cerebellar mouse models further validating the ladder as a 'phenotyper' tool in genetic screens. The Erasmus ladder remains to be evaluated and standardized across different laboratories.

L8. HOW TO STUDY NEURONAL CORRELATES OF EMOTIONAL CONTAGION IN RATS?

Knapska E

Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland

Emotional contagion means sharing negative or positive emotional states between individuals. It is considered the simplest form of empathy, from which more elaborated forms evolved. Tuning one's emotional state to that of another increases the probability of similar behavior, which thereby allows for a rapid adaptation to environmental challenges. Emotional contagion, commonly observed in animals, including rodents, is well described at the behavioral level, but the neuronal circuits necessary for sharing emotions are not well understood. To study role of neuronal circuits involved in this phenomenon, we need simple animal models. We have developed such models in rats for both negative and positive social emotions. In these models, rats either interact with a fearful partner, observe a partner in danger or are reunited after social isolation. We showed that such models can be used to study the role of neuronal circuits in the amygdala in control of socially transferred emotions. To manipulate activity of neurons involved during social interaction, we use c-fos-driven expression of light-activated opsins that are expressed only in neurons that were activated by social interaction. Then, by optogenetic activation and inhibition of these neurons combined with observations of the resulting behavioral changes, we can draw conclusions about the function of the neurons activated in different parts of the amygdala by social interaction. We hope that the methods we have developed will help to shed some light on mechanisms of social behaviors, which are still largely unknown.

L9. MODERN APPROACHES OF REGENERATIVE MEDICINE – PRODUCTION OF CORNEAL LIMBAL CELLS AS AN EXAMPLE

Łos M

Department of Clinical & Experimental Medicine, Integrative Regenerative Med. Center (IGEN), Linköping University, Linköping, Sweden

Current regenerative medicine technologies allow for in vitro-production of organs and tissues and their therapeutic replacement. Modern techniques offer a vide range of natural and synthetic biomaterial that fulfill the biomechanical requirements of tissue scaffolds. The cellular component of tissues and organs is typically individually made from recipient's own cells, so that the histocompatibility is perfectly matched (isografty-type transplantation). The corneal epithelium is maintained by a small pool of tissue stem cells located at the limbus. Through certain injuries or diseases this pool of stem cells may get depleted. This leads to visual impairment. Standard treatment options include autologous or allogeneic limbal stem cell (LSC) transplantation, however graft rejection and chronic inflammation lowers the success rate over long time. Transdifferentiation technology as well as induced pluripotent stem (iPS) cells have opened new possibilities for treating various diseases with patient specific cells, eliminating the risk of immune rejection. In recent years, several protocols have been developed, aimed at the differentiation of iPS cells into the corneal epithelial lineage by mimicking the environmental niche of limbal stem cells. However, the risk of teratoma formation associated with the use of iPS cells hinders most applications from lab into clinics. We have optimized the protocol for the differentiation of iPS cells into corneal epithelial cells. Such obtained cells express corneal epithelial markers showing a successful differentiation, however the process is long and the level of gene expression for the pluripotency markers does not vanish completely. Therefore we have also developed a direct transdifferentiation approach to circumvent the intermediate state of pluripotency (iPS-stage). The resulting cells,

obtained by direct transdifferentiation of fibroblasts into limbal cells, exhibited corneal epithelial cell morphology and expressed corneal epithelial markers. The transdifferentiation protocol appears to work much more efficiently than reprogramming with the subsequent differentiation into corneal limbal cells. Direct transdifferentiation of human dermal fibroblasts into the corneal epithelial lineage may serve as source for corneal epithelial cells for transplantation approaches.

L10. NEURAL MECHANISMS UNDERLYING FLOW-FIELD SELECTIVITY AND VISUALLY GUIDED COURSE CONTROL IN DROSOPHILA

Mauss A
Max Planck Institute of Neurobiology, Martinsried, Germany

We perceive many aspects of our visual environment effortlessly yet this requires sophisticated signal processing in complicated brain circuits. A vital source of information is provided by visual motion, which is encoded in the temporal sequence of signals across the retina. It is commonly thought that retinal image slip is used by animals to counteract unintended body movements. In line with this view, many animals turn syn-directionally with moving patterns, termed "optomotor response". Work in the past decades in a variety of animals has identified wide-field motion-sensitive neurons potentially important in this context. However, due to complex and distributed circuitry especially in vertebrates, it has been challenging to determine how they obtain their flow-field selectivity and how their activity is causally related to optomotor behavior.

Apart from vertebrates, arthropods represent another major animal group with sophisticated visual systems. Especially the fruit fly Drosophila has emerged as a fascinating model system: first, it offers an extensive genetic tool box developed over many decades enabling us to visualize and manipulate neurons selectively in many ways; second, neurons are generally uniquely identifiable, allowing their unequivocal comparison across experimental conditions, which greatly facilitates data interpretation; and third, the Drosophila brain is of moderate numerical complexity, holding promise of reaching an integrative understanding of brain function at all levels, from sensory perception to motor action, in a single species.

I will focus on how wide-field motion-sensitive neurons in Drosophila obtain their response properties. One important presynaptic element are cholinergic local motion sensors, so-called T4/T5 cells, which carry retinotopic excitatory signals of the same directional preference to wide-field cells. In addition, we have identified a new local cell type receiving oppositely tuned T4/T5 input and conveying it to wide-field cells via glutamatergic inhibitory synapses. These two synaptic inputs give rise to motion-opponent responses in wide-field cells, i.e. preferred direction excitation and anti-preferred direction inhibition. Our neurogenetic silencing experiments suggest that such motion opponent subtraction, as observed in many species, is essential to ensure flow-field selectivity. I will further describe our ongoing efforts to define the causal roles of wide-field motion sensors in visually guided behavior.

L11. PARENT-INFANT INTERACTIONS AND INFANT COGNITIVE DEVELOPMENT

Niedźwiecka A
Neurocognitive Development Lab, Faculty of Psychology, University of Warsaw, Warsaw, Poland

The quality of parent-infant interactions during the first year of life affects long-term developmental outcomes. By interacting with their parents, infants learn how to regulate their attention, emotion and behavior. I will outline the main approaches to the analysis of parent-infant interactions and discuss their methodological constraints. I will present results of studies demonstrating the associations between interactive behaviors in parent-infant dyads and infant cognitive development in low-risk infants and in infants at risk for autism. I will conclude by presenting prospective studies using recurrence quantification analysis of gaze and movement data obtained during parent-infant play.

L12. DEVELOPING A PREVENTIVE PHARMACOTHERAPY FOR NEONATAL ABSTINENCE SYNDROME

Oberdick J
Department of Neuroscience, The Ohio State University, Columbus, USA

Prolonged fetal exposure to opioids results in neonatal abstinence syndrome (NAS), a major medical problem requiring intensive care and increased hospitalization times for NAS newborns. Using mass spectrometry our lab has recently shown that 6β-naltrexol (6BN), a mu opioid antagonist, can cross the placenta and enter the fetal brain of mice, while it is relatively excluded from the maternal brain by virtue of the blood brain barrier (BBB). In this same study we showed that the BBB of mice remains undeveloped until after postnatal day 14. This finding has allowed

us to use the postnatal mouse model to show that in the absence of the BBB, co-administration of 6BN with morphine can prevent morphine-induced withdrawal behaviors at extremely low doses. These data suggest that 6BN or derivatives could be co-delivered with opioids during pregnancy and used to prevent NAS at doses that should, in principle, have no effect on maternal pain and/or opioid maintenance therapy. In addition, 6BN is the main metabolite of naltrexone in humans, which has been approved for treatment of alcoholism. Therefore, we expect that the safety profile of 6BN favors its rapid development as a NAS pharmacotherapy.

L13. THE L7 GENE AND CONTROL OF EMOTIONAL BEHAVIORS BY THE CEREBELLUM

Oberdick J

Department of Neuroscience, The Ohio State University, Columbus, USA

The primary functions of the cerebellum are i) motor coordination, achieved by integration of sensory input with motor output, and ii) motor learning. Nevertheless, many studies both in humans and in animals have suggested non-motor functions of the cerebellum primarily in the cognitive and emotional realms. Genetic analysis of the cerebellar role in non-motor behaviors is complicated by the fact that inactivation of most genes relevant to cerebellar function results in motor defects, which can obscure the scoring of non-motor behaviors, and in addition very few genes are expressed exclusively in the cerebellum necessitating complex conditional approaches for cerebellum-specific gene inactivation. The L7 (Pcp2, Gpsm4) gene encodes a bidirectional modulator of Gi/oPCR's (GPCR's that signal through the Gi/o subfamily of Galpha heterotrimeric subunits). These receptors, when activated, act to activate GIRK-type potassium channels and inhibit voltage-dependent calcium channels thereby damping electrical activity of neurons. L7 has the unique property of highly restricted expression in cerebellar Purkinje cells. When the gene is globally inactivated in mice there are no observable motor coordination defects; rather there are significant changes in locomotion-based anxiety behaviors and the dynamics of tone-cued fear conditioning. Oddly these changes are sexually dimorphic. We also have shown that L7 is a direct transcriptional target of the autism gene, RORA, which encodes a transcription factor in the nuclear receptor superfamily. Based on these studies we suggest that L7 and the Gi/o pathway, and by implication both α2-adrenergic and selected serotonin receptors in Purkinje cells, are important components of an emotional control function of the cerebellum.

