3rd International Seminar on Behavioral Methods

SZCZYRK, POLAND, 18-20.09.2014

Various species - similar behaviors

Seminar venue:

HOTEL META, 4 Skosna St, 43-370 Szczyrk, Poland

More information available on the website:

www.behavioral-methods.pl

Speakers:

Prof. Helmut Adelsberger, Institute of Neuroscience, Technische Universität München, Munich, Germany
Sarah Balder, NOLDUS Information Technology, Wageningen, The Netherlands
Prof. Hannah Buchanan-Smith, University of Stirling, Scotland
Dr. Paweł Boguszewski, Nencki Institute of Experimental Biology, Warsaw, Poland
Dr. Laurens W. J. Bosman, Erasmus MC, Rotterdam, The Netherlands
Dr. Fabio Canneva, Experimental Therapy, Preclinical Experimental Center, University of Erlangen, Germany
Dr. Anita Cybulska-Klosowicz, Nencki Institute of Experimental Biology, Warsaw, Poland
Dr inż. Rafał Głaszczka, Shim-Pol, Izabelin, Poland
Prof. Ewa Godzińska, Nencki Institute of Experimental Biology, Warsaw, Poland
Dr. Paul Hocking, Roslin Institute, University of Edinburgh, Scotland
Dr. Eliza Kiepura, Institute of Mother and Child, Warsaw
Prof. Patric Pageat, Research Institute in Semiochemistry and Applied Ethology, Toulouse, France and E.I. Purpan, Toulouse, France
Dr. Mark Rutter, Harper Adams University, England
Dr. Maria-Luisa Scattoni, Instituto Superiore di Sanita', Rome, Italy
Prof. Beat Schwaller, University of Fribourg, Fribourg, Switzerland
Dr. Marta Soares, Université de Neuchâtel, Switzerland
Piotr Trzaska, Central Europe Manager – JoVE, London, Great Britain
Florent Varenne, senior Consultant - View Point, Lyon, France
Szymon Wyrwicki, Sales Director AnimaLab, Poznan, Poland
Dr. Markus Wöhr, Philipps-Universität Marburg, Germany
Dr. Dora Zelena, Institute of Experimental Medicine, Budapest, Hungary

Organizer:

Center for Experimental Medicine Medical University of Silesia in Katowice,4 Medyków St, 40-752 Katowice, Poland, tel.: +48 32 208 85 93, fax +48 32 252 4275, 693 304 447 e-mail: dchojnacka@sum.edu.pl, jbarski@sum.edu.pl

Business Service Galop 22 Jesionowa St, 40-1591 Katowice, Poland, tel. +48 32 253 00 69, e-mail: a.czornik@kongresy.com.pl, www.kongresy.com.pl

CDM

Business Service galop

Organizer:

Center for Experimental Medicine, Medical University of Silesia in Katowice

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

Scientific board:

Chairperson:	Jarosław-Jerzy Barski, head of the Center for Experimental Medicine
Vice-chairpersons:	Dominika Chojnacka Paweł Boguszewski
Members:	Helmut Adelsberger Roman Aleksiewicz Beat Schwaller Agnieszka Sergiel

Organizing Committee:

Chairperson:	Dominika Chojnacka
Vice-chairperson:	Jarosław-Jerzy Barski

Sponsors and financial support:
AnimaLab – Strategic partner

Noldus Information Technology
AnimaLab Szymon Wyrwicki
Vivari Ewa Głowacka Regina Nowak
BIOGENET Grzegorz Chorzewski
Eppendorf Poland Sp. z o.o.
Meranco Sp. z o.o.
Arco System Sp. z o.o.
UGO BASILE S.R.L.
SHIM-POL A.M. Borzymowski
BMT Sp. z o.o.
Stowarzyszenie Śląska Poliklinika Weterynaryjna
STEFANIA Kempski i Wspólnicy Sp. J.
JoVE
Wytwórnia Pasz Morawski Zofia Połczyńska
Merazet S.A.
GL Center Sp. z o.o.

Special sponsorship for Prof. Beat Schwaller:
European Brain and Behaviour Society EBBS

PROGRAMME

Thursday, September 18th 2014

06:15 PM	Opening lecture **Prof. Ewa Godzińska, Nencki Institute of Experimental Biology, Warsaw, Poland** Some mortal and venial sins in the analysis of behavior
07:00 PM	Welcome cocktail

Friday, September 19th 2014
I. Morning session
RODENT MODELS I

09:00–09:30 AM	**1. Dr. Fabio Canneva, Experimental Therapy, Preclinical Experimental Center, University of Erlangen, Germany** Longitudinal intra-homecage phenotyping of a transgenic rat model of Alzheimer's disease
09:30–10:00 AM	**2. Dr. Paweł Boguszewski, Nencki Institute of Experimental Biology, Warsaw, Poland** New and "cool" methods in behavioral neuroscience
10:00–10:30 AM	**3. Florent Varenne, senior Consultant – View Point, Lyon, France** New trends in behavior analysis
10:45–11:00 AM	Coffee break
11:00–11:30 AM	**4. Prof. Helmut Adelsberger, Institute of Neuroscience, Technische Universität München, Munich, Germany** Phenotyping and *in vivo* calcium imaging using a mouse model of Alzheimer´s disease
11:30–12:00 AM	**5. Dr. Dora Zelena, Institute of Experimental Medicine, Budapest, Hungary** The vasopressin deficient Brattleboro rat as a good animal model of schizophrenia
12:00–12:30 PM	**6. Szymon Wyrwicki, Sales Director AnimaLab** AnimaLab – comprehensive source of products *in vivo* and *in vitro* research
01:00–02:00 PM	Lunch
02:00–03:00 PM	Poster session

II. Afternoon session
BEHAVIORAL RESEARCH IN HUMAN AND OTHER PRIMATES

03:00–03:30 PM	**1. Sarah Balder, NOLDUS Information Technology, Wageningen, The Netherlands** Innovations in behavioral data collection

03:30–04:00 PM	**2. Dr inż. Rafał Głaszczka, Shim-Pol, Izabelin, Poland** Shimadzu LABNIRS – a functional Near-Infrared Spectroscopy System for studies of brain activity
04:00–04:30 PM	**3. Dr. Maria-Luisa Scattoni, Instituto Superiore di Sanita', Rome, Italy** Non invasive tools for early detection of autism spectrum disorders
04:45–05:00 PM	Coffee break
05:00–05:30 PM	**4. Prof. Patric Pageat, Research Institute in Semiochemistry and Applied Ethology, Toulouse, France and E.I. Purpan, Toulouse, France** Attachment-related pheromones in human: their effects on confidence during social interactions
05:30–06:00 PM	**5. Prof. Hannah Buchanan-Smith, University of Stirling, Scotland** Behavioural methods for welfare assessment in non-human primates and dogs used in laboratory research and testing
06:00–06:30 PM	**6. Dr. Eliza Kiepura, Institute of Mother and Child, Warsaw, Poland** Social interactions and vocal behaviors of mothers and preterm *versus* full term babies: combining micro and macro approaches to behavior analysis
06:30–07:30 PM	Poster session
07:30 PM	Gala dinner

Saturday, September 20th 2014
I. Morning session
RODENT MODELS II

09:00–09:30 AM	**1. Dr. Laurens W.J. Bosman, Erasmus MC, Rotterdam, The Netherlands** Sensory, motor and cognitive aspects of cerebellar control over the whisker system
09:30–10:00 AM	**2. Prof. Beat Schwaller, University of Fribourg, Fribourg, Switzerland** A comprehensive behavioural analysis reveals an autism-like phenotype in parvalbumin-deficient mice. EBBS-lecture
10:00–10:30 AM	**3. Dr. Markus Wöhr, Philipps-Universität Marburg, Germany** Ultrasonic communication in rodents
10:45–11:00 AM	Coffee break
11:00–11:30 AM	**4. Dr. Maria-Luisa Scattoni, Instituto Superiore di Sanita', Rome, Italy** Modeling social communication deficits in mouse models of autism
11:30–12:00 AM	**5. Dr. Anita Cybulska-Klosowicz, Nencki Institute of Experimental Biology, Warsaw, Poland** The role of dopamine transporter in executive control function of attention

12:00–12:30 PM	**6. Piotr Trzaska, Central Europe Manager – JoVE, London, Great Britain** JoVE - increasing reproducibility of experiments – how to make it easier?
01:00–02:00 PM	Lunch
02:00–02:30 PM	Poster session and oral presentation

II. Afternoon session
NOAH'S ARK

02:30–03:00 PM	**1. Dr. Mark Rutter, Harper Adams University, England** The automatic recording of foraging in ruminants: Research into practice
03:00–03:30 PM	**2. Dr. Marta Soares, Université de Neuchâtel, Switzerland** Using the marine cleaning mutualisms to study the physiological bases of cooperative and deceptive behavior
03:30–04:00 PM	**3. Dr. Paul Hocking, Roslin Institute, University of Edinburgh, Scotland** Analysing animal welfare by sequence analysis of behavior
04:00–04:30 PM	**4. Prof. Ewa Godzińska, Nencki Institute of Experimental Biology, Warsaw, Poland** Behavioral techniques and bioassays used in the research on social insects
04:45–05:00 PM	Break
05:00–06:00 PM	Poster session
06:00 PM	Good-bye meeting

LECTURE SESSION

L1. Phenotyping and *in vivo* calcium imaging using a mouse model of Alzheimer´s disease

Adelsberger H.
Institute of Neuroscience, Technische Universität München, Munich, Germany

Alzheimer's disease (AD) is characterized by the development of learning and memory deficits. Particularly at later states of the disease, patients also progressively exhibit cognitive impairments and deficits in central sensory processing. Accumulation of amyloid-β in the brain of patients is thought to play a direct role in the initiation of AD. We use double transgenic APP23 x PS45 mice, who overexpress both β-amyloid precursor protein and mutant presenilin 1 under the control of Thy-1 promoter as a model system to study the consequences of enhanced β-amyloid levels. With two-photon based fluorometric calcium imaging we detected age-dependent impairments of both spontaneous and stimulus evoked neuronal activity on the level of individual neurons. In addition we also found dramatic changes in the long-range coherence of wave-like calcium transients with CCD-camera based calcium imaging. These transients reflect synchronous activity of populations of neurons and are involved in the coordination of brain networks. Phenotypical analysis of mutant mice showed age-dependent impairments in spatial and working memory tasks as well as in a test for visual acuity. These findings indicate a correlation between changes in the neuronal activity and the behavior.

L2. New and "cool" methods in behavioral neuroscience

Boguszewski P.M., Puścian A., Knapska E.
Department of Neurophysiology, Nencki Institute of Experimental Biology, Warsaw, Poland

Behavioral tests in laboratory rodents play an essential role in basic and applied biomedical research. Development of new animal models for neurological and psychiatric disorders, as well as preclinical phase of drug research require a „proof-of-concept" testing on a system level. Since most of the behavioral procedures are not rigorously standardized, it is difficult to obtain replicable results between laboratories. One of the approaches to solve this problem is designing more ethologically-relevant behavioral tasks, in which behavioral expression is more voluntary and manifold behavioral measures are collected over long periods. Collecting large amounts of data requires automatic control of all stages of a study – experimental cage/system manipulation, data gathering and analysis. Such automated systems offer important advantages. They increase level of standardization that results in more coherent data, save time and manpower, as well as reduce animal numbers required. Automated monitoring, although new and often sophisticated, could be cheap as well. The inspiration comes from growing popularity of amateur robotics, accessibility of 3D printing and progress in electronics. The aim of the lecture is to present new tests and solutions we are developing in Nencki Institute, including RFID tags for social experiments, automatic vocalization classification, video image animals detection and recognition software and even Mindstorm Lego robots.

L3. Sensory, motor and cognitive aspects of cerebellar control over the whisker system

Bosman L.W.J.[1], Lindeman S.[1], Spanke J.K.[1,2], Ju C. [1], Owens C.B.[1], Negrello M.[1], Koekkoek S.K.E.[1], De Zeeuw C.I.[1,2]
[1]Department of Neuroscience, Erasmus MC, Rotterdam, The Netherlands; [2]Netherlands Institute for Neuroscience, Royal Academy of Arts and Sciences, Amsterdam, The Netherlands

Mice rely on whiskers to orient themselves in the environment and to determine position, size and texture of objects. They have large whiskers that can be moved rhythmically. In line with its behavioral importance, a substantial part of the brain is devoted to the whisker system. Most attention has been paid to ascending trigemino-thalamo-cortical pathways and descending pathways from motor cortex to brainstem, but many other brain regions are also part of the whisker system: I will discuss sensory, motor and cognitive roles of the cerebellum, a crucial organ for sensorimotor control. The cerebellum receives strong whisker input, directly *via* the brainstem and indirectly *via* the cerebral cortex. The spatial and temporal organization of whisker input to the cerebellum will be discussed as well as the cerebellar impact on whisker movement. This will be shown in electrophysiological recordings in awake mice. Finally, we demonstrate that mutant mice specifically impaired in synaptic and intrinsic plasticity of cerebellar Purkinje cells have severe problems learning a whisker based object localization task, showing that the cerebellum is also important for cognitive aspects of behavior.

L4. Behavioural methods for welfare assessment in non-human primates and dogs used in laboratory research and testing

Buchanan-Smith H.M.[1], Tasker L.[1], Hall L.[1], Robinson S.[2], Ash H.[1], Lynn D.[1]
[1]Psychology, School of Natural Sciences, Stirling, Scotland; [2]AstraZeneca, Alderley Park, England

For animals that can suffer, we have an ethical and legal obligation to ensure their welfare is maximised. Given the link

between animal welfare and scientific output we need to ensure animals are "fit for purpose", and develop reliable tools that can measure welfare accurately and practically. These welfare tools should be developed using a battery of measures. Behaviour is the most accessible and practical measure of welfare, and one of the most informative. Behaviour is the ultimate phenotype – it is the result of the animal's own individual decision making processes, is the expression of emotions, and may reflect underlying physiology. Measuring behaviour is non-invasive and in many cases also non-intrusive. In this presentation, I shall describe a battery of behavioural measures used to assess welfare in non-human primates and dogs. This includes behaviour in home enclosure, in response to challenge and in anticipation of an event, cognitive bias to assess an animal's emotional state, and the relationship between behaviour and cardiovascular and hormonal measures. Examples of how behaviour and welfare is influenced by laboratory husbandry and procedures will be given. Developing and using practical behavioural welfare tools promotes both Refinement and Reduction, two of the 3Rs.

