12th International Congress of the Polish Neuroscience Society

September 6-8, 2015
Gdańsk, Poland

12th International Congress of the Polish Neuroscience Society

Honorary Committee

LECH WAŁĘSA INSTITUTE foundation

Ministerstwo Nauki i Szkolnictwa Wyższego

PAN POLSKA AKADEMIA NAUK

POLSKA AKADEMIA UMIEJĘTNOŚCI

Patronage

MIECZYSŁAW STRUK
MARSZAŁEK
WOJEWÓDZTWA POMORSKIEGO

Prezydent
Miasta Gdańska
Paweł Adamowicz

Prof. Janusz Moryś, M.D., Ph.D.,
Rector
of the Medical University of Gdańsk

OKRĘGOWA IZBA LEKARSKA

UNIWERSYTET GDAŃSKI

Sponsors & Exhibitors

REMONTOWA HOLDING

shim-pol

ANIMA LAB
wyposażenie zwierzętarni i laboratoriów • modele zwierzęce

BECKMAN COULTER

CED
CAMBRIDGE ELECTRONIC DESIGN LIMITED
DATA ACQUISITION & ANALYSIS

CELLLAB

EUROIMMUN

Jackson ImmunoResearch Europe Ltd

SCIENCE PRODUCTS GmbH
for Research in Life Sciences

nata aqua

SIGMA-ALDRICH®

Supporting Institutions

Institute of Pharmacology
Polish Academy of Sciences

International Institute of Molecular and Cell Biology in Warsaw

nencki institute
of experimental biology

Organizing Committee

Chairperson: Agnieszka Jankowska-Kulawy

Vice-chairperson: Anna Ronowska

Members: Hanna Bielarczyk, Aleksandra Dyś, Dorota Bizon-Zygmańska, Beata Gapys, Katarzyna Grużewska, Sylwia Gul-Hinc, Ewa Kiszka, Ilona Klejbor, Joanna Klimaszewska-Łata, Przemysław Kowiański, Beata Ludkiewicz, Katarzyna Majak, Natalia Melka, Anna Michno, Anna Raszeja-Specht, Marlena Zyśk

Scientific Committee

Chairperson: Andrzej Szutowicz

Vice-chairperson: Małgorzata Skup

Members: Jan Celichowski, Marian Lewandowski, Joanna Lewin-Kowalik, Anna Ronowska, Maria Stankiewicz, Wioletta Waleszczyk, Daniel Wójcik

http://www.pns2015.gumed.edu.pl

Organizer: Polish Neuroscience Society (PNS)

Co-organizers: Department of Laboratory Medicine, Medical University of Gdańsk
Department of Anatomy and Neurobiology, Medical University of Gdańsk

Scientific Program Committee:
Chairperson: Andrzej Szutowicz – President of Polish Neuroscience Society
Vice-chairperson: Małgorzata Skup
Members: Jan Celichowski
Marian Lewandowski
Joanna Lewin-Kowalik
Anna Ronowska
Maria Stankiewicz
Wioletta Waleszczyk
Daniel Wójcik

Local Organizing Committee:
Chairperson: Agnieszka Jankowska-Kulawy
Vice-chairperson: Anna Ronowska
Members: Hanna Bielarczyk
Aleksandra Dyś
Dorota Bizon-Zygmańska
Beata Gapys
Katarzyna Grużewska
Sylwia Gul-Hinc
Ewa Kiszka
Ilona Klejbor
Joanna Klimaszewska-Łata
Przemysław Kowiański
Beata Ludkiewicz
Katarzyna Majak
Natalia Melka
Anna Michno
Anna Raszeja-Specht
Marlena Zyśk

Honorary Committee:
Lech Wałęsa Institute Foundation
Prof. Lena Kolarska-Bobińska, Minister of Sciences and Higher Education
Prof. Jerzy Duszyński, President of the Polish Academy of Sciences
Prof. Andrzej Białas, President of the Polish Academy of Art and Sciences Authorities

Supporting Institutions:
Institute of Pharmacology, Polish Academy of Sciences, Kraków
International Institute of Molecular and Cell Biology in Warsaw
Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw

Patronage:
Mieczysław Struk, The Marshal of the Pomorskie Voivodeship
Paweł Adamowicz, The Mayor of the City Gdańsk
The Regional Medical Chamber
Prof. Janusz Moryś, Rector of the Medical University of Gdańsk
Prof. Bernard Lammek, Rector of the University of Gdańsk

Sponsors and financial support:
Ministry of Science and Higher Education of Poland
International Brain Research Organization (IBRO)
Remontowa Holding
Shim-pol
Animalab
Cellab
Euroimmun
Jackson Immuno Research
Science Products
Lonza-Nata
Beckmann Coulter
Cambridge Electronic Design Ltd
Sigma Aldrich

12TH INTERNATIONAL CONGRESS OF THE POLISH NEUROSCIENCE SOCIETY

PROGRAMME
Sunday, September 6th, 2015

11.00 AM – 07.00 PM	Registration
01.00 PM – 02.45 PM	General Assembly of the Polish Neuroscience Society
03.00 PM – 03.15 PM	Opening ceremony
03.15 PM – 04.00 PM	**Opening lecture** Leszek Kaczmarek (Warszawa, Poland) **PL1. Molecular linking brain to mind** Introduced by: Andrzej Szutowicz
04.00 PM – 04.15 PM	Coffee break

Sunday, September 6th, 2015
(Afternoon sessions)

04.15 PM – 06.15 PM	**SYMPOSIA** **S1. Lipid rafts – sphingolipids in neurodegeneration and neuroprotection** Chairpersons: Robert P. Strosznajder (Warszawa, Poland), W. Gibson Wood (Minneapolis MN, USA) Ongoing activation of sphingosine 1-phosphate receptors mediates maturation of exosomal multivesicular endosomes Shun-ichi Nakamura (Kobe, Japan) Protein Prenylation, Synaptic Function and Aging W. Gibson Wood (Minneapolis MN, USA) Sphingolipid biology, lipid rafts and their roles in neurodegeneration and neuroprotection Alessandro Prinetti (Milan, Italy) Brain sphingolipids in hyperglycemia Halina Car (Białystok, Poland) Sphingosine-1-phosphate and its receptors signaling in neurodegeneration /neuroprotection Joanna B. Strosznajder (Warszawa, Poland) **S2. Different models, one goal: Understand Parkinson's disease mechanisms to find new therapeutic targets** Chairperson: Danuta Jantas (Kraków, Poland) Early Parkinson's disease and mechanisms compensating for dopaminergic neurons loss – an energy metabolism point of view Katarzyna Kuter (Kraków, Poland) Glycation disrupts proteostasis and promotes neurodegenerative alterations in synucleinopathies Tiago Outeiro (Goettingen, Germany) Human neuroblastoma SH-SY5Y cells – *in vitro* model to study neurodegeneration and neuroprotection in relation to Parkinson's disease Danuta Jantas (Kraków, Poland) From modeling to treating Parkinson's disease: The importance of viral vectors Ludivine Breger (Lund, Sweden) **S3. Epigenetics and chromatin structure in brain function** Chairperson: Bożena Kamińska (Warszawa, Poland)

	Epigenetics and biomarkers in neuropsychiatric diseases
	Robeto Carlos Agis-Balboa (Vigo, Italy)
	Alterations in methylome in three experimental models of epilepsy – differences and similarities
	Katarzyna Łukasiuk (Warszawa, Poland)
	Genomic landscape of lysine acetylation in the adult hippocampus: Implications in neuronal plasticity and brain disorders
	Angel Barco (Alicante, Spain)
	Epigenetic control of microglia polarization in brain pathologies
	Bożena Kamińska (Warszawa, Poland)
	Architecture of the neuronal nucleus
	Grzegorz Wilczyński (Warszawa, Poland)
06.15 PM – 06.30 PM	Coffee break
06.30 PM – 06.45 PM	**Konorski Award Presentation**
	Marcin Szczot (Wrocław, Poland)
	α1F64 residue at $GABA_A$ receptor binding site involved in gating by influencing the receptor flipping transitions
	Introduced by: Jan Celichowski
06.45 PM – 07.30 PM	**Plenary lecture**
	Andrew J. Fuglevand (Tucson, AZ, USA)
	PL2. Neural control of the hand
	Introduced by: Jan Celichowski
07.30 PM – 09.30 PM	Welcome reception

Monday, September 7th, 2015
(Morning sessions)

08.30 AM – 08.40 AM	**Konorski Distinguished Work**
	Jakub Włodarczyk (Warszawa, Poland)
	Genetically encoded FRET-based biosensor for imaging MMP-9 activity
	Introduced by: Jan Celichowski
08.40 AM – 09.30 AM	**Plenary lecture**
	Jan Krzysztof Blusztajn (Boston, MA USA)
	PL3. Choline in neuronal development and neuroprotection
	Introduced by: Andrzej Szutowicz
09.30 AM – 11.30 AM	**SYMPOSIA**
	S4. Amyloid-β – cause or outcome of neurodegeneration
	Chairpersons: Grzegorz A. Czapski (Warszawa, Poland), Anna Ronowska (Gdańsk, Poland)
	Pyroglutamate modification of Abeta – a pathogenic mechanism in Alzheimer's disease
	Steffen Rossner (Leipzig, Germany)
	Perspectives of the neurochemical dementia diagnostics: From the pathophysiologic mechanisms to the applications in the early diagnosis
	Piotr Lewczuk (Erlanden, Germany)
	Genetics and transcriptomics of Alzheimer's disease
	Michalina Kosiorek (Warszawa, Poland)
	Amyloid beta at the crossroad of neurodegeneration and neuroinflammation
	Grzegorz A. Czapski (Warszawa, Poland)
	S5. Biology and function of oligodendrocyte precursor cells, the CNS multipotent cells in health, disease and regeneration
	Chairperson: Małgorzata Zawadzka (Warszawa, Poland)

Heterogeneity and balance between proliferation and maturation in the oligodendrocyte progenitor pool
Annalisa Buffo (Turin, Italy)
Intrinsic regulators of OPC activation during CNS remyelination – role of Sox2
Chao Zhao (Cambridge, UK)
Identification of factors modulating oligodendrocyte precursor cells differentiation and maturation
Małgorzata Zawadzka (Warszawa, Poland)
Myelin, oligodendrocytes and axonal pathology in St8sia2-/- mice, a model for schizophrenia research
Marta B. Wiśniewska (Warszawa, Poland)
The neuroprotective effect exerted by oligodendroglial progenitors on ischemically impaired hippocampal slices
Joanna Sypecka (Warszawa, Poland)

S6. A multiview of current Spanish neuroscience
Chairperson: Daniel Wójcik (Warszawa, Poland)
Transcriptome and proteome remodeling through alternative splicing in the vertebrate brain
Manuel Irimia (Barcelona, Spain)
Cerebral cortex activity and network computations
Mavi Sanchez Vives (Barcelona, Spain)
The bilingual brain: Plasticity and processing from cradle to grave
Manuel Carreiras (Bilbao, Spain)
Down syndrome: Unravelling the genetic players of a disorder of neuronal plasticity
Mara Dierssen (Barcelona, Spain)

11.30 AM – 01.30 PM **LUNCH POSTER SESSION 1**

Monday, September 7th, 2015
(Afternoon sessions)

01.30 PM – 02.00 PM **Special Lectures**
Krzysztof Turlejski (Warszawa, Poland)
SL1. A CARE lecture. Ethical and legal aspects of experimenting on animals
Introduced by: Małgorzata Skup
Georg Reiser (Magdeburg, Germany)
SL2. Brain energy metabolism – neurotoxicity, neuroprotection
Introduced by: Andrzej Szutowicz

02.00 PM – 04.00 PM **SYMPOSIA**

S7. Neurobiology of aging brain
Chairperson: Monika Liguz-Lęcznar (Warszawa, Poland)
The influence of aging on context reasoning
Grzegorz Sędek (Warszawa, Poland)
A window on the ageing brain: *In vivo* imaging of structural changes at synaptic resolution
Vincenzo de Paola (London, UK)
Age-related plasticity impairments-presynaptic mechanisms
Monika Liguz-Lęcznar (Warszawa, Poland)
Neurobiology of aging brain
Otto Witte (Jena, Germany)

S8. Central control of reproduction
Chairperson: Joanna H. Śliwowska (Poznań, Poland)

KNDy neurons, peptides, and the control of mammalian reproduction
Michael N. Lehman (Jackson, MS, USA)
Participation of salsolinol in the regulation of the secretory activity of the pituitary gland in sheep during lactation
Tomasz Misztal (Jabłonna, Poland)
Neuronal feedback within the network regulating GnRH secretion
Imre Kalló (Budapest, Hungary)
Diet, alcohol and reproduction – lessons from animal models
Joanna H. Śliwowska (Poznań, Poland)

S9. Calcium as a key regulator of neural function
Chairperson: Jarosław J. Barski (Katowice, Poland)
Reduced expression levels of parvalbumin may lead to an ASD-like phenotype in mice
Beat Schwaller (Fribourg, Switzerland)
Role of STIM1 in neuronal Ca^{2+} signaling and synaptic transmission
Jana Hartmann (Munich, Germany)
Role of store operated calcium entry in neurons
Jacek Kuźnicki (Warszawa, Poland)
The role of calbindin D-28k in cerebellum
Jarosław J. Barski (Katowice, Poland)

04.00 PM – 04.15 PM Poster Coffee Service

04.00 PM – 06.00 PM **AFTERNOON POSTER SESSION 1**

06.00 PM – 06.10 PM **Young Investigator Award**
Magdalena Kusek (Kraków, Poland)
Glutamatergic and GABAergic transmission in rat PVN is altered after acute restraint stress
Introduced by: Marian Lewandowski

Workshop
Marcin Gawryś (Warszawa, Poland)
Roxy system – tool for mimicking drug metabolism: Oxidative metabolism of amadiaquine (phase I) and adduct formatting (phase II).

06.10 PM – 07.00 PM **Plenary lecture**
Robin Franklin (Cambridge, UK)
PL4. CNS remyelination – from mechanisms to medicines
Introduced by: Magdalena Zawadzka

Tuesday, September 8th, 2015
(Morning sessions)

08.30 AM – 08.40 AM **Young Investigator Award**
Alicja Puścian (Warszawa, Poland)
Chronic fluoxetine treatment disrupts appetitively motivated learning and central amygdala structural plasticity
Introduced by: Marian Lewandowski

08.40 AM – 09.30 AM **Plenary lecture**
Jacopo Annese (San Diego, CA, USA)
PL5. Brain banking as a shared neuroimaging resource: MRI, microscopy, and Web services
Introduced by: Daniel Wójcik

09.30 AM – 11.30 AM **SYMPOSIA**

S10. Coenzyme A and its derivatives in brain metabolism and neurodegeneration

Chairpersons: Andrzej Szutowicz (Gdańsk, Poland), Jan K. Blusztajn (Boston, MA, USA)
Alteration of Coenzyme A biosynthetic pathway in neurodegeneration with brain iron accumulation syndromes
Valeria Tiranti (Milan, Italy)
Regulation of Coenzyme A biosynthesis and homeostasis in health and disease
Ivan Gout (London, UK)
Precursors of coenzyme A – panthenol and pantethine – in the mechanisms of neuroprotection
Andrey G. Moiseenok (Grodno, Belarus)
Disturbances in Acetyl-CoA metabolism a key point in mechanisms of cholinergic degeneration
Andrzej Szutowicz (Gdańsk, Poland)

S11. Motor control
Chairperson: Jan Celichowski (Poznań, Poland)
Spinal cord, reticular formation and cerebellum – there and back again
Ingela Hammar (Göteborg, Sweden)
Is motoneuron hyperexcitability harmful in ALS?
Daniel Zytnicki (Paris, France)
The role of selected serotonergic receptors in the control of locomotor movements in intact and paraplegic rats
Urszula Sławińska (Warszawa, Poland)
Adaptation of motoneurons to altered physical activity
Piotr Krutki (Poznań, Poland)

S12. Structure, function and plasticity of $GABA_A$ receptors
Chairperson: Jerzy W. Mozrzymas (Wrocław, Poland)
Structural mechanism underlying $GABA_A$ receptor gating and drug modulation
Cynthia Czajkowski (Madison, WI, USA)
Plasticity of gabaergic synapses
Andrea Barberis (Genova, Italy)
Contribution of the GABAergic system to cortical plasticity
Małgorzata Kossut (Warszawa, Poland)
Linking agonist binding to $GABA_A$ receptor opening transition
Jerzy W. Mozrzymas (Wrocław, Poland)

11.30 AM – 01.30 PM **LUNCH POSTER SESSION 2**

Tuesday, September 8th, 2015
(Afternoon sessions)