L14. RUNNING INTERFERENCE: PHYSICAL ACTIVITY PREVENTS METHAMPHETAMINE-INDUCED ABERRANT NEUROGENESIS

Park M[1], Levine H[1], Toborek M[1,2]

[1] Department of Biochemistry and Molecular Biology, University of Miami School of Medicine, Miami, FL, USA, [2] The Jerzy Kukuczka Academy of Physical Education, Katowice, Poland

While no effective therapy is available for the treatment of methamphetamine (METH)-induced neurotoxicity and cognitive dysfunction, behavioral interventions, including aerobic exercise, are being proposed to improve depressive symptoms and substance abuse outcomes. The present study focuses on the effect of exercise on METH-induced aberrant neurogenesis in the hippocampal dentate gyrus (DG) in the context of the blood-brain barrier (BBB) pathology. Mice were administered with METH or saline (vehicle) by i.p. injections three times per day for 5 days with an escalating dose regimen in 4 h intervals, starting from 0.2 mg/kg. One set of mice was sacrificed 24 h post last injection of METH, and the remaining animals were either subjected to voluntary wheel running (exercised mice) or remained in sedentary housing (the sedentary group). METH administration resulted in decreased expression of tight junction (TJ) proteins and increased BBB permeability in the hippocampus. These changes were preserved post METH administration in sedentary mice and were associated with the development of significant aberrations of neural differentiation. Exercise protected against these effects by enhancing the protein expression of TJ proteins, stabilizing the BBB integrity, and enhancing differentiation of progenitor cells to neuronal lineage. In addition, exercise protected against METH-induced systemic increase in inflammatory cytokine levels. These results suggest that exercise can attenuate METH-induced neurotoxicity by protecting against the BBB disruption and related microenvironmental changes in the hippocampus. This work was supported, in whole or in part, by National Institutes of Health Grants DA039576, DA027569, HL126559, MH098891, MH072567, and NSC 2015/17/B/NZ7/02985.

L15. NEW PSYCHOACTIVE SUBSTANCES

Pawlas N

Institute of Occupational Medicine and Environmental Health, Sosnowiec, Poland

Recently we witness of development of large amount of new psychoactive substances. They are known as designer drugs, legal highs, herbal highs, party pills, boosters and smart drugs. They are defined as

"A new psychoactive substance (NPS) is defined as a new narcotic or psychotropic drug, in pure form or in preparation, that is not controlled by the United Nations drug conventions, but which may pose a public health threat comparable to that posed by substances listed in these conventions" according to European Monitoring Centre for Drugs and Drug Addiction. In Poland it appeared to be a problem in 2008, but in 2015 we observed and enormous amount of poisoning, including lethal ones. Most of those new psychoactive substances consists of phenylethamines – their chemical structure is similar to amphetamines and their derivatives and synthetic cannabinoids. There is a large number of web pages presenting the recipes for home synthesis as well as impressions of users. The legislation process in Poland is inefficient in banning the production and marketing. Users may become addicted to those substances very easily and fast due to their properties. The review of current NPSs, their mechanisms of action will be presented.

L16. MOUSE MODELS FOR THE STUDY OF BEHAVIOURAL STATES CORRESPONDING TO RDOC PSYCHOPATHOLOGIES

Pryce C

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The research domain criteria (RDoC) project, a new framework for classifying mental disorders based on observable behaviours and neurological measures, has provided fresh impetus for animal model research. Domains-dimensions include: negative valence e.g. fear, anxiety; positive valence e.g. effortful motivation for reward, reward learning; cognitive systems e.g. control; systems for social processes e.g. social affiliation and communication; and arousal/modulatory systems e.g. biological rhythms. These behavioural dimensions can be quantified in humans, and they also provide the opportunity to establish objective, translational tests in mice. For negative valence in mice, in collaboration with TSE we developed the Multi Conditioning system, which allows for the simultaneous testing of motor activity, Pavlovian fear learning/memory, anxiety, and active avoidance, escape and loss of control. For positive valence in mice, we have applied TSE operant chambers and IntelliCage to establish tests of effortful reward motivation, learned non-reward, probabilistic reversal learning, and circadian distribution of reward motivation. With respect to the regulation of these translational behavioural dimensions, we used pharmacological depletion of nucleus accumbens dopamine to demonstrate its importance in the regulation of effortful operant responding for: reward, a stimulus recently associated with non-reward, and to escape footshock recently experienced as uncontrollable. That is, accumbens dopamine depletion results in motivation pathologies under effortful conditions. Interestingly, chronic psychosocial stress resulted in these same motivation pathologies, as well as leading to increased Pavlovian fear learning and decreased reward motivation in the home cage. Chronic psychosocial stress induced immune-inflammation, including in the dopamine system, and its behavioural effects could be reduced (but not entirely reversed) by the antidepressants fluoxetine and agomelatine, the dopamine reuptake inhibitor vanoxerine, and an inhibitor of indoleamine 2,3-dioxygenase.

L17. INTER- VERSUS INTRA-LABORATORY COMPARISON OF HOME CAGE BEHAVIOUR

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Preclinical and especially behavioural neuroscience is under increasing scrutiny due to the growing perception of irreproducibility of recorded and published data. The recent summary of Michael Jarvis and Michael Williams in TINS (2016) is the recent summary of a multitude of reports and efforts to improve this issue many of which have concentrated on the improvement and standardisation of reporting practices. A critical issue is the question of time during which experiments are conducted and whether it is appropriate for rodent experiments to be undertaken in the light phase of the cycle when rodents are typically asleep. While this typically applies to tests conducted in recording units different from the home cage, one would predict that independent whether a normal or inverted circadian rhythm is run in the holding unit, home cage observations should generate reproducible results. Experiments here describe a binary approach, comparing circadian activity between two laboratories running on an inverted day/night cycle followed by a comparison of normal versus inverted cycle home cage activity within the same animal unit.

While the overall circadian activity was reproduced in both laboratories there were considerable differences in the absolute levels of activity for C57BL/6JOlaHsd and DBA/2OlaHsd mice. Even the comparison between normal and inverted cycle within the same facility returned several anomalies in activity levels, which counter the expectation that harmonisation of experiments can achieve identical experimental outcomes. Here, we purposefully maintained identical

study designs and apart from small differences in feeding and standard holding regimes, this standardisation was not sufficient to produce identical data. We feel that one of the more important factors, the global environment of the test facility, work surrounding the test rooms etc. are of greater importance if one wants to increase robustness of experimental results within and between laboratories.

L18. MULTIMODAL SENSORY STIMULATION IN SEVERE DISORDERS OF CONSCIOUSNESS

Sarzyńska-Długosz I

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Survival and the outcome of patients in severe disorders of consciousness have improved significantly over the last two decades. Adequate medical care and neurorehabiltiation interventions are mandatory to cope with severe disorders of consciousness such as vegetative state/unresponsive wakefullness syndrome or minimally conscious state and to help promote the recovery. Treatment in persons in vegetative state or minimally conscious state remains very much empirical at present, however the principles and basic techniques of multisensory stimulation – especially the general rules of visual, auditory, smell, taste, touch, movement and position stimulation will be discussed. Recent research developments, scientific evidence of benefit of pharmacotherapy and neural stimulation and future directions for research in severe disorders of consciousness will be presented.

L19. QUANTIFYING AND INTERPRETING BEHAVIOR: MAKING SENSIBLE USE OF BASIC STATISTICS

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In several behavioral tests, subjects are confronted with a limited set of predefined choices they may make. Preferences are then quantified by counting how often a given choice is taken, or measuring the time spent on that choice. The sensible interpretation of such data requires to consider how alternatives were valued. This relative perspective implies that individual measurements are highly correlated; in technical parlance, such data are referred to as compositional.

This special data structure has important consequences on how measurements may be best analyzed and interpreted: Specifically, the fact that we do not deal with absolute, independent measures precludes their efficient and reliable analysis with standard parametric methods such as t-tests or ANOVA - which nonetheless are typically applied to them.

I will briefly sketch, from a biomedical perspective, and using widely familiar examples, the problems inherent in traditional statistical approaches to data resultant from limited choice behavioral experiments, and I will outline methodology and procedures appropriate for such data. The methods presented have a solid theoretical footing, and they are widely used in fields such as geology or social sciences, which routinely deal with data of compositional structure. Lastly, they are readily implemented in standard (and free) statistical software, notably R, the lingua franca of modern statistics. A key advantage of the approach suggested is that it takes advantage of, and allows to probe, the intrinsically multivariate structure of the data. Consequently, it entails a much more efficient use and interpretability of the data than traditional approaches.

L20. DUAL INTERACTIVE EYE TRACKING WITH VIRTUAL AVATARS: INTERACTION DYNAMICS PREDICTING BEHAVIORAL OUTCOMES

Timmermans B

School of Psychology, King's College, Aberdeen, UK

Recent interactionist views on social cognition suggest that the most under-studied and important aspect of social cognition may be interaction dynamics. However, it has hitherto proven extremely difficult to devise a controlled setup in which social cues, such as eye gaze, are subject to unconstrained interaction.

To address these issues, we use a Dual interactive eye-tracking with Virtual anthropomorphic Avatars (DiVA). Participants are presented with the face of an anthropomorphic avatar, the eye movements of which are linked in real-time to another participant's eye-gaze. This allows for control of interaction aspects that are not related to the experience of gaze contingency.