L5. The role of dopamine transporter in attentional functions

Cybulska-Klosowicz A.
Laboratory of Neuroplasticity, Nencki Institute of Experimental Biology, PAS, Warsaw, Poland

Attentional functions are regulated by a dopaminergic (DA) system. Dopamine transporter (DAT), regulating DA neurotransmission, likely plays a role in controlling the influence of DA in cognitive processes. The aim of the study was to examine the role of DAT in attention. Mice with DAT gene genetically deleted (DAT+/- heterozygotes) were compared with the wild type (WT) mice in several tests of attention. Locomotor activity and non-selective attention were tested in a Làt-maze. Attentional set-shifting was tested in intradimensional shift (IDS) and in extradimensional shift (EDS) phases of the attentional set-shifting task (ASST). Associative and reversal learning were tested in a simple discrimination (SD), compound discrimination (CD) and reversal (Rev) phases of ASST task. Sustained attention was tested in an operant chamber. DAT level in the striatum of mice was compared using DAT immunohistochemistry. Neuronal activity during ASST was visualised with the egr-1 and egr-2 immunohistochemistry. The results obtained show that DAT+/- mice have significantly higher scores of locomotor activity in comparison with WT mice. DAT+/- mice do not differ in simple associative learning and non-selective attention scores when compared with WT mice. However, they are impaired in tests that tax executive control function of attention. The results suggest that normal DAT activity level is required for optimal attentional functioning.

NSC Grant 2011/01/D/NZ4/04958.

L6. Some mortal and venial sins in the analysis of behavior

Godzińska E.J.
Laboratory of Ethology, Department of Neurophysiology, Nencki Institute of Experimental Biology, PAS, Warsaw, Poland

In 1997 Manfred Milinski published an influential paper entitled "How to avoid seven deadly sins in the study of behavior" in which he described in detail seven major errors relatively frequently committed during behavioral experiments and discussed their negative consequences. The list of mortal and venial sins in the analysis of behavior is, however, much longer and includes major and minor errors made at all successive steps of behavioral research: designing the experiment, selection and treatment of experimental animals, quantification of their behavior and statistical analysis of behavioral data. If unchecked, such errors may have extremely negative consequences and lead to incorrect conclusions. I will discuss a wide spectrum of examples of such errors taken from literature and from my own experience and discussions with other researchers.

L7. Behavioral techniques and bioassays used in the research on social insects

Godzińska E.J.
Laboratory of Ethology, Department of Neurophysiology, Nencki Institute of Experimental Biology, PAS, Warsaw, Poland

Social insects are increasingly frequently used as experimental subjects by researchers investigating such questions as neurobiological mechanisms underlying learning and memory processes, alcohol and drug addiction and various types of aggression, ageing processes and their reversal, and other forms of phenotypic plasticity. Behavioral methods used in that research are surprisingly rich and sophisticated. I will describe various manipulations of social context known as techniques of social engineering (mostly various forms of partial or complete social deprivation and other modifications of social group size and structure) and their consequences (in particular, expression and/or suppression of specific behavior patterns, and acceleration, retardation or reversion of individual development). I will also discuss some classical and new methods of administration of neuroactive compounds and a wide array of bioassays used to study responses of social insects to unanimate objects, and their aggressive and non-aggressive interactions with conspecific and allospecific individuals. I will also provide some examples showing how these techniques can be used in innovative interdisciplinary research.

L8. Analysing animal welfare by sequence analysis of behavior

Hocking P.M.
Division of Genetics and Genomics, Roslin Institute and R(D) SVS, University of Edinburgh, Eater Bush, Midlothian, Scotland

The analysis of behaviour as the proportion of time spent on particular behaviours (an ethogram) has been widely used as a proxy for assessing the welfare, or the internal mental state, of domestic poultry. New techniques using detailed analysis of ehavioural sequences are based on the premise that an unpleasant experience or condition will affect the normal pattern of behaviour. The major techniques are detrended fluctuation analysis (DFA), real-time patterns (T-patterns) and social network analysis (SNA). DFA uses binary measures (e.g. active vs. non-active) and assesses change over a long period. However, welfare implications are difficult to interpret as there is no simple relationship with stress. T-patterns are unique sequences of behaviour (e.g. walk-stand) separated by a specific period of time, that occur more often than expected. Sequence information is retained but several arbitrary input parameters are required for the analysis. SNA estimates transition frequencies between different behaviours and can be used at the individual or group level. In a study of feed restricted boiler parent chickens fed on different quantities of two feeds, T-patterns complemented an ethogram approach whereas DFA was less useful. In an earlier investigation both T-patterns and SNA enriched the comparisons of different broiler genotypes fed *ad libitum* or restricted.

L9. Social interactions and vocal behaviors of mothers and preterm *versus* full term babies: Combining micro and macro approaches to behavior analysis

Kiepura E.[1], Kmita G.[1,2]
[1]Institute of Mother and Child, Warsaw, Poland; [2]Faculty of Psychology, University of Warsaw, Warsaw, Poland

With relatedness being one of the most fundamental human motives and needs, infants are born with progressive capacity to communicate with others. Social interactions are the building blocks of human development. In particular, they can be considered as a very important "port of entry" in the early psychological intervention addressed to preterm infants and their families. Authors will present a unique approach to the psychological observation of infants and their parents that combine micro- and macroanalysis of behavior. The results of own research on the parent and three-month-old infant interactions will be presented. Protodialogues between 30 mother-full term as compared to 30 mother- preterm infant dyads will be described on the level of the microanalysis of vocal interactions (time, prosody and content analysis) as well as on macroanalytic level of patterns of interactive behaviors. The presentation will be also illustrated with the results of the microanalysis of interactional behaviors of the same babies aged 6 and 12 months. The study was conducted with the use of the Praat software and Observer XT 9.0 by Noldus. Theoretical and practical implications will be discussed.

L10. Attachment-related pheromones in human: Their effects on confidence during social interactions

Pageat P., Lecuelle C., Cozzi A.
IRSEA Research Institute, Saint-Saturnin-les-Apt, France

In 1999, our team has identified the first mammalian maternal appeasing pheromone, in sows and female dogs. We then identified a similar secretion that we have named HAP (Human Appeasing Pheromone), released by Montgomery glands from the areolae and a synthetic analogue of it was prepared. This paper describes the effects of HAP in children (18 to 36 months-old), experiencing a routine physical examination in a pediatric hospital. 100 children, 18 to 36 months old, presented for routine examination, have been enrolled in the Department of Pediatric Medicine of the Hospital of La Seyne-sur-Mer (France). The parents were informed about the study and after they accepted to participate in it, their children were enrolled in the study. Treatment, HAP or placebo, was allocated at random. The semiochemical was delivered by the mean of a tissue doll stuffed with a biopolymer (MaterBi®) containing 10% of synthetic HAP. Placebo doll were stuffed with pure MaterBi®. The doll was given 5 min before the examination. Two principle parameters were studied: the Mean Heart Rate (MHR), calculated from 5 measurements (waiting room, beginning and end of examination by the nurse, beginning and end of examination by the pediatrician); and the mean behavioural score (MBS from 0, easy examination, to 5 examination requires someone to help in controlling the child) measured in the same time as the heart rate. The groups were compared using Student *t* test for "age" and χ^2 for "sex". Having two principle parameters, a Bonferroni correction was applicable and the alpha risk was 2.5%. Two factors variance analysis (treatment and pediatrician) was used for MHR and MBS. For MBS, we also used Scheirer Ray Hare test. The two groups were comparable regarding sex and age. In HAP group, both MHR and MBS were very significantly lower ($P<0.001$) and this difference was confirmed using Scheirer Ray Hare test. Interaction between "pediatrician" and "treatment" was not significant. As observed in veterinary medicine with homologous maternal semiochemicals, the use of synthetic analogues of maternal odorous secretions,

appears to be a promising technique. Both autonomic and behaviours are affected in presence of maternal semiochemicals, controlling the severity of the clinical signs displayed by patients facing stressing situations.

L11. Longitudinal intra-homecage phenotyping of a transgenic rat model of Alzheimer's disease

Canneva F.
Experimental Therapy, Preclinical Experimental Center, University of Erlangen, Germany

A comprehensive behavioral screening was carried out in the McGill-R-Thy1-APP rat model of Alzheimer's disease, over 11 months. Classical behavioral read-outs were performed parallel to intra-homecage metabolic measurements (PhenoMaster®) and operant conditioning (IntelliMaze®), once every 3 months. The collection of data gathered in this study offers a measure of the sensitivity and throughput of automated phenotyping as compared to classical paradigms, for the detection of cognitive and non-cognitive alterations in transgenic rats.

L12. The automatic recording of foraging in ruminants: Research into practice

Rutter S.M.
The National Centre for Precision Farming, Harper Adams University, Newport, Shropshire, United Kingdom

Grazing is a cheap and sustainable way to feed ruminant livestock, and is important in maintaining the open grassland landscapes that dominate much of Europe. Researchers have devised several methods to record when and where livestock graze in response to the spatial and temporal heterogeneity of grassland swards. Advances in technology mean that these approaches have the potential for application in decision-support systems to help farmers monitor and manage grazing. Although Global Navigation Satellite System receivers work well in hilly terrain, their high power consumption means that local radio-based positioning systems are potentially more viable for on-farm use. A bioacoustics approach to grazing (i.e. recording and analysing the sound of grazing) has the potential to record when animals are grazing, the plant species being eaten and the amount of herbage consumed. A bioacoustic sensor will probably need to be combined with an accelerometer to detect head movements to overcome the problem of the sound of one animal's grazing interfering with the analysis of that from a nearby conspecific. The precise monitoring of grazing behaviour and intake should provide farmers with practical tools that help them improve the economic efficiency and environmental sustainability of livestock production.

L13. Noninvasive tools for early detection of autism spectrum disorders

Scattoni M.L.
Department of Cell Biology and Neuroscience, Istituto Superiore di Sanità, Rome, Italy

Autism spectrum disorders (ASDs) are often not diagnosed until children reach 3–4 years of age. Early identification of young children with ASDs, possibly through a set of behavioral and neurophysiological indexes, is crucial in light of findings indicating that early intervention is much more effective than interventions starting in later childhood. Aim of our study is to identify early diagnostic markers through the assessment of neurobiological and developmental patterns in infant siblings of children with ASDs. We focused on age-specific motor and vocal repertoires which are known to be later impaired in ASD children and that have been found altered in other neurodevelopmental disorders. Analysis of infant crying revealed that high risk infants have a lower frequency of fundamental frequency and of the two resonance frequencies F1 and F2 as compared to full term subjects. General movements' analysis revealed an unusual motor pattern in infants at high risk. Moreover, five high risk infants showed no responses to name, deficits in emotion recognition, and poor motor development. Our preliminary results showed the importance of monitoring high-risk infant development during the first six months of life and suggest the usefulness of these non-invasive tools to identify early diagnostic markers. However, the sample size, primarily of high risk subjects, needs to be increased.

L14. Modeling social communication deficits in mouse models of autism

Scattoni M.L.
Department of Cell Biology and Neuroscience, Istituto Superiore di Sanità, Rome, Italy

Male and female mice emit ultrasonic vocalizations during infancy when pups are separated from mother and littermates, as well as at adulthood in different experimental/social contexts. Mouse ultrasonic vocalizations had become now a popular assay for behavioral phenotyping throughout the life-span of models of autism since this response represents the best option to detect deficits within the social communication domain in the mouse species. In the present talk, I will describe the available methods to elicit and record mouse ultrasonic vocalizations in different social contexts and at different ages and behavioral data collected on Autism animal models in these paradigms/contexts. Thanks to the progresses of computer technology, researchers can now perform detailed analyses of the vocal repertoire (clas-

sifying ultrasonic vocalizations into different categories). Recently, these analyses have revealed unusual vocal patterns in selected mouse lines. This innovative approach allows detecting also qualitative alterations in the social communication repertoire usually not identified with the standard analysis of emission rate. Future studies should be aimed at performing quantitative and qualitative analyses of vocalization patterns also in preclinical studies evaluating potential treatments in validated autism mouse models.

L15. A comprehensive behavioural analysis reveals an autism-like phenotype in parvalbumin-deficient mice

Schwaller B.
Anatomy, Dept. of Medicine, University of Fribourg, Fribourg, Switzerland

Mutations in several genes and gene copy number variants are associated with neurodevelopmental disorders including autism spectrum disorders (ASD). Several gene products putatively implicated in ASD are frequently part of signaling networks involved in synapse formation and/or function leading to changes in the balance between excitation and inhibition (E/I balance). The network of parvalbumin (PV)-expressing interneurons has gained particular attention in ASD, yet not much is currently known on PV's possible role with respect to ASD. PV-knockout mice were subjected to a battery of behavioral experiments testing for ASD-like, schizophrenia-like and anxiety-related behavior. PV-/- mice display the three core symptoms present in ASD patients: impaired social interactions, reduced communication and repetitive and stereotyped behavior. In addition, they also show ASD-associated developmental neuroanatomical changes in the neocortex and the cerebellum. Using electrophysiology, we observed that the E/I balance is altered by modification of both, inhibitory and excitatory synaptic transmission. Based on the reported changes in PV expression pattern in numerous mouse ASD models, we suggest a convergent pathway in ASD, where mutations in ASD-linked genes may lead to (homeostatic) adaptations causing a down-regulation of PV that ultimately results in the ASD phenotype.