01.30 PM – 02.00 PM **Special Lecture**
Bogdan Sadowski (Warszawa, Poland)

SL3. Pain – an old and a new problem
Introduced by: Wioletta Waleszczyk

02.00 PM – 04.00 PM **SYMPOSIA**

S13. Regenerative neurobiology of the eye
Chairperson: Joanna Lewin-Kowalik (Katowice, Poland)
The theory of inflammaging
Anu Kauppinen (Kuopio, Finland)
Age-related macular degeneration (AMD): Alzheimer's disease in the eye?
Kai Kaarniranta (Kuopio, Finland)
Schwann cells induced neuroprotection and regeneration of retinal ganglion cells
Joanna Lewin-Kowalik (Katowice, Poland)

	Spontaneous and inducible reinervation of human cornea
	Adrian Smedowski (Katowice, Poland)
	S14. Paying attention to attention towards better understanding of its mechanisms and neuronal correlates
	Chairperson: Anita Cybulska-Kłosowicz (Warszawa, Poland)
	Stimulus-driven and anticipatory attention differentially modulate primary visual cortex
	Marek Bekisz (Warszawa, Poland)
	Influence of attention an instructions on tactile processing
	C. Elaine Chapman (Montreal, Canada)
	Finding the psycho in psychomotor stimulation: Differential effects of amphetamine and KW6002 on response preparation
	Verity J. Brown (Oxford, UK)
	Deficits in attentional functions and dopamine transporter polymorphisms
	Anita Cybulska-Kłosowicz (Warszawa, Poland)
	S15. Computational challenges in imaging at different scales
	Chairperson: Daniel Wójcik (Warsaw, Poland)
	Activity-dependent dendritic spine neck changes are correlated with synaptic strength
	Tim Vogels (Oxford, UK)
	Quantitative analysis of shapes of dendritic spines
	Szymon Łęski (Warszawa, Poland)
	Imaging populations in the awake animal
	Jason Kerr (Bonn, Germany)
	Optical control of network context in hippocampal synaptic plasticity
	Upinder Bhalla (Bangalore, India)
04.00 PM – 04.15 PM	Poster coffee service
04.00 PM – 06.00 PM	**AFTERNOON POSTER SESSION 2**
06.00 PM – 06.10 PM	**Young Investigator Award**
	Kacper Ptaszek (Gdańsk, Poland)
	The influence of electrical stimulation of the raphe magnus on rat behaviours
	Introduced by: Marian Lewandowski
06.10 PM – 07.00 PM	**Plenary lecture**
	Amiram Grinvald (Rehovot, Israel)
	PL6. Real-time VSD based optical imaging revealed the bidirectional interaction of ongoing activity and sensory-evoked activity
	Introduced by: Wioletta Waleszczyk
08.00 PM	**Gdańsk Shipyard farewell party**

PLENARY LECTURES

PL1
MOLECULAR LINKING BRAIN TO MIND

Leszek Kaczmarek
Laboratory of Neurobiology, Nencki Institute of Experimental Biology, PAS, Warsaw, Poland

The Merriam-Webster Dictionary derives term "Mind" from Old English *gemynd*; akin to Old High German *gimunt* meaning memory. Over the last quarter of century we have followed molecular roots of the memory in a hope to identify also building blocks of the mind. Initially, we have identified increased *c-fos* mRNA levels in memory formation, thus discovering phenomenon of gene expression in learning. Following c-Fos protein function as transciptional regulator, we have focused on its gene targets: TIMP-1 and MMP-9 (tissue inhibitor of matrix metalloproteinases-1 and matrix metalloproteinase-9), composing extracellular proteolytic system that we and others have implicated as a major player in the synaptic plasticity, learning and memory. MMP-9 has been shown first to be activated in dendritic remodeling, accompanying epileptogenesis. Then, functional studies demonstrated MMP-9 role in learning and memory as well as their cellular models and finally epileptogenesis. At the subcellular level, MMP-9 localization and activity helps to explain this role, as the enzyme, its protein and mRNA are all available at the or near excitatory synapses located at the dendritic spines to allow for a rapid, local unleash of the enzymatic activity in response to synaptic stimulation. Furthermore, MMP-9 was shown to directly affect the dendritic spine morphology and excitatory neurotransmitter receptor function and trafficking. In aggregate, the pivotal role of MMP-9 in the synaptic plasticity underlying brain physiology has been firmly established. The present research challenge is to explain possible contribution of the enzyme to such human neuropsychiatric conditions as epilepsy, drug addiction, schizophrenia and autism spectrum disorders to name just those for which such a link has been demonstrated. By these virtues, MMP-9 emerges indeed as a molecular link to the brain molecular underpinning of the mind.

PL2
NEURAL CONTROL OF THE HAND

Andrew J. Fuglevand
Department of Physiology and College of Medicine, University of Arizona, Tucson, AZ, USA

A large expanse of the primate cerebral cortex is dedicated to formulating the motor commands that control the complex mechanical apparatus of the hand. Conventional views about how the motor cortex controls the hand, however, are being overturned. For example, it is now clear that the hand region of the motor cortex is not topographically subdivided into distinct regions associated with the control of individual digits. Furthermore, it appears that multiple muscles are engaged even during the simplest movements of a single finger. Consequently, we have to reconsider how a spatially-distributed motor cortex might orchestrate the activities of multiple muscles in the elaboration of finger movements. One possibility is that descending pathways diverge to act collectively upon several motor nuclei to enlist sets of muscles into functional groups (synergies). This type of organization, while relatively inflexible, might underlie the assemblage of muscles into synergistic groups needed to generate the elemental movements that are the building blocks of the behavioral repertoire of the hand. I will present evidence both supporting the presence and absence of such fixed synergies among different groups of hand muscles and the functional significance associated with both forms of multi-muscle coordination.

PL 3
CHOLINE IN NEURONAL DEVELOPMENT AND NEUROPROTECTION

Jan Krzysztof Blusztajn
Department of Pathology & Laboratory Medicine, Boston University School of Medicine, Boston, MA, USA

Choline is as an essential nutrient for humans. Because the fetus and neonate have high demands for choline, its dietary intake during pregnancy and nursing is particularly important for normal development of the offspring. Previous studies in rodents have shown that high choline intake during gestation or the perinatal period improves cognitive function in adulthood, prevents memory decline of old age, and protects the brain from damage and cognitive and neurological deterioration associated with epilepsy. This presentation will focus on our recent studies showing that high choline intake in early life alleviates deficits in social interaction and improves anxiety-like behaviors in a mouse model of autism and is effective in reducing brain amyloidosis in a mouse model of Alzheimer's disease. The behavioral changes evoked by choline nutrition are accompanied by altered patterns of expression of multiple cortical and hippocampal genes including those encoding key proteins that contribute to the biochemical mechanisms of learning and memory. One of the biochemical fates of choline is to serve as a donor of methyl groups and the effects of choline correlate with cerebral cortical changes in DNA and histone methylation, thus suggesting an epigenomic mechanism of action of perinatal choline.

PL4

CNS REMYELINATION – FROM MECHANISM TO MEDICINE

Robin J.M. Franklin

Wellcome Trust-MRC Cambridge Stem Cell Institute, University of Cambridge, Cambridge, UK

Remyelination, the process by which new myelin sheaths are restored to demyelinated axons, represents one of the most compelling examples of adult multipotent stem cells contributing to regeneration of the injured CNS. This process can occur with remarkable efficiency in multiple sclerosis (MS), and in experimental models, revealing an impressive ability of the adult CNS to repair itself. However, the inconsistency of remyelination in MS, and the loss of axonal integrity that results from its failure, makes enhancement of remyelination an important therapeutic objective. There is now compelling evidence that ageing is the major contributor to the declining efficiency of remyelination and that this is largely due to a failure of stem cell differentiation. This talk will review recent studies we have undertaken aimed at obtaining a detailed understanding of the mechanisms of regulating differentiation during remyelination and hence identifying novel therapeutic targets.

PL5

BRAIN BANKING AS A SHARED NEUROIMAGING RESOURCE: MRI, MICROSCOPY, AND WEB SERVICES

Jacopo Annese

The Brain Observatory, San Diego, CA, USA

By preserving the brain of his aphasic patient Leborgne, the French neurologist Paul Broca inaugurated the most effective instrument of human neuroscience before Magnetic Resonance Imaging (MRI); that is: the *post-mortem* documentation of neurological damage in patients with discrete behavioral deficits. Following this illustrious tradition, but applying modern digital technology, The Brain Observatory is currently engaged in the development of a permanent digital archive for images and data produced from donated human brains. The archive is meant to represent the phenotypical spectrum of normal brain maturation, aging, and neurological disease. The resource is designed to support remote collaboration, future comparisons, as well as retrospective studies, as exemplified by the databases created for amnesic patients H.M. Individual anatomical atlases representing each donor include MRI and large-scale microscopic images. The atlases are linked to MRI-based morphometrics, quantitative neuropathology and scores derived from neuropsychological tests. Some donors also consent to the recording of biographical audio- and video-interviews, effectively personalizing their datasets. Our digital curation strategy is based on multiple, interrelated object identifiers (DOI) that describe diverse elements of the database and statistical analyses can be performed on line. Web technologies (such as Google Maps APIs) maximize the exploration and interoperability of the collection at multiple levels of expertise.

PL6

REAL-TIME VSD BASED OPTICAL IMAGING REVEALED THE BIDIRECTIONAL INTERACTIONS OF ONGOING ACTIVITY AND SENSORY-EVOKED ACTIVITY

Amiram Grinvald

Department of Neurobiology the Weizmann Institute of Science, Rehovot, Israel

To better understand cortical processing, principles underlying the neural code and higher brain functions, many technical requirements must be met by tools used to measure brain electrical activity, all the way from the single cell level to the entire brain. Remarkable progress has been accomplished with the development of genetically engineered calcium and voltage-sensitive probes, and two photon imaging technologies. However, all available tools are currently falling short in meeting one or more of the technological requirements. Here we describe an advance in optical imaging of spatio-temporal pattern of coherent population neuronal activity, millisecond by millisecond, *in-vivo*. We integrated this technique with other neurophysiological techniques in order to explore the role of ongoing activity on brain function and behavior.

We designed and synthetized a superior organic voltage sensitive dye (VSDs) offering remarkable signal-to-noise ratio in a single trial with sub-millisecond time resolution and detection both subthreshold and spiking coherent activity, distinctly.

Exploring the dynamics of ongoing cortical activity we discovered that ongoing activity accounts for the large variability of the evoked activity. In exploring anesthetized and behaving non-human primates we found similarity and differences. We also explored the interaction in the opposite direction and found that sensory evoked activity diminished ongoing activity.

Imaging tools to detect both the input to and output produced by coherently active neuronal populations, ms by ms, over a large cortical area are available for revolutionizing our current understanding of neuronal processing. Intrinsic cortical activity and extrinsic sensory-evoked activity interacts. These activities are composed of coherent spatio-temporal patterns of synaptic potentials and Coherent Population Spikes rather than by the same individual cells.

SPECIAL LECTURES

SL1
ETHICAL AND LEGAL ASPECTS OF EXPERIMENTING ON ANIMALS – A CARE LECTURE

Krzysztof Turlejski
Laboratory of Neurobiology of Development and Evolution, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland

In my presentation I am going to review the present the state of law concerning research on animals in the European Union and Poland in particular. I am going to refer relate the status of implementation of the EU Directive 2010/63 in Poland and other EU. Next I will review the major changes in standards of the laboratory animal husbandry, process of ethical evaluation and control over scientific experiments that implementation of the new Directive 2010/63/UE of the European Union Council and European Parliament imposes. Lastly, I will compare numbers of animals used for animal research in various European countries, the spectrum of species used and the most problematic directions and objects of scientific research on animals from the point of view of the new Directive. In conclusion, I will try to describe the possible impact of the new Directive on animal research in Poland and Europe.

SL2
BRAIN ENERGY METABOLISM – NEUROTOXICITY, NEUROPROTECTION

Georg Reiser
Institute of Inflammation and Neurodegeneration, Faculty of Medicine, Otto-von-Guericke University Magdeburg, Magdeburg, Germany

Paradigms of cerebral metabolism claim that mitochondrial β-oxidation of activated fatty acids (FA) has only minor importance for brain energy homeostasis. In contrast to other organs with high energy demand, long-chain FA (LCFA) play minor roles as hydrogen source in brain. There are clear disadvantages of the use of free fatty acids as brain fuel. These shortcomings could have exerted evolutionary pressure to lower expression in brain of β-oxidation enzymes in brain mitochondria to favor glucose oxidation. Otherwise, accumulated fatty acids exert detrimental activities on mitochondria, to trigger mitochondrial route of apoptosis. Pathological tissue accumulation of LCFA impairs mitochondrial physiology, ATP regeneration, and sensitizes mitochondria for permeability transition and stimulates oxidative stress. In contrast to LCFA, mitochondria do not degrade branched-chain phytanic acid (PA) and very long chain fatty acids (VLCFA). Elevated serum levels are biochemical hallmarks of inherited neurodegenerative diseases, (i) of PA for the adult form of the Refsum disease and, (ii) of VLCFA for X-linked adrenoleukodystrophy (X-ALD). The severe neurodegenerative disorder X-ALD results from defective ABCD1 transporter protein. Clinical symptoms are manifested in neural tissues and adrenal gland. VLCFA increase the vulnerability of Abcd1$^{-/-}$ astrocytes more than that of astrocytes from wild-type mice. In VLCFA-exposed Abcd1$^{-/-}$ astrocytes, the reduction of a tetrazolium electron acceptor was severely diminished. Exposure of isolated mitochondria to PA impairs the inner membrane integrity and energy-dependent functions, electron transport in the respiratory chain and cellular physiology of hippocampal astrocytes. Activation of an intracellular Ca^{2+} signaling pathway by PA and pristanic acid suggests that a membrane receptor coupled to intracellular Ca^{2+} release might be involved. We propose that inhibition of β-oxidation does not seem to be a promising therapeutically helpful strategy to lower cerebral oxidative stress, as shown by ROS measurements in brain mitochondria *or in-situ* in astrocytes. They contradict proposals to cure consequences of oxidative stress in cerebral tissue by β-oxidation inhibition. For fatty acids physiology, we also consider the 3 PPAR isoforms (α, β/δ and γ), which represent a tightly interconnected array of ligand-activated transcription factors, for which we coined the term PPAR triad.

SL3
PAIN – AN OLD AND A NEW PROBLEM

Bogdan Sadowski
Institute of Genetics and Animal Breeding, Polish Academy of Sciences, Jastrzębiec, Poland

Pain has been interpreted as a specific sensation in response to potentially or actually noxious agents. Actually, this classical view is supplemented by a distinction between nociception, i.e. the ability to sense noxious stimuli, and emotional appreciation of nociceptive experience. Nociceptive information is targeted mainly to the sensorimotor cortex and the insula, whereas the emotional evaluation occurs in the anterior cingulate cortex. The intensity of pain perception is often disproportional to the nociceptive input. This phenomenon has fundamental adaptive value, whenever sensing more or less pain in some vital situations is important for organism's survival. Intense or prolonged noxious stimulation causes sensitization of peripheral nociceptors and/or pain centers, leading to hyperalgesia, i.e. sensing more pain to mild noxious stimuli, or to allodynia, as innocuous stimuli become painful. The sensitization involves an activity-driven molecular process, based on heterosynaptic non-Hebbian facilitation. This condition usually subsides along with the progression of the healing process, or transforms into chronic pain. Neuroimaging studies in patients suffering from chronic pain revealed activation of the medial prefrontal cortex and amygdala. Chronic pain, when prolonged, exerts a deleterious action. Repetitive co-occurrence of pain epizodes and incidental environmental cues leads to pathological Pavlovian conditioning of pain. This maladaptive plastic process involves activation of glutamatergic synapses and is based on a Hebbian LTP-like learning mainly in the cingulate and in dorsolateral prefrontal cortex. A poorly understood problem of the pain story is the central pain

without obvious tissue damage, following brain stroke or other central lesions. Its background may by an imbalance between glutamate and GABA-ergic neurotransmission in thalamocortical pain circuits, or a lack of inhibition from peripheral cold receptors on pain-processing brain areas.