Participants have to choose which one out of two spheres on either side of the avatar face is the largest. These spheres can have a medium, small, and no difference. Gaze dynamics guide choices. Using cross-recurrence quantification, we analyse the time course of the gaze interactions and look at how this predicts individual and joint decisions about sphere size, which participant will follow the other. Application of the DiVA technique for studying social interaction will be discussed.

L21. MEASURING AND TRAINING ATTENTION CONTROL IN INFANCY WITH EYE-TRACKING

Tomalski P

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Research in the area of developmental cognitive neuroscience has greatly benefitted from recent advancements in eye-tracking methodology.
It allows to measure the developmental change in many aspects of developing attention networks and to collect additional psychophysiological data on pupil dilation.
During the talk I will present some of our recent work with infants as young as 6 months of age to show how eye-tracking can be reliably used to study the development of specific aspects of attentional selection and disengagement. I will also discuss the use of eye-tracking in clinical assessment and training of attention in infants and young children.

L22. BEAR-LY SCRATCHING THE SURFACE OF BEAR COGNITION

Vonk J

Departmen of Psychology, Oakland University, Rochester, MI, USA

I will present data from studies of natural category discrimination, quantity estimation, object/picture recognition and perhaps some new data on behavioral flexibility and innovation. Most of the data will come from black bears although we have now tested sun bears, grizzly bears and giant pandas on a multi-access box problem that involves inhibition and innovation.

L23. NEW INSIGHTS IN BEHAVIORAL PHENOTYPING: TOOLS AND INSTRUMENTATIONS

Wenzler E

TSE Systems GmbH, Bad Homburg, Germany

Characterization of animal models of human diseases often faces substantial data variability due to different test environment, suboptimal equipment, experimenter interference and animal stress. In recent years, TSE Systems has pioneered a home cage based research approach to increase animal welfare, reduce experimenter interference and increase throughput. I will present the audience a short overview over tools and instruments, developed by TSE Systems for the behavioral characterization of rats and mice:
1) complex phenotyping instrumentation PhenoMaster for behavioral and metabolic experiments,
2) Intellicage – unique high throughput behavioral phenotyping tool in which animals can be investigated within their social context,
3) MultiConditioning system for evaluation of learning and memory, as well as emotion and stress-related behaviors in mice and rats,
4) Stellar telemetry system allows the parallel measurement of physiological parameters such as blood pressure, heard rate as well as ECG under group housing conditions during experiments.
A special focus of the talk will be dedicated to the novel kinematic analysis system MotoRater, which enables characteristic locomotor phenotypes in mouse and rat models of neurodegenerative movement disorders. Movement analysis is not restricted to foot prints and gait analysis. The MotoRater observes the animal from 3 sides simultaneously and provides sensitive, specific and detailed readouts for movements of all relevant body parts, i.e. limbs, joints, tail, etc. – this makes MotoRater a unique translational tool given that motor (dys)function in humans is assessed the same way.

L24. COLD-BLOODED COGNITION: COMPARISONS ACROSS CLASSES

Wilkinson A

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Very little is known about the cognitive abilities of reptiles. They have traditionally been considered to be "sluggish and unintelligent creatures" and have largely been ignored in the study of animal cognition. However, more recent research has revealed an impressive suite of cognitive abilities in this group. To gain an understanding of the evolution of cognition in amniotes, it is necessary to carry out direct experimental investigations of the learning and memory abilities of reptiles that parallel the extensive work already available in mammals and birds. Therefore, examination of the cognitive mechanisms underlying the behaviour of these animals can provide crucial information about the evolution of the brain. This talk will present some recent research on the cognitive abilities of reptiles and compare them to what is known about these processes in other animals.

L25. THE EFFECT OF LONG-TERM USE OF PSYCHOACTIVE SUBSTANCES ON PERIPHERAL SENSORY NEUROPATHY: A PRELIMINARY STUDY

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Peripheral neuropathy means a group of symptoms which develop as a result of peripheral nerve damage. These symptoms accompany some systemic diseases or represent a specific disease itself. The most commonly occurring

neuropathies are diabetic neuropathy, compression neuropathy and toxic neuropathy, in which alcoholic and after-narcotic can be distinguished. In peripheral neuropathy, there are some subjective symptoms such as pain, paraesthesia, numbness, tingling, and some sensory and motor disturbances. So many symptoms may not only decrease quality of life but increase the risk of skin damage and diminish hand dexterity in professional life and the activities of daily living. To date, there have been no studies assessing the effect of long-term use of psychoactive substances (narcotics, legal highs) on the two-point discrimination sensation (2PD) of fingers, the strength kinaesthetic sensation (SKS) of hand and thumb flexors, and the motion kinaesthetic sensation (MKS) of the radiocarpal joint in addicted participants compared to control (non-addicted) subjects.

The null hypothesis was that long-term use of psychoactive substances has no effect on 2PD, SKS and MKS.

Overall, 38 male subjects participated in the study. Participants were divided into two groups. In the first (A) group were 20 addicted subjects, whereas in the second (N) group comprised 18 non-addicted subjects. Participants in A group took part in addiction treatment: hence they were free of narcotic substances during examination. The mean addiction period and treatment period were 48 months and 2.7 months, respectively. The mean age in A and N groups was 26.9 and 25.7, respectively. There were no statistical differences in age, body mass, body height and BMI in the examined groups (in all cases $p>0.05$). The standard Dellon discriminator (Baseline Discrim-A-Gon Discriminator) was used to assess 2PD. The two dynamometers were used to assess SKS (pincer and cylindrical grip). The digital Saunders inclinometer was used to assess MKS in the radiocarpal joint. Each measurement was performed three times and, for further analysis, mean values were used. In the case of SKS and MKS, the sensation error was assessed. During analysis, the mean values, standard deviations, p value from independent t-test and chi-squared were assessed. Significant p value was set at $p=0.05$.

2PD in all fingers on both hands was significantly lower ($p<0.001$) in A group (2PD values were 3.85–4.55 mm in right hand fingers and 3.8–4.48 mm in left hand fingers) compared to N group (2PD values were 2.3–2.58 mm in right hand fingers and 2.28–2.48 mm in left hand fingers). The SKS error in pincer grip was significantly higher ($p<0.001$) in A group compared to N group. The mean error in A group was 34.4 and 38.5 per cent for right and left hand separately, whereas in N group the error was 5.08 and 7.46 per cent for right and left hand separately. The SKS error in cylindrical grip was also significantly higher ($p<0.001$) in A group compared to N group. The mean error in A group was 36.8 and 34 per cent for right and left hand separately, whereas in N group the error was 4.92 and 8.84 per cent for right and left hand separately. With regard to MKS, it was shown that for extension and flexion in the radiocarpal joint, the error was significantly higher ($p<0.001$) in A group compared to N group. The MKS mean error values were as follows: a) extension: A group, 37.9 per cent (right hand) and 43.4 per cent (left hand); N group, 18.8 per cent (right and left hand); b) flexion: A group, 34.2 per cent (right hand) and 31.2 per cent (left hand); N group, 15.9 per cent (right hand) and 13.6 per cent (left hand). The long-term use of psychoactive substances (narcotics, legal highs) have an effect on different kinds of sensation disturbance in the hands. This aspect should be taken into account when determining a rehabilitation programme for people with a narcotics addiction history.

POSTER SESSION

P1. INFLUENCE OF ELECTROMAGNETIC FIELDS AT FREQUENCIES OF 2,4 GHZ ON THE CHICK EMBRYOS HATCHING BEHAVIOR

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The use of wireless computer networks (Wi-Fi) occasionally raises concerns regarding health and safety issues related to radiofrequency electromagnetic radiation. This study attempts to determine the effects of the 2400 MHz electromagnetic field (EMF) commonly used by Wi-Fi devices on chicken embryogenesis. The chicken eggs (n=120) were randomly divided into two equal groups and incubated under standard conditions. Control group was incubated in the incubator without an EM field generator, experimental group – chicken embryos were subjected to exposure to magnetic field (2400 MHz) 10 hours every day. From 430h of incubation hatch times were observed. The study showed that the chicks in experimental group started internal piping 8 h earlier than in the controls and shame group. While, the process of external piping in experimental group started 10 h earlier than in the control and shame group. Has been observed decline in liver glycogen and fat content and increased concentration of corticosterone. The pipping and hatching of chicks can be accelerated by stressful impact of EMF, which is confirmed by increase in plasma corticosterone concentrations and decrease fat and glycogen respectively, in the liver of chicks exposed

during embryogenesis on the electromagnetic field with a frequency of 2400 MHz. This study was performed under the project NN311536340 and DS-3263/ZWRiDZ/2015.

P2. THE INFLUENCE OF ARTIFICIAL GEOMAGNETIC FIELD DEPRIVATION ON THE OCCURRENCE OF ANXIETY BEHAVIOR IN LABORATORY RATS

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The geomagnetic field (GMF) is a component of the Earth's environment and may be subject to natural or artificial disturbances. Available data show that plants and many animal species react to changes of GMF that may have a negative impact to them. The aim of this work was to study the influence of artificially disturbed GMF on the behavior of laboratory rats.
The research was conducted in two stages (in the light-dark cycle compatible with natural and in the reversed one) lasting 60 days each. In each of the stages the experimental material consisted of 36 male Wistar rats divided randomly into two equal groups: the control (housed in natural, non-disturbed GMF, ca. 40 μT) and the experimental group (housed in GMF deprivation conditions, ca. 12 μT). At the end of the experiment the following behavioral tests were performed: open field test, Y maze test, elevated plus maze test and light/dark box test.
In elevated plus maze test rats from experimental group spent less time in open arms, also entered them fewer times, but passed more times through the central area than rats from control group. In Y maze test animals from experimental group entered more times into all arms than animals from the control group. In the parameters measured in open field test and light/dark box test there were no statistically important differences between groups. The obtained results show that the GMF deprivation has impact on the behavior of laboratory rats and it is mainly manifested by the increased anxiety in animals from the experimental group.
This work was financed from DS-3263.