L16. Using the marine cleaning mutualisms to study the physiological bases of cooperative and deceptive behaviour

Soares M.
University of Neuchatel, Neuchâtel, Switzerland

Cleaner fish inspect the surface, gills and sometimes the mouth of so called 'client' reef fish, eating ectoparasites, mucus, scales and dead or infected tissue. Individuals of the best studied species, the Indo-Pacific bluestreak cleaner wrasse Labroides dimidiatus, may engage in as many as 2000 such interactions per day, and individual clients often visit cleaners several times during the same period, the highest recorded number of visits exceeding 100. While there is evidence that both cleaners and clients typically gain from their encounters, there are several conflicts of interest that lead to sophisticated behavioural strategies: predatory clients may try to eat cleaners, cleaners prefer the protective mucus layer of clients over ectoparasites, and sometimes two or more clients simultaneously seek the service of the same cleaner. In addition, cleaners are known to recognize individual clients, to distinguish between client categories (predators – non predators, residents – visitors), to reconcile and manipulate client decisions by providing a form of tactile stimulation with their pelvic fins, to adjust their service quality to the presence or absence of potential clients, and to use predators as social tools to stop other clients from punishing after a cheat. During this talk I will focus on the methodology applied to collect empirical data on these systems, as to answer both functional and mechanistic questions.

L17. Innovations in behavioral data collection

Balder S.
Noldus Information Technology, Wageningen, the Netherlands

Behavioral research has developed strongly over the last years. In human behavior research, we moved from asking for opinions and experiences to observing and measuring. At the same time the location of research is shifting from the lab to the real world. This presentation gives an overview of existing solutions and innovative developments for observing and measuring behavior, including real examples from research conducted with these tools. Noldus Information Technology is an international company that develops innovative solutions for behavioral research. Within the research and innovation lab, Noldus InnovationWorks, novel products and prototypes are being developed by participating in research projects. [Sarah Balder works as Sales Consultant within Noldus Information Technology and as Business Developer within Noldus InnovationWorks. Within these roles she is responsible for consultancy and sales of solutions for behavioral research and for introducing new products and prototypes.]

L18. Ultrasonic communication in rodents

Wöhr M.
Behavioral Neuroscience, Philipps-University of Marburg, Marburg, Germany

Rats emit distinct types of ultrasonic vocalizations (USV), which serve as situation-dependent affective signals with important communicative functions. Low-frequency 22-kHz USV typically

occur in aversive situations, such as social defeat or predator exposure, whereas high-frequency 50-kHz USV can be observed in appetitive situations, like social play in juveniles or mating in adults. Importantly, the two main USV types serve distinct communicative functions and induce call-specific behavioral responses in the receiver. While 22-kHz USV probably serve as alarm calls and induce freezing behavior in the receiver, 50-kHz USV lead to social approach behavior, indicating a pro-social, affiliative communicative function. The opposite behavioral responses are paralleled by distinct patterns of brain activation. Freezing elicited by 22-kHz USV is accompanied by increased neuronal activity in brain areas regulating fear and anxiety, such as the amygdala. In contrast, social approach behavior evoked by 50-kHz USV is paralleled by reduced activity levels in the amygdala, but enhanced activity and dopamine release in the nucleus accumbens, a brain area implicated in reward processing. Together, this indicates that affective USV might be an important tool for studying the neurobiology underlying socio-affective communication, which is particularly relevant for rodent models of neuropsychiatric disorders characterized by social and communication deficits, such as autism and schizophrenia.

L19. The vasopressin deficient Brattleboro rat as a good animal model of schizophrenia

Zelena D., Varga J., Fodor A., Klausz B.
Laboratory of Behavioural and Stress Studies, Institute of Experimental Medicine, Budapest, Hungary

Schizophrenia is a devastating disorder for the afflicted people and very costly for the society. Therapy is not solved and preclinical testing requires animal models. Several studies suggested that vasopressin is important in schizophrenia. Patients have lower plasma vasopressin levels, which was normalized after antipsychotic treatment. Prepulse inhibition (PPI) is reduced in unmedicated schizophrenic patients, which correlates with the degree of thought disorder. Therefore the vasopressin-deficient Brattleboro rat might be an appropriate model. Indeed, they have a number of cognitive and behavioral abnormalities that are analogous to those seen in schizophrenia patients. Beside the reduced PPI, their object, as well as social discrimination abilities are also poor. In connection with social communication deficit pups emit less ultrasound upon maternal separation. EEG analysis showed circadian rhythm alterations in vasopressin-deficient rats; they spent more time awake during their inactive phase. After antipsychotic treatment all of these changes was normalized. Although these alterations might be observable in other mental disorders, but all data together and the response to antipsychotic treatment suggest that Brattleboro rats are unique model of schizophrenia having a natural (nonpharmacological) deficit in all above mentioned tests.

L20. Shimadzu LABNIRS – a functional Near-Infrared Spectroscopy System for studies of brain activity

Głaszczka R.
Shim-Pol, Izabelin, Poland

Just how the human brain functions remains one of the greatest unsolved puzzles. To solve this mystery, brain-function imaging for visualization of brain functions has developed rapidly in recent years. In particular, *in vivo* optical imaging by functional near-infrared spectroscopy (fNIRS) has attracted attention as a technique that supports next-generation brain science. Utilizing its leading-edge science and technology, Shimadzu has developed the LABNIRS, thereby contributing to the still growing field of brain science. This system is capable of registering signal of interest in 6 ms. The studied object can be monitored in more than 140 channels. It can work simultaneously with EEG, fMRI, PET and MEG methods. [Rehabilitation Research (Movement, Work, Language, Hearing); Drug development and Medical Research (Neuroscience and Psychiatry Brain Function of Newborn Babies); Basic Research (Brain Functional Network Research, Multi-Modality Research)]

L21. AnimaLab – comprehensive source of products *in vivo* and *in vitro* research

Wyrwicki S.
AnimaLab – Animal facility and laboratory equipment

AnimaLab is a comprehensive provider of products and services for *in vitro* and *in vivo* research in Poland, Czech and Slovakia region. The company, founded in 2004, is engaged in the supply of laboratory animal models, automated testing equipment for behavioral, metabolic and physiological studies. We specialize in the design of Vivarium and the comprehensive furnishing them. We launch innovative research techniques, such as systems for the analysis of neural networks and cardiomyocytes on the dish, telemetry measurements of physiological parameters or test systems for measurement of oxygen level in tissue. We invite you to visit our website www.animalab.pl to learn more about our dedicated solutions for your research.

L22. JoVE - increasing reproducibility of experiments – how to make it easier?

Trzaska P.
JoVE, London UK

Use of modern, popular technologies for knowledge transfer between scientific and research institutions worldwide. Increasing the reproducibility of experiments means measurable savings of time, money, animal's lives and your nerves. How to make yours and other's researches easier?

L23. Pioneer in Videotracking analysis system, the company Viewpoint exists since 25 years. The heaed office is in France and our branches in Canada and China

Varenne F.
ViewPoint, Lissieu, France

ViewPoint provides innovate tools to assist researchers in their experimental projects. Rodent behavior: GaitLab is an automated quantitative gait analysis system for rodents, based on the CatWalk Method. The GaitLab turnkey system allows evaluation of many locomotor defects, such as those occurring in Parkinson's disease, arthrosis, spinal cord injury. The Marlau Cage purpose is to develop brain plasticity using different mazes and stimulate locomotor activity with 3 wheels. Then with this environmental enrichment, rodents are less sensitive to stress and they will provide you more significant result. PhenoRack, home cage monitoring system really easy to use. Detecting locomotions, behaviors, freezing, global activity. Then your results can be easily transfer to your spreadsheet or statistical software. Explore different ways to use VideoTrack software to run your tests such as openfields, all kind of mazes, light and dark boxes, Morris Water Maze, Forced Swim Test, EPM, CPP and much more... SleepDeprivation system. SleepScore is a software for the acquisition of electrophysiological signal necessary to the analysis of sleep in animals. Zebra Fish behaviour & screening: ZebraLab is the leading solution for zebra fish screening, zebralab is a state of the art automated observation and videotracking solution designed by Viewpoint. Zebralab can be deployed to track zebrafish larvea in multi-well plates.

POSTER SESSION

P1. Behavioral responses to amygdaloid stimulation

Myślińska D., Ruciński J., Czerwiec K., Wądołowska A., Ciepielewski Z., Kurowska E., Majkutewicz I., Plucińska K., Podlacha M., Ptaszek K.
Department of Animal and Human Physiology, University of Gdańsk, Gdańsk, Poland

The central nucleus of the amygdala (CeA) is involved in fear conditioning and participates in euroendocrine response to stressful stimuli. In our previous study, we found that CeA also influences immune function, especially innate antitumore response. In the present study, we investigated the influence of 14-day electrical stimulation of the CeA on corticosterone level (CORT; determined by radioimmunoassay) and behavioral response: locomotor activity in the new environment (measured in the Opto Varimex Minor actometer), explorative and anxiety-like behavior [assessed in the open-field (OF) and in the elevated plus-maze (EPM) tests]. Male Wistar rats implanted with stimulating electrodes into the CeA were divided into groups: CeA 14-day electrical stimulation (n=20) and CeA sham (n=18). Current intensity (70–120 μA; 50 Hz) was raised incrementally in 30-s trials until behavioral reaction in the Opto Varimex Minor actometer was observed. OF and EPM tests were performed after termination of stimuation procedure. Electrical stimulation of the CeA caused augmentation of CORT level (P<0.001) correlated with an increase in the average number of movements in horizontal and vertical plane imitating escape behavior. These effects were accompanied by an increase in anxiety-related behavior in the EPM and decreased locomotor activity and exploration in the OF. We suggest the hormonal response to of the CeA stimulation plays a crucial role in the regulation of the behavioral response.

Supported by The National Science Centre, grant number: NN303819040.

P2. An efficient method of digital analysis of spatial behavior modified by olfactory stimuli in *A. diaperinus*

Baran B.[1], Cup M.[2], Bednarek A.[1], Sawadro-Wieczorek M.[1], Francikowski J.[1], Dziewięcka M.[1]
[1]Department of Animal Physiology and Ecotoxicology, University of Silesia, Katowice, Poland; [2]University Association for Science, University of Silesia, Katowice, Poland

The beetle *Alphitobius diaperinus* (Coleoptera: Tenebrionidae), known as lesser mealworm or darkling beetle is a cosmopolitan pest and a crucial problem in poultry production. Consequently, deterioration of animals' health and fitness is observed. Hence, an effective deterrent against lesser mealworm is highly desired. As the model repellent, propionic acid was used. Only one paper treats on the effect of pure propionic acid, without additional substances. Dose-dependent repellent effects on adult stored-grain pests: *Sitophilus granarius* and *S. Oryzae* was observed. In our experimental setup each insect was placed separately in a custom-made rectangular chamber made of clear Lucite with constant air flow sources at the opposite sides of each chamber. The insect was allowed to move freely between the side supplying the chamber with pure air, and that with propionic acid odored air. The locomotion of insects was digitally recorded and analyzed with SwisStrack software. As the effect of analysis a pattern of spatial preferences was obtained. Used method allowed to determine dynamic changes of spatial behavior modified by olfactory stimulus. Developed protocol will be useful as an assay to test potential repellent agents against *A. diaperinus*.

P3. Influence of selected kinds of stress on the behavior of young and mature rats

Wesołowska B., Babińska I., Kowalski A.
University of Warmia and Mazury, Olsztyn, Poland

The reaction to stress stimuli occurs at the mental and somatic level, and it is why the behavioral changes may be a good predictor for assessment of such states. Many studies show that the age of animal influences the adaptation abilities to a significant level. The aim of the study was to compare adaptation processes in response to short-lasting stressors by the analysis of behavior of young (2-month-old) and mature (12-month-old) rats. The experiment was performed on 60 rats which were treated with the following stressors: food deprivation, water deprivation, isolation and bacterial lipopolysaccharide (LPS) injection. Then the behavioral tests of „open field" and „eight arms" were performed. The obtained results showed significant decrease of the activity of mature rats in comparison to young ones in both behavioral tests, especially in response to water deprivation („open field") and isolation („eight arms"). Only in the group treated with LPS young rats showed lower activity, however, in this group the lowest activity was seen on both age groups. Animals showed significant decrease of motor activity. Higher activity of mature rats in response to LPS treatment may be due to the higher resistance to pyrogen and the lower energy demand to fight the inflammation.

P4. The role of hypothalamic glycine receptors in regulation of the male rat sexual behavior

Zhuravleva Z.[1], Volnova A.[2], Mukhina I.[1,3], Druzin M.[4], Lebedeva A.[1]
[1]Lobachevsky State University of Nizhny Novgorod, Nizhny Novgorod, Russia; [2]Saint Petersburg State University, Saint Petersburg, Russia; [3]Nizhny Novgorod State Medical Academy, Nizhny Novgorod, Russia; [4]Umeå University, Umeå, Sweden

Membrane-bound glycine receptors (GlyRs) mediate inhibition in many central neurons including hypothalamic neurons from the medial preoptic area (MPOA), known to be critically involved in reproductive behavior of all vertebrate species. To study the contribution of the abundant GlyRs present in this area to the male rat sexual behavior we used intracranial bilateral microinjection of the GlyR-targeting compounds delivered into MPOA through the chronically implanted stainless steel microcannulae. Several behavioral patterns such as session duration, duration of postejaculatory period, number of intromissions and ejaculations were routinely monitored using video registration. The data analysis revealed that the bilateral microinjection of glycine (1 mM) into the male rat MPOA reliably decreased the ejaculation latency period, the duration of the postejaculatory period and the number of intromissions. Interestingly, the bilateral microinjection of GlyR antagonist strychnine (20 μM) had similar effect on the ejaculation latency period and the number of intromission but not on the postejaculatory period. Thus, our results provide the first experimental evidence for the involvement of GlyRs expressed in MPOA in the regulation of the male sexual behavior and improve our understanding of the mechanisms underlying such behavior.