SL4

KONORSKI AWARD LECTURE

A_1F64 RESIDUE AT $GABA_A$ RECEPTOR BINDING SITE IS INVOLVED IN GATING BY INFLUENCING THE RECEPTOR FLIPPING TRANSITIONS

Marcin Szczot, Magdalena Kisiel, Marta M. Czyżewska, Jerzy W. Mozrzymas

Laboratory of Neuroscience, Department of Biophysics, Wrocław Medical University, Wrocław, Poland

[Published in: J Neurosci 34(9): 3193–3209]

SYMPOSIUM S1. LIPID RAFTS – SPHINGOLIPIDS IN NEURODEGENERATION AND NEUROPROTECTION

Chairpersons: *Robert P. Strosznajder (Warsaw, Poland), W. Gibson Wood (Minneapolis MN, USA)*

S1.A

ONGOING ACTIVATION OF SPHINGOSINE 1-PHOSPHATE RECEPTORS MEDIATES MATURATION OF EXOSOMAL MULTIVESICULAR ENDOSOMES

Shun-ichi Nakamura, Taketoshi Kajimoto, Taro Okada, Satoshi Miya, Lifang Zhang

Division of Biochemistry, Department of Biochemistry and Molecular Biology, Kobe University Graduate School of Medicine, Kobe, Japan

Exchange of information through extracellular vesicles, including exosomes, has emerged to be an important tool of intercellular communication necessary for the physiology of neural systems. Exosomes also have a sinister role in the propagation of toxic amyloid proteins in neurodegenerative conditions, including Alzheimer's and Parkinson's diseases. However, mechanisms underlying biogenesis of exosomes and the cargo sorting remain unclear. We present here the mechanism of regulation of exosome biogenesis by sphingosine 1-phosphate (S1P) signaling. During late endosome maturation, cargo molecules are sorted into intralumenal vesicles (ILVs) of multivesicular endosomes (MVEs), and are either delivered to lysosomes for degradation or fused with the plasma membranes for exosome release. We have recently found that inhibitory G protein (Gi)-coupled S1P receptors regulate exosomal MVE maturation. Gi-coupled S1P receptors on MVEs are constitutively activated through a constant supply of S1P *via* autocrine activation within organelles. We also found that the continuous activation of Gi-coupled S1P receptors on MVEs is essential for cargo sorting into ILVs destined for exosome release. Our results reveal a mechanism underlying ESCRT-independent maturation of exosomal MVEs. These findings shed light on the understanding as to how cargo molecules are sorted into exosomal ILVs and provide an important clue to the development of molecule-based treatment of exosome-mediated intractable diseases including neurodegenerative disorders.

Kajimoto T, Okada T, Miya S, Zhang L, Nakamura S (2013) Ongoing activation of sphingosine 1-phosphate receptors mediates maturation of exosomal multivesicular endosomes. Nat Commun 4: 2712. doi: 10.1038

S1.B

PROTEIN PRENYLATION, SYNAPTIC FUNCTION AND AGING

S. Afshordel[1], G.P. Eckert[1], D.A. Hottman[2], L. Li[2], U. Igbavboa[3], W.G. Wood[3]

[1]Department of Pharmacology, Biocenter, University of Frankfurt, Frankfurt, Germany; [2]Department of Experimental and Clinical Pharmacology, [3]Department of Pharmacology, School of Medicine, University of Minnesota, Minneapolis, MN, USA

Synaptic plasticity is the cellular basis of learning and memory that declines with increasing age. Dendritic structure plays a key role in synaptic plasticity that is impaired in aged brain. Rho family small GTPases, including Rac1 and RhoA play distinct roles in the development and modification of dendritic spines, axons and synapses. We recently found that levels of prenylated Rac1 and RhoA were significantly lower in aged mouse brain as compared with younger mice. This reduction was associated with a decrease in abundance of the synaptic markers synaptophysin and GAP43. Rac1 stimulates spine formation and RhoA impedes spine formation. Previous studies have proposed that there needs to be a balance in the activity of those proteins for optimal spine function. This balance appears to be disrupted in aged brain which could contribute to age-related spine dysfunction. Rac1 and RhoA, are prenylated by GGTase-I whereas Rab proteins are prenylated by GGTase-II. Prenylated Rab protein levels were similar in the young and aged mice raising the possibility that the lower Rho-GTPase prenylated protein levels were the result of a deficiency in GGTase-I. We found that mRNA and protein levels of GGTase-Iβ were significantly lower in brain tissue of aged mice as compared with younger mice. Further support for the critical role of GGTase-I in synaptic plasticity comes from two novel mouse models: (1) a heterozygous GGTase-Iβ gene knockout; and (2) a homozygous floxed GGTase-Iβ brain neuron specific Cre recombinase. Data from those mice revealed diminished long-term potentiation in the CA1 region of the hippocampus and loss of dendritic spines. We propose that impaired GGTase-Iβ regulation in aged brain causes a reduction in Rac1 and RhoA protein prenylation

resulting in disruption of the dynamic balance between those proteins. That disruption triggers aberrant downstream effector function causing a loss of synaptic plasticity and impaired cognitive function.

S1.C

SPHINGOLIPID BIOLOGY, LIPID RAFTS AND THEIR ROLES IN NEURODEGENERATION AND NEUROPROTECTION

Alessandro Prinetti

Department of Medical Biotechnology and Translational Medicine, University of Milan, Milan, Italy

Sphingolipids are polar membrane lipids present as minor components in eukaryotic cell membranes. Sphingolipids are highly enriched in nervous cells, where they exert important biological functions. They deeply affect the structural and geometrical properties and the lateral order of cellular membranes and modulate the function of several membrane- associated proteins. Sphingolipid metabolism is regulated along the differentiation and development of the nervous system, and the expression of a peculiar spatially and temporarily regulated sphingolipid pattern is essential for the maintenance of the functional integrity of the nervous system: sphingolipids in the nervous system participate to several signaling pathways controlling neuronal survival, migration and differentiation, responsiveness to trophic factors, synaptic stability and synaptic transmission, and neuron-glia interactions, including the formation and stability of central and peripheral myelin. In several neurodegenerative disease, sphingolipid metabolism is deeply deregulated, leading to the expression of abnormal sphingolipid patterns and altered membrane organization, that participate to several events related to the pathogenesis of these diseases. Targeting sphingolipid metabolism and lipid rafts organization represents today an underexploited but realistic opportunity to design novel therapeutic strategies for the intervention in this diseases.

S1.D

BRAIN SPHINGOLIPIDS IN HYPERGLYCEMIA

Halina Car[1], Anna Fiedorowicz[2], Sławomir Prokopiuk[1], Kamil Bienias[1], Małgorzata Żendzian-Piotrowska[3], Adrian Chabowski[3], Irena Kasacka[4], Rotem Tidhar[5], Anthony H. Futerman[5], Katarzyna Głombik[6], Agnieszka Basta-Kaim[6]

[1]Department of Experimental Pharmacology, [3]Department of Physiology, [4]Department of Histology and Cytophysiology, Medical University of Białystok, Białystok, Poland; [2]Department of Experimental Oncology, Ludwik Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Białystok, Poland; [5]Department of Biological Chemistry, Weizmann Institute of Science, Rehovot, Israel; [6]Department of Experimental Neuroendocrinology, Institute of Pharmacology, Polish Academy of Science, Kraków, Poland

Ceramide (Cer) and sphingomyelin (SM) are members of sphingolipid (SL) family. Their concentrations in the brain undergo substantial changes in many pathologies. They are also important players in diabetes-linked brain dysfunctions, in which increased content of ceramides can be toxic to neurons. The aim of the study was to evaluate selected parameters of sphingolipids and insulin pathways in prefrontal cortex (PC) and hippocampus (H) of rats with experimentally induced hyperglycemia. STZ-rat model of type 1 diabetes and high fat diet model of insulin resistance were used. Analyses of studied parameters were performed by GLC, IHC and Elisa.

We found the augmented levels of ceramides in H and PC and only minor in striatum and cerebellum of rats with STZ-induced diabetes. Similar expressions of Cer were confirmed by IHC. Myriocin, an inhibitor of an enzyme of ceramide de novo synthesis pathway, reduced ceramide generation in hyperglycemic brains, particularly in PC, which was reflected in altered Cer synthase activities. In addition, we reported the fluctuations in sphingomyelin levels in investigated structures. The level of insulin did not change in H and PC of STZ-treated rats. An expression of insulin receptor and its phosphorylated form decreased in both structures, but was restored after myriocin administration. Similarly, Akt and phosphorylated Akt changed in these structures suggesting important role of *de novo* Cer synthesis in intracellular pathways of insulin. In the rat model of high fat diet, which leads to insulin resistance, the sphingolipid pattern (Cer and SM) was altered in H and PC as well. Metformin, the drug of choice in diabetes type 2, influenced the content of the above SLs in these structures, suggesting the additional central activity of antidiabetic treatment. We conclude that ceramide and SM may be important mediators of diabetes- accompanied brain dysfunction.

S1.E

SPHINGOSINE-1-PHOSPHATE AND ITS RECEPTORS SIGNALING IN NEURODEGENERATION / NEUROPROTECTION

Joanna B. Strosznajder[1], Joanna Motyl[1], Kinga Czubowicz[2], Robert Strosznajder[2]

[1]Department of Cellular Signaling, [2]Department of Neurosurgery, Mossakowski Medical Research Centre, Polish Academy of Sciences, Warsaw, Poland

Sphingosine -1-phosphate (S1P) is synthesized by sphingosine kinases (SphK1/2E.C. 2.7.1.91) and exerts its function as intracellular messenger or acts in an autocrine or paracrine fashion through specific G protein operated receptors (S1P1-S1P5). Depending on SphK type and its localization S1P may influence different cell functions. S1P synthesized by SphK1 is involved in cell survival while produced by SphK2 may activate death signaling. S1P is degraded by phosphohydrolyses and irreversibly by S1P lyase (SPL, E.C.4.1.2.27) which appears to be

very important in sphingolipid homeostasis. The alterations of sphingolipid rheostat is suggested to be crucial in pathogenesis/pathomechanism of neurodegenerative disorders. In our study we have evaluated the SphKs and SPL expression/activity as well as the role of S1P in different types of oxidative stress involved in neurodegenerative disorders. Moreover, the implications of SphK/S1P in the cell models of Alzheimer's disease induced by amyloid peptides (AB) and alfa synuclein (ASN) were determined. Oxidative stress alters SphKs and SPL expression, activity and cells viability. In AD model significant decrease of SphK expression and activity/lower S1P synthesis leads to series of the following consecutive events: oxidative stress, down regulation of anti-apoptotic protein Bcl-2, up-regulation of pro-apoptotic BAX and HrK and finally to cell's death. Exogenous S1P and the agonist(s) of S1P1 or S1P3 receptors exert cytoprotective effects which are mediated by PI3/Akt signaling pathway and by regulation of Bcl2 proteins. Summarizing, our data suggest that S1P, its receptor(s) agonists and inhibitors of SPL should be considered in therapy of neurodegenerative disorders.
Supported by NCN grant 5870/P01/2011/40

SYMPOSIUM S2. DIFFERENT MODELS, ONE GOAL: UNDERSTAND PARKINSON'S DISEASE MECHANISMS TO FIND NEW THERAPEUTIC TARGETS

Chairperson: *Danuta Jantas (Kraków, Poland)*

S2.A
EARLY PARKINSON'S DISEASE AND MECHANISMS COMPENSATING FOR DOPAMINERGIC NEURONS LOSS – AN ENERGY METABOLISM POINT OF VIEW

Katarzyna Kuter[1,2]

[1]Physical Biochemistry, Department of Chemistry, Darmstadt University of Technology, Germany; [2]Department of Neuropsychopharmacology, Institute of Pharmacology, Polish Academy of Sciences, Kraków, Poland

Degeneration of dopaminergic neurons in substantia nigra (SN) is underlying cause of movement disorder observed in Parkinson's disease. At its early stages motor deficits are masked by compensatory mechanisms. Our aim was to describe how prolonged metabolic dysfunction of astrocytes would influence processes of compensation for the dopaminergic neurons degeneration. Rat model of selective nigrostriatal dopaminergic system degeneration was induced by intracerebral injection of 6-OHDA into medial forebrain bundle. Astrocytes metabolic dysfunction was induced by 7-days infusion of fluorocitrate (FC) into SN. Dopaminergic neurons lesioning as well as astrocytes dysfunction induced motor deficits that were reversed with time, despite progressing neuronal degeneration after 6-OHDA. Inhibition of astrocytes metabolism by FC caused tendency to decrease performance of not-assembled complex I and IV. Double toxicity of 6-OHDA and FC also decreased performance in complex I and IV especially 4 weeks after operations and FC discontinuation, causing also significant decrease in specific activity of complex IV. Along with those changes, we observed decreased mitochondrial membranes viscosity. Results from aconitase activity showed that when neurons were devoid of astrocytes support, their aconitase activity increased drastically. Presented research shows that prolonged dysfunction of astrocytes influences dopaminergic cells metabolism and vulnerability. Surprisingly, changes in oxidative phosphorylation system were shown very small even after loss of 33% to 61% of dopaminergic neurons – what would suggest extensive adaptive possibilities, maybe in mitochondria volume.
Supported by the Statutory Funds of the Institute of Pharmacology, PAS, Poland; Technische Universität Darmstadt, MOBILNOŚĆ PLUS MNiSW scholarship and NCN grant nr 2012/05/B/NZ4/02599.

S2.B
GLYCATION DISRUPTS PROTEOSTASIS AND PROMOTES NEURODEGENERATIVE ALTERATIONS IN SYNUCLEINOPATHIES

Tiago Outeiro

University Medical Center Goettingen, Goettingen, Germany

Alpha-synuclein (aSyn) aggregation in Lewy bodies is a pathological hallmark of Parkinson's disease (PD) and other synucleinopathies. Glycation, an age-dependent protein modification, is present in Lewy bodies. Here, we investigated the effect of the natural glycating agent methylglyoxal on aSyn biology and found that glycation increased aSyn aggregation and toxicity. Notably, striatal injection of methylglyoxal in mice caused neuronal loss. Genetic and pharmacological manipulation of methylglyoxal increased aSyn-dependent toxicity in human LUHMES cells and in PD patient-derived iPSCs, and decreased motor performance and survival in aSyn transgenic flies. Furthermore, glycated aSyn impaired synaptic transmission in rat hippocampal slices. Methylglyoxal promoted aSyn oligomerization by affecting its N-terminal structure and impairing lipid-binding ability. Glycation disrupted proteostasis, reducing aSyn turnover, aggregation, and release, likely the mechanistic link underlying the phenotypes observed. In total, our study uncovers glycation as a novel player in synucleinopathies, opening novel avenues for the design of therapeutic strategies.

S2.C
HUMAN NEUROBLASTOMA SH-SY5Y CELLS – *IN VITRO* MODEL TO STUDY NEURODEGENERATION AND NEUROPROTECTION IN RELATION TO PARKINSON'S DISEASE

Danuta Jantas

Department of Experimental Neuroendocrinology, Institute of Pharmacology, Polish Academy of Sciences, Cracow, Poland

Human neuroblastoma SH-SY5Y cells are widely used in neurotoxicity and neuroprotection research. Since this cell line, derived from peripheral nervous system, possesses a dopaminergic phenotype and is sensitive to dopaminergic specific toxins (e.g. MPP(+), 6-OHDA), it has been extensively utilized not only to study Parkinson's disease (PD) pathology but also to test new putative neuroprotective agents. During the presentation the advantages and limitations associated with the use of this cellular model for the study of PD will be presented with discussion on its translational potential. Finally, data from the study performed in SH-SY5Y cells aimed to assess the neuroprotective potential of metabotropic glutamate receptors group II and III (mGluR II/III) activators will be demonstrated. There are many experimental evidences demonstrating the importance of normalizing glutamatergic and GABAergic pathways in the basal ganglia circuitry, using for example agonists of mGluR II and III. With the emergence of new mGluR III specific agents, there is a need for efficient cellular screening platform to assess both neuroprotective effects and mechanisms of action.

S2.D

FROM MODELING TO TREATING PARKINSON'S DISEASE: THE IMPORTANCE OF VIRAL VECTORS

Ludivine Breger, Luis Quintino, Anders Björklund, Cecilia Lundberg

Department of Experimental Medical Science, Wallenberg Neuroscience Centre, Lund, Sweden

Over the years, viruses have been manipulated and became a common tool in the field of molecular biology. They have played a key role in the development of gene delivery and modulation of gene expression, which are widely used today to model neurodegenerative disorders, *in vitro* as well as *in vivo*. With over 6 million people affected worldwide, Parkinson's disease is the second most common neurodegenerative disorder. It is characterized by a lack of dopamine in the striatum, resulting from neurodegeneration of the substantia nigra. The mechanisms underlying the development of the disease remain unclear and current treatments are only symptomatic. There is therefore a crucial need for the development of more reliable animal models, which better reproduce the disease's features, including cell loss and protein aggregation. Viral vector can be used to obtain stable expression of causal genes, in various brain regions, thus expending animal modeling beyond genetically engineered mice. Furthermore, gene therapy has been considered as a promising approach for the treatment of neurodegenerative disorders. Different approaches have been studied, from slowing down neuronal death using neurotropic factors to delivering the genes required for dopamine production in the striatum. Whether it is for disease modeling or for therapeutic purposes, gene transfer requires the use of viral vectors. This talk will therefore explore how viral vectors, by taking advantage of the virus properties, have revolutionized Parkinson's disease research, from disease modeling to therapeutic treatments currently in the clinic.