IBRO SUPPORT

P3. STEREOTYPIC BEHAVIOUR PATTERNS IN CAPTIVE BEARS

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Bears in captivity are highly susceptible to the development of stereotypies. In the wild they spend most of their time foraging while displaying a wide variety of exploratory and manipulatory behaviours. While free-ranging bears devote a lot of time and effort to forage, in captivity their natural foraging behaviour is frustrated both regarding quantity and variety. Unfulfilled foraging and other natural motivations, enclosure size, lack of proper stimulation and enclosure structuring contribute to high levels of stereotypy.
Stereotypic behaviour of ten individuals of four species (brown bears – five individuals, Asiatic black bears – three individuals, polar bear – one individual, and Andean bear – one individual) was scanned at constant intervals and recording was continuous (=all occurrences). Bears were observed from 7.30 a.m. till 5.30 p.m. Research lasted two seasons (April through September) in 2006 and 2007. Multifactorial analysis and general linear modelling were employed to assess the external factor's influences on specific features of the behaviour, for example bout duration, movement speed and path choice. Other behaviour features analysed were the following: 1) time devoted for stereotypical movements (as the proportion of the observation time), 2) frequency, 3) intensity, 4) number of sequences per bout, 5) variations in number of episodes of stereotypical movements, 6) number of sequences and 7) variations in sequence duration on particular paths, as well as 8) speed and its variations pear each path. Additionally, the correlation and covariance analysis (ANCOVA) of the stereotypic behaviour parameters with external factors, that may potentially affect the level of expression of stereotypies, were conducted. Parameters of behavioural indicator of stress were confronted with an objective and quantitative physiological parameter of stress – faecal cortisol metabolites (FCM) concentration.

P4. BINGE ETHANOL EXPOSURE DURING ADOLESCENCE: ORG 24598, SELECTIVE GLYCINE REUPTAKE INHIBITOR REVERSED MEMORY DISTURBANCE IN ADULT MALE AND FEMALE RATS

Filarowska J, Lopatynska M, Lupina M, Marszalek-Gabska M, Gibula-Bruzda E, Kotlinska JH
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In the brain, glycine plays an important role, being a co-agonist of the NMDA receptors. Glycine concentration is regulated by glycine transporters (GlyT-1, GlyT-2). Administration of GlyT-1 inhibitors has been suggested so as to improve memory performance when impaired by a relative NMDA receptor blockade. We performed the study to indicate

if Org24598 (a GlyT-1 inhibitor) when given to adult rats, reverses the memory impairment induced by ethanol. Male and female Wistar rats were exposed to either ethanol (5 g/kg, i.g.) or to a vehicle, between PND-30 and PND-46, in a binge-drinking model. Subsequently, a reversal learning trial of the Barnes maze test was used to assess memory flexibility between PND-70 and PND-78. Prior to the trial, animals were placed within 4 groups: saline-saline, ethanol – saline, ethanol – Org24598, ethanol – L-701,324 (4 mg/ml, i.p.) +Org24598 (12 mg/ml, i.p.). L-701,324, a selective antagonist of glycine site at the NMDA receptor was used to confirm an involvement of this glycine site in Org24598 effects. Intermittent adolescent ethanol administration has no significant impact on learning and spatial memory, but it was found to impair memory flexibility in adult male and female rats. Org24598 administration had a positive effect on spatial memory performance and reversed memory deficits induced by ethanol. Pretreatment with L-701,324 abolished Org24598 action. In both male and female rats, modulation of the glycine site at the NMDA receptor improves adaptive learning and enhances the behavioral flexibility that is disturbed by ethanol.

IBRO SUPPORT

P5. MODIFICATION OF A HIGH FAT KETOGENIC DIET ALLEVIATES ITS STUNTING EFFECT IN RODENTS

Liśkiewicz A[1], Kasprowska-Liśkiewicz D[2], Sługocka A[1,3], Wiaderkiewicz J[4], Jędrzejowska-Szypułka H[1], Barski JJ[1,3], Lewin-Kowalik J[1]

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The high fat, low carbohydrate diet is extensively studied within the fields of numerous diseases including cancer and neurological disorders. Since most studies incorporate animal models, ensuring the quality of ketogenic rodent diets is important, both in the context of laboratory animal welfare as well as for accuracy of obtained results. In this study we implemented a modification to a commonly used classical ketogenic rodent chow.

We assessed the effects of a month long treatment with either the classical or the modified ketogenic diet on the growth and development of young male mice and rats of both sexes. This data was compared to control animals fed with standard rodent chow. Daily body weight, functional performance and brain morphometric parameters were assessed to evaluate the influence of both applied diets on rodent development and general well-being.

Our results revealed that the supplementation of classical ketogenic diet with a plant origin component mitigates the stunting effect of this diet on laboratory rodents.

Classical ketogenic chow induced strong side effects that included weakness, emaciation and brain undergrowth concomitant to total growth inhibition. However, application of the modified ketogenic chow suppressed these adverse side effects and significantly improved animal welfare. These results indicate that our ketogenic diet modification allows for its application even in young animals without causing detrimental side effects.

IBRO SUPPORT

P6. TRAIT 'PESSIMISM' DETERMINES VULNERABILITY OF RATS TO THE STRESS-INDUCED MOTIVATIONAL DEFICITS – UNEXPECTED RESULTS FROM THE ATTENTIONAL SET SHIFTING TASK

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We have demonstrated recently that cognitive judgment bias (optimism/pessimism) may determine different aspects of rat's behavior. In the present study we extended these studies and investigated the effects of traits optimism/pessimism on cognitive flexibility. For this, initially, the rats were trained and tested in a series of ambiguous-cue interpretation tests, what allowed to classify them as 'optimistic' or 'pessimistic'. Subsequently the animals were re-trained and re-tested in the attentional set shifting task (ASST) what allowed to evaluate the differences between optimists and pessimists in the cognitive flexibility. Additionally, half of the optimistic and half of the pessimistic rats were subjected to chronic (2 weeks) restraint stress. Although we did not observe statistically significant effects of the investigated traits and stress on cognitive flexibility, the 'pessimistic' animals subjected to chronic stress showed significantly longer latencies to approach experimental rewards than their 'optimistic' conspecifics. This effect may indicate stress-induced motivational deficits that are specific to 'pessimistic' animals. Results of the present study along with our previous reports indicate, that trait 'pessimism' could determine animals' vulnerability to stress.

This work was supported by NCN grant: OPUS-2014/13/B/NZ4/00214.

IBRO SUPPORT

P7. KETAMINE DECREASES SENSITIVITY OF MALE RATS TO NEGATIVE FEEDBACK IN THE PROBABILISTIC REVERSAL-LEARNING TASK

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Depression is characterized by an excessive attribution of value to infrequent negative feedback. This imbalance in feedback sensitivity can be measured using the probabilistic reversal-learning (PRL) task. This task was initially designed for clinical research, but recently an operant rodent version was described by Bari, providing a new and much needed translational paradigm to evaluate potential novel antidepressants. In the present study, we evaluated the effects of ketamine (KET) on the sensitivity of rats to positive and negative feedback in the rodent version of the PRL paradigm.

For successful completion of the PRL task, the subjects had to learn to ignore infrequent and misleading feedback arising from the probabilistic (80:20) nature of the discrimination. We tested the effects of three doses of KET (5, 10 and 20 mg/kg) on feedback sensitivity 1, 24 and 48 hours after administration.

We report that acute administration of the highest applied dose of KET (20 mg/kg) rapidly and persistently decreases proportion of lose-shift responses made by rats after receiving negative feedback.

Present results suggest that KET could decrease negative feedback sensitivity and that changes in this basic neurocognitive function might be responsible for its antidepressant action.

This work was supported by NCN grant: OPUS-2014/13/B/NZ4/00214.

IBRO SUPPORT

P8. NEUROANATOMICAL AND NEUROCHEMICAL BACKGROUND OF COGNITIVE JUDGMENT BIAS IN RATS

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Neuroimaging studies in humans have recently shown that the medial prefrontal (mPFC) and orbitofrontal (OFC) cortices mediate bias in judgment of forthcoming events. In the present study, we assumed a challenge to determine whether cognitive judgment bias was also dependent on one or the other of these prefrontal regions in non-human animals.

For this, we trained a cohort of rats in the ambiguous-cue interpretation (ACI) paradigm, subjected them to selective excitotoxic lesions of mPFC and OFC, and evaluated the effects of lesions in the ACI test. Additionally, to determine the neurochemical correlates of cognitive judgment bias, we subjected another cohort of rats to neurochemically specific (5–6 DHT and 6-OHDA) lesions within the mPFC and OFC cortices and subsequently tested them with the ACI paradigm.