P5. Detour test in mice

Kokocińska A.[1], Juszczak G.[2], Korwin M.[2]
[1]Warsaw University of Life Sciences – SGGW, Warsaw, Poland; [2]Institute of Genetics and Animal Breeding, Jastrzębiec, Poland

A common test of intelligence is the detour test where a forward movement, motivated usually by a reward, is prevented by transparent barrier. In order to solve the task, the subject has to move initially away from the target and therefore the performance depends on the ability to inhibit the prepotent but ultimately counterproductive responses driven by visual stimulus. Up to date, detour abilities have been shown in different species including humans, monkeys and dingoes, but there is no rodent detour test applying transparent barrier. Therefore, we have developed a mouse detour test based on water-escape paradigm. Mice were first trained to swim toward the visible platform and next were tested to detour the V shaped glass barrier placed in front of the platform. We have found that mice from different strains are able to learn how to solve the task although there are between-subject and between-strain differences. We have also found that mice display behavioral lateralization in the detour test.

P6. The effect of caffeine on the activity of NMDA receptor ligands in the forced swim test in mice

Poleszak E., Szopa A., Serefko A., Wośko S.
Chair and Department of Applied Pharmacy, Medical University of Lublin, Lublin, Poland

Depression is one of the most frequent chronic and life-debilitating health problems in the world. Though its epidemiology, risk factors and development have been continuously studied for decades, the optimal treatment of the depressed patients still remains an important clinical dilemma. The conventional antidepressant therapy is not sufficient due to the common ineffectiveness and adverse reactions. The search for better alternatives seems to be crucial. Recently, much consideration has been given to both totally novel chemical compounds as well as to the unique combinations of the well-known drugs. The use of the NMDA receptor ligands, particularly in co-administration with other agents exerting the antidepressant activity, emerged amongst the new ideas. The main objective of our study was to evaluate the influence of caffeine on the antidepressant-like activity of various NMDA receptor modulators. The antidepressant-like effect

was assessed by the forced swim test in mice. The obtained results demonstrated the significant interaction between caffeine (5 mg/kg) and the following NMDA receptor ligands: CGP 37849 (0.312 mg/kg), L-701,324 (1 mg/kg) and D-cycloserine (2.5 mg/kg). Interaction between caffeine and the inorganic modulators, i.e. Zn^{2+} (2.5 mg/kg) and Mg^{2+} (10 mg/kg), was not considered as significant. The antidepressant-like potential of the NMDA receptor ligands (except for Zn^{2+} and Mg^{2+}) given concomitantly with caffeine was reinforced. The possible mechanism of the observed interaction may involve the influence of caffeine on the glutamatergic system.

P7. Antipsychotic activity of the positive allosteric modulator of mGlu2R in mice model of schizophrenia

Belousova I.I.[1,2], Shalnov A.V.[1], Ěukhina I.V. [1,2]

[1]Nizhny Novgorod State Medical Academy, Nizhny Novgorod, Russia; [2]Lobachevsky State University of Nizhny Novgorod, Nizhny Novgorod, Russia

Glutamatergic dysfunction has been implicated in psychiatric disorders such as schizophrenia. Stimulation of the metabotropic glutamate (mGlu) 2/3 receptor have been shown to be effective in a number of animal models of schizophrenia. For modelling symptoms of schizophrenia a selective and non-competitive NMDA receptor antagonist MK801 were used. The administration of MK801 evoked hyperactive locomotor behavior with increases in distance travelled, speed, and clockwise/anticlockwise locomotion, and a marked decrease in rearing behavior and disruption of PPI in mice. The intraperitoneal administration of a positive modulator mGlu2-receptor Biphenylindanone A (BINA) reversed disrupted PPI evoked by MK801 administration and improve locomotion impairment. These findings indicate that the stimulation of the mGlu2 receptor improved locomotion impairment elicited by MK-801, and such manipulations could be effective approaches for the treatment of behavioral dysfunctions observed in schizophrenic patients.

P8. Repeated intra-hippocampal and intra-accumbal infusions of phosphodiesterase 4 inhibitor rolipram have opposite effects on depressive-like behavior in rats

Bollen E.[1], De Vry J.[2], Vanmierlo T.[2], Steinbusch H.[2], Prickaerts J.[2]

[1]Department of Behavioral Neuroscience, Institute of Pharmacology, Polish Academy of Sciences, Cracow, Poland; [2]School for Mental Health and Neuroscience, Maastricht University, Maastricht, The Netherlands

Phosphodiesterase 4 (PDE4) is an important regulator of cyclic adenosine monophosphate (cAMP) levels in the body and brain. Specific inhibitors of this PDE subtype, such as rolipram, are reported to have antidepressant effects *via* increasing cAMP signaling. In the present study, we investigated depressive-like behavior in rats after region-specific upregulation of cAMP signaling by intra-hippocampal and intra-accumbal infusions of rolipram. Our results demonstrate a dissociative role for cAMP signaling in the nucleus accumbens and hippocampus in depressive-like behavior. That is, rats showed an increase in anhedonic behavior when rolipram was administered in the nucleus accumbens, while hippocampal administration reduced anhedonia. This is in line with previous reports which described an analogous dissociative effect after altering hippocampal and accumbal expression of the transcription factor cAMP responsive element binding protein (CREB). Although CREB is generally regarded as an important downstream target of cAMP, in the present study CREB phosphorylation was unaffected by the repeated rolipram infusions. Thus, the exact underlying mechanism of the region-specific pro- and antidepressant effects of rolipram remains to be elucidated.

P9. Behavioral flexibility in mice with selective inactivation of NMDA receptors in the dopamine system

Cieślak P.E., Łopata K., Rodriguez Parkitna J.

Laboratory of Transgenic Models, Department of Molecular Neuropharmacology, Institute of Pharmacology, Polish Academy of Sciences, Cracow, Poland

Goal-directed behavior requires flexibility, the ability to adjust behavior in response to changing environmental circumstances. This flexibility is mediated by the prefrontal cortex and striatum of the brain and is strongly influenced by neurotransmitter dopamine, which modulates the balance between persistence and shifting of behavioral strategies. Here, we investigated behavioral flexibility in genetically modified mice, NR1DATCreERT2 and NR1D1CreERT2, lacking functional NMDA receptors in dopaminergic and dopaminoceptive (D1 expressing) neurons, respectively. We used a T-maze based task, which permits to examine the ability to learn and switch between two spatial maze tasks requiring different response strategies. In a visual cue task, mice had to make a turn toward the arm of the maze where the visual cue was placed to obtain food reward. After the switch to response direction task, animals had to make a turn based on direction (left or right, regardless of the visual cue). Loss of NMDA receptors in dopamine but not D1 expressing neurons disrupted shifting between strategies. Furthermore, analysis of arm choice errors revealed that the deficit was not due to perseveration of a strategy previously learned but due to impairments in acquisition of a new strategy.

Supported by the grant OPUS 2011/03/B/NZ4/02211

P10. Phenotyping reward-driven behaviors in transgenic mice

Łopata K., Cieślak P.E., Turbasa M., Rodriguez Parkitna J.
Department of Molecular Neuropharmacology, Institute of Pharmacology, Polish Academy of Sciences, Cracow, Poland

The dopamine neurons of the ventral midbrain form the core of the brain's reward system and the plasticity of these neurons are essential in mediating the effects of positive reinforcement. To study the underlying neuronal mechanisms we used genetically modified mice (NR1DATCreERT2) that lacked NMDA glutamate receptors on dopamine neurons and thus had impaired plasticity on excitatory synapses. We found that mutant mice performed similarly as controls in food-self administration test under fixed, progressive or variable interval schedules. However, NR1DATCreERT2 mice failed to acquire instrumental responding for sensory stimuli in the operant sensation seeking test. Furthermore, when mice were tested for sweet taste preference under instrumental access (FR3) in an IntelliCage, mutants did not prefer the saccharine solution over pure water (52±11%) while control animals reached 98±13% preference. The anhedonia-like phenotype prompted us to test the animals in forced swimming test and we found that NR1DATCreERT2 animals spent more time immobile than controls. In summary, we believe that the loss of NMDA receptors on dopamine neurons caused decreased sensitivity to sensory stimuli, anhedonia and increased learned helplessness, without causing obvious impairments in instrumental learning.

Supported by the grant OPUS 2011/03/B/NZ4/02211

P11. The use of unbiased conditioned place preference procedure as the predictive analysis of vulnerability to psychostimulant addiction in rats

Niedzielska E., Pomierny-Chamiolo L., Filip M.
Department of Toxicology, Faculty of Pharmacy, Collegium Medicum, Jagiellonian University, Cracow, Poland

As in humans, also in laboratory animals, addiction phenotype including drug craving and relapses, develops only in about 20% subjects following initial drug abuse. The aim of our study was to verify if the conditioned place preference (CPP) paradigm can serve as a model for determination of the variable vulnerability to drug addiction. We used male Wistar rats (250–300 g) housed under standard laboratory conditions. Animals were exposed to either vehicle (n=20) or to the psychostimulant cocaine (15 mg/kg, ip; n=64) during 10 days with CPP. As a result, 15 animals (i.e., 23.4% of all experimental animals) that performed unbiased version of CPP, spent more time in cocaine-paired chamber in test in comparison to pretest (P<0.0005 vs. control); those rats were classified as addiction vulnerable. In conclusion, our results suggest that unbiased conditioned place preference procedure can serve as an animal model for predictive analysis of vulnerability to psychostimulant addiction in rats. Moreover, it also gives the opportunity to study the phenotype of animals (so called addiction-resistant) which did not develop drug-associated environment-linked craving.

P12. Analgesic-like activity of piperazine derivatives of 3,3-disubstituted pyrrolidine-2,5-diones in mice

Jastrzębska-Więsek M.[1], Pabian M.[1], Partyka A.[1], Obniska J.[2], Wesołowska A.[1]
[1]Department of Clinical Pharmacy, [2]Department of Medicinal Chemistry, Jagiellonian University Medical College, Cracow, Poland

Serotonin (5-HT) and its receptors play a multifaceted role in pain modulation. Among the many subtypes of 5-HT receptors potentially contributing to medullo-spinal modulation of pain, 5-HT1A and 5-HT7 receptors have significant role, as it is expressed both in supraspinal and spinal areas. A series of newly synthesized 3,3-disubstituted pyrrolidine-2,5-diones with affinity for 5-HT1A and 5-HT7 receptors was investigated in some behavioral tests in mice. Previous studies showed antiepileptic activity of these compounds in experimental models of seizures, i.e. induced by pentylentetrazole, maximal electroshock, and psychomotor ones (6-Hz). Many antiepileptic drugs (e.g. carbamazepine, valproinic acid) possess analgesic activity, especially in neuropathic pain treatment. Hence it was an intriguing question, if these new structures may be useful as an analgesics. An antinociceptive effect of four new 3,3-disubstituted pyrrolidine-2,5-diones (JOP364, JOP362, JOP346, and JOP315) was determined in model of acute pain: the hot-plate (phasic pain model) and their activity was compared with effects of the reference drugs tramadol and morphine. Measured antinocicepitve latency of two investigated compounds (JOP364 and JOP 362) in the hot plate test was comparable with tramadol, while the compound JOP315 was inactive in this test. The obtained results entitle to lead further examinations to establish mechanism(s) of analgesic activity of these new derivatives and to estimate their potential therapeutic value.

P13. Sexual behavior of male rats with moderate depression – a preliminary study

Biały M.[1], Nikolaev E.[2], Boguszewski P.M.[2]
[1]Department of Clinical and Experimental Physiology, Medical University of Warsaw, Poland; [2]Nencki Institute of Experimental Biology PAS, Warsaw, Poland

Sexual behavior of male rats is well described type of appetitive behavior. This behavior is described by at least five independent factors: anticipatory, initiation, copulatory rate, hit rate and intromission count. Each factor has specific neuronal networks. The aim of our

study was to analyze which factor is the most informative to describe sexual deficit in rats with depressive like syndrome. We investigate 6–8 month old WAG/Rij rats with absent epilepsy commodity with moderate depression and compare sexual behavior with control Crl:Han Wistar and Sprague-Dawley males. Sexually naïve WAG/Rij rats started to copulation less frequently (only 6 from 20 display mounting) compare to Crl:Han Wistar (6 from 6) and Sprague-Dawley (4 from 6). Sexually experienced WAG/Rij rats displayed longer mount latency (initiation factor) compare to both control strains. Surprisingly, sexually experienced WAG/Rij rats vocalized very intensively at 50-kHz band during five minutes before introduction of female. This anticipatory precontact vocalizations did not correlate with initiation of copulation. Latency to initiation of copulation (initiation factor) seems to be the most sensitive parameter describing depressive like syndrome during male rat sexual behavior and seems to be useful to investigation depressive disorders. It correspond to lower libido in men depressive patients.

P14. The influence of divergent selection for body weight on social behavior of mice (*Mus musculus*)

Góral-Radziszewska K.
Department of Genetics and Animal Breeding, Faculty of Animal Science, Warsaw University of Life Sciences – SGGW, Warsaw, Poland

It was proved many times that the selection leaded in the direction of one trait can also influence on other. One of the most frequently selected trait is body weight which is easy to measure. In this experiment, mice from three lines were used. Two of them were divergently selected on body weight at the postnatal day 21 (light and heavy lines) for over 135 generations. The third line were the control line without any selection. The animals were observed in the social testing apparatus which is used for perform two behavioral tests: sociability and preference for social novelty tests. The results show that selection on body weight influenced the social behavior of laboratory mice. Individuals from the heavy line (both sexes) showed minor modifications of the natural behavior in the sociability test ($P<0.05$). Additionally results of females from the heavy line demonstrated some disorders in social novelty test ($P<0.05$).

P15. Efficacy of cat synthetic facial pheromones in reducing stress during examination in veterinary clinic compared to placebo

Bidzińska B., Góral-Radziszewska K.
Department of Genetics and Animal Breeding, Faculty of Animal Science, Warsaw University of Life Sciences – SGGW, Warsaw, Poland

The study was conducted in order to verify the hypothesis of the sedative effect of the preparation FELIWAY® in the prophylactic examination in a veterinary clinic. It was a double-blind placebo controlled study. The examined population consisted of 48 cats, of different breeds, sexes and ages. Analysis of the results showed that preparation had no effect on limiting the stress and animals were calmer during the test phase with placebo ($P=0.028$). Stratification of the results showed that the degree of stress during the test was related only to the age of the cat ($P<0.001$).