SYMPOSIUM S3. EPIGENETICS AND CHROMATIN STRUCTURE IN BRAIN FUNCTION

Chairperson: *Bożena Kamińska (Warsaw, Poland)*

S3.A

EPIGENETICS AND BIOMARKERS IN NEUROPSYCHIATRIC DISEASES

Roberto Carlos Agis-Balboa

IBI – Institute of Biomedical Research Vigo, Vigo, Italy

Our group studies the epigenetic mechanisms associated to psychiatric (e.g. PTSD, depression and schizophrenia), neurodegenerative (e.g. Alzheimer's disease) and autoimmune (e.g. multiple sclerosis) diseases. We search for biomarkers and therapeutic targets for diagnosis, prognosis and treatment of such devastating diseases. Our findings provide a novel molecular insight to the mechanisms underlying fear extinction and cognitive impairment and highlight the potential of targeting the IGF2/IGFBP7 pathway as therapeutic avenue to treat some neuropsychiatric diseases. Furthermore, SERT clustering in peripheral lymphocytes can be used to identify patient response to antidepressant therapy.

S3.B

ALTERATIONS IN METHYLOME IN THREE EXPERIMENTAL MODELS OF EPILEPSY – DIFFERENCES AND SIMILARITIES

Katarzyna Łukasiuk

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

According to the WHO, 60 million people in the world have epilepsy, and in 30% of patients are drug resistant. Finding new mechanisms involved in development of epilepsy that could be a targets for new therapies is an unmet medical need. Recent evidence points to epigenetic mechanisms which occur during epileptogenesis, including acute changes in DNA methylation, histone modifications, and microRNA expression which have been reported in different epilepsy models and in human tissue. In our work we tested the hypothesis that epileptogenesis is accompanied by specific changes in DNA methylation that are common for different epilepsies regardless of etiology. Using Methyl-capture and massive parallel sequencing (Methyl-Seq), we compared alterations in genomic DNA methylation in the hippocampal CA1/CA3 fields at 3 months following epileptogenic injury in three experimental models of epilepsy: focal amygdala stimulation, systemic pilocarpine injection, or lateral

fluid-percussion induced TBI in rats. We have found that in all three injury models methylation status differentiates controls from injured animals. All three models are characterized by markedly increased methylation in gene bodies and by hypomethylation in non-genic areas. However, analysis of the precise locations of methylation events in the genome did not identify any regions with altered methylation which had been common to all three models, and only a few regions common to any two models. We found only modest association of altered methylation and gene expression. Changes in methylation status occur in experimental models of epilepsy, however the methylation pattern is dependent on etiology and can underlie model specific mechanisms or stage of epileptogenesis.

S3.C

GENOMIC LANDSCAPE OF LYSINE ACETYLATION IN THE ADULT HIPPOCAMPUS: IMPLICATIONS IN NEURONAL PLASTICITY AND BRAIN DISORDERS

Angel Barco

Laboratory for Transcriptional and Epigenetic Mechanisms of Neuronal Plasticity, Miguel Hernández University – CSIC, Alicante, Spain

[Abstract not received]

S3.D

EPIGENETIC CONTROL OF MICROGLIA POLARIZATION IN BRAIN PATHOLOGIES

Bożena Kamińska

Laboratory of Molecular Neurobiology, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland

Microglia are the myeloid cells residing in the central nervous system, quickly responding to pathological alterations. Microglia polarization refers to establishment of a specific phenotype which can produce detrimental or beneficial effects. The inflammatory (M1) and alternative, anti-inflammatory (M2) phenotype are the extreme phenotypes. It is poorly understood how microglia are reprogrammed in a responses to challenges and how signals are converted into sustained patterns of gene expression in brain pathologies. Transcriptome analysis of microglial cultures exposed to glioma (GCM) or lipopolysaccharide (LPS) shows activation of distinct signaling and metabolic pathways resulting in different patterns of gene expression. Studies of activating and repressive histone marks revealed the early decrease of histone acetylation in microglia exposed to GCM, while changes in repressive histone modifications after GCM or LPS were delayed and correlated to transcription down-regulation. HDAC inhibitors blocked morphological changes associated with GCM or LPS treatment and reduced GCM-induced gene expression. Those results demonstrate that the inflammatory genes are epigenetically "primed" and easy to be induced in microglia, while the erasure of histone acetylation marks is prerequisite to put the repressive marks on M1 inflammatory genes and induce activating histone acetylation at the M2 genes. Studies of epigenetic patterns in sorted microglia were extended to a cerebral ischemia (MCAo) model, in which microglia activation is associated with the prolonged inflammation and subsequent neurodegeneration. Our results demonstrate that microglial polarization is mediated by alterations in gene expression and changes in their epigenetic patterns. Epigenetic modifications provide an additional step for the control of long-lasting changes in transcriptional programs in stimulated microglia.

S3.E

ARCHITECTURE OF THE NEURONAL NUCLEUS

Grzegorz Wilczyński

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

Studies in cultured cells have demonstrated the existence of higher-order epigenetic mechanisms, determining the relationship between expression of the gene and its position within the cell nucleus. It is unknown, whether such mechanisms operate in post-mitotic, highly differentiated cell types, such as neurons *in vivo*. Accordingly, we examined whether the intranuclear positions of Bdnf and Trkb genes, encoding the major neurotrophin and its receptor respectively, change as a result of neuronal activity, and what functional consequences such movements may have. In a rat model of *status epilepticus* we found that elevated neuronal expression of *Bdnf* after seizures is associated with its detachment from the nuclear lamina, and translocation toward the nucleus center. In contrast, the position of stably expressed *Trkb* remains unchanged after seizures. Our study demonstrates that activation-dependent architectural remodeling of the neuronal cell nucleus *in vivo* contributes to activity-dependent changes in gene expression in the brain.

SYMPOSIUM S4. AMYLOID-β – CAUSE OR OUTCOME OF NEURODEGENERATION

Chairpersons: *Grzegorz A. Czapski (Warsaw, Poland), Anna Ronowska (Gdańsk, Poland)*

S4.A

PYROGLUTAMATE MODIFICATION OF Aβ – A PATHOGENIC MECHANISM IN ALZHEIMER'S DISEASE

Steffen Rossner

Paul Flechsig Institute for Brain Research, University of Leipzig, Leipzig, Germany

N-terminally truncated and pyroglutamate (pGlu)-modified Abeta peptides (pGlu-Abeta) are major constituents of Abeta deposits in

brains of Alzheimer's disease (AD) patients. There is ample biochemical and cell biological evidence for the formation of pGlu-Abeta peptides by the enzymatic activity of glutaminyl euriti (QC). However, a thorough histopathological association between the spatial presence of QC and the formation of pGlu-Abeta deposits in human brain is still lacking. The role of QC in the formation of different types of pGlu-Abeta aggregates in neocortex and hippocampus of AD subjects was studied by immunohistochemistry. Live cell imaging and enzymatic activity assays were used to reveal secretion of QC from primary neurons. In many cases, there was a clear co-localization or spatial association between QC expressing neurons and pGlu-Abeta aggregates. Additionally, pGlu-Abeta aggregates were found to be present in brain regions with only few QC immunoreactive neurons. However, such brain regions are known to receive afferents from QC expressing neurons that are affected early by Abeta pathology in the course of the disease. This points towards a mechanism in which pGlu-Abeta and/or QC are being transported along euritis and released from synapses of projection neurons at their terminal fields. Indeed, live cell imaging and enzymatic activity assays demonstrated the secretion of QC from neurons in a constitutive and regulated manner. We conclude that pGlu-Abeta aggregates may develop through different mechanisms: intracellularly at sites of somatic QC activity as well as extracellularly through seeding at terminal fields of QC expressing projection neurons.

S4.B

PERSPECTIVES OF THE NEUROCHEMICAL DEMENTIA DIAGNOSTICS: FROM THE PATHOPHYSIOLOGIC MECHANISMS TO THE APPLICATIONS IN THE EARLY DIAGNOSIS

Piotr Lewczuk

Laboratory for Clinical Neurochemistry and Neurochemical Dementia Diagnostics, Erlangen University Hospital, Department of Psychiatry and Psychotherapy, Erlangen, Germany

In the current diagnostics of neurodegenerative disorders, such as Alzheimer's disease (AD), Neurochemical Dementia Diagnostics (NDD) plays extremely important role. Two groups of biomarkers in the cerebrospinal fluid (CSF) are taken currently into consideration: amyloid β (Aβ) peptides and Tau proteins, along with the hyperphosphorylated forms of the latter (pTau). The analyses of these two groups of biomarkers can reveal pathologic alterations as early as twenty years before the onset of the first clinical symptoms. In pre-clinical stages, like mild cognitive impairment (MCI), NDD can reliably predict increased risk of progression into dementia stage of AD. The role of biomarkers of amyloid β deposition in the brain tissue (including the CSF concentrations of $A\beta_{42}$), as well as the biomarkers of neurodegeneration (including the CSF concentrations of Tau/pTau proteins), is reflected in the currently proposed diagnostic criteria for AD and MCI. Current further directions in the development of NDD include: (1) search for novel biomarkers with improved analytical or diagnostic performance, (2) optimization of the analysis of the biomarkers already available (for example, by improved quality control and inter-laboratory comparison of results), (3) applications of novel technologies enabling better management of patients samples, for example application of multiplexing technologies, and (4) search for biomarkers in the blood.

S4.C

GENETICS AND TRANSCRIPTOMICS OF ALZHEIMER'S DISEASE

Michalina Kosiorek[1], Michał Kabza[2], Magdalena Skrzypczak[3], Krzysztof Ginalski[3], Izabela Makałowska[2], Maria Barcikowska[1], Cezary Żekanowski[1]

[1]Laboratory of Neurogenetics, Mossakowski Medical Research Centre, Polish Academy of Sciences, Warsaw, Poland; [2]Laboratory of Bioinformatics, Faculty of Biology, Adam Mickiewicz University, Poznań, Poland; [3]Laboratory of Bioinformatics and Systems Biology, University of Warsaw, Warsaw, Poland

Alzheimer's disease is the most common form of dementia characterized by a progressive deterioration of cognitive functions and by overproduction of toxic form of β-amyloid (Aβ) and intracellular accumulation of the microtubule-associated protein tau into neurofibrillary tangles (NFTs). In the past few years, our research team has investigated the genetic variability of PSEN1, PSEN2 and APP genes in AD patients, especially familial early-onset AD (fEOAD). We have identified mutations in PSEN1 and PSEN2, including novel ones located in exons coding for the large cytosolic loop of presenilin 1. To test functional nature of the aforementioned mutations we have performed analysis of the whole transcriptome using RNA sequencing method and total RNA isolated from primary fibroblasts cultures derived from fEOAD patients. Using RNA-Seq data we have performed differential gene expression (DGE) analysis, which was estimated by three independent bioinformatic tools (i.e. Cuffdiff, EgdeR and Deseq2). Further DGE enrichment analysis revealed a number of signaling pathways significantly altered in the samples from fEOAD patients, which varied depending on identified mutations in PSEN1 or PSEN2 genes. Next to cell cycle, pro-apoptotic, TNF, adherent junction, p53, or Wnt signaling pathways, we have found several changes in the pathways that have not been previously linked to AD. Among the aforementioned pathways we have focused on HIF-1 signaling, Hippo signaling as well as DNA mismatch repair, base excision repair, and transcriptional misregulation mechanisms. Interestingly, two novel PSEN1 mutations changing the amino acid sequence of the large cytoplasmic loop have been linked to TNF and HIF-1 signaling pathways, sug-

gesting induction of proinflammatory response and opening future directions of the research on fEOAD pathomechanism.
Supported by NCN G1119-2013/09/D/NZ3/01348.

S4.D

AMYLOID-β AT THE CROSSROAD OF NEURODEGENERATION AND NEUROINFLAMMATION

Grzegorz A. Czapski

Mossakowski Medical Research Centre, Polish Academy of Sciences, Warsaw, Poland

An increasing body of evidence demonstrated that oligomers of Amyloid beta (Aβ) are the most toxic form, which is responsible for neuronal dysfunction, neurodegeneration and cognitive impairment in Alzheimer's disease (AD). Among many pathological alterations evoked by Aβ, modulation of innate mechanisms of inflammatory response seems to be especially important, in both early and late stages of AD. Misfolded Aβ peptides stimulate Toll-like receptors and activate phagocytic activity of microglia, leading to clearance of Aβ. However, prolonged activation may divert immune system from its beneficial functions and evoke sustained release of inflammatory mediators and reactive oxygen species responsible for degeneration of neurons. On the other hand, inflammation-related processes promote Aβ formation and another AD-related pathological alterations, leading to initiation of self-propagating cycle. Our studies focused on the role of cyclin-dependent kinase 5 in molecular mechanisms of toxicity of Aβ. Experiments on mouse model of AD demonstrated that this kinase may regulate expression of inflammation-related genes in the brain. Depending on age, disease stage and other environmental conditions, neuroinflammatory processes may be beneficial, promoting neuroprotection and neuroregeneration, or can result in neuronal damage. The data suggest that modulation of immune system is a promising protective strategy in AD.
This study was supported by The National Science Centre Grant 2011/03/B/NZ3/04549.

SYMPOSIUM S5. BIOLOGY AND FUNCTION OF OLIGODENDROCYTE PRECURSOR CELLS, THE CNS MULTIPOTENT CELLS IN HEALTH, DISEASE AND REGENERATION

Chairperson: *Małgorzata Zawadzka (Warsaw, Poland)*

S5.A

HETEROGENEITY AND BALANCE BETWEEN PROLIFERATION AND MATURATION IN THE OLIGODENDROCYTE PROGENITOR POOL

Annalisa Buffo

Department of Neuroscience Rita Levi Montalcini, Neuroscience Institute Cavalieri Ottolenghi (NICO), University of Turin, Turin, Italy

Oligodendrocyte progenitor cells (OPCs) persist in the adult Central Nervous System (CNS) and guarantee oligodendrocyte turnover and myelin repair throughout life. It remains obscure how OPCs avoid exhaustion during adulthood and whether, similar to neural stem cells, they include distinct populations or functional stages intrinsically committed to self-maintain or to produce a differentiating progeny. To address these issues, by *in vivo* fate-mapping approaches we examined the phenotype of OPCs during mitosis and the fate of their daughter cells at distinct time points after cell birth. We found that distinct transcription factors are expressed in segregated subsets of dividing OPCs in the mouse adult and juvenile cerebral cortex. Such subsets produce cell progenies with different fates. Further, we showed that a fraction of dividing OPCs gives rise to asymmetric daughter cell pairs including sister OPCs with diverse early immunophenotypic profiles and fates (i.e. acquisition of premyelinating markers or maintenance of progenitor features, including the expression of NG2, PDGFRa and the ability to undergo re-proliferation). Sister OPC heterogeneity appears as early as cells exit cytokinesis, suggesting that, similar to stem cells, a subset of the dividing OPCs can undergo asymmetric division. Interestingly, although molecules such as NG2 and PDGFRa expressed in the mother cells do not segregate asymmetrically during OPC mitosis, OPCs express a repertoire of classical molecular regulators of the cell division modality operating in neural stem cells, including cell fate determinants and polarity machineries. Our data point to the existence of mechanisms that finely tune the OPC turn-over to preserve the progenitor pool while assuring the production of new oligodendrocytes in the intact adult brain. Alteration of these mechanisms may contribute to the hamper oligodendrogenesis and myelin repair in the aged CNS.

S5.B

INTRINSIC REGULATORS OF OPC ACTIVATION DURING CNS REMYELINATION – ROLE OF SOX2

Chao Zhao

Wellcome Trust-Medical Council Cambridge Stem Cell Institute and Department of Clinical Neuroscience, University of Cambridge, Cambridge, UK

Understanding the mechanisms of CNS remyelination is central to developing effective means by which this process can be therapeutically enhanced in chronic demyelinating disease such as multiple sclerosis. Progression of oligodendrocyte progenitor cells (OPCs) to mature oligodendrocytes in response to signals in injury environment holds the key for successful myelin regeneration. Here we discuss the role of Sox2, a transcription factor widely implicated in stem cell biology, in CNS myelination and

remyelination. We show that Sox2 is expressed in most OPCs at active period of developmental CNS myelination at early postnatal stage but diminished in adults in oligodendrocyte lineage cells. When demyelinating injury occurs the expression of Sox2 in OPCs is transiently increased but down-regulated upon differentiation. Using genetic fate mapping, gain of function and loss of function experiments, we demonstrated that Sox2 sustains the recruitment of OPCs and its up-regulation is essential for CNS remyelination. These findings suggest that Sox2 and its downstream regulatory factors may play an important role in the activation of OPCs following CNS demyelination and subsequent remyelination.