Results of our study demonstrate that excitotoxic lesions of OFC cause pessimistic judgment bias in rats. Similar effect (although not entirely yet verified) was observed following depletion of dopamine but not serotonin within the OFC. We did not observe statistically significant effects of neither excitotoxic nor neurochemically specific lesions within mPFC.

The results of our study indicate that orbitofrontal dopamine might be one of the neurochemical mediators of cognitive judgment bias in rats.

This work was supported by the National Science Centre (Research grant: Sonata bis dec-2012/07/E/NZ4/00196).

P9. THE COMBINATION OF MEMANTINE AND GALANTAMINE ENHANCE MEMORY IN RATS: THE ROLE OF THE A7 NICOTINIC ACETYLCHOLINE RECEPTOR

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The search for effective treatments of cognitive dysfunctions associated with Alzheimer's disease (AD) or schizophrenia is one of the challenges facing present medicine. Promising therapeutic strategy is provided by the combination of memantine and the acetylcholinesterase inhibitor, galantamine. Galantamine is of particular interest because it has a dual mechanism of action: it is also an allosteric modulator of nicotinic receptors, including α7 nicotinic acetylcholine receptor (α7 nAChR). Interestingly, α7 nAChR is involve in interaction between glutamatergic and cholinergic system.

The aim of the present study was to evaluate the role of α7 nAChRs in the pro-cognitive effects of this drug combination. We used novel object recognition (NOR) task to assess the cognitive performance, particularly recognition memory.

Co-administration of inactive doses of memantine (1 mg/kg, IP) with galantamine (0.3 mg/kg, IP) enhanced recognition memory. Combined treatment with memantine and α7 nAChRs positive allosteric

modulators, CCMI (0.3 mg/kg, IP) or PNU120596 (1 mg/kg, IP) was equally effective. Procognitive effects were blocked by the α7 nAChR antagonist methyllycaconitine, suggesting that the observed cognitive enhancement is α7 nAChR dependent.
Stimulation of α7 nAChRs may underlie the pro-cognitive effects of combining memantine and galantamine. Our results suggest that memantine, when given with enhancers of α7 nAChRs, may represent an effective strategy for cognitive improvement.
This study was supported by the Polish National Science Centre grant NCN 2012/07/B/NZ/01150

P10. BEHAVIOUR OF SOWS HOUSED IN SMALL STATIC GROUPS

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According to Council Directive EU 2008/120/EC sows and gilts have to be hopused in groups. In the herd of pigs a social hierarchy is a natural occurrence. The stabilization of the hierarchy in the newly created groups involves higher aggression and low animal welfare level.
The aim of the study was to compare the behavioural profiles of two groups of sows (12 sows each) housed in static groups which means the composition of the group does not change throughout the production cycle. The first group (group A) was treated with commonly used sedative Stresnil®. The second group (group B) did not receive any pharmacological remedy. The behaviour of experimental sows was video-recorded. Observation began when sows entered the groups and lasted for the next four days. The recordings were then analyzed taking into account the resting and activity phases in particular time and frequency of aggressive behavior.
In the first day of observation sows from the group B showed higher levels of aggression (0.64%) than sows in group A (0.03%). Similar results were found in case of the frequency of aggressive behaviors. The mean number of such behavior in group A was 1.33/head and was statistically significantly lower ($P<0.05$) than in group B – 13.50/head. The differences were statistically significant ($P<0.05$). The duration of aggressive behaviour decreased in subsequent days of observation in comparison to the first day in both groups.
The above results show that the stable hierarchy was achieved just in the first day of research. Furthermore the injection of sedatives had a positive impact on the decrease of aggression in sows housed in groups.

P11. THE EFFECT OF COMBINED TREATMENT WITH MEMANTINE, GALANTAMINE AND PNU-120596 ON THE COGNITIVE IMPAIRMENT IN THE NOVEL OBJECT RECOGNITION TEST

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Impaired cognition constitutes an integral part of CNS disorders including Alzheimer's disease, depression and schizophrenia. Alpha 7 nicotinic acetylocholine receptors (α7-nAChRs) play important role in the regulation of cognitive functions and represent useful targets for cognitive improvement. Combined therapy with the well know drugs, such as memantine or galantamine and a positive allosteric modulator (PAM) of α7-nAChR, PNU-120596, might be a promising strategy in the treatment of cognitive impairment.
The aim of the present study was to evaluate the efficacy of type II α7-nAChR PAM (PNU-120596) with and without memantine and galanatamine, against 1.25 mg/kg scopolamine-induced cognitive deficits in the novel object recognition test (NORT) in rats.
Galantamine (0.3 and 1 but not 0.1 mg/kg), memantine (1 and 3 but not 0.3 mg/kg) and PNU-120596 (1, but not 0.1 or 0.3 mg/kg), dose-dependently reversed the scopolamine-induced object recognition deficit. Furthermore, co-administration of inactive doses of galantamine (0.1 mg/kg) with PNU-120596 (0.1 mg/kg), or memantine (0.3 mg/kg) with PNU-120596 (0.1 mg/kg), facilitated cognitive performance. The drugs and scopolamine were given 60 and 30 minutes, respectively, before trial 1 in NORT. The inter-trial interval (ITI) was 1 h.
Present observations suggest that combined treatment with memantine or galantamine with positive allosteric modulator of α7-nAChRs could be a promising strategy for the cognitive deficits.
This study was supported by the NSC Grant No 2012/07/B/NZ/01150 (A. Nikiforuk) and by the Statutory Funds of the Institute of Pharmacology (PAS).

P12. THE COGNITIVE DEFICITS DURING NICOTINE WITHDRAWAL IN RATS

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Nicotine withdrawal is associated with a variety of symptoms including elevated anxiety, depressed mood, increased appetite and cognitive deficits. The withdrawal-related cognitive deficits are likely associated with the prefrontal functions.

The aim of the present study was to develop an animal model of the recognition memory deficits in the novel object recognition (NOR) test during nicotine withdrawal. Nicotine was administered in a conditioned place preference (CPP) apparatus to assure the compound produced rewarding effects. The memory was assessed during nicotine withdrawal.
In an unbiased CPP procedure, male Sprague-Dawley rats were conditioned for 4–5 times with nicotine, for 40 minutes. Six days after the last conditioning with nicotine, rats were adapted to the open field arena for 5 minutes. On the next day, the test comprised of two, 3-min trials separated by an inter-trial interval of 1h. During the first trial (familiarisation), two identical objects were presented in the opposite corners of the apparatus. In the second trial (retention), one of the objects was replaced with a novel one.
Nicotine produced a significant place preference for the drug-paired compartments. During withdrawal, rats displayed memory deficits that depended on the number of nicotine conditionings. Present findings suggest the utility of nicotine withdrawal to induce cognitive deficits rats.
This study was supported by the Statutory Funds of the Institute of Pharmacology, Polish Academy of Sciences.

P13. EFFECT OF BRILLIANT BLUE G, A SELECTIVE P2X7 RECEPTOR ANTAGONIST, IN SOME ACUTE SEIZURE MODELS IN MICE

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Epilepsy is one of the most common neurological disorders which is diagnosed in around 50 million people worldwide. Clinically available AEDs fail to control epileptic activity in about 30% of patients and they are merely symptomatic treatments and cannot cure or prevent epilepsy. There remains a need for searching new therapeutic strategies for epileptic disorders. The $P2X_7$ receptor ($P2X_7R$) has been recently investigated as a new target in epilepsy treatment. Preclinical studies revealed that $P2X_7R$ antagonists have anticonvulsant properties in models of *status epilepticus*.
We aimed to investigate whether $P2X_7R$ antagonist – brilliant blue G (BBG) – is able to change seizure threshold in three acute seizure models in mice, i.e., the intravenous pentylenetetrazole seizure threshold, maximal electroshock seizure threshold and 6-Hz psychomotor seizure threshold tests. BBG was administered acutely (50–200 mg/kg, 30 min before the tests) and subchronically (25–100 mg/kg, once daily for 7 days). Moreover, the chimney and grip strength tests were used to estimate influence of BBG on the motor coordination and muscular strength in mice, respectively.
Our results revealed only a week anticonvulsant potential of the studied $P2X_7R$ antagonist because it showed anticonvulsant action only in the 6 Hz seizure test, both after acute and subchronic administration. BBG did not significantly influence seizure thresholds in the remaining tests. Motor coordination and muscular strength were not affected by the studied $P2X_7R$ antagonist.
In summary, BBG does not possess any remarkable anticonvulsant potential in acute seizure models in mice.

P14. EFFECT OF SCOPOLAMINE IN THE RAT IOWA GAMBLING TASK

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Pathological gambling (PG) is a form of behavioral addiction. Both substance and behavioural addictions are characterized by the impairment in decision-making processes and impulsive responding. These components of PG can be investigated in a rat Iowa Gambling Task (rIGT).
Because the central cholinergic systems are implicated in cognitive domains, including cost/benefin decision-making, we investigated whether scopolamine, the muscarinic receptor antagonist, could affect gambling behaviour in rats.
We employed a novel model of PG in rodents, called the rat Iowa Gambling Task. In this task, the rats are trained in the skinner boxes. The animals choose among four nose-poke holes, which differ in the amount of reward they provide, and in the probability and duration of punishing time-out periods, during which the reward cannot be earned. Subjects were trained to earn as many sugar pellets as possible within 30 min. After reaching a stable baseline, the test was performed. Scopolamine was administered at 0,1 mg/kg, S.C., 30 minutes before the test.
We report that scopolamine at a dose of 0,1 mg/kg did not influence gambling behaviour. The compound decreased the choice of the most optimal (P2) and the most disadvantageous (P4) nose-poke apertures, suggesting non-specific effects on gambling. In addition, scopolamine decreased the number of premature responses and completed trials, while increased the number of omissions, suggesting a sedative action.
Dominik Rafa is a holder of scholarship from the KNOW sponsored by the Ministry of Science and Higher

Education, Republic of Poland. Supported by the Statutory funds.