P16. Effects of lithium and valproate on cognitive judgment bias of rats in the ambiguous-cue interpretation paradigm

Rygula R., Gołębiowska J., Kręgiel J.
Affective Cognitive Neuroscience Lab, Department of Behavioral Neuroscience and Drug Development, Institute of Pharmacology Polish Academy of Sciences, Cracow, Poland

Mania is a debilitating psychiatric condition and a cardinal feature of Bipolar Disorder. One of the critical features of mania is over-optimistic judgment bias leading to high-risk behaviors. As both; lithium and valproate are well-established treatments for mania in humans, in the present study, using recently developed ambiguous-cue interpretation test (ACI) we assessed the effects of treatment with these two drugs on cognitive judgment bias in rats. To accomplish this goal, in two separate experiments, previously trained animals were treated with 3 different doses of either valproate (100, 200 and 400 mg/kg) or lithium chloride (10, 50 and 100 mg/kg) and were subsequently tested with the ACI paradigm. The drugs were administered using fully randomized Latin square designs. Control animals received physiological saline injections. We report that neither valproate nor lithium chloride had significant effects on ambiguous-cue interpretation in rats.

Supported by Polish Ministry of Science and Higher Education (Research grant: Iuventus Plus IP2011047271 to RR) and the statutory funds of the Institute of Pharmacology PAS.

P17. Effects of acute administration of L-DOPA on cognitive judgment bias of rats in the ambiguous-cue interpretation paradigm

Kręgiel J., Gołębiowska J., Rygula R.
Affective Cognitive Neuroscience Lab, Department of Behavioral Neuroscience and Drug Development, Institute of Pharmacology, Polish Academy of Sciences, Cracow, Poland

Recent research has shown that pharmacological enhancement of dopaminergic function by acute administration of L-DOPA increases an optimism bias in humans. In the present study, we investigated whether L-DOPA have similar effects in rats. To accomplish this goal, the animals were trained in the ambiguous-cue interpretation (ACI) paradigm. In this paradigm the rats must

press one lever in response to one tone to receive a reward and to press another lever in answer to a different tone to avoid punishment. Cognitive judgment bias is than tested by measuring the pattern of animals‘ responses to a tone of intermediate frequency (ambiguous-cue). After initial behavioral training, the rats received single injections of L-DOPA and were subsequently tested with the ACI paradigm. The drug was administered in 3 doses using a fully randomized Latin square design. Control animals received injections of physiological saline. L-DOPA at all tested doses (2, 4 and 8 mg/kg, co-administered with benserasidine 12.5 mg/kg) significantly biased animals towards negative interpretation of the ambiguous cue. The results are discussed in relation to human studies.

Supported by the National Science Centre (Research grant: Sonata bis dec-2012/07/E/NZ4/00196) and the statutory funds of the Institute of Pharmacology PAS.

P18. Cognitive judgment bias in the cocaine-induced model of mania in rats

Kubik J., Gołębiowska J., Kręgiel J., Rygula R.
Affective Cognitive Neuroscience Lab, Department of Behavioral Neuroscience and Drug Development, Institute of Pharmacology, Polish Academy of Sciences, Cracow, Poland

Chronic psychostimulant administration is a frequently used, however unspecific, model of mania in rodents. In our study, we investigated whether chronic administration of cocaine may produce in rats, also more specific, cognitive symptoms of mania, such as the hyperoptimistic cognitive judgment bias. To accomplish this goal, after initial behavioral training, 2 groups of rats were subjected either to 2 weeks of chronic cocaine treatment or physiological saline injections. Before and after the treatment the animals were tested with ambiguous-cue interpretation paradigm. We report that chronic cocaine administration does not produce optimistic judgment bias in rats.

Supported by the National Science Centre (Research grant: Sonata bis dec-2012/07/E/NZ4/00196) and the statutory funds of the Institute of Pharmacology PAS.

P19. Effects of acute administration of escitalopram on cognitive judgment bias of rats in the ambiguous-cue interpretation paradigm

Gołębiowska J., Kregiel J., Rygula R.
Affective Cognitive Neuroscience Lab, Department of Behavioral Neuroscience and Drug Development, Institute of Pharmacology, Polish Academy of Sciences, Cracow, Poland

The monoamine serotonin (5-HT) has long been implicated in the modulation of affective and cognitive processing and has been consistently linked to depression, anxiety and negative mood. In the present study, we investigated the effects of acute administration of selective serotonin reuptake inhibitor – escitalopram (S- enantiomer of citalopram) on the valence of cognitive judgment bias of rats in the ambiguous-cue interpretation (ACI) paradigm. In this paradigm the rats are trained to press one lever in response to one tone to receive a reward and to press another lever in answer to a different tone to avoid punishment. Cognitive judgment bias is than tested by measuring the pattern of animals‘ responses to a tone of intermediate frequency (ambiguous-cue). After initial behavioural training, the animals received single injections of 3 different doses of escitalopram and were subsequently tested with the ACI paradigm. The drug was administered using fully randomised Latin square design. Escitalopram (0.5, 1 and 2 mg/kg) had no significant effects on the interpretation of the ambiguous cue by rats. The results are discussed in terms of pharmacological action of escitalopram.

Supported by the National Science Centre (Research grant: Sonata bis dec-2012/07/E/NZ4/00196) and the statutory funds of the Institute of Pharmacology PAS.

P20. Anxiodepressive state induced by neonatal administration of the dipeptidyl peptidase-IV inhibitors with different mechanisms of action: A model of neurodevelopmental disorder

Khlebnikova N., Krupina N., Kushnareva E.
Institute of General Pathology and Pathophysiology, Moscow, Russia

At present emotional and motivational diseases (including anxiety and depression) are often believed to be neurodevelopmental disorders. Anatomical and functional brain systems responsible for emotional reactions are formed at the second half of pregnancy and early postnatal period, when synaptogenesis of major neurotransmitter systems involved in emotions is realized. Experimental and clinical data testify to the involvement of proline-specific peptidase dipeptidyl peptidase IV (DP-IV, EC 3.4.14.5) in the genesis of anxiety and depressive disorders. In the developing rat brain DP-IV activity increases up to 4th postnatal week and then decreases during maturation. Pharmacological intervention in early ontogeny can affect brain development and cause behavioral alterations in adult animals. In our studies, neonatal rat pups were treated (5–18 postnatal days) with DP-IV inhibitors with different mechanisms of action – non-competitive irreversible inhibitor methionyl-2(S)-cyano-pyrrolidine (1 mg/kg), competitive high selective inhibitors diprotin A (2 mg/kg) and sitagliptin (4 mg/kg); control rats were treated with saline. At the age of 1, 2, 3 and 6 months experimental rats demonstrated anxiety- and depression-like behavior in the elevated plus maze, forced swimming test, sucrose consumption/preference test and some others. Sitagliptin was the least efficient in the induction of behavioral alterations. Data

prove the development of anxiety-depressive state in adolescent and adult rats postnatally exposed to inhibitors of DP-IV. This experimental approach is effective in order to study the development of affective disorders.

P21. Neonatal administration of the dipeptidyl peptidase-IV inhibitor methionyl-2(S)-cyano-pyrrolidine affects learning capability and memory retention in adult Wistar rats

Krupina N., Khlebnikova N., Kushnareva E.
Institute of General Pathology and Pathophysiology, Moscow, Russia

Previous studies demonstrated emotional and motivational differences in males and females adult rats subjected to the neonatal administration (5–18 postnatal days) of irreversible synthetic inhibitor of dipeptidyl peptidase IV (DP-IV, EC 3.4.14.5) methionyl-2(S)-cyano-pyrrolidine (1 mg/kg). Males and females with anxiety-depressive state showed the difference in the dynamics of proline-specific peptidase activities – DP-IV and prolyl endopeptidase (PEP, EC 3.4.21.26) in brain structures involved in learning and memory that is, in frontal cortex and hippocampus. In rat models of experimental retrograde amnesia, PEP activities were increased in frontal cortex and hippocampus. The purpose of the present study was to investigate learning capability and memory in rats with anxiety-depressive state induced by neonatal administration of DP-IV inhibitor using a two-way active avoidance learning procedure in a shuttle box (one training session of 100 trials; a footshock as an unconditioned stimulus, 0.5 mA, 8 s; combined auditory and light signals as a conditioned stimulus, 10 s). As compared with control, experimental males showed the tendency to worse data acquisition and better memory retention 24 hours after learning session while females demonstrated worse data acquisition and memory retention 2 months after learning session. These data indicate that neonatal administration of DP-IV inhibitor in rats impairs normal functioning of neural circuits of learning and memory in males and females with emotional behavior deficits which may be related to alterations in activities of proline-specific peptidases in brain structures.

P22. Rats that 'gamble' demonstrate impaired cognitive flexibility in the attentional set shifting task

Rafa D., Potasiewicz A., Nikiforuk A., Popik P.
Department of Behavioral Neuroscience and Drug Development, Institute of Pharmacology, Polish Academy of Sciences, Cracow, Poland

Pathological gambling (PG), a behavioral addiction resembling substance abuse disorder can be modeled in laboratory rats using slot machine task (rSMT). Animals that respond to the cues suggesting, but not warranting food reward ('near miss' responses) can be classified as 'gamblers'. This pathological response apparently persists in some animals and could be viewed as a behavioral trait. The correct response in this task involves a number of cognitive processes, including cognitive flexibility, i.e., an ability to adapt to the changing rules. This phenomenon may be assessed in rodents in the attentional set shifting task (ASST). We investigated whether 'gamblers' differed from 'non-gamblers' in cognitive flexibility. Animals were trained in rSMT by responding to a series of three flashing lights. A winning trial was signaled when all three lights were illuminated. At the end of each trial, rat chose between responding on the ‚collect' lever (that on the 'win' trials resulted in reward delivery, and on the 'loose' trials in a time penalty), or responding on the 'roll' lever that initiated the next trial. Then, the 'gamblers' and 'non-gamblers' were tested in the ASST. Animals exhibiting a 'gambling' trait required more trials to reach criterion at the Reversal and Extra Dimensional (ED) phases of ASST, that require animals to switch their attention to previously irrelevant stimulus exemplars or stimulus dimension, respectively. These results suggest that impairment of cognitive flexibility may play an important role in pathological gambling phenomenon.

Supported by the grant NR 72/H/E/13 from the Ministry of Health

P23. Marble burying test in mice – validation of test method in the laboratory of Department of Clinical Pharmacy

Partyka A., Cetera M., Wasik A., Wesołowska A.
Department of Clinical Pharmacology, Jagiellonian University Medical College, Cracow, Poland

Marble burying test (MBT) is an animal model of anxiety and obsessive-compulsive disorder, used as a screening test for new compounds in modern drug development. The aim of this study was to introduce that method to experimental workshop of the Clinical Pharmacy Department. MBT was carried out according to the procedure of Broekkamp et al. (1986). The first stage of the work was to determine the relevant parameters such as time of observation, age and body weight of mice. It was assumed that a 30-minute observation period and higher body weight of mice, i.e. 26–30 g are favorable parameters for the assessment of anxiolytic-like activity in MBT. The next step was to investigate properties of six reference compounds from different pharmacological groups: benzodiazepines (BZD), antidepressants (SSRI, TCA), typical and atypical antipsychotics. The selection was based on the literature data indicating anxiolytic-like activity of those drugs in MBT. The results show that diazepam (BZD) and escitalopram (SSRI) were active at both doses of 2.5 and 5.0 mg/kg; imipramine (TCA) was active at a dose of 30 mg/kg; olanzapine and aripiprazole (atypical antipsychotics)displayed anxiolytic-like activity at both doses of 2.5 and 5.0 mg/kg; chlorpromazine (typical antipsychotic) was active at doses of 1.25 and 2.5 mg/kg. To verify the impact of locomotor activity on animals' behavior in MBT, the effect of

active doses on spontaneous locomotor activity was also examined. Olanzapine, aripiprazole and chlorpromazine, given at active doses decreased locomotor activity of mice whereas other drugs had no effect on that parameter.

P24. Synthesis and pharmacological evaluation of some novel thiourea derivatives incorporating 3-(trifluoromethyl)phenyl moiety

Kędzierska E.[1], Fidecka S.[1], Bielenica A.[2], Struga M.[3]

[1]Department of Pharmacology and Pharmacodynamics, Faculty of Pharmacy with Division of Medical Analytics, Medical University of Lublin, Lublin, Poland; [2]Chair and Department of Biochemistry, Medical University of Warsaw, Warsaw, Poland; [3]Department of Pharmacogenomics, Faculty of Pharmacy, Medical University of Warsaw, Warsaw, Poland

Despite the great progress in the treatment of pain, depression, anxiety, and other central nervous system (CNS) disorders, the used drugs do not fulfil all expectations. And there is still a need to search for new, more effective therapies as well as need to know the mechanisms of action of existing drugs. The tested compounds are a group of thiourea derivatives with expected potential central activity in the CNS. Numerous thiourea-derived compounds are selective ligands for 5-HT2 receptors family (Forbes et al. 1995) and also exhibit a broad spectrum of biological activities as analgesic, antidepressant, anticonvulsant, antiviral, anti-HIV, antibacterial, and HDL-elevating properties (Struga et al. 2007, Wardakhan et al. 2008, Karakus et al. 2009). Modification of the chemical structure makes it possible to obtain new compounds with pharmacological activity, higher selectivity and fewer adverse effects. Compounds were studied in behavioral tests used to predict a potential influence on the CNS in mice (after their ip or sc administration). The results of the pharmacological studies showed that new compounds exerted substantial impact on the CNS in mice. The most important seems to be their influence on the transmission of serotonin (activity in the head-twitch test, changes in body temperature) as well as antinociceptive effects. These compounds caused no coordination disorders, and do not affect locomotor activity, but slightly decreased the hyperactivity caused by administration of amphetamine. Obtained results indicate the possibility of the involvement of serotonin in the action of the majority of the compounds.