S5.C

IDENTIFICATION OF FACTORS MODULATING OLIGODENDROCYTE PRECURSOR CELLS DIFFERENTIATION AND MATURATION

Małgorzata Zawadzka

Department of Molecular and Cellular Neurobiology, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland

Remyelination is a regenerative process, driven by oligodendrocyte precursors (OPC), resulting in new myelin sheaths production and restoring to axons that have been demyelinated as a result of oligodendrocyte death. An understanding of the mechanisms that control the response of OPCs, the CNS multipotent cells, to the pathological conditions and how these cells differentiate and finally support the functional recovery is particularly important for the development of therapeutic strategies targeting both enhancement of endogenous remyelination and tissue regeneration after transplantation of exogenous cells.

We have shown that there are two endogenous regenerative processes taking place simultaneously in response to the CNS white matter injury – remyelination and blood vessels reconstruction. Moreover, oligodendrocyte precursor cells differentiate in response to white matter injury giving rise to oligodendrocytes as well as to Schwann cells and the alternative fate is closely depended on the settling of the precursors within the vascular niche. We have found that the vascular niche substantially differs functionally from tissue that does not contain blood vessels by distinct pattern of gene expression coding for the proteins that belong to the key signaling pathways such as BMP, WNT, and Hedgehog. They are differentially involved in alternative differentiation of oligodendrocyte precursors, which suggests that OPCs fate decision depends on the specific microenvironmental clues within injured tissue.

Our results show that interactions between cells that respond to damage in nervous system and reside in different areas of tissue, regulate the injury-induced plasticity of OPCs

S5.D

MYELIN, OLIGODENDROCYTES AND AXONAL PATHOLOGY IN *St8sia2*-/- MICE, A MODEL FOR SCHIZOPHRENIA RESEARCH

Marta B. Wiśniewska[1,2], Lukasz Szewczyk[1,2], Herbert Hildebrandt[3], Jacek Kuźnicki[1]

[1]International Institute of Molecular and Cell Biology, Warsaw, Poland; [2]Centre of New Technologies, University of Warsaw, Warsaw, Poland; [3]Hannover Medical School, Hannower, Germany

Growing body of evidence implicates myelin and axon abnormalities in schizophrenia. Using a proteomic approach, we detected a decrease in myelin proteins in the hippocampus of *St8sia2*-/- mice that display behavioral and neuroanatomical features of schizophrenia. ST8SIA2 adds polysialic acid to neural cell adhesion molecule (NCAM), and this posttranslational modification is vital for development and plasticity in the brain. Polymorphisms in the *ST8SIA2* gene and NCAM hyposialylation have been associated with schizophrenia. To gain an insight into the relationship between polysialylation state of NCAM and myelin we performed phenotypic analysis of *St8sia2*-/- mice, focusing on: myelin formation and maintenance, oligodendrocyte differentiation, and ultrastructure of axons. We applied several imaging techniques ranging from histological staining to electron microscopy, several immunodetection methods, and *in vitro* differentiation of oligodendrocyte precursors. Myelin formation was not delayed in the knockout mice, yet the levels of major myelin proteins were decreased from the early beginning (in 15-day-old mice) and it was accompanied by a lower number of oligodendrocytes. Moreover, *in vitro* differentiation of oligodendrocyte precursors was less efficient in the case of *St8sia2*-/- cells. Ultrastructure analysis of the nerve fibers showed thinning of the myelin sheath in 3-month-old mice. This phenotype was more severe in 8-month-old mice, with clear signs of axon degeneration and even lesions. We suppose that these late axonal pathologies are secondary to oligodendrocyte and myelin dysfunction. We conclude that the ST8SIA2-mediated polysialylation of NCAM plays a role in maintaining myelin and axonal integrity. The myelin phenotype in *St8sia2*-/- mice resembles white matter abnormalities in schizophrenia. ST8SIA2-deficient mice are a suitable model for better understanding schizophrenia-associated myelin and axonal pathology and to identify novel therapeutic targets.

S5.E

THE NEUROPROTECTIVE EFFECT EXERTED BY OLIGODENDROGLIAL PROGENITORS ON ISCHEMICALLY IMPAIRED HIPPOCAMPAL SLICES

Joanna Sypecka, Anna Sarnowska

NeuroRepair Department, Mossakowski Medical Research Centre, Polish Academy of Sciences, Warsaw, Poland

The role of oligodendrocytes is supposed to go beyond the process of myelinating nerve fibers in the central nervous system. Pre-clinical studies based on oligodendrocyte progenitor cell (OPCs) therapies revealed a significant behavioral improvement in spite of failure in the remyelination process in animal models of demyelinating diseases. To address the issue of potential trophic support conferred by OPC to diseased nervous tissue, co-culture experiments with neonatal rat OPCs and organotypic hippocampal slices were designed. For this purpose, rat organotypic hippocampal slices were exposed to a brief oxygen and glucose deprivation (OGD) which allowed mimicking an ischemic injury *in vitro*. Soon after the OGD procedure, the hippocampal slices were co-cultured together with differentiating oligodendroglial progenitors. Cell survival and their proliferation rates in the injured slices were estimated by immunohistochemical methods. After acknowledging the beneficial influence of the neighboring oligodendrocytes on the injured tissue, a set of molecular and biochemical experiments were carried out with the aim of determining the mechanism(s) of the observed profound neuroprotective effect. The results of both molecular studies and functional assays revealed that the oligodendrocyte-derived BDNF is the major factor promoting neuronal survival in OGD-subjected hippocampal slices, while SCF and IL-10 strongly promote the cell proliferation. Among the newly-born cells, neuroblasts and microglia could most frequently be found pointing to both the neuroprotective and neuroimmunomodulatory role of differentiating oligodendrocytes. In conclusion, the presented study revealed that oligodendrocytes are able to secret BDNF and other active factors, thus providing trophic support for other neural cells. The presented study confirms the hypothesis concerning the complex functions of oligodendrocytes in the nervous tissue.
Supported by MMRC statutory funds.

SYMPOSIUM S6. A MULTIVIEW OF CURRENT SPANISH NEUROSCIENCE

Chairperson: *Daniel Wójcik (Warsaw, Poland)*

S6.A
TRANSCRIPTOME AND PROTEOME REMODELING THROUGH ALTERNATIVE SPLICING IN THE VERTEBRATE BRAIN

Manuel Irimia
Centre for Genomic Regulation (CRG), Barcelona, Spain

Mirroring their unparalleled morphological and cellular richness, vertebrate brains are built by uniquely complex transcriptomes and proteomes. Multiple post-transcriptional mechanisms contribute to expand this molecular complexity; in particular, alternative splicing (AS) – the differential processing of introns and exons to generate multiple mRNA isoforms from a single gene – is believed to be the largest contributor to proteomic diversification in animals. With the advent of next generation sequencing, we can now comprehensively identify the isoforms that are unique to neural cells and start elucidating their functions. For example, neural-specific alternative exons in proteins involved in neurogenesis and synaptic functions have been shown to often impact protein-protein interactions, contributing to rewire interaction networks in a cell type-specific manner. Moreover, we have recently uncovered in mammalian brains a large number of microexons (exons as short as 3–27 nucleotides) that display the most striking evolutionary conservation and switch-like regulation during neuronal regulation. These microexons modulate the function of interaction domains of proteins involved in neurogenesis, and we are only starting to glimpse their multiple biological roles. Remarkably, they are frequently misregulated in the brains of individuals with autism spectrum disorder, providing a new perspective to investigating the molecular bases of this complex disease.

S6.B
CEREBRAL CORTEX ACTIVITY AND NETWORK COMPUTATIONS

Mavi Sanchez Vives
Institute of Biomedical Research August Pi y Sunyer, Barcelona, Spain; Catalan Institute for Research and Advanced Studies, Barcelona, Spain

Understanding complex systems like brain networks is a challenge. Cortical networks can perform computations of remarkable complexity, accounting for a large variety of behaviours and cognitive states. At the same time, the same networks can engage in stereotypical patterns of spatio-temporal activation, such as the ones that can be observed during sleep, anaesthesia and in cortical slice. Collective phenomena emerging from activity reverberation in cortical circuits at different spatio-temporal scales results in a rich variety of dynamical states. Slow (around or below 1 Hz) and fast (15–100 Hz) rhythms are spontaneously generated by the cortical network and propagate or synchonize populations across the cortex. This is the case even in isolated pieces of the cortical network, or *in vitro* maintained cortical slices, where both slow and fast oscillations are also spontaneously generated. We understand that these emergent patterns provide information on the structure, dynamics and function of the underlying cortical network and their alterations in neurological diseases reveal the circuits dysfunction. In particular in this talk we will concentrate in cortical columnar activation and wave propagation.

S6.C
THE BILINGUAL BRAIN: PLASTICITY AND PROCESSING FROM CRADLE TO GRAVE

Manuel Carreiras
Basque Centre on Cognition, Brain and Language, Donostia-San Sebastián, Spain; Basque Foundation for Science, Bilbao, Spain; University of the Basque Country, Bilbao, Spain

Most people either learn more than one language from birth or invest quite a lot of time and effort learning a second language. Bilingualism and second language learning is an interesting case for investigating cognitive and brain plasticity. In this talk I will describe behavioral and neuroimaging evidence on the cognitive and brain mechanisms adults and infants (monlinguals, bilinguals and second language learners) use for processing language. In particular I will address whether proficient second language learners use similar or different brain mechanisms during processing and what are the neural consequences (structural and functional) of dealing with two languages.

S6.D

DOWN SYNDROME: UNRAVELLING THE GENETIC PLAYERS OF A DISORDER OF NEURAL PLASTICITY

Mara Dierssen

Center for Genomic Regulation (CRG) and CIBERER, Barcelona, Spain

Intellectual disabilities (ID) are chronic diseases that place a disproportionate burden on medical and social care, and educational systems, and rates of ID are on the increase. People with ID are vulnerable and many need lifelong assistance in most aspects of daily life. Cognitive deficits are a prominent feature and severely compromise their quality of life. The reduction in health inequalities and the improvement of health for people with intellectual disability has become a priority worldwide. In spite of the broad spectrum of genetic and environmental aetiologies, the disruption of neural plasticity can be viewed as a sign of cognitive impairment across ID. Our observations that brain plasticity is effective in the recovery from some of the cognitive deficits associated with ID open a window of opportunity for a novel therapeutic concept: therapies that improves and stabilizes physiological (experience-dependent) plasticity in genetic ID syndromes. Thus, targeting core molecular substrates of neuroplasticity with specific drugs, in combination with non-pharmacological interventions. We have identified an extremely powerful target for drug development, DYRK1A (dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 1A), a serine/threonine kinase that plays key roles in cell proliferation, survival and neural plasticity.

This work was supported by grants from Fondation Jérôme Lejeune (Paris, France), Instituto de Salud Carlos III FEDER, (PI11/00744), MINECO (SAF2013-49129-C2-1-R), EU (Era Net Neuron PCIN-2013-060), DIUE de la Generalitat de Catalunya (SGR 2014/464 SGR 2014/1125).

SYMPOSIUM S7. NEUROBIOLOGY OF AGING BRAIN

Chairperson: *Monika Liguz-Lęcznar (Warsaw, Poland)*

S7.A

THE INFLUENCE OF AGING ON CONTEXT REASONING

Grzegorz Sędek

University of Social Sciences and Humanities, Warsaw, Poland

In my talk I will discuss results and implications of published and unpublished studies on influence of aging on higher order cognitive processes like context reasoning. The first studies demonstrate that although aging and depression impaired transitive reasoning the mechanisms of these limitations are quite different. In the case of aging these deficits in reasoning might be derived from limitations in more basic cognitive processes like mental speed or working memory capacity, whereas in the case of depression there seem to be genuine constraints in integration of premises into more coherent mental structures like mental array. The next studies on transitive reasoning across adult life span (compared and young adults, middle-aged adults and older adults) show intriguing interactions between age and modal forms of reasoning. There were visual formal, visual narrative, and auditory narrative forms of presenting premises for transitive reasoning. The additional manipulation concerned the motivation to avoid cognitive closure, motivation to promote cognitive closure, and control group. Older adults showed the pattern of increasing accuracy for solving transitive problems from visual formal, by narrative text, to auditory narrative form while the young adults showed the exactly opposite pattern. Hence, there were striking differences between age groups in visual formal reasoning and the age group differences were diminished for the auditory narrative reasoning especially under the manipulation to avoid cognitive closure. In conclusion I will present more general implications (including applications for effective cognitive trainings among older adults) for the findings that older adults are especially efficient in extracting logical inferences from narrative materials.

S7.B

A WINDOW ON THE AGEING BRAIN: *IN VIVO* IMAGING OF STRUCTURAL CHANGES AT SYNAPTIC RESOLUTION

Vincenzo de Paola

MRC Clinical Science Centre, Imperial College London, London, UK

Synaptic dysfunction is a prevalent and early hallmark of a vast array of neurocognitive and age-related diseases. Ageing is a major risk factor for cerebrovascular and neurodegenerative diseases and is characterised by performance decline in specific sensory and cognitive tasks. Age-dependent learning deficits are thought to arise from a progressive loss of synapses and detriments to synaptic plasticity, but *in vivo* evidence for this has been lacking. We have imaged different types of excitatory axons and their boutons in the

somatosensory cortex of aged (>22 months) mice with impaired long-term recognition memory. Interestingly, the aged cortex shows circuit-specific increased rates of axonal bouton addition, elimination and destabilization. Compared to the young adult brain, large (i.e. strong) boutons in the aged cortex show 10-fold higher rates of destabilization and 20-fold higher turnover. Size fluctuations of persistent boutons, believed to encode long-term memories are more pronounced, while bouton size and density are not affected. Our study suggests that increased synaptic plasticity in specific cortical circuits represents a novel mechanism for age-related cognitive decline (Grillo et al. 2013, Proc Natl Acad Sci U S A 110: 1514).

S7.C

AGE-RELATED PLASTICITY IMPAIRMENTS-PRESYNAPTIC MECHANISMS

Monika Liguz-Lęcznar

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

The lecture will focus on age-related changes in expression of presynaptic proteins necessary for the neurotransmitter release as well as alterations of inhibitory/excitatory neurotransmitter balance and their influence upon the functional cortical plasticity. Age-related cognitive impairments may be linked to molecular changes in the synapse resulting in plasticity decline. In somatosensory cortex of aging mice we observe a decline of functional cortical plasticity induced by 3-days lasting classical conditioning, which could be linked to the altered excitatory-inhibitory balance that controls cortical excitability. This decline can be overcome by extended training. Analysis of mRNA and protein expression of several presynaptic markers controlling neurotransmission revealed decrease of synaptophysin, vesicular glutamate transporter-vglut2, glutamic acid decarboxylase-GAD67 and vesicular GABA transporter-VGAT protein, as well as mRNA for VGAT in aged mice barrel cortex. Moreover, due to significant decrease of glutamate concentration, the glutamate/GABA ratio, measured with HPLC, was decreased in the somatosensory cortex of aged animals. Importantly, the impairment of conditioning-induced plasticity in aged animals coincided with the lack of GABA and GAD 67 increase after training. It may reflect the inefficiency of inhibitory mechanisms in aging brain to control increased excitation after training and to shape proper signal to noise ratio essential for the appropriate stimuli processing. We propose that the disequilibrium of excitation and inhibition in favour of inhibition may be the decisive factor for weakening the plastic potential of aging neurons and combined with insufficient GABA response to conditioning, contribute to the impairment of learning-dependent cortical plasticity.

Supported by National Science Centre Grant: 2013/09/B/NZ3/00540.

S7.D

PLASTICITY OF THE AGING BRAIN

Otto W. Witte

Hans Berger Department of Neurology, University Hospital Jena, Jena, Germany

Aging is associated with a reduction of brain plasticity and learning ability. Using a translational approach with investigations on animals, and on human subjects we investigate whether and how plasticity in the aged brain can be preserved. For this purpose, we use different approaches (telomere length, transcriptomic profile, MRI brainage score, neuropsychological parameters) to determine the biological age of subjects. Aging experiments with rodents show that prenatal stress induces an epigenetic alteration of the stress axis which enhances the inflammation level in the brain. Likewise, strong intermittent inflammatory disorders accelerate brain aging. Antiinflammtory drugs may partially reverse the age-associated disturbances of brain plasticity. Similarly, enriched environment partially re-juvenates the transcriptomic profile of the brain. Based on these observations we currently investigate the role of brain microglia for brain ageing. In the presentation, the methodological approach as well as results from several studies which address interventions to reduce the age-associated decline in brain plasticity will be presented.