P15. MATERNAL RESPONSIVENESS OF SOWS MEASURED IN BEHAVIOURAL TESTS

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Maternal responsiveness of sows is an individual trait and depends on housing system, however, there have been no behavioural tests used to measure the maternal responsiveness of sows. Aim of the study was to develop and assess efficiency of developed behavioural tests for sows.
24 sows (12 in crates and 12 in pens) were tested with two behavioural tests during first three days after farrowing. Test procedure: Test 1. Recorded 15 s. squeak of the crushed piglet was played when sows were laying. The reaction of the sow was observed during 30 seconds after playing the squeak. Scale of reaction: 0 – no reaction of the sow, 1 – slight movement of the sow's head, 2 – rise of the head, 3 – rise of the front legs, 4 – quick rise of the whole body and quick lying down, 5 – the rise of the whole body, the sow keeps a lookout for piglets, sniffs out. Test 2. Squeak of the crushed piglet played when the sow was changing the body position from standing to laying. Scale: 0 – the sows quickly lays down, 1 – the sow stops laying and stands up after a short while, 3 – the sow stands up quickly. Sows housed in farrowing pen were more susceptible for piglets' sounds: 4,21 (pen) *vs.* 3.86 (crate) ($P<0,05$). Test 2 – 2.38 *vs.* 2.04, respectively for farrowing pen and crate ($p<0.05$).
Tests showed higher maternal responsiveness of sows which were able to turn around, sniff and look at their offspring. The behavioural tests could help to assess the maternal responsiveness of sows after first parturition regardless of real results of rearing piglets. It could help breeders to eliminate very early sows with poor maternal abilities

P16. PHEROMONES – DOES HUMANS USES THEM? FACTS AND MYTHS

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Pheromones are chemical signals used to communication within animal species. There are some controversies on whether pheromones exist and are used for chemical communication in humans. It is known that the sense of smell plays a minor role in human biology. There is no robust bioassay evidence that the androstadienone and estratetraenol are really human pheromones, as supposed, and there are no studies that clearly demonstrate their use by people for olfactory communication. Pheromones are thought to be detected mainly by the vomeronasal organ (VNO). The existence and function of the VNO in humans is being questioned. There are several theories about the role of human pheromones . Most authors claim that the human VNO is a chemosensory structure which probably degenerates and disappears during prenatal development and is not functional in adults. However, in the literature there are contradictory opinions on that. For the study on human pheromones, similar chemical and behavioral research methods are applied as in other species.
This poster is a review of research methods used in the olfactory study on animal, which can be used in human studies.

P17. HOW A VARIETY OF STIMULI MAY AFFECT ON BRAIN FUNCTIONING

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A variety of environmental stimuli promotes the development of the nervous system and ensure its better functioning. In the many mental disorders the brain activity is reducted. Therefore we decided to show how enrichment of environment may influence behavioral deficits such as memory, learning or social interactions.
We conducted behavioral tests on 2 groups of mice, one were kept in standard laboratory cages without external stimuli and second group in cages equipped with variety of three-level cages, such as tunnels, reels and other toys. Each group was divided into 2 subgroups (control and treated with MK-801). We used Social Interactions Test (SIT) and Novel Object Recognition Test (NORT) to investigate the influence of enrichment environment on social behaviour and working memory. In the second part of study, we measured certain proteins in the brain, which are considered as surrogate markers of schizophrenia or bipolar disorders (GAD_{65}, GAD_{67}, $5\text{-}HT_{1A}$).
The mice kept for 4 months in enriched environment showed higher level of social episodes and better performance in novel object recognition test than animals without any additional stimuli. The administration of MK-801 decreased the time of interactions (in SIT) and recognition index (in NORT) with the same susceptibility in both groups. In biochemical studies a reduced level of $5\text{-}HT_{1A}$, GAD_{65} and GAD_{67} was observed.

Our research indicate that animals kept standard laboratory conditions may have poorer ability to develop social behavior and cognition. The level of chosen proteins is also change in their brains.

P18. BEHAVIOURAL STUDY ON NEW MONOACYLGLYCEROL LIPASE INHIBITOR {4-[BIS-(BENZO[D][1,3]DIOXOL-5-YL) METHYL]-PIPERIDIN-1-YL}(1H-1,2,4-TRIAZOL-1-YL) METHANONE (JJKK-048)

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Monoacylglycerol lipase (MAGL) is a serine hydrolase that acts as a principal degradative enzyme for the endocannabinoid 2-arachidonoylglycerol (2-AG). Given the roles of the endocannabinoid system in the regulation of many processes, enzymes targeting cannabinoid catabolism provide valuable tools for characterization of MAGL and 2-AG signaling pathways and are potentially important targets for pharmaceutical development. It was previously reported piperidine triazole urea, {4-[bis-(benzo[d][1,3] dioxol-5-yl)methyl]-piperidin-1-yl}(1*H*-1,2,4-triazol-1-yl) methanone (JJKK-048), to be an ultrapotent and highly selective inhibitor of MAGL *in vitro*. Here we present the results of bahavioural study of JJKK-048.

Mice were evaluated in the tetrad test (locomotor activity, nociception, catalepsy and hypothermia) for cannabimimetic effects. Locomotor activity was measured by means of a photocell apparatus, nociception in the writhing and tail-immersion tests, catalepsy in the bar test and rectal temperature with a thermistor thermometer.

Acute administration of JJKK-048 promoted significant analgesia in writhing test with the low dose that did not cause cannabimimetic side effects. Higher doses of JJKK-048 induced analgesia both in writhing test and in tail immersion test, as well as hypomotility and hyporthermia but not catalepsy. Among these in vivo effects, only antinociceptive effect of JJKK-048 was blocked by rimonabant.

Based on the results, JJKK-048 provides a potent pharmacological tool for the further functional characterization of MAGL.

P19. POSITIVE ALLOSTERIC MODULATOR OF M4 MUSCARINIC RECEPTOR DISPLAYS ANTIPSYCHOTIC-LIKE EFFECT IN ANIMAL MODELS OF SCHIZOPHRENIA

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Schizophrenia, a devastating disease that affects young adults, is characterized by the presence of positive, negative and cognitive symptoms. Currently prescribed drugs have low efficacy in treating both negative and cognitive symptoms. Recent studies and preclinical data suggest that muscarinic receptors have potential therapeutic effects on treating CNS disorders. Thus the aim of this study was to evaluate the antipsychotic effect of positive allosteric modulator of M4 muscarinic receptor (VU152100).

DOI-induced head twitches, social interaction test and novel object recognition test were used in order to characterize the effect of selective positive allosteric modulator of M4 muscarinic receptor on positive, negative and cognitive symptoms, respectively.

VU152100 at a dose of 10 mg/kg decreased the number of head twitches induced by DOI administration in comparison to the control group. It was observed that VU152100 in a dose dependent manner reversed the deficits induced by MK-801 in novel object recognition test. Moreover, at a dose of 4 mg/kg, it attenuated social withdrawal induced by MK-801 by increasing both the number and the duration of social episodes.

Collected data suggest that positive allosteric modulation of M4 muscarinic receptors has an antipsychotic-like effect in animal models of schizophrenia, thought to be predictive of positive, negative and cognitive symptoms. M4 muscarinic receptor could be a potential target of novel therapeutic strategies to cure schizophrenia.

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P20. SEARCHING FOR MOLECULAR MECHANISMS UNDERLYING RAT ADDICTION-VULNERABLE PHENOTYPE: FOCUS ON SOME GLUTAMATE TRANSPORTERS, MGLUR2/3 RECEPTORS AND THEIR TRANSCRIPTION FACTORS

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The development of new medications treating cocaine addiction requires identification of its targetable molecular mechanisms. Repeated cocaine treatment results in impairment of glutamate system homeostasis with a crucial role of glutamate transporters the EAAT2 and xCT as well as presynaptic mGluR2/3 receptors that influence glutamate release. Both EAAT2 and xCT expression can be regulated by transcription factors NF-κB or/and N-myc for EAAT2 and Nrf2 for xCT. Based on this information we examined the level of EAAT2, xCT, NF-κB,

N-myc, Nrf2 and mGluR2/3 as potential drug targets in animals showing addiction-vulnerable phenotype (AV). For this purpose, male Wistar rats (240–280 g) underwent an unbiased 10-day conditioned place preference with cocaine (15 mg/kg, i.p.) after which animals showing AV were selected. Protein expression was determined using Western Blot in prefrontal cortex (PFC), hippocampus (HIP), nucleus accumbens (NAc) and dorsal striatum (DSTR). Data were analyzed using one-way ANOVA followed by the Bonferroni test. We found a decrease in the EAAT2 and NF-κB expression in the PFC and a rise in EAAT2 and NF-κB in the DSTR. No changes in N-myc expression were found, whereas xCT in the NAc and Nrf2 in the PFC and DSTR increased. Moreover, mGluR2/3 level was diminished in the PFC, NAc, and DSTR. In conclusion, our findings indicate that cocaine administration diminishes the level of mGluR2/3 in brain areas crucial for addiction development and that changes of EAAT2 level depend on NF-κB but not N-myc. Finally, it appears that Nrf2 do not influence the expression of xCT at least not in this model of addiction.