P25. The effect of adenosinergic system on development of sensitization to morphine-induced withdrawal signs in rats

Listos J., Talarek S., Orzelska J. Fidecka S.

Department of Pharmacology and Pharmacodynamics, Medical University, Lublin, Poland

In the presented study the effect of continuous and sporadic treatment with morphine (MPH) on the severity of MPH withdrawal signs was examined in rats. Also, the effect of A1 and A2A adenosine agonists (N6-cyclopentyladenosine and 2-p-(2-carboxyethyl) phenethylamino-5'-N- ethylcarboxamidoadenosine hydrochloride, respectively) on development of this sensitization was studied. Rats were treated with increasing doses of MPH and were divided into groups. First group received MPH for 8 consecutive days (continuous group), the second, received MPH in four two-day periods with three 36-hour breaks (sporadic group). Control group received saline. Adenosine drugs were administered during three MPH-free periods (6 injections). Last day, to induce withdrawal signs, 1 hour after MPH injection, naloxon was administered. The number of jumps was recorded for a period of 30 min. Jumps were significantly greater in sporadic group in compare with continuous group, which confirm that sensitization to MPH-induced withdrawal signs has been developed. There were not any jumps in control rats. Rats showed that stimulation of A2A receptors inhibited the withdrawal signs. Less significant effect was observed after stimulation of A1 receptors. The findings showed that repeated withdrawal episodes intensified the severity of withdrawal signs and adenosinergic system, mainly by A2A receptors, was able to inhibit the development sensitization to MPH withdrawal signs.

P26. Participation of NO:sGC pathway in the development of tolerance to mephedrone in mice

Talarek S., Listos J., Orzelska J., Fidecka S.

Department of Pharmacology and Pharmacodynamics, Medical University, Lublin, Poland

Mephedrone is a cathinone derivative that possesses powerful psychostimulant and hallucinogenic effects. It has been speculated that mephedrone may act by increasing release and reuptake inhibition of serotonine and dopamine. However, the precise mechanisms underlying its psychomimetic action remain unclear. Nitric oxide (NO), synthesized from L-arginine by a reaction catalyzed by NO synthase, is involved in different central function and can participate in the mechanisms of drug tolerance and dependence. NO acts as an activator of soluble guanylyl cyclase (sGC) and thereby increases the level of an intracellular second messenger, cGMP. The purpose of the present study was to determine the role of NO in the development of tolerance to mephedrone. Tolerance to hyperlocomotor activity was induced by chronic administration of mephedrone (5 mg/kg ip, 6 days) in male albino Swiss mice. The following drugs were used to modify the NO:cGMP pathway: NG-nitro-L-arginine methyl ester (L-NAME; 25, 50 mg/kg, ip) – NO synthase inhibitor and methylene blue (5, 10 mg/kg ip) – sGC inhibitor. Locomotor activity was measured for 10 and 30 min, 20 min after administration of drugs. The present experiments demonstrated that chronic coadministration of L-NAME and methylene blue with mephedrone attenuates the development of tolerance to mephedrone. These

findings suggest that NO:sGC pathway may be involved in the tolerance to mephedrone in mice.

P27. Antidepressant- and anxiolytic-like properties of ADN-1319, a new analogue of aripiprazole

Wasik A.[1], Partyka A.[1], Jastrzębska-Więsek M.[1], Kołaczkowski M.[2], Pawłowski M.[2], Wesołowska A.[1]

[1]Department of Clinical Pharmacy, [2]Department of Pharmaceutical Chemistry, Jagiellonian University Medical College, Cracow, Poland

ADN-1319 is a new indoloamine analogue of aripiprazole which displays antipsychotic-like properties in mouse models of psychosis, i.e. MK-801- and amphetamine-induced locomotor hyperactivity. In order to examine its potential antidepressant and anxiolytic activity, the forced swim test, and the four-plate and the marble burying tests, respectively, in mice were performed and a minimum effective dose is presented. ADN-1319 (2.5 mg/kg) significantly decreased the immobility time. Moreover, it increased the number of punished crossings (5 mg/kg) and reduced (10 mg/kg) the number of buried marbles. ADN-1319 up to a dose of 10 mg/kg had no influence on spontaneous locomotor activity in mice. Obtained data suggest that the tested compound displays specific antidepressant- and anxiolytic-like properties in mice and might find an application in treatment of symptoms of depression, anxiety or obsessive–compulsive behavior concomitant in schizophrenia.

P28. Does olfaction play the most important role in learning and memory processes in pigs?

Nowicki J.

University of Agriculture in Cracow, Cracow, Poland

Many results report poor visual acuity in pigs, while olfaction is presented as a major sense in adaptation to the pig lifestyle. There have been very little results which showed the importance of these senses in learning processes in pigs. It is the reason why the aim of the study was to assess the effect of visual and olfactory stimulation on learning abilities in pigs. The experiment was conducted on three experimental groups consisted of 8 weaners each. The duration of the experiment was 30 days and two hour training daily with 10 trials and tidbit reward was performed. The task of the first group was to choose (among 4 shapes) and indicate (touch with the nose) the proper BW shape after seeing it on hand-held board. In the 2nd group the task was the same but instead of shapes, 4 aromas in containers were used. In the 3rd group, each of 4 shapes had assigned its unique aroma. The number of proper indications increased in subsequent days in all groups, however, animals which had to choose among aromatized boards with shapes started to indicate them more properly from the beginning and achieved better results during the whole experiment. Average daily number of successful indications of adequate shapes with attached aromas was 7.9 vs. 5.03 in group with shapes only and 6.1 in aromas only ($P<0.05$). Olfactory and visual cues working together during training seem to make the learning process more efficient.

P29. The effect of selective 5HT2A, 5HT6 and 5HT7 receptor antagonists on ketamine-induced social deficits in rats

Holuj M., Nikiforuk A., Popik P.

Institute of Pharmacology, Polish Academy of Sciences, Cracow, Poland

Social withdrawal is one of the negative symptoms of schizophrenia, which often precedes the onset of the first psychotic episode and persists for most of patient‘s life. Current antipsychotics are relatively ineffective in normalize this social dysfunction. There are only a few studies addressing the efficacy of antipsychotic drugs in animal models of social deficits. The aim of the present study was to evaluate the effects of the selective serotonin 5HT2A, 5HT6 and 5HT7 receptor antagonists in ketamine-induced schizophrenia-like social deficit in rats. After 5 days of social isolation male Sprague-Dawley rats were individually adapted to the open field arena for 7 minutes. On the next day, two unfamiliar rats of matched body weight received the same treatment and were placed in the open field arena for 10 minutes. The active, non-agressive social behaviors were scored: sniffing, social grooming , following, mounting and climbing. Ketamine caused significant reduction of the time of active social behavior. Administration of 5HT7 but not 5HT6 or 5HT2A receptor antagonist reversed ketamine-induced social deficits. Present findings suggest the importance of 5HT7 receptor antagonism in ameliorating the negative symptoms of psychoses. It is noteworthy that most recently synthesized second and third generation antipsychotic exhibit a high, nano-molar affinity for 5HT7 receptor.

Supported by the Statutory Funds of the Institute of Pharmacology, Polish Academy of Sciences

P30. Positive allosteric modulation of nicotinic receptors: A new hope to treat cognitive deficits?

Potasiewicz A., Nikiforuk A., Popik P.

Department of Behavioral Neuroscience and Drug Development, Institute of Pharmacology, Polish Academy of Sciences, Cracow, Poland

Alpha-7 nicotinic acetylcholine receptors (α7-nAChRs) represent the promising target for the development of new therapies of cognitive dysfunctions associated with schizophrenia and Alzheimer’s

disease. Activation of these receptors produces procognitive effects. Recent data suggest that the positive allosteric modulators (PAMs) of α7-nAChRs may demonstrate a more favorable pharmacological profile than the orthosteric agonists. However, little is known about the potential efficacy of α7-nAChRs PAMs on cognitive processes. The aim of the present study was to evaluate the effects of the PNU 120596, an α7-nAChRs PAM (1 and 3 mg/kg, ip), on cognitive processes in rats. We used odor span test (OST), novel object recognition (NOR) task and 5-choice serial reaction time task (5-CSRTT) to assess the working memory capacity, episodic memory and attention, respectively. The compound enhanced episodic memory tested 24 h following its administration, however, PNU-120596 did not affect rat's performance in OST and 5-CSRTT. The present study demonstrates the beneficial effects of PNU 120596 on some aspects of cognition in rats tested in cognition-unimpaired conditions. Further studies are now ongoing to evaluate the efficacy of this compound in cognition-deficit based models.

Supported by the Polish National Science Centre grant NCN 2012/07/B/NZ/01150.

P31. SB 742,457, a 5-HT6 receptor antagonist, reverses cognitive impairment induced by scopolamine in the Novel Object Recognition Test in rats

Kos T.[1], Nikiforuk A.[1], Zajdel P.[2], Popik P.[1]

[1]Department of Behavioral Neuroscience, Institute of Pharmacology, Polish Academy of Sciences, Cracow, Poland; [2]Department of Medicinal Chemistry, Pharmaceutical Faculty, Medical College, Jagiellonian University, Cracow, Poland

Serotonin (5-HT) type 6 antagonists have been regarded as cognition-enhancing principles associated with schizophrenia and Alzheimer's disease. It has been proposed that these favorable effects are mediated through a blockade of excitatory tonic action of 5-HT6 receptors located at GABAergic neurons in the hippocampus and cortex. This blockade is leading to an indirect enhancement of cholinergic and glutamatergic neurotransmission. To evaluate the cognitive effects of SB 742,457, the novel object recognition test (NOR) was performed following scopolamine-administration. As a positive control, galantamine, a competitive and reversible cholinesterase inhibitor, was used. We report that compound SB 742,457 reversed scopolamine-induced memory impairment in a dose-dependent manner. The effect of higher dose of 1 mg/kg was as strong as an effect of a positive control, galantamine. Present data confirm that antagonists of 5-HT6 receptors exhibit pro-cognitive effects purportedly useful in the treatment of cognitive deficits associated with Alzheimer's disease and schizophrenia.

Supported by statutory funds from the Institute of Pharmacology, Polish Academy of Sciences (Cracow, Poland)

P32. Agmatine inhibits flunitrazepam-induced memory impairment in the mEPM in mice

Orzelska J., Talarek S., Listos J., Fidecka S.

Chair and Department of Pharmacology and Pharmacodynamics, Medical University of Lublin, Lublin, Poland

Benzodiazepines (BZ) have long been known to impair memory as a result of their action at γ-aminobutyric acid (GABA)A receptors. But it is not clear what stages of memory (acquisition, consolidation or retrieval) are affected by BZ. Flunitrazepam (FNZ) – one of the BZ drug – has highly ability to cause amnesia and it is known as a date-rape drug. Recent evidence suggests that agmatine (AGM), the metabolite of L-arginine, exists in the mammalian brain and can modulate behavior function, including learning and memory. The mechanism of AGM action has not been completely explained. Many studies showed that AGM regulates the L-arginine:nitric oxide(NO):cGMP pathway because AGM is a metabolite of L-arginine and AGM can inhibit neuronal NO synthase. Our previous research has indicated that modulators of L-arginine:NO:cGMP affected BZ-induced memory impairment. The aim of this study was to assess the role of AGM in the amnesic effects of FNZ in the modified elevated plus-maze task (mEPM) in mice. Our experiments showed that FNZ (0.05 and 0.1 mg/kg, sc) disrupted acquisition and consolidation of memory in mice. The amnesic properties of FNZ were prevented by AGM (20 mg/kg, ip – the acquisition stage) and (5, 10 and 20 mg/kg, ip – the consolidation stage). The above results suggest that AGM may be involved in the amnesic effects of FNZ.

P33. Open Field Test: Comparing square and round arenas

Grabovska S., Salyha Y.

Institute of Animal Biology, Lviv, Ukraine

Open Field Test is one of the most popular and widely used behavioral tests studying emotional rate, especially anxiety, and exploration activity of animals. There are two main modifications of the Open Field Test apparatus – round and square arena shape; possible differences between them have not been studied enough. The purpose of our study was to compare round and square shape of the apparatus analyzing the same parameters. We used square and round test arenas with equal area, surface material, illumination, and other conditions. 10 adult white Wistar rats, 5 male and 5 female, were tested in both arenas 3 times with 7 days interval. Studied parameters were: inner and outer horizontal activity, vertical activity, long and short grooming, defecation, and hole reflex. Statistically significant differences (at $P<0.05$) were observed only in "hole reflex" parameter and its time dynamics. In round arena, this parameter was firstly significantly lower, then rapidly increased, and decreased a little at the third testing. In square arena the dynamics was quite opposite: significantly higher at day 1, it rapidly decreased at the second test,

and increased slightly in the last test. In other parameters, no significant changes were observed. As "hole reflex" is just an additional parameter of exploration activity, this difference wouldn't be crucial. In main parameters of exploration activity (inner and outer horizontal activity, vertical activity) and anxiety (long and short grooming, defecation) no significant changes were observed. So, we can claim that both shapes of arenas can be used in Open Field test equally.

P34. The involvement of the CB2 agonist in the behavioral and biochemical effects in the experimental animal models

Kruk-Slomka M.[1], Budzyńska B.[1], Boguszewska-Czubara A.[2], Biała G.[1]

[1]Department of Pharmacology and Pharmacodynamics,
[2]Department of Medical Chemistry, Medical University of Lublin, Lublin, Poland

Cannabinoids are implicated in regulation of variety emotions through the type 1 (CB1) and type 2 (CB2) receptors. Our pharamcological interests have been focused on the effects of CB2 receptor selective ligands in the treatment of Alzheimer disease (AD) which is associated with memory-loss, phobia, depression as well as oxidative processes in brain. We examined the impact of CB2 agonist: JWH-133 in mice using passive avoidance (PA) test to measure memory-related responses, elevated plus maze (EPM) test to measure anxiety-related behavior and forced swimming test (FST) to measure depression-related responses. Total antioxidant capacity (TAC) and lipids peroxidation level, expressed as malondialdehyde (MDA) concentration, were measured in homogenates of brain. Our findings revealed that a single injection of JWH-133 improved memory in PA, had an anxiogenic effects in EPM, antidepressant effects in FST and slightly increased total antioxidant capacity but did not affect lipids peroxidation level in brain. CB2 ligands could become a new pharmacological alternative in treatment of AD which is associated not only with emotional-related disorders but also with oxidative stress.