SYMPOSIUM S8. CENTRAL CONTROL OF REPRODUCTION

Chairperson: *Joanna H. Śliwowska (Poznań, Poland)*

S8.A

KNDY NEURONS, PEPTIDES, AND THE CONTROL OF MAMMALIAN REPRODUCTION

Michael N. Lehman

Department of Neurobiology and Anatomical Sciences, University of Mississippi Medical Center, Jackson, MS, USA

In mammals, reproduction is governed by intricate neural and hormonal communication between the brain, pituitary gland and gonads. At the top of this hierarchy are gonadotropin-releasing hormone (GnRH) neurons that are responsible for the pulsatile secretion of luteinizing hormone (LH) from the anterior pituitary gland. Changes in the frequency of GnRH and LH pulses have a profound effect on the reproductive system, but identification of the "GnRH pulse generator" in the brain has remained a major unanswered question. Our research has identified a subset of neurons in the arcuate nucleus of the hypothalamus that appear to play a key role in the generation of GnRH pulses and their control by endogenous hormones and other signals. We have called these cells, KNDy neurons based on their unique co-expression

of three neuropeptides (kisspeptin, neurokinin B, and dynorphin) each of which is functionally important for reproduction. In this talk, we will review current evidence for KNDy neurons as a core component of the "GnRH pulse generator", as well as findings suggesting they play a major role in the control of reproduction by gonadal steroids and other stimuli. Finally, we will discuss recent evidence that KNDy neurons are altered in reproductive disorders characterized by defects in the feedback control of GnRH secretion.

S8.B

PARTICIPATION OF SALSOLINOL IN THE REGULATION OF THE SECRETORY ACTIVITY OF THE PITUITARY GLAND IN SHEEP DURING LACTATION

Tomasz Misztal, Małgorzata Hasiec, Konrad Górski, Elżbieta Marciniak, Katarzyna Romanowicz The Kielanowski Institute of Animal Physiology and Nutrition, Polish Academy of Sciences, Jabłonna, Poland

Salsolinol is an endogenous derivative of dopamine (DA), previously known for its involvement in the progression of a disease characterized by dysfunctional dopaminergic neurons. In the last decade, more attention has been focused on the possible physiological role of salsolinol as a neuromodulator within the central nervous system. This DA-originated compound was found to be present in the pituitary gland, as well as in median eminence (ME) extracts and hypothalamic perfusates of rats and ruminants. Salsolinol is currently believed to be one of the hypothalamic factors stimulating both the synthesis and release of prolactin, especially during lactation. The stimulus that releases salsolinol during lactation is sucking. The high concentration of salsolinol in the hypothalamus and ME of lactating sheep suggests a role for this compound in mechanisms regulating other processes in which involvement of DA has been found. Studies using exogenous salsolinol or its antagonistic analogue (1-MeDIQ) conducted on lactating sheep showed that salsolinol also participated in the regulation of oxytocin secretion. It upregulated both oxytocin gene expression in the supraoptic and paraventricular nuclei and the content of this hormone in the posterior pituitary and stimulated its release into the circulation. Moreover, salsolinol infused into the third ventricle (IIIv) of the brain reduced the isolation stress-induced elevation of plasma ACTH and cortisol concentrations. Therefore, salsolinol may be considered to be one of the factors responsible for the adaptive inhibition of the HPA axis during lactation. Recent reports indicate that salsolinol may modulate (induce or inhibit) the activity of the GnRH/LH axis, depending on the site of administration (IIIv or ME). Further investigations are necessary to understand the molecular action of salsolinol and to elucidate the mechanism controlling the corticotropic and gonadotropic axes in sheep during lactation.

S8.C

NEURONAL FEEDBACK WITHIN THE NETWORK REGULATING GNRH SECRETION

Imre Kalló

Laboratory of Endocrine Neurobiology, Hungarian Academy of Sciences, Budapest, Hungary

Besides forming the final output pathway regulating the reproductive functions of the anterior pituitary gland, GnRH neurons provide neuronal input to sites within the hypothalamus. The aim of these studies was to reveal, whether GnRH neurons interact with neuronal systems that mediate estrogenic, circadian, metabolic, lactational and/or stress-related signals to GnRH neurons. The neuronal circuitry regulating GnRH secretion was investigated by confocal and electron microscopic immunohistochemistry in rodent and human brains. In addition, interaction of GnRH neurons with other neurons within the circuitry was studied by *in vitro* electrophysiological experiments. Based on the observation that cannabinoids suppress fertility *via* reducing hypothalamic GnRH output, the presence of type 1 cannabinoid receptors in the afferents of GnRH neurons and cannabinoid release from GnRH neurons were tested in GnRH-GFP mice. The obtained morphological and electrophysiological evidence supports that retrograde endocannabinoid signaling reduces GABAergic afferent drive onto GnRH neurons *via* the activation of presynaptic CB1 receptors. In addition to local interactions, GnRH neurons send axonal projections to neurons of the preoptic area and the arcuate nucleus, which have particular significance due to their critical role played in the control of GnRH and prolactin secretion. It was tested whether GnRH-immunoreactive (IR) projections establish morphological and functional connections with kisspeptin (KP) and dopaminergic neurones at these sites. Synaptic connections were revealed between GnRH axon terminals and KP-IR and/or tyrosine hydroxylase (TH)–IR neurons; in addition, membrane effects of GnRH were demonstrated on preoptic neurons identified post hoc the recordings. The functional significance of the discovered cannabinoid signaling, and the GnRH input to KP and dopaminergic cell populations, and the translational aspects of the rodent studies will be discussed.

S8.D

DIET, ALCOHOL AND REPRODUCTION – LESSONS FROM ANIMAL MODELS

Joanna H. Śliwowska

Laboratory of Neurobiology, Institute of Zoology, Poznań University of Life Sciences, Poznań, Poland

Metabolic stressors (e.g. under- or overnurition, alcohol) acting in the mother's womb as well as in adulthood influence reproductive functions governed by the hypothalamic-pituitary-gonadal (HPG)

axis. Studies link the intrauterine environment with later obesity, diabetes and polycystic ovarian syndrome. Data presenting reproductive dysfunctions associated with diet and alcohol will be discussed. Animal models of inadequate nutrition and alcohol exposure will be presented.

Females prenatally exposed to alcohol (PAE) have delayed puberty, alterations in hormonal profile (estradiol, progesterone, prolactin, luteinizing hormone), irregular estrus cycles. Changes in hormonal profile (e.g. decrease in secretion of testosterone and luteinizing hormone) were also reported in PAE males. Inadequate diet may also leads to reproductive abnormalities (e.g. altered levels of sex steroids, hypogonadotropic hypogonadism, premature child birth or infertility) observed in obese and diabetic patients. In a variety of obese and diabetics animal models imbalance in hormonal profile was also confirmed. Kisspeptins, coded by KiSS 1 gene, acting *via* GPR54 (KISS1 R) receptor, are important in regulation of the HPG axis. Kisspeptin acts together with neurokinin B (NKB) and dynorphin, which are co-express in KNDy neurons in the arcuate nucleus of the hypothalamus (ARC). Importantly, in the ARC integration of metabolic and reproductive functions occurs. Abnormalities observed in animal models of PAE, obesity and diabetes may be related to kisspeptin system. Moreover, in animal models of high fat diet-induced obesity and steptozotocin-induced diabetes dysregulations in hormonal profile and alterations in KNDy neurons were reported. Potential therapeutic intervention to improve reproductive functions in obese and diabetic patients will be presented.

Supported by NCN grant 2011/01/B/NZ4/04992.

SYMPOSIUM S9. CALCIUM AS A KEY REGULATOR OF NEURAL FUNCTION

Chairperson: *Jarosław J. Barski (Katowice, Poland)*

S9.A

REDUCED EXPRESSION LEVELS OF PARVALBUMIN MAY LEAD TO AN ASD-LIKE PHENOTYPE IN MICE

Beat Schwaller

Anatomy, Department of Medicine, University of Fribourg, Fribourg, Switzerland

Parvalbumin (PV) is a calcium-binding protein expressed in a subpopulation of mostly GABAergic neurons in several brain regions, e.g. cortex and striatum. A reduction in the number of PV-immunopositive (PV^+) neurons or a decrease in the intensity of PV immunoreactivity was reported in several mouse models of autism spectrum disorders (ASD) including mice with mutations in *Shank* genes. We have previously shown that the absence or reduction of PV in PV-/- and PV+/- mice, respectively, leads to a robust ASD-like phenotype, evidenced by all core symptoms including impairment in social interaction and communication, as well as repetitive and stereotyped patterns of behavior. We investigated whether the "reduction in PV^+ neurons" in knockout mice for Shank1, Shank3 and PV+/- mice was the result of a decrease in PV expression levels and/or partial loss of the PVergic neuron subpopulation. We applied stereological methods to estimate the number of PVergic neurons in ASD-associated brain regions of PV-/-, PV+/-, Shank1-/- and Shank3B-/- mice. *Vicia Villosa* Agglutinin (VVA) was used to identify the specific extracellular matrix components enwrapping PVergic neurons. Quantitative levels of PV protein and *Pvalb* mRNA were analyzed by Western blot analyses and qRT-PCR, respectively. The analyses of cell numbers in different brain regions revealed that the observed "reduction of PV^+ neurons" resulted from a reduction in *Pvalb* mRNA and PV protein. The unaltered numbers of VVA^+ neurons are not compatible with a PV cell decrease/loss in these ASD models. We hypothesize that the down-regulation of PV in PVergic networks leads to an impairment of the excitation/inhibition balance and might signify a convergent downstream endpoint for some forms of ASD. Restoring of normal PV protein levels and/or of PV^+ neuron function might serve as a novel therapeutic strategy to avoid and/or possibly reverse the ASD phenotype.

S8.B

ROLE OF STIM1 IN NEURONAL CA^{2+} SIGNALING AND SYNAPTIC TRANSMISSION

Jana Hartmann[1,2], Arjan Dijke[1], Arthur Konnerth[1,2]

[1]Technical University Munich, Institute for Neuroscience, Munich, Germany; [2]Center for Integrated Protein Science Munich, Munich, Germany

The metabotropic glutamate receptor type 1 (mGluR1) is highly expressed in cerebellar Purkinje cells (PCs). At parallel fiber-PC synapses, activation of mGluR1 evokes a complex synaptic response consisting of IP_3 receptor-dependent Ca^{2+} release from endoplasmic reticulum (ER) Ca^{2+} stores and a slow depolarizing potential involving the transient receptor potential (TRPC) channel subunit TRPC3. We have shown recently that the ER Ca^{2+} sensor stromal interaction molecule 1 (STIM1) is important for cerebellar function by analyzing a PC-specific *Stim1* knockout ($STIM1^{pko}$) mouse line. Using whole-cell recordings in combination with confocal Ca^{2+} imaging in acute cerebellar slices we demonstrated that STIM1 is a link of mGluR1 to its downstream effectors and regulates Ca^{2+} homeostasis in PCs (Hartmann et al. 2014, Neuron). In $STIM1^{pko}$ mice both TRPC3-mediated synaptic potentials and agonist-evoked inward currents were absent in PCs at resting membrane potential. Moreover, ER Ca^{2+} release was found to be strongly attenuated. However, activation of voltage-gated Ca^{2+} channels (VGCCs) transiently filled ER Ca^{2+} stores and rescued, for the filling period, TRPC3-mediated currents in the absence of STIM1. Next, we tested how TRPC3 gating depends on the filling state of ER Ca^{2+} stores. In control mice, we found

that TRPC3-mediated currents are not altered when ER Ca^{2+} stores are emptied by blocking SERCA pumps using cyclopiazonic acid (CPA). Moreover, opening of VGCCs by short depolarizing pulses in $STIM1^{pko}$ mice allows the transient activation of TRPC3 when store filling was prevented by the presence of CPA in the bath. Inclusion of 25 mM BAPTA into the pipette solution, however, in control mice strongly attenuates TRPC3-mediated currents and prevents their depolarization-evoked rescue in $STIM1^{pko}$ mice. Together, these results establish that STIM1 couples mGluR1 and TRPC3 in cerebellar PCs through the regulation of cytosolic rather than ER Ca^{2+} content.

S9.C

ROLE OF STORE OPERATED CALCIUM ENTRY IN NEURONS

Jacek Kuźnicki

International Institute of Molecular and Cell Biology in Warsaw, Warsaw, Poland

The number of people with neurodegenerative and psychiatric diseases is expanding globally. Unfortunately, there are no treatments, which can delay the progress of Alzheimer's disease (AD), no new drugs with fewer side-effects on Parkinson's disease (PD), Huntington's disease (HD) and depression (MDD), no drugs for patients resistant to the existing MDD therapies. Moreover, in most diseases there are no reliable biomarkers for presymptomatic diagnosis and monitoring of effects of therapeutic treatments. Therefore, there is a growing need for better understanding of the basis of neurodegenerative and psychiatric diseases, identification of drug targets and new biomarkers, and development of new treatments and drugs. Altered Ca^{2+} homeostasis in neurons is proposed to be one of the early events responsible for AD. Disturbances in Ca^{2+} signaling are found in SAD patients before any obvious extracellular Aβ pathology, and Ca^{2+} dysfunction augments Aβ formation and Tau hyperphosphorylation. Dysregulation of Ca^{2+} homeostasis and signaling has been also observed in PD, HD and some psychiatric diseases. GWAS analysis identified specific SNPs in two voltage-gated calcium channels associated with a range of psychiatric disorders. We detected the enhanced magnitude of Ca^{2+} influx during Store Operated Calcium Entry (SOCE) in human lymphocytes from SAD patients, and decreased level of STIM2 from FAD patients in parallel to an attenuation of SOCE. The decreased level of STIM2 in AD models and brains of AD patients was confirmed by other authors. We also showed that the cytoplasmic resting Ca^{2+} level in cultured neurons can be modulated by overexpression of SOCE proteins (STIM1, STIM2 or Orai1). Based on our and literature data I will describe the role of SOCE in neurons and its perturbation in neurological diseases. To conclude, the SOCE proteins can be considered as new drug targets for some of these diseases and that dysregulation of SOCE might be used for diagnostic purposes.

S9.D

THE ROLE OF CALBINDIN D-28K IN CEREBELLUM

Jaroslaw Jerzy Barski

Center for Experimental Medicine, Department of Physiology, Medical University of Silesia, Katowice, Poland

Calbindin-D28k (calbindin) is a calcium-binding protein expressed in many neuronal populations of the central nervous system. It is generally thought of as calcium buffer, but its cell type-specific physiological roles are unknown. We show that cerebellar Purkinje cell-specific calbindin null mutant mice exhibit a distinct alteration in motor coordination which is primarily due to impaired processing of sensory information. This occurs independent of alterations of synaptic long-term depression but concomitant with selective alteration of synaptically-evoked Ca^{2+} transients. Whereas AMPA-receptor-mediated transients in Purkinje cell spines and dendrites have larger amplitudes and decay faster, the delayed mGluR-mediated synaptic Ca^{2+} signal is unaffected. We conclude that calbindin within Purkinje cells, possibly through direct modulation of dendritic Ca^{2+}, is a critical molecular constituent of sensorimotor integration.

SYMPOSIUM S10. COENZYME A AND ITS DERIVATIVES IN BRAIN METABOLISM AND NEURODEGENERATION

Chairpersons: *Andrzej Szutowicz (Gdańsk, Poland), Jan K. Blusztajn (Boston, MA, USA)*

S10.A

ALTERATION OF COENZYME A BIOSYNTHETIC PATHWAY IN NEURODEGENERATION WITH BRAIN IRON ACCUMULATION SYNDROMES

Valeria Tiranti

Unit of Molecular Neurogenetics, Foundation IRCCS Neurological Institute "Carlo Besta", Milan, Italy

Neurodegeneration with brain iron accumulation (NBIA) comprehends a heterogeneous group of neurodegenerative diseases having as a common denominator iron overload in specific brain areas, mainly basal ganglia and globus pallidus. In the last decade a bunch of disease genes have been identified, but NBIA pathomechanisms are still not completely clear. Pantothenate kinase-associated neurodegeneration (PKAN), an autosomal recessive disorder with progressive impairment of movement, vision and cognition, is the most common form of NBIA. It is caused by mutations in the pantothenate kinase 2 gene (PANK2), coding for a mitochondrial enzyme that phosphorylates vitamin B_5 in the first reaction of the coenzyme A (CoA) biosynthetic pathway. A distinct form of NBIA, denominated CoPAN, is caused by mutations in Coenzyme A synthase gene (COASY) coding for a bi-functional mitochondrial enzyme, which catalyses final steps of the CoA biosynthesis. These two inborn er-

ror of CoA metabolism further supports the concept that dysfunctions in CoA synthesis may play a crucial role in the pathogenesis of NBIA.