P21. INFLUENCE OF CHRONIC ADMINISTRATION OF SILVER NANOPARTICLES ON BEHAVIOR OF ADULT RATS

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Silver nanoparticles (AgNPs) are one of the most commonly used metal-nanomaterials, which have broad-spectrum antibacterial and antifungal properties. What is more, one of the main aims of nanomedicine is to enhance drug availability within the central nervous system (CNS) by providing a mechanism for delivery of drugs past the blood-brain-barrier to increase therapeutic efficacy. Currently, it is known that AgNPs induce neurotoxicity after oral, systemic and inhalatory exposures which was shown in a few rodent studies. Despite of these toxicological reports, in vitro and in vivo, the exact influence of AgNPs on CNS processes and the underlying mechanism(s) of the action are not well understood.
The aim of the present study was to investigate the possible behavioural changes of rats following 14 days repeated oral exposure of 10 nm AgNPs, using a wide range of behavioural assessments. Measurements of body weight and body temperature and tests of locomotor activity, motor coordination, nociceptive reaction, memory performance and anxiety-like behaviour, generally accepted in investigations of the central activity of new compounds were performed.
Although no behavioural differences were observed in locomotor activity, motor coordination, anxiety, memory and pain responses after chronic administration of nanoAg, the decrease in body temperature and body weight of rats was noticed.
These results suggest that the CNS may be a target of low-level toxicity of nanoAg and warrant further studies.

P22. ENCEPHALISATION QUOTIENT AND RELATIVE BRAIN PART SIZES PROVIDE LITTLE EVIDENCE THAT MACHIAVELLIAN BEHAVIOUR IN CLEANER WRASSE SELECTS FOR COMPLEX BRAINS

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The Machiavellian Intelligence Hypothesis – at least in its original form – provides a wide permissive perspective on the variety of socio-cognitive adaptations through which an individual may exploit the potential benefits of its social world, as well as dealing with the hostile aspects of it. Thus, in addition to socio-cognitive abilities like perspective-taking and knowledge of social hierarchy, they explicitly included social play, social curiosity, social learning and teaching, and thus cultural transmission, as well as social influenced flexibility, problem solving and innovation. The aim of this experiment is to use a different approach to study potential links between social complexity and brain size. First analysis focus on brain size relative to body size. We therefore use available data on the sizes of fish brain parts to explore the relative sizes of various brain parts: the % of weight of telencephalon, diencephalon, mesencephalon and cerebellum in relation to the entire brain. Our own data set and the data extracted from fishbase.org are consistent in suggesting that the relative brain size of Labroides species is not particularly large compared to other wrasse species, that could be explained by the fact that service quality are either not particularly cognitively demanding or that cleaners responded to selection for increased cognitive abilities with a restructuration of the brain rather than with an increase in brain size. Thus, the next step will be to look at the cleaners' brain architecture.

IBRO SUPPORT

P23. POSTEJACULATORY 22-KHZ VOCALIZATION AS A REFLECTION OF POSITIVE EMOTIONAL STATE

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Ultrasonic vocalization (USV) in rodents attracts attention as a model of communication, expression of emtions, or

changes of arousal level during social interactions including also sexual behavior. Although now we understand more and more about role of ultrasonic vocalizations, many aspects still remains unexplained. One of question without good answer concern the function of the 22-kHz vocalizations emitting by male after ejaculation. The aim of the study was to werify the hypothesis that postejaculatory vocalizations reflects a positive rather than a negative emotional state. To that end we measured the postejaculatory 22-kHz USV parameters in response to the conditioning process to new environmental cues in males after ejaculation. Additionally, we investigated the effect of cues which potentially increased anxiety in males (odor cues from unfamiliar males) on the 22-kHz vocalization.
We used 12 male and 13 female Sprague-Dawley rats in 7 behavioral sessions.
At the beginning of the experimental training 3-month-old rats were sexually naive and before tests all animals were intensively acclimated to all behavioral procedures. Postejaculatory behavior was recorded by Noldus EthoVision observation system simultaneously with recording USV by Metris Sonotrack system. We analyzed: latency to first vocalizations (time from ejaculation to first call at 22-kHz), vocalization duration (time from first to last call at 22-kHz), and number of rearing.
We found that during first CPP session only half males vocalized for a few seconds with intensive rearing behavior. Since second session in CPP chamber, vocalization duration had been increased and rearing number had been decreased. In well-established CPP odor cues from foreing males (which we assumed increase anxiety level) inhibit 22-kHz vocalization duration and increased rearing behavior. On the other hand, a male exposition to all cues present during copulation – copulatory chamber and female, enhanced postejaculatory 22-kHz vocalization and reduced number of rearing even over the level observed in CPP session in clean chamber.
Our results shown that the postejaculatory 22-kHz vocalizations depends on cues associate with positive emotional state after ejaculation. We found that ejaculation alone is not sufficient to evoke fully expressed postejaculatory vocalization. Our experiment proves that the simply division for 50-kHz – positive and 22-kHz – negative USV is not fully comprehensive.

IBRO SUPPORT

P24. UP-REGULATION OF MTOR SIGNALING IN FOREBRAIN NEURONS LEADS TO IMPROVEMENT OF COGNITIVE FUNCTIONS IN PTEN KNOCK-OUT MICE

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Mammalian target of rapamycin (mTOR) is a serine/threonine kinase that is involved in the control of cell growth and proliferation. mTOR participates in various functions of the brain, such as synaptic plasticity, adult neurogenesis, memory and learning. It was shown that activation of PI3K-Akt-mTOR pathway plays important roles in neuronal plasticity, memory formation, feeding behavior as well as circadian rhythm. In normal conditions activity of this pathway is controlled by Pten. Pten is phosphatase and tensin homolog that negatively regulates intracellular levels of phosphatidylinositide 3-kinases (PI3K) in cells and functions as a tumor suppressor by negatively regulating Akt/PKB signaling pathway. We used Pten knock-out mouse model to study the cognitive functions in IntelliCage system. We showed that loss of Pten gene in forebrain neurons is involved in up-regulation of PI3K-Akt-mTOR signaling which strengthened learning and memory and decreased life span.
Mice model: The model of up-regulation of mTOR activity were mice with deletion of Pten gene restricted to forebrain neurons induced by tamoxifen. To restrict mutation to forebrain neurons, the CaMKCreERT2 mouse line was used and crossbred with mice having the Pten genes surrounded by loxP sites.
Behavioral testing: Following induction of the mutation with tamoxifen, Pten mutant mice and respective controls were tested in learning and memory test in IntelliCage. It is a fully automated system for the behavioral assessment of mice that live in social groups. We measured spatial learning with appetitive reinforcement in place learning task. The test measures the abililty of mouse to learn that water is only available in a specific location in the cage. Each mouse had dedicated corner where it could drank, while other corners were inaccessible. The increasing preference for the rewarded corner was a measure of learning.
Life span: Long-term activity of PI3K-Akt-mTOR pathway led to increased mutant mice mortality. Pten mutants were able to survive until the 13 weeks after the induction of mutation.
Place learning in IntelliCage: In the IntelliCage, housing and testing occur in the same cage that is a familiar environment, thus creating a unique opportunity to test behavior for a long-term period in relatively low-stress conditions without handling or social isolation. Using the system, we were able to discover better performance of Pten mutants in place learning task. Moreover, the memory improvement was detectable even 24 hours before death.
Pten knock-out mice as a model of up-regulation of mTOR signaling pathway in forebrain neurons:
1) maintain the memory of rewarded place better than control mice,

2) show shorten life span,
3) surprisingly, superior performance of Pten mutants in place learning task persists until their death.
We plan to repeat the experiments with up-regulation and in the next step, with down-regulation of the pathway using different mouse model.
Behaviorally, we have shown that IntelliCage is suitable tool to monitor learning abilities and their changes during life span. The place learning task may be used for measuring progression of neurodegenerative disorders.