P35. Behavioral and biochemical effects of acutely administered MDMA in animal models

Budzyńska B.[1], Boguszewska-Czubara A.[2], Kruk-Słomka M.[1], Biała G.[1]

[1]Department of Pharmacology and Pharmacodynamics,
[2]Department of Medical Chemistry, Medical University of Lublin, Lublin, Poland

3,4-Methylenedioxymethamphetamine (MDMA, 'ecstasy'), a potent monoamine-releaser that is widely used as a recreational drug. Hence, understanding the effects and mechanisms of MDMA is of immense importance for the public health in the world. We examined how an acute dose of MDMA affects memory and learning processes, anxiety- and depressive-like behaviors in female Swiss mice as well as oxidative processes in brain. The aim of the present study was to examine the effects of MDMA (20–0.1 mg/kg, ip) in the forced swim test (FST), elevated plus maze (EPM) and passive avoidance (PA) paradigm in mice. At first, we showed that MDMA at the doses of 10 and 2.5 mg/kg exhibited a significant reduction of immobility time in the FST, indicating the antidepressant-like activity. In the second series of our experiment, we demonstrated that MDMA at the doses of 5 and 2.5 mg/kg improved consolidation of memory processes in PA test. Also, this compound showed strong anxiogenic effect at the range of doses (20–0.5 mg/kg). MDMA at the higher doses (20-5 mg/kg) enhanced oxidative stress, expressed as superoxide dismutase activity, ascorbic acid concentration and malondialdehyde level, within brain, whereas its lower doses did not affect measured parameters. Taken together, these data suggest an anxiogenic-like, antidepresive and procognitive in consolidation trial effects of acute MDMA treatment. The results of our study also showed the influence of MDMA on the oxidative stress processes in the brain.

P36. Impact of long-term weakening of the geomagnetic field on the mental state of rats

Tombarkiewicz B., Pawlak K., Lis M., Kanik W., Lutnicka H., Niedziółka J.

Department of Poultry, Fur Animal Breeding and Animal Hygiene, University of Agriculture in Cracow, Cracow, Poland

Geomagnetic field (GMF) is one of omnipresent, essential for normal development, factors of the environment. Human activity, among others steel construction elements and stock buildings equipment attenuates GMF. Geomagnetic shielding resulted in the anatomical and physiological abnormalities during early stages of growth in and mammals, as well as increase depressive episodes and suicides and are potential reason for development of schizophrenia in individuals born after periods of increased geomagnetic activity. In the present study using an animal model of depression-chronic exposure to mild stressors we investigated if prolonged weakening of the GMF would cause decreased sucrose intake, and if it will affect the level of ACTH and corticosterone which reflects the stress status. The experiment was carried out on male Wistar rats, which were divided on three groups: group A – rats maintained in normal conditions, group B – rats were kept in weakened GMF from 2 months, and group C rats were kept in weakened GMF from two generations. Results of sucrose test could suggest greatest immunity on stress of rats kept in hypogeomagnetic conditions, but higher level of corticosterone in group of rats kept in shielding GMF can suggest, that lasting weakening the GMF was the higher stress than which was caused by other stressors used in an animal model of depression.

This study was performed under project DS 3210/KHDZFiZ

P37. Chronic social stress and LPS-induced inflammation have a synergistic influence on BDNF in the hypothalamus and pituitary of female rats

Nowacka M.[1,2,3], Paul-Samojedny M.[4], Bielecka A.[3], Obuchowicz E.[3]

[1]Laboratory of Molecular Biology, Academy of Physical Education, Katowice, Poland; [2]Center for Experimental Medicine, [3]Department of Pharmacology, Medical University of Silesia, Katowice, Poland; [4]Department of Medical Genetics, Medical University of Silesia, Sosnowiec, Poland

Chronic stress, by initiating changes in the hypothalamic-pituitary-adrenal (HPA) axis and the immune system, acts as a trigger for neuropsychiatric disorders. Brain-derived neurotrophic factor (BDNF) is highly involved in regulation of HPA activity. The aim of the study was to investigate the influence of acute immunostimulation on the of BDNF in the hypothalamus and pituitary of rats subjected to chronic stress. Female Sprague-Dawley rats were subjected to 4-week stress, including phases of isolation and crowding, in an unpredictable manner. On the last day of the experiment rats being at the estrus phase were injected ip. with LPS (1 mg/kg/2 ml) or saline. Six hours later the brain structures were rapidly isolated. QRT-PCR experiments were performed using TaqMan Gene Expression Assays. The BDNF concentration was measured with a conventional ELISA assay. In the hypothalamus and pituitary of LPS-treated stressed rats BDNF mRNA expression was decreased in comparison to saline-treated stressed group. We concluded that chronic stress and inflammation have synergistic deleterious influence on BDNF in the studied structures.

P38. Ultrasonic vocalization (USV) patterns in Eker rat newborns

Sługocka A.[1,2], Chojnacka D.[1], Grabowska M.[1,2], Barski J.J.[1,2]

[1]Center for Experimental Medicine, [2]Department of Physiology, Medical University of Silesia, Katowice, Poland

Tuberous sclerosis complex (TSC) is one of the most frequent neurocutaneous disorders mostly present in pediatric patients. In TSC development we can observe neuropsychiatric signs with symptoms of epilepsy, mental retardation, and autism. Molecular mechanism of this disease involves two genes TSC1 and TSC2. Expression of both of them results in two proteins tuberin and hamartin negatively regulating mTOR signaling pathway. Mutations in TSC1 or TSC2 disturb their inhibitory function, what impairs the proliferation machinery downstream of mTOR. It results in growth of tumors (tubers) in many organs including the brain structure. Eker rat is a strain, that carries a spontaneously occurring germline mutation in the TSC2 gene. By the age of 1 year all TSC2Ek/- animals develop renal epithelial tumors. Tumors also appear in the brain tissue and make the strain a suitable model of autism spectrum disorders (ASD). One of the symptoms specific for autistic-like behaviors is the impaired communication between mother and child. Many rodent species including mouse and rat emit at the ultrasonic range (USV) sounds – a vocal communication between mother and offspring. This phenomenon is a good indicator of social interactions and alterations of the USV can be analyzed by means of specialized recording systems. In our study we attempted to check if there are any differences between TSC2Ek/- genotype newborns and wild type ones in patterns of USV using the standard isolation test. We analyzed sonographic structure of calls, number of calls per minute, duration of single call, etc.

P39. Stimulated locomotor activity of *Spodoptera exigua* larvae after bioinsecticide (spinosad) application

Płachetka A., Francikowski J., Baran B., Kafel A., Augustyniak M.

Department of Animal Physiology and Ecotoxicology, University of Silesia, Katowice, Poland

Spodoptera exigua, beet armyworm is an agricultural insect pest in tropical and subtropical areas of the world. Long-lasting contact with heavy metals could change sensitivity of insects to other stressor, such as insecticide. Spinosad is a nerve poison and it kills pests that it contacts and/or ones that consume it. Spinosad overstimulates nerve cells by prolonging electrical impulses by acting like acetylcholine. The aim of our study was to compare activity of larvae of *S. exigua* and sensitivity of them before and after bioinsecticide (spinosad) application. We used two strains of *S. exigua*: exposed over 100 generations to sub-lethal concentration of cadmium (44 mg Cd/kg dry weight of larval diet) and kept in standardised conditions. Stimulated locomotor activity was recorded during open-field test. Five insects, placed in separate holes in transparent lucite plate, were simultaneously observed. The analysis was made with freely available SwisTrack software that allows simultaneous tracking of more than one insect. For measurement of acetylcholinesterase activity we used acetylthiocholine iodide as a substrate. We found no differences in insect activity between control group and groups treated with spinosad in both breeding strains of *S. exigua*. We found differences in the changes of the acetylcholinesterase activity between the treated and no treatment groups in control strain of *S. exigua*, but not in the cadmium strain.

P40. The influence of electromagnetic field emitted by mobile telephony on chicken embryos hatching behavior

Pawlak K., Tombarkiewicz B., Lis M., Lutnicka H., Niedziółka J.

Laboratory of Animal Hygiene, Department of Poultry and Fur Animals Breeding and Animal Hygiene, University of Agriculture in Cracow, Cracow, Poland

Artificial electromagnetic fields, which are a consequence of human progress especially generated by mobile telephony, begin to

play a considerable role in shaping the Earth's electromagnetic environment. This study attempts to determine the effects of the 1 800 MHz electromagnetic field on chicken embryogenesis. The chicken eggs (*n*=180) were randomly divided into three equal groups and incubated under standard conditions. Control group was incubated in the incubator without an EM field generator, sham group was set in the incubator with a switched off EM field generator, experimental group – chicken embryos were subjected to exposure to magnetic field (1 800 MHz) 10 time per 4 minutes every day. From 430 h of incubation hatch times were observed. The study showed that the chicks in experimental group started internal piping 32 h earlier than in the controls and shame group. While, the process of external piping in experimental group started 26 h earlier than in the control and shame group. The results show that electromagnetic field emitted by mobile telephony does not affect the number of obtained healthy chicks. Only in few unhatched embryos from the experimental group, lack of eyeball was observed.

This study was performed under project DS 3210/KHDZFiZ

P41. Changes in the repetitive stereotyped characteristic of calcium binding proteins (CaBPs) knockout mice in the autistic-like behavior

Grabowska M.[1,2], Sługocka A.[1,2], Barski J.J.[1,2]

[1]Center for Experimental Medicine, Medical University of Silesia, Katowice, Poland, [2]Department of Physiology, Faculty of Medicine in Katowice, Medical University of Silesia, Katowice, Poland

Autism is a multifactorial disorder that involves impairments in social interactions and communication, as well as restricted and repetitive behaviors. Recent whole-genome exon sequencing studies of ASD (autism spectrum disorders) samples estimate that as many as 100 to 1 000 genes may be involved. So different candidate genes have been tested in mouse models by knocking them out. Calbindin D-28k (CB) and parvalbumin (PV) are cytosolic calcium-binding proteins expressed in many neurons without general preference for functionally and morphologically defined subpopulations. Deletion of CB and/or PV alters intracellular calcium signaling, and physiological properties of affected neurons. General knockouts for both proteins display a distinct and permanent motor impairment which is revealed only when adaptation of movement to novel environmental conditions is required. In order to determine whether the absence of CB and PV influences locomotor properties and behavior we compared mouse lacking CB, PV, or both with wild type controls. The mice were compared in the open field task and in the light-dark compartment tests to measure activity, exploratory behavior, and restricted and repetitive behaviors (TruScan, Coulbourn, USA). Relative to wild-type mice, the transgenic mice exhibited much more stereotypic movements in the open field test. Multiple mouse behavioral effects suggest that the CB and PV genes may play a role in modulating behaviors relevant to psychiatric disorders.

P42. Vitamin E, C and Astaxanthin pretreatment prevents high-fat diet induced memory deficits in passive avoidance learning

Karimi S.A.[1, 2], Komaki A.[2], Salehi I.[2], Sarihi A.[2], Zarei M.[2], Shahidi S.[2]

[1]Department of Physiology and Neuroscience Research Center, Faculty of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran; [2]Neurophysiology Research Center, Faculty of Medicine, Hamadan University of Medical Sciences, Hamadan, Iran

Learning and Memory are impaired by imbalanced diet consumption. The objective of this study was to determine the relation between the chronic consumption of High-fat diet (HFD) and antioxidants on passive avoidance learning (PAL) in male rats. Forty male Wistar rats were randomly assigned into five groups (*n*=6–8): Control group; consumed an ordinary diet, HFD group; received high-fat diets only, ANO group; which received HFD plus antioxidants (vitamin E, C and Astaxanthin), RHFD; Received the HFD was restricted, RANO; which received restricted HFD plus antioxidants. Following 6 months of controlled dietary in each experimental group, the PAL was assessed using shuttle box apparatus. Our results showed that HFD caused a decrease in step through latency in retention test (STLr) compared to the control group. And antioxidant supplementation caused an increase in STLr in comparison to the control group. According to our results, HFD impairs PAL and the combination of vitamin E, C and Astaxanthin improve PAL deficits in HFD group.

P43. A comment on fellatio in captive brown bears (*Ursus arctos*): Seeking satisfaction of frustrated suckling?

Sergiel A.[1], Maślak R.[2], Zedrosser A.[3,4], Paśko Ł.[2], Garshelis D.L.[5], Reljić S.[6], Huber D.[6]

[1]Department of Wildlife Conservation, Institute of Nature Conservation, Polish Academy of Sciences, Cracow, Poland; [2]Department of Evolutionary Biology and Conservation of Vertebrates, Institute of Environmental Biology, University of Wroclaw, Wroclaw, Poland; [3]Department of Environmental and Health Studies, Faculty of Art and Sciences, Telemark University College, Bø, Norway; [4]Institute for Wildlife Biology and Game Management, University for Natural Resources and Life Sciences, Vienna, Austria; [5]Minnesota Department of Natural Resources, Grand Rapids, Minnesota, USA; [6]Department of Biology, Faculty of Veterinary Medicine, University of Zagreb, Zagreb, Croatia

Little is known about sexually-oriented behaviors unrelated to reproduction in non-human mammals. Fellatio has been

observed in a number of mammals, including bears, but the role and cause of this behavior remains unclear. We investigated the mechanisms and determinants of regular and persistent fellatio in two captive male brown bears, both orphaned as cubs. The roles of the males, one as the provider and the other as receiver of fellatio, never changed during the observation period. Humming vocalizations used by suckling bear cubs, were clearly audible during 18 fellatio acts, suggesting that the providing bear retained infantile behavior. To our knowledge, this constitutes the first study of this behavior in bears. Bear cubs suckle their mothers for milk, bonding, and comfort for at least the first year of their life. Orphaned bear cubs may suckle own or their sibling's body parts as a substitute for nipples. Forced premature weaning and subsequent deprivation of proper and sufficient stimulation of the suckling reflex can result in teat-searching behavior persisting into adulthood. Our data suggest that the fellatio behavior observed in the captive bears may have emerged from frustrated suckling reflex in individuals orphaned as cubs.

P44. Cross-institutional study on captive bears' behaviour and welfare

Sergiel A.[1], Maślak R.[2], Bowles D.[3], Paśko Ł.[2]
[1]Department of Wildlife Conservation, Institute of Nature Conservation, Polish Academy of Sciences, Cracow, Poland; [2]Department of Vertebrates Evolutionary Biology and Conservation, Institute of Environmental Biology, University of Wrocław, Wrocław, Poland; [3]Royal Society for Prevention of Cruelty to Animals, Horsham, UK

In the study conducted in Poland (2007–2009) data on animal welfare were collected using questionnaires based on the Five Freedoms concept. The ranking of keeping conditions was created, based on the total count of evaluation points for each enclosure. The behaviour was scanned with all-occurrences recording up to 6 hours per enclosure. Each location was visited three times giving the total number of 342 observation hours. Occurrences of stereotypic behaviours were observed in all individuals in the amount ranging from 0.5 to 88.3% of observation time. The results showed a significant difference in time devoted to stereotypies in relation to species (P=0.04). Brown bears (*Ursus arctos*) were more likely to perform stereotypic behavior than Asiatic black bears (*Ursus thibetanus*). There was a significant correlation between space and stereotypies (r=−0.43, P=0.03). A lower level of stereotypic behavior accompanied higher values of space variable. The correlation between the level of stereotypies and the overall assessment of the keeping conditions was r=0.58, statistically significant for $P \leq 0.05$.

P45. Changes in behaviour of spectacled bears (*Tremarctos ornatus*) following relocation and dental treatment

Maślak R.[1], Sergiel A.[2], Hill S.P.[3]
[1]Department of Evolutionary Biology and Conservation of Vertebrates, Institute of Environmental Biology, University of Wroclaw, Wroclaw, Poland; [2]Department of Widlife Conservation, Institute of Nature Conservation, Polish Academy of Sciences, Cracow, Poland; [3]Conservation Medicine Division, Chester Zoo, Chester UK

The behaviour of two spectacled bears kept in a very small enclosure was studied at Wroclaw Zoo (Poland), and then following transfer to a large, naturalistic exhibit at Chester Zoo (UK). In part one of the study, the median amount of time devoted to stereotypical movements was 57 min/h. Shortly after arrival at Chester Zoo, abnormal behaviours were eliminated from the behavioural repertoire of one bear. The other one still performed high levels of stereotypic behaviour. Signs of dental problems were observed, and a dental examination with multiple extractions was carried out. Following the treatment, the amount of time that the bear spent stereotyping reduced significantly. Before dental treatment the bear spent 55% of the time on stereotypies, which reduced to 16% in the first month post-treatment PT1 (P<0.000). There were statistically significant increases in time spent feeding during the two post-treatment periods, compared with the baseline (PT1 feeding: P<0.000; PT2 feeding: P<0.000). Whilst there are several factors that could have contributed to the observed reduction in stereotypy, it is clear that further investigation is needed into the effects of pain and physical condition on stereotypy and other abnormal behaviours in bears and other animals.

P46. Complex social behavior and brain morphology of *Labroides dimidiatus*

Chojnacka D.[1], Barski J.J.[1,2]
[1]Center for Experimental Medicine in Katowice, Medical University of Silesia, Katowice, Poland; [2]Department of Physiology, Medical University of Silesia, Katowice, Poland

Labroides dimidiatus (Labridae) is a model fish in research concerning cleaner fish mutualism. Recent studies shows that cleaners are capable to build up complicated relationships (Bshary and Würth 2001). Cleaners recognize clients individually, they remember past interactions with clients, they may cooperate, reconcile, manipulate, cheat, produce signals out of context, and use predatory clients as social tools against aggressive clients (Bshary 2011). The apparent complexity of cleaner fish behavior makes them a particularly interesting object of comparative analysis of morphology and cytoarchitecture of brain and brain centers in

telencephalon, optic tectum and cerebellum, parts of the brain potentially involved in cognitive functions related to behaviors in complex social environment (Demski and Beaver 2001). A cyto-architectonic study shows in the telencephalic regions responsible for the control of the processes related to visual information, a complex cortical structure; a columnar arrangement of cells in the region responsible for the integration of multimodal information; and duplication of nuclei in vetral part of the telencephalon. Nuclei connected with olfactory information were poorly developed. Proportions, complexity and size of the optic tectum layers, tori semicircularis, tori longitudinalis, and the cerebellum proved diversified importance of visual organs, small role of hearing and lateral line, and complex and well developed skills associated with the spatial mapping and complex locomotion.

P47. Food neophobia in wild and laboratory rats (multi-strain comparison)

Modlińska K., Stryjek R., Pisula W.
Institute of Psychology, Polish Academy of Sciences, Warsaw, Poland

A few decades have passed since most of studies on neophobia in rats were conducted. In the case of such fast-breeding animals this period of time could have led to substantial changes in rat's physiological and behavioral functions. What is more, there are the inconsistencies in research findings with respect to comparisons between wild and laboratory rats, and within domesticated strains. The procedure was designed to isolate specific fear of novel food from generalized fear of a novel object (i.e. container). Rats accustomed to one type of food served in a specific location and in a familiar container were given a different type of food. Each test trial was preceded by food deprivation. The following variables were measured: feeding latency, pace of eating, number of approaches to container, number of times food was sampled for each trial. The amount of food consumed in each trial was weighed and also taken into account. Grooming time served as the measure of stress in rats in the experiment. The analysis of differences between groups showed that rats in all groups achieved comparable values in analyzed variables at the start of the experiment. However, certain differences between strains emerged during the course of the experiment. It turned out that wild rats consumed less amount of food than Brown Norway and Sprague-Dawley in a few trials, demonstrated greater eating latency than Brown Norway, and when novel food was introduced, their number of approaches to food container was higher than those of Long Evans. WWCPS rats demonstrated significantly more of grooming behavior in nearly all trials. The results of the experiment did not confirm the assertion of some authors that wild rats avoid eating unfamiliar foods. All groups demonstrated only a temporary decrease in the amount of food consumed, the magnitude of which was similar in all strains. No evidence of particularly low neophobia in albino rats was found. However, the behavioral symptoms indicated higher level of stress in wild rats compering to the other groups.

P48. Socialization assessment of TSC1 knockout mice

Wiaderkiewicz J.[1,2], Barski J.J.[1,2]
[1]Center for Experimental Medicine, [2]Department of Physiology, Medical University of Silesia, Katowice, Poland

Tuberous sclerosis (TSC) is a genetic disorder, linked to the TSC1 gene and characterized by the emergence of multi-system benign tumors. Symptoms related to central nervous system disruptions include seizures, developmental delay and a varying range of behavioral disorders. These disorders can be identified by analyzing various parameters, many of which can be observed during social interactions. Cre recombinase mediated TSC1 gene knockout mice were constructed and analyzed for sociability and preference for social novelty. The test was performed in a standard three-chamber sociability cage and results were obtained and analyzed using automated tracking software Ethovision XT from Noldus.

P49. A framework for analysis of mice behavior in Python

Kowalski J.M., Puścian A., Mijakowska Z., Radwańska K., Łęski S.
Nencki Institute of Experimental Biology, PAS, Warsaw, Poland

An abundance of behavioral data can be obtained with the IntelliCage system. Basic analysis of the data can be performed with a tool provided with the system, however more advanced analyses have to be performed manually. One of disadvantages of manual analysis is its extreme unefficiency since it requires significant effort. Another disadvantage is that it is vulnerable to human errors. The analysis process can be easily automated with a programmable workflow. In this approach a researcher focuses his (or her) effort to design the workflow and let the computer do the work in which it performs better (in aspect of speed, precision and robustness) than a human. Once the workflow has been defined it is possible to reproduce the analysis on data acquired from other application of the experimental protocol facilitating reproducible research. A possibility of convenient access to data is crucial for programming a workflow. To facilitate it we are developing a Python framework which allows for easy loading of recorded data as well as accessing it in an intuitive manner. With the framework we develop a methodology for advanced analysis of these data. Developed analysis methods are being included into the framework providing its users with a powerful tool for their scientific work.

P50. Impact of tuberin (TSC2) gene knockout on locomotor activity, motor coordination and behavioral patterns in mice

Głowacka M.[1,2], Barski J.J.[1,2]

[1]Center for Experimental Medicine, Medical University of Silesia, Katowice, Poland; [2]Department of Physiology, Medical University of Silesia, Katowice, Poland

Tuberin (TSC2) is one of the proteins involved in autism spectrum disorders' pathogenesis (ASD), which together with hamartin (TSC1), is responsible for cellular growth, protein synthesis, and cellular proliferation. The highest concentration of Tuberin has been observed in the cerebellum, particularly in the Purkinje cells. In view of the physiological role of the cerebellum, lack of this protein may impair both locomotor activity and motor coordination. Being one of the factors responsible for ASD, tuberin might also play a role in early behavioral pattern formation. In this work we try to prove these two hypotheses. The study involved three groups of mice: 12 heterozygous TSC (+/-) and 8 mutant TSC (-/-) mice were compared with a control group of 7 wild-type C57BL/6 mice. All the mice were introduced into the study at the age of 6 weeks. Each mice underwent the following standardized tests: the RotaRod test, the Elevated Runway test, the Open Field test. All the tests were performed three times- at the age of 6, 12 and 28 weeks. Both the RotaRod and the Elevated runway tests revealed significantly impaired motor coordination skills in the mutant mice compared with both the control and the heterozygous group. Furthermore, the TSC (-/-) mice exhibited a noticeably lower level of explorative activity and higher levels of fear.

P51. Long term treatment of ketogenic diet increases kidney tumor growth by p53-dependent manner

Liśkiewicz A.[1], Kasprowska-Liśkiewicz D.[2], Lewin-Kowalik J.[1], Jędrzejowska-Szypułka H.[1]

[1]Department of Physiology, [2]Depatment of Pharmacology School of Medicine, Medical University of Silesia, Katowice, Poland

The Eker rat is a useful model of tuberous sclerosis, genetic disease with leads to tumor growth in many tissues. Ketogenic diet (KD) is already used in treatment of epilepsy in tuberous sclerosis patients, but its influence on tumor growth is still not clear. Here we reported how KD affects growth of kidney tumors in Eker rats, subjected to the diet for 4, 6 and 8 months. Data were compared to animals fed with standard diet. Obtained results showed that long term treatment of ketogenic diet promotes tumor growth in kidneys. Additionally, biochemical analysis of renal tissue indicate a possible mechanism in growth promoting influence of KD by lowering p53 protein, guardian of the genome.

P52. Role of parvalbumin-positive interneurons in the prefrontal cortex: A link to schizophrenia?

Wołoszynowska-Fraser M.U.[1], Wulff P.[2], Riedel G.[1]

[1]University of Aberdeen, Aberdeen, Scotland; [2]Christian Albrechts University, Kiel, Germany

Alterations in the inhibitory circuitry of the pre-frontal cortex (PFC) are thought to underlie some of the cognitive deficits observed in schizophrenia. These alterations particularly concern a subset of GABAergic interneurons that express the calcium-binding protein parvalbumin (PV) as shown in post mortem studies in patients and animal models of the disease (Lewis et al. 2005). To assess the contribution of PV+ interneurons to PFC- dependent behaviours, we selectively blocked the output from those cells via virus-mediated expression of tetanus toxin light chain (TeLC). We found that functional removal of PV+ neurons causes specific impairments in working memory and cognitive flexibility, which represent key cognitive deficits in schizophrenia. As oscillatory brain activity in the theta (4-8 Hz) and gamma (20-80 Hz) frequency range correlates with working memory performance and patients suffering from schizophrenia show alterations in these frequency bands, we measured local field potential oscillations in the PFC and simultaneously in the hippocampus, a brain region implicated in working memory. Preliminary data indicate a significant decrease in PFC theta and gamma activities in the test mice relative to control animals. Interestingly hippocampal activity in the theta and gamma range was also diminished. These results show that PV+ interneurons in the PFC control task relevant neuronal activity in different brain regions engaged with working memory. Impaired signalling from PFC PV+ interneurons may thus underlie the neurophysiological alterations and concurrent cognitive deficits found in schizophrenia.

P53. Behavior and Metabolism Research Laboratory at Mossakowski Medical Research Centre

Olszyński K.H., Filipkowski R.K.

Behavior and Metabolism Research Laboratory, Mossakowski Medical Research Centre, Warsaw, Poland

Our laboratory provides a venue to conduct behavioral and metabolism research on mice and rats. We offer all levels of help concerning designing appropriate behavioral/metabolism experiments, training or conducting the experiments as well as the analysis, discussion and presentation of the results. Various forms of cooperation within our institution and with external investigators are possible. Please visit http://www.imdik.pan.pl/pl/pracownie-srodowiskowo-uslugowe/srodowiskowe-laboratorium-behawioralno-metaboliczne for the full list of equipment, behavioral models and description of metab-

olism unit. The equipment assembled allows us to run sensorimotor, motor & anxiety (open field, elevated-plus maze), learning & memory (e.g., fear conditioning, avoidance, Morris water maze) and social tests. Additionally, Automated Blood Sampling and Telemetry system for full automated, chronic metabolic and telemetric experiments on rodents provides: (i) automated system for blood and other body fluids *in vivo* sampling and collecting in cooling collector, (ii) automated system of solution infusions to vessels or other selected locations in animal and (iii) telemetry able to measure various physiological parameters (blood pressure, biopotentials, etc.). Behavior and Metabolism Research Laboratory equipment is located in air-conditioned, partly noise-attenuated rooms with regulated day/ night cycle.

AUTHORS' INDEX