S10.B

REGULATION OF COENZYME A BIOSYNTHESIS AND HOMEOSTASIS IN HEALTH AND DISEASE

Ivan Gout

Institute of Structural and Molecular Biology, University College London, London, UK

Coenzyme A (CoA) is an essential cofactor in all living organisms. CoA and its thioesters (Acetyl-CoA, Malonyl-CoA, HMG-CoA etc) are implicated in a diverse range of metabolic pathways, such as oxidation of carbohydrates, lipids and proteins, the citric acid cycle, lipid and cholesterol biosynthesis, as well as for covalent modification of proteins and regulation of gene expression. The level of CoA/CoA derivatives is not constant in cells and tissues and changes in response to cellular metabolites, nutrients, diet (e.g. high fat diet), and hormones (insulin, glucagon, glucocorticoids). These changes are important for the metabolic adaptation of cells to different physiological and pathological conditions. Abnormal CoA/CoA derivatives levels have been observed in diverse human pathologies including cancer, metabolic disorders and neurodegeneration. CoA is synthesised in prokaryotic and eukaryotic cells from pantothenate (vitamin B_5), ATP and cysteine by a five-step biosynthetic pathway. There are two rate-limiting steps in CoA biosynthesis, involving pantothenate kinase (PANK) and CoA synthase (CoAsy). Our efforts have been mainly focused on elucidating the regulation of CoAsy by signalling pathways and revealing the mechanisms by which the CoA biosynthetic complex is formed in response to extracellular stimuli and stresses. Recent advances on these studies will be presented.

S10.C

PRECURSORS OF COENZYME A – PANTHENOL AND PANTETHINE – IN THE MECHANISMS OF NEUROPROTECTION

Andrey G. Moiseenok

Institute of Biochemistry of Biological Active Compounds, NAS of Belarus, Grodno, Belarus

The results of clinical studies on coenzyme A (CoA) biosynthetic precursors carried out over 30 years revealed and confirmed neuroprotective properties of D-panthenol (PL) and D-pantethine (PT). The efficiency of all these drug formulations was demonstrated both in the alcohol withdrawal syndrome and delirium tremens, during involution psychosis, for prevention and treatment of the brain post-ischemic syndrome and surgery of arterial aneurisms. Parallel preclinical studies revealed high efficiency of the CoA precursors in focal brain ischemia, aluminum neurotoxicosis, bacterial lipopolysaccharide and cholinotoxin exposures and modulation of the effects of nootropic pharmacologic agents eliminating neurologic deficiency. The participation of CoA-dependent mechanisms on administration of amyloid P-peptide and m-anticholinergic drugs was shown. The ability of CoA precursors to prevent development of oxidative stress (OS), endogenous intoxication and immobilization stress was determined. A protective effect of PT was found in vagotomy. Comparative pharmacokinetic studies on bioavailability of PT and PL in the albino rat CNS showed the presence of differences in the processes of "uptake", transport, deposition and biotransformation in individual neurostructures with preferential accumulation of 4'- phosphopantothenic acid (PPA). The [^{3}H]JJ-PT perfusion of hippocampus sections was also accompanied by accumulation of PPA as a result of possible hydrolysis by phospho-PT pantethinase (Vanin-1). The role of PPA is unclear, but it can be related to the balance of the Fe^{2+}/Fe^{3+} and cysteine/cystine redox pairs. It has been established that the neuroprotective effect of the CoA precursors is realized through stabilization of neuromembranes, as well as the glutathione and acetylcholine systems in the CNS and is possibly mediated through the cellular redox potential and redox signaling by OS products and the processes of post-translation protein modification (S-glutathionylation).

S10.D

DISTURBANCES IN ACETYL-CoA METABOLISM A KEY POINT IN MECHANISMS OF CHOLINERGIC DEGENERATION

Andrzej Szutowicz, Hanna Bielarczyk, Anna Ronowska, Sylwia Gul-Hinc, Joanna Klimaszewska-Łata, Aleksandra Dyś, Marlena Zyśk

Chair of Clinical Biochemistry, Department of Laboratory Medicine, Medical University of Gdańsk, Gdańsk, Poland

Acetyl-CoA synthesized from glucose-derived pyruvate by pyruvate dehydrogenase complex (PDHC) is a main substrate, for mitochondrial energy production and cytoplasmic synthetic pathways in all types of brain cells. Activities of mitochondrial PDHC, and several enzymes of acetyl-CoA metabolism, and ZnT1 transporter level in cholinergic septal SN56 cells were from 2 to 8 times higher than those in microglial (N9) or astroglial (C6) cells. Differentiated cholinergic SN56 cells were highly susceptible to various neurotoxic signals: Zn, amyloid-β or NO excess. They decreased their viability and acetyl-CoA/ATP contents, due to inhibition or inactivation of PDHC and other enzymes of energy metabolism. Such conditions suppressed synthesis of acetyl-CoA, N-acetyl-L-aspartate, acetylcholine as well as its quantal release. Significant correlations existed between mitochondrial acetyl-CoA levels and SN56 viability in those conditions. On the other hand, nondifferentiated SN56, microglial (N9) or astroglial (C6) cells were more resistant to

same detrimental insults. SN56 cells were resistant to high concentrations of lipopolysaccharide (LPS). On the contrary, in N9 cells low concentrations of LPS caused several-fold activation of NO and IL-6 and TNF-α synthesis/release, along with inhibition of PDHC, KDHC and aconitase activities yielding depression of acetyl-CoA, ATP contents but relatively small losses in their viability. Also, Zn and NO caused relatively weak inhibition enzymes of energy metabolism in N9 and C6 cells. Lipoic acid and L-carnitine rescued cells by preventing inhibition some of those enzymes by neurotoxins and alleviating acetyl-CoA and ATP deficits. Presented data indicate that particular types of brain cells constitute compartments of different levels and rates of acetyl-CoA metabolism, variably influencing their functional properties and viability both under neurodegenerative and cytoprotective conditions.
Supported by MN59, MN58, MN108, ST57 GUMed funds.

SYMPOSIUM S11. MOTOR CONTROL

Chairperson: *Jan Celichowski (Poznań, Poland)*

S11.A
SPINAL CORD, RETICULAR FORMATION AND CEREBELLUM – THERE AND BACK AGAIN

Ingela Hammar
Department of Physiology, Institute of Neuroscience and Physiology, Sahlgrenska Academy, Göteborg University, Göteborg, Sweden

Information on the activity within spinal premotor circuitry is vital for the performance of correct movements. This feedback is provided by ascending tract neurons forwarding information to the cerebellum either directly (*via* spinocerebellar pathways) or indirectly (*via* the reticular formation) and used by the cerebellum to modulate descending motor commands, thereby shaping the final motor output. We investigated whether ascending tract neurons also inform the cerebellum about descending motor commands, relayed by corticospinal- and reticulospinal tract neurons or the mesencephalic locomotor region (MLR). In a series of *in vivo* experiments axons of descending tract neurons were stimulated within the pyramids, the medial longitudinal fasciculus and the MLR. Responses evoked in individual spinal neurons of different subpopulations of spinocerebellar tracts were recorded both extracellularly and intracellularly. Descending motor commands are monitored by a number of spinocerebellar tracts. Monosynaptic excitation evoked by descending tract neurons was found in ventral spinocerebellar tracts neurons while only polysynaptic actions were found in dorsal spinocerebellar tract neurons and neurons of the indirect spinocerebellar tract projecting *via* the lateral reticular nucleus. Specific subpopulations of spinocerebellar projections forward information concerning the degree of activity in spinal circuitry related to centrally initiated and voluntary motor commands as well as the local neuronal circuitry [1–3]. Information forwarded between neurons in the spinal cord, cerebellum and the reticular formation may thus serve not only to correct but also to predict and prevent motor errors.
1. Hammar et al. (2011) J Physiol 589: 653–665; 2. Jankowska et al. (2011) J Physiol 589: 5709–5725; 3. Hammar et al. (2013) Society for Neuroscience Annual Meeting, San Diego, USA.

S11.B
IS MOTONEURON HYPEREXCITABILITY HARMFUL IN ALS?

Daniel Zytnicki
Center for Neurophysics, Physiology and Pathology – Paris Descartes University, Paris, France

It has long been suggested that hyperexcitability of motoneurons induces excitotoxicity in Amyotrophic Lateral Sclerosis. However this assumption had received little support so far. In my talk, I will review recent data that we have obtained in our laboratory. We have studied the properties of spinal motoneurons in ALS mouse models: first in adult animals, using an *in vivo* preparation that we have recently developed and which allows us to perform intracellular recordings of type-identified motoneurons; and then in neonatal animals, using whole cell-recordings of motoneurons in lumbar slices. Our data indicate that intrinsic hyperexcitability is confined to neonatal S-type motoneurons, which are resistant in ALS. In sharp contrast FF and FR types motoneurons that degenerate in ALS tend to become hypoexcitable in adults in the days that precede their degeneration. Our results show that, as far as intrinsic hyperexcitability is concerned, it is unlikely to trigger motoneuron degeneration. However, firing does not depend solely on intrinsic properties but also on the synaptic inputs received by the cell. Indeed, motoneuron excitotoxicity might still arise, if a strong unbalance of excitatory versus inhibitory inputs exists in ALS mice. An unbalance towards more excitation could overcome the intrinsic hypoexcitability of vulnerable motoneurons and force them to discharge more than usual. In this perspective, I will also present preliminary experiments in which we have started investigating whether excitatory and inhibitory pathways to motoneurons are dysfunctional or not in ALS.

S11.C
THE ROLE OF SELECTED SEROTONERGIC RECEPTORS IN THE CONTROL OF LOCOMOTOR MOVEMENTS IN INTACT AND PARAPLEGIC RATS

Urszula Sławińska
Interinstitute Laboratory of Neuromuscular Plasticity, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland

Serotoninergic neurons projecting from the brainstem to the spinal cord are engaged in initiation and control of the locomotor move-

ments. This effect is exerted by actions on motoneurons as well as on the spinal cord neuronal network for locomotion, the Central Pattern Generator (CPG). The serotonergic neurons send their axons to specific neuronal target in the spinal cord where the different types of serotonergic receptors allow the serotonergic system to play multiple roles in the control of locomotion. Using defined serotonergic agonists and antagonists we demonstrated in intact and in paraplegic adult rats that the 5-HT2 receptors control CPG activation as well as motoneuron output, while 5-HT7 receptors activate the locomotor CPG and control interneurons responsible for intra-and interlimb coordination. The combined use of agonists of the 5-HT2 and 5-HT7 receptors in a low dose, that is not effective when applied by either drug alone, results in production of well-coordinated weight supported locomotion with a reduced need for exteroceptive stimulation. Next we found that in adult paraplegic rats intraspinal grafting of different populations of 5-HT neurons dissected from embryonic brainstem can activate the spinal cord circuitry below the total transection and enhance recovery of plantar hindlimb stepping. However, the locomotor recovery differed depending on the source of the grafted cells. The best effect of motor recovery was obtained using a combination of B1, B2 and B3 serotonergic neurons for grafting. In addition, we confirmed using defined serotonergic antagonists that the action of reestablished serotonergic innervation responsible for locomotor recovery is mediated by 5-HT2 and 5-HT7 receptors, as it is in the normal condition of himdlimb locomotor control. Our investigations demonstrate the marked potential of the remaining spinal cord circuitry below the total transection to enhance recovery of plantar hindlimb stepping in paraplegic adult rats.

S11.D

ADAPTATION OF MOTONEURONS TO ALTERED PHYSICAL ACTIVITY

Piotr Krutki

Department of Neurobiology, University School of Physical Education in Poznań, Poznań, Poland

The study shows that different models of an increased muscular activity may induce measurable effects on electrophysiological properties of motoneurons (MNs). Three types of altered motor activity were compared in rats: chronic compensatory muscle overload, whole-body vibration (WBV), and strength training. Intracellular recordings from spinal motoneurons were made to measure membrane and firing properties of MNs.Muscle overload was induced in the rat medial gastrocnemius by bilateral tenotomy of its synergists. Adaptive changes in passive and threshold properties were observed only in fast-type MNs innervating the overloaded muscle. The data suggest their higher excitability, and a shift towards electrophysiological properties of slow-type MNs. The WBV training was performed 5 days a week, for 5 weeks, and each daily session consisted of four 30-second runs of vibration at 50 Hz. No significant changes in the passive membrane properties of MNs were found after the WBV program. However, lower values of rheobase current and a leftward shift of the frequency-current relationship were observed for fast-type MNs. This indicates their ability to become recruited earlier (and possibly more frequent), and to achieve the same or higher firing rates at lower stimulus intensities. During strength training rats were nutritionally conditioned in order to make weightlifting put on their shoulder in a special apparatus with progressively increasing load. After 5 weeks adaptive changes in several membrane properties were revealed in fast and slow-type MNs. Increased maximum frequencies of rhythmic firing of MNs, and higher susceptibility of MNs to an increased or decreased intensity of a stimulus were observed. Adaptations of MNs to various modes of chronic activation of muscles were relatively quick, but different with respect to extent and dynamics of the effects.

Supported by the National Science Center grants: 2011/01/N/NZ4/04901, 12/04/M/NZ4/00190 and 2013/11/B/NZ7/01518.

SYMPOSIUM S12. STRUCTURE, FUNCTION AND PLASTICITY OF $GABA_A$ RECEPTORS

Chairperson: *Jerzy W. Mozrzymas (Wrocław, Poland)*

S12.A

STRUCTURAL MECHANISM UNDERLYING $GABA_A$ RECEPTOR GATING AND DRUG MODULATION

Cynthia Czajkowski

School of Medicine and Public Health, Madison, WI, USA

[Abstract not received]

S12.B

PLASTICITY OF GABAERGIC SYNAPSES

Andrea Barberis

Italian Institute of Technology (IIT), Genova, Italy

Postsynaptic long-term potentiation of inhibition (iLTP) can rely on increased $GABA_A$ receptors ($GABA_A$Rs) at synapses by promoted exocytosis. However, the molecular mechanisms that enhance the clustering of postsynaptic $GABA_A$Rs during iLTP remain obscure. This study demonstrates that, during iLTP, $GABA_A$Rs are immobilized and confined at synapses, as revealed by single particle tracking of individual $GABA_A$Rs in cultured hippocampal neurons. iLTP expression requires the synaptic recruitment of the scaffold protein gephyrin from extrasynaptic areas, which in turn is promoted by CaMKII-dependent phosphorylation of $GABA_A$R-β3-Ser^{383}. We also report that gephyrin moderately contributes to the maintenance of

$GABA_AR$ synaptic clustering in basal conditions, whereas it is essential for the postsynaptic rearrangements underlying receptor accumulation at synapses during iLTP. Indeed, impairment of gephyrin assembly prevents iLTP and, in parallel, blocks the accumulation and immobilization of $GABA_ARs$ at synapses. Importantly, increases of synaptic $GABA_ARs$ and gephyrin similar to those observed during iLTP in culture are found in the rat visual cortex following an experience-dependent plasticity protocol that potentiates inhibitory transmission *in vivo*. Thus, phosphorylation-dependent accumulation of gephyrin at synapses and $GABA_AR$ immobilization are crucial for iLTP and are likely to have a strong impact on network excitability.

S12.C

CONTRIBUTION OF THE GABAERGIC SYSTEM TO CORTICAL PLASTICITY

Malgorzata Kossut

Department of Molecular and Cellular Neurobiology, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland

Experience-induced plastic changes in the cerebral cortex are accompanied by alterations in excitatory and inhibitory transmission. Increased excitatory drive, necessary for plasticity, precedes the occurrence of plastic change, while decreased inhibitory signaling often facilitates plasticity. However, an increase of inhibitory interactions was noted in some instances of experience-dependent changes. We found upregulation of the number of inhibitory markers in the barrel cortex of mice after classical conditioning engaging vibrissae, observed concurrently with enlargement of the cortical representational area of the row of vibrissae receiving conditioned stimulus (CS). We also observed that an increase of GABA level accompanied the conditioning. Moreover, Npas4, a transcription factor important for structural and functional neuronal plasticity and identified as an element of the program controlling inhibitory synapse development, was affected by conditioning. With real-time PCR on laser-dissected individual rows of barrels we found that Npas4 mRNA level was upregulated following conditioning. In order to find whether unaltered GABAergic signaling is necessary for learning-dependent rewiring in the murine barrel cortex, we locally decreased GABA production in the barrel cortex or reduced transmission through $GABA_A$ receptors at the time of the conditioning. Both treatments prevented learning-induced enlargement of the conditioned vibrissae representation. At the behavioral level, consolidation of the conditioned response (cessation of head movements in response to CS) was impaired. These results show that appropriate functioning of the GABAergic system is required for both manifestation of functional cortical representation plasticity and for the development of a conditioned response.

S12.D

LINKING AGONIST BINDING TO $GABA_A$ RECEPTOR OPENING TRANSITION

Jerzy W. Mozrzymas[1,2], Magdalena Kisiel[1], Magdalena Jatczak[1,2], Marta Czyżewska[1], Marek Brodzki[1,2]

[1]Laboratory of Neuroscience, Department of Biophysics, Wrocław Medical University; [2]Laboratory of Cellular Neurobiology, Department of Animal Molecular Physiology, Wrocław University, Wroclaw, Poland

$GABA_A$ receptors are responsible for mediating inhibition in the adult mammalian CNS. These receptors are greatly diversified but the most common type is alpha1beta2gamma2. Intriguingly, GABA binding sites on $GABA_A$ receptor are remarkably distant (ca. 5 nm) from the channel gate. This structural feature raises the question about molecular mechanisms underlying the energy transfer from binding process to conformational transitions. Recently, we found that mutation of binding site residue alpha1F64 affects not only binding but also conformational transitions. Extensive experimental data and model simulations indicated that the major mechanism underlying alpha1F64 mutation is to affect so called preopening (channel remains closed but increases its propensity to open) and desensitization (Szczot et al. 2014). Single-channel revealed that, additionally, this mutation shortens the channel opening time, indicating increase in the closing rate. Interestingly, alpha1F64 mutation was found to affect $GABA_AR$ proton sensitivity (Huang et al. 2004). We thus checked the impact of pH changes on WT and mutated alpha1beta2gamma2 receptors and found that protons modulate gating by altering mainly preactivation and desensitization. Kinetic analysis of alpha1beta2gamma2 receptors with mutation at a different location within agonist binding site (beta2E155) suggested again involvement of this residue in preactivation transition. Taking altogether, preactivation transition emerges as a key conformation transition which affects both kinetics and pharmacological sensitivity of currents mediated by alpha1beta2gamma2 $GABA_A$ receptors.

Supported by NCN grant DEC-2013/11/B/NZ3/00983.

SYMPOSIUM S13. REGENERATIVE NEUROBIOLOGY OF THE EYE

Chairperson: *Joanna Lewin-Kowalik (Katowice, Poland)*

S13.A

THE THEORY OF INFLAMMAGING

Anu Kauppinen[1,2]

[1]University of Eastern Finland, Kuopio, Finland; [2]Kuopio University Hospital, Kuopio, Finland

Inflammation is a fast defense response to any danger factor encountered by a cell. Its function is to eliminate foreign material or damaged cells and to help to restore the homeostasis. Aging causes

decreased functionality of adaptive but increased reactivity of innate immune system. Age-related senescence also deteriorates cellular housekeeping processes, such as autophagic capacity. Chronic low-grade inflammation accompanied by age-related changes has been termed as inflammaging. Elevated pro-inflammatory status in association with genetic susceptibility contributes to the pathogenesis of age-related disorders, such as Alzheimer's disease and age-related macular degeneration (AMD).

S13.B

AGE-RELATED MACULAR DEGENERATION (AMD): ALZHEIMER'S DISEASE IN THE EYE?

Kai Kaarniranta

Group of Immuno-Ophthalmology, Department of Ophthalmology, University of Eastern Finland and Kuopio University Hospital, Kuopio, Finland

Alzheimer´s disease (AD) and age-related macular degeneration (AMD) are the most common age-related neurodegenerative diseases in the western countries. They share similar environmental risk factors, comprising of smoking, hypertension, hypercholesterolemia, atherosclerosis, obesity, and unhealthy diet. In both diseases, cellular pathology is associated with increased oxidative stress, inflammation, and impaired proteolysis that evoke formation of intra- and extracellular deposits; plaques in AD and drusen in AMD. Autophagy is a lysosomal catabolic clearance mechanism that is triggered as an adaptive response during AD- and AMD-associated stress conditions. Autophagy dysfunction is currently discussed as an important factor in the development of neurodegenerative diseases. Failure of autophagy in aged post mitotic cells, including neurons or retinal pigment epithelial cells (RPE), can result in accumulation of aggregate-prone proteins, cellular degeneration and finally cell death. Since both diseases have different genetic background, but have similar environmental risk factors and cellular pathology we believe that impaired autophagy is a key player in the pathogenesis of these diseases.

S13.C

SCHWANN CELLS INDUCED NEUROPROTECTION AND REGENERATION OF RETINAL GANGLION CELLS

Joanna Lewin-Kowalik, Adrian Smedowski, Marita Pietrucha-Dutczak

Department of Physiology, Medical University of Silesia, Katowice, Poland

To investigate neuroprotective effect of intravitreally applied Schwann cells therapy towards Retinal Ganglion Cells (RGCs) in rat experimental glaucoma. Twenty male Wistar rats were included to this study. Experimental glaucoma was induced in the left eye of each rat by intraocular pressure (IOP) elevation using intracameral injection of polystyrene microbeads. The right eye served as a healthy control. Ten animals received intravitreal injection of 5 µl Schwann cells suspension (about 10^6 cells), another 10 received injection of equivallent volume of PBS. Animals were breaded for 6 weeks and IOP was monitored using laboratory tonometer once a week. After 6 weeks animals were sacrificed, eyes with optic nerves were enucleated and processed for histology and immunohistochemistry. RGCs survival was compared by counting RGCs bodies and optic nerve axons from control eyes (healthy and PBS) and Schwann cells treated. Mean 6 weeks IOP in ocular hypertension eyes was significantly higher in comparison to healthy contralateral eyes (31.02±5.5 mmHg and 10.32±0.54 mmHg, mean±SD, Wilcoxon paired test, $P<0.05$). There were significant differences between RGCs bodies and optic nerve axons numbers in Schwann cell-treated vs. PBS-treated vs. healthy control eyes ($P<0.05$, Kruskall-Wallis test). Mean 6-weeks loss of RGCs bodies was 21.7% in glaucoma eyes treated with Schwann cells and 45% in glaucoma eyes treated with PBS. Immunofluorescent staining with GAP43 showed neurites outgrowth within optic nerves from eyes treated with Schwann cells. Applied cellular therapy using predegenerated Schwann cells showed neuroprotective and regenerative effect towards RGCs in rat glaucoma model.

S13.D

SPONTANEOUS AND INDUCIBLE REINERVATION OF HUMAN CORNEA

Adrian Smedowski, Edward Wylęgała

Department of Ophthalmology, Faculty of Medicine and Division of Dentistry in Zabrze, Medical University of Silesia, Katowice, Poland

Correct innervation of the ocular surface is, in addition to the endothelial barrier, a leading mechanism conditioning the transparency of the cornea. Corneal nerves are involved in perception of pain, as well as touch, thermal and chemical stimuli. Moreover, they are involved in modulating the blink reflex and tears production. Corneal nerve plexus insufficiency might occur as a primary or secondary sign of ocular or systemic disorders. Neuropathic keratopathy is a corneal disorder due to the partial or complete denervation of the eye surface tissues associated with damage or dysfunction of ophthalmic nerve branches originating from the trigeminal ganglion (V cranial nerve). This damage leads to a complete or partial corneal analgesia, destabilization of corneal epithelium and production of tear film, resulting in the development of corneal epithelial erosion, ulceration or melting. Total denervation of corneal tissue is observed following corneal grafting, while within weeks after transplant procedure, spontaneous innervation of graft might be observed using

in vivo confocal microscopy technology. Neuropathy associated with viral keratitis (HSV and HZV), chemical burn, contact lenses wearing, corneal dystrophies or diabetes are rather unusual to be associated with spontaneous nerves growth. In these cases novel therapeutic approaches, which are still at the stage of development, represent a promising alternative for patients with neurotrophic keratopathy. Topical application of recombinant human Nerve Growth Factor (NGF) is a recent achievement of regenerative medicine for ocular surface disorders. Currently it is undergoing Phase II Clinical Study for treatment of neurotrophic keratopathy. The latest possible therapeutic option to induce corneal innervation is a topical supplementation of extracellular matrix components supporting the healing process of the cornea. The combination of clinical and basic science knowledge results currently developing novel therapeutic options, which in the future may contribute to a better prognosis for neuropathic keratopathy treatment.

SYMPOSIUM S14: PAYING ATTENTION TO ATTENTION TOWARDS BETTER UNDERSTANDING OF ITS MECHANISMS AND NEURONAL CORRELATES

Chairperson: *Anita Cybulska-Kłosowicz (Warsaw, Poland)*

S14.A

STIMULUS-DRIVEN AND ANTICIPATORY ATTENTION DIFFERENTIALLY MODULATE PRIMARY VISUAL CORTEX

Marek Bekisz, Wojciech Bogdan, Anaida Ghazaryan, Wioletta J. Waleszczyk, Ewa Kublik, Andrzej Wróbel

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

Only a fraction of the visual information can be consciously processed. The mechanism by which stimuli are selected for mindful processing is based on current behavioral situation and involves selective attention. Attention can be focused either volitionally by anticipatory signals, derived by task demands, or automatically by bottom-up signals from salient stimuli. Since brain mechanisms underlying these two processes of attention, are poorly understood, we performed electrophysiological experiments with chronic recordings from visual cortex of behaving cats engaged in delayed visual and auditory spatial discrimination task. Our previous results showing that both attentional tasks were accompanied by enhanced activity in beta frequency band in visual cortical areas 17 and 18 led us to the hypothesis that beta signals serve as attention carrier. We have now searched whether attention-related beta activity would influence cortical responses to electrical peripheral stimulation. We compared potentials (EPs) evoked by the optic chiasm stimulation in primary visual cortical areas during ongoing high or low amplitude beta signals. Under anticipatory attentional task peripheral stimuli preceded by a 200 ms high-amplitude beta signals evoked larger amplitude EPs as compared to those which followed low-amplitude beta activity. In contrast, during bottom-up attentional condition cortical EPs preceded by high beta oscillation, were on average smaller than those following low beta signals. Correlation analysis between beta signals at different recording sites showed that bottom-up task activated cortical beta frequency maps in a mosaic-like pattern whereas anticipatory modulation resulted in spatially homogeneous beta excitation. Taken together, our results suggest that enhanced beta activity during the two attentive conditions shape the functional organization of cortical networks into substantially different spatial patterns.

S14.B

C. Elaine Chapman

Department of Neuroscience, University of Montréal, Montréal, Canada

[Abstract not received]

S14.C

FINDING THE PSYCHO IN PSYCHOMOTOR STIMULATION: DIFFERENTIAL EFFECTS OF AMPHETAMINE AND KW6002 ON RESPONSE PREPARATION

Verity J. Brown[1], Martin O'Neill[2]

[1]School of Psychology and Neuroscience, University of St Andrews, St. Andrews, UK; [2]Department of Experimental Psychology, Oxford University, Oxford, UK

The neurotransmitters dopamine and adenosine play a key role in motor control, with drugs affecting these systems strongly stimulating motor processes. The issue we address here is the nature of the cognitive stimulating properties of psychostimulants. Expectation of a stimulus in time and space results in anticipatory preparation of a response, improving both speed and accuracy. Evidence for this comes from the observation that response speed and accuracy is tightly modulated by the prior probability of the stimulus. We were interested in establishing whether psychostimulation modulated the calculation of stimulus prior probability and/or the expression of this calculation. We manipulated dopamine (by administration of amphetamine) and adenosine (using the adenosine A_{2A} antagonist, KW6002). Reaction time and accuracy of rats was measured using a two-choice spatial discrimination task. We manipulated the spatial probability of a stimulus as a function of time, to tease apart the effects on motor preparation of stimulus prior probability, as opposed to absolute preparation time. Reaction times were determined by the time preceding a stimulus (absolute preparation time) as well as

prior likelihood of the stimulus (stimulus prior probability). Movement times and anticipatory responses were particularly strongly influenced by both spatial and temporal probability of stimuli, irrespective of absolute preparation time. Both amphetamine and KW6002 stimulated motor activity, as expected. Amphetamine also enhanced the effects of both absolute preparation time and prior probability, whereas KW6002 particularly enhanced the effect of prior probability.

The stimulatory effects of psychostimulants are on cognitive processes, implicating the dopamine system, particularly *via* the dopamine D_2 receptor, in the translation of stimulus likelihood into expectation and anticipation for the determination of motor output.

S14.D

DEFICITS IN ATTENTIONAL FUNCTIONS AND DOPAMINE TRANSPORTER POLYMORPHISMS

Anita Cybulska-Kłosowicz, Katarzyna Giertuga

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

Attention, its executive function in particular, is regulated by the dopaminergic (DA) system. Dopamine transporter (DAT), regulating DA neurotransmission, likely plays a role in controlling the influence of DA in cognitive processes. We examined the role of DAT in attention in mice and humans. Mice with DAT gene genetically deleted (DAT+/- heterozygotes) were compared with the wild type (WT) mice in several tests measuring attention. The effect of DAT inhibition was examined in mice submitted to repeated administration of selective DAT inhibitor – GBR 12909 and tested after 10 days of withdrawal. Locomotor activity and non-selective attention were tested in a Làt-maze, while attentional set-shifting, associative and reversal learning – in Attentional Set-Shifitng task (ASST). DAT level in the striatum was assessed using DAT immunohistochemistry. Neuronal activity in medial prefrontal cortex (mPFC) during ASST was visualized with the egr-1 and egr-2 immunohistochemistry and with [14C]-2-deoxyglucose autoradiography. Results have shown that DAT+/- mice had significantly higher scores of locomotor activity in comparison with WT mice. Heterozygotes did not differ from WT mice in respect of non-selective attention and associative learning measures. However, they were significantly impaired in more demanding tasks that tax the executive control function of attention. Also, neuronal activity level in mPFC of DAT+/- mice was significantly lower when compared with WT mice. These observations correspond well with behavioral results of children with attention deficit hyperactivity disorder (ADHD) examined in Attention Network Test (ANT), Sustained Attention to Response Task (SART) and in Test of Everyday Attention for Children (TEA-Ch) which revealed substantial deficits in executive function of attention. Measures of attention evaluated in attentional tests were analyzed in terms of relation with DAT1 gene polymorphisms. National Science Centre Grant 2011/01/D/NZ4/04958.

SYMPOSIUM S15. COMPUTATIONAL CHALLENGES IN IMAGING AT DIFFERENT SCALES

Chairperson: *Daniel Wójcik (Warsaw, Poland)*

S15.A

ACTIVITY-DEPENDENT DENDRITIC SPINE NECK CHANGES ARE CORRELATED WITH SYNAPTIC STRENGTH

Tim Vogels

Department of Physiology, Anatomy and Genetics, University of Oxford, Oxford, UK

[Abstract not received]

S15.B

QUANTITATIVE ANALYSIS OF SHAPES OF DENDRITIC SPINES

Szymon Łęski

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

Dendritic spines are targets of excitatory synaptic inputs. The morphology of a spine is linked to its function. For example, a mature dendritic spine resembles a mushroom, with a wide spine head connected to the dendrite by much thinner neck. Several studies link dimensions of that mushroom to the functioning of the synapse, for example it has been shown that the amplitude of uncaging potential at the soma correlates with spine head volume and negatively correlates with spine neck length. To study the shapes of dendritic spines quantitatively we have developed a set of computational methods for analysis of microscopy images of individual spines. The first method allows for semi-automatic classification of spine shapes in a dataset. First, spine shapes are grouped in an unsupervised way (that is, without assuming any a priori structure of the dataset) using a clustering algorithm. Then the groups can be manually segragated according to criteria set by the researcher. The second method has been designed to automatically measure dimensions of heads and necks of spines in large datasets. The idea is to first represent the shape of a spine as a sum of building blocks: an ellipsoid for the spine head, a cylinder for the spine neck, and possibly a cone for the part of the spine closest to the dendrite. Then widths and lengths of heads and necks can be defined based on dimensions of the fitted blocks. As an example we show the results of application of these methods to a large collection of dendritic spine shapes – several

thousands of individual spines – obtained from confocal microscopy images.

S15.C
IMAGING POPULATIONS IN THE AWAKE ANIMAL
Jason Kerr
Research Center, Max Planck Institute for Biological Cybernetics, Bonn, Germany

Multiphoton-imaging allows unambiguous access to neuronal populations and neuronal substructures located well below the cortical surface. In combination with genetically encoded activity indicators this approach can be used to infer spiking activity from neuronal populations in the awake animal, with single cell and single action-potential accuracy. For this lecture I will present imaging tools that are necessary to accurately record activity from neuronal populations in the awake behaving animal using the multi-photon excitation principle. I will also outline strategies that have allowed access to neuronal activity in the freely moving animal and in deep cortical layers. In addition, I will outline recent strategies to simultaneously track the precise head and eye positions of freely behaving animals.

S15.D
OPTICAL CONTROL OF NETWORK CONTEX IN HIPPOCAMPAL SYNAPTIC PLASTICITY
Upinder Bhalla
National Centre for Biological Sciences, Bangalore, India

We wish to understand plasticity mechanisms in a context designed to approximate the stimuli and synaptic input *in vivo*. While plasticity in the mouse hippocampal slice preparation has been extensively studied, the lack of background activity is a major difference between slice and *in-vivo* contexts. We approximated steady as well as rhythmic