IBRO SUPPORT

P25. A NOCICEPTION ASSAY IN TRANSGENIC MICE LACKING PARVALBUMIN AND CALBINDIN – AN EVALUATION OF VON FREY FILAMENTS AND HOT-COLD PLATE METHODS

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The study is part of the project considering eventual involvement of calcium-binding proteins in allodynia in mice lacking calbindin and parvalbumin.
An allodynia is a clinical feature of many diseases associated with neuropathic pain. It is a condition of central pain sensitization when a stimulus which normally do not cause the pain is recognized as a painful one. The allodynia can be divided into mechanical (tactile) and thermal one.
Calbindin and parvalbumin are intracellular calcium binding proteins widely distributed in muscle fibers and GABA-ergic inhibitory neurons and interneurons. Recent investigations have shown a relationship between the insufficient activity of those neurons and common neurological diseases such as Alzheimer disease, autism and schizophrenia. There is little known about mechanism by which calbindin or parvalbumin knockouts increase pain threshold. The aim of the study was to shed light on mechanism of allodynia and role of calbindin and parvalbumin in central nervous system.
In the study we used two transgenic mouse lines – mice lacking calbindin D-28k protein, parvalbumin knockout mice and standard Bl6 line as a control.
Tactile nociception was measured by means of von Frey filaments. Von Frey method is based on set of filaments with different diameter and so with different flexibility and pressure's force. Applying different type filaments on animal's foot we can determine pain threshold by observing animal's reaction.
Thermal nociception was examined on hot-cold plate. The hot-cold plate is a flat surface heated to 52°C. An animal is placed in a center of plate and time to reaction is counted. The latency to reaction is a measure of the allodynia.
In the articles we can find a clue to start test at the hair of 1.4 g. In contrast our mice had reacted at the hair of 1.0 g.
The average size of the filament which cause the reaction is 2.0 g. However, some mice reacted when we used 1.0 g filament.
Used methods have couple disadvantages: are not easy to apply, quite subjective and are characterized by wide range of inter-individual variability. Changes can be so subtile that even unnoticeable. There is a need to look for new much more objective methods to evaluate pain threshold in rodents.
Adjusting the aforementioned protocols to examined mouse lines may lead to unification of used methods to make them much more objective than they are in present form.
Studies on potencial role of calcium binding proteins in allodynia in mice lacking parvalbumin and calbindin can shed light on mechanism underlying allodynia and nociception with particular focus on calcium binding proteins.

IBRO SUPPORT

P26. EFFECTS OF IN UTERO EXPOSURE TO RETINOIC ACID ON INNATE BEHAVIOUR OF YOUNG AND ADULT RATS

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Retinoic acid is a metabolite of vitamin A, which is necessary for correct development and growth of individuals. But a few studies indicate that children exposed to retinoids during embryonic development have reduced intelligence and this impairment is not always associated with detectable malformation. Damages caused by prenatal retinoids exposure are developmental stage specific. Sensitive stage of brain development is the embryonic days 11–21 in the rats and embryonic weeks 4–16 in humans, in which intensive proliferation and differentiation of neural cells occur. Exposure of fetal brain to retinoic acid or other noxious factors during this stage can lead to the damages of brain, which can subsequently manifest as behavioural disorders in the postnatal period. The focus of our study was to investigate if in utero exposure to increased doses of retinoic acid will influence innate behaviour of young rats and if potential behavioural changes will persist into adulthood.

Wistar strain rats were used in our experiment. Retinoic acid was administered to pregnant females on the 14.–16. day of gravidity. The dose of retinoic acid (RA) was 1 mg RA/kg body weight. Three weeks old and adult offspring were tested in two behavioural tests: the open field test and plus maze test. Locomotor activity and exploratory behaviour were evaluated in the open field test. Plus maze test was used to monitor anxiety-like behaviour.
Results. Prenatal exposure to retinoic acid increased locomotor activity of young rats in the open field test. Also anxiety-like behaviour was significantly increased in these animals in the plus maze test. Retinoic acid did not affect the exploratory behaviour. Behavioural testing of adult animals produced the same results. Retinoic acid exposure adult rats showed enhanced locomotor activity in the open field test and an increase in anxiety-like behaviour in the plus maze test. Changes of exploratory behaviour were not recorded in the adult rats.
Our findings confirm a fact that developing nervous system is very sensitive to action of various factors. Also slightly increased doses of retinoic acid applied during sensitive stage of brain development are able to make changes of innate behaviour in young animals without physical signs of abnormal brain development. Behavioural changes observed in juvenile can persist into the adulthood. This work indicates that retinoic therapy or excessive intake of vitamin A during gravidity may present a risk of neurobehavioural impairment, therefore it is important to heed the proper dosing of vitamin A and avoid to preparation containing retinoids during pregnancy.

IBRO SUPPORT

P27. KETOGENIC DIET BUT NOT KETONE BODIES INCREASES SOCIAL ACTIVITY IN YOUNG MALE RATS

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High fat ketogenic diet (HFKD) is increasingly considered as an alternative or add-on therapy in Autism spectrum disorders (ASD). Given the beneficial effect of HFKD on epilepsy, mitochondrial function, carbohydrate metabolism and inflammation, the treatment with HFKD has potential to reduce some of the ASD-associated symptoms including abnormal social interactions. It is not known whether HFKD influences social interactions by reducing the pathological processes underlying ASD or through some independent mechanism. The aim of our study was to evaluate the influence of HFKD on social activity in healthy young male rats.
Four weeks old male Long Evans rats were treated with HFKD or ketone bodies for following 4 weeks. Subsequently behavioral tests were performed in order to evaluate social interactions, locomotor activity, working memory and anxiety-related behavior.
Considering that young animals fed with HFKD are much smaller than animals fed with standard rodent chow we performed the social interaction test in two settings. In the first case the guest animal was smaller but younger than the host animal. In the second case the guest was bigger but in the same age as the host animal. Additionally we performed a third test in which both animals (host and guest) were fed with HFKD. Moreover to check if the observed effect is transient or permanent animals after one month of HFKD treatment were fed with standard rodent chow for one more week and the level of social interaction was measured again. Ketogenic properties of modified diet was confirmed by the measurement of the level of ketone bodies in blood serum.
We have observed that rats fed with HFKD showed increased social exploration in all three experimental settings. The effect was no longer observed when rats were fed with SD for one week after one month of HFKD treatment. We did not observed any changes in the level of social interactions in animals treated with KB.
In order to check if increased activity is limited to social interactions or it results from overall hyperactivity or reduced anxiety we have performed locomotor activity, elevated plus maze (EPM) and novel object recognition (NOR) tests. The results didn't show any difference in mobility or anxiety-related behaviors or working memory of animals fed with HFKD or standard diet.
In conclusion we have showed that HFKD has prosocial effect on healthy young male rats, which is not associated with general hyperactivity or reduced anxiety. Exogenous administration of ketone bodies does not cause similar effect. The results suggest that HFKD may influence social activity not only by reducing pathophysiological processes associated with ASD but also through some independent mechanisms.

IBRO SUPPORT

P28. EFFECTS OF EXERCISE TRAINING ON REGENERATION OF THE SCIATIC NERVE IN MICE WITHOUT EFFECTS OF EXERCISE TRAINING ON REGENERATION OF THE SCIATIC NERVE IN MICE WITHOUT BCL-2 GENE

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The problem of repairing damaged peripheral nerves has long been the subject of intensive studies of many laboratories around the world. Despite many studies and the involvement of specialists from many fields of science, research results are still not satisfactory. Most of the research into the proteins Bcl-2 is based on the results obtained in vitro using isolated cell lines. Very important is the determination of the role of Bcl-2 in the processes associated with regeneration and development of the organism and the maintenance of homeostasis in vivo, especially since the impact of Bcl-2 protein for the processes of axonal growth in CNS has been demonstrated. The research material consists of 180 mice, weighing 20–25 g. Surgical procedures are performed under sterile conditions. The animals are damaged right sciatic nerve under anesthesia. Neuropraxia damages are performed using vascular clips. After the treatment for 3 days in all groups of animals are administered anti-inflammatory drug – paracetamol (suspension of 120 mg/5 ml) in drinking water. All animals are subjected to physical training on a treadmill at a sufficiently long acclimatization. Then they are functional tested using a Catwalk – device enabling the gait analysis. Tests are carried out: one day before the intersection of peripheral nerve and every 7 days of injury: 7, 14, 21 and 28 days after surgery. These tests are used to assess the regeneration of the sciatic nerve fibers. After the experiment, the nerves are subjected to histological and immunohistochemical analysis. The collected experimental data are verified in Excel and Statistica. Microscoping images are subjected to spatial computer analysis.

Mice genotyping was performed. As a result, homo- and homozygotes were extracted. Optimal model of sciatic nerve injury was developed using the vascular clip. Finally the effectiveness of nerve damage was confirmed by histological immunohistochemical and gait analyzing.

Mice reproduce without problems, and thanks to the genotyping may extract heterozygotes. It is worth saying that method of sciatic nerve injury using vascular clip in this experiment is reproducible and gives reliable results. At the end it is worth noting that studies of pro- and anty-apoptotic proteins gene expression using knock out technology may be helpful in the treatment of patients suffering from neurodegenerative diseases.

Encephalisation Quotient and Relative Brain Part Sizes Provide Little Evidence that Machiavellian Behaviour in Cleaner Wrasse Selects for Complex Brains

The Machiavellian Intelligence Hypothesis – at least in its original form – provides a wide permissive perspective on the variety of socio-cognitive adaptations through which an individual may exploit the potential benefits of its social world, as well as dealing with the hostile aspects of it. Thus, in addition to socio-cognitive abilities like perspective-taking and knowledge of social hierarchy, they explicitly included social play, social curiosity, social learning and teaching, and thus cultural transmission, as well as social influenced flexibility, problem solving and innovation. The aim of this experiment is to use a different approach to study potential links between social complexity and brain size. First analysis focus on brain size relative to body size. We therefore use available data on the sizes of fish brain parts to explore the relative sizes of various brain parts: the % of weight of telencephalon, diencephalon, mesencephalon and cerebellum in relation to the entire brain. Our own data set and the data extracted from fishbase.org are consistent in suggesting that the relative brain size of Labroides species is not particularly large compared to other wrasse species, that could be explained by the fact that service quality are either not particularly cognitively demanding or that cleaners responded to selection for increased cognitive abilities with a restructuration of the brain rather than with an increase in brain size. Thus, the next step will be to look at the cleaners' brain architecture.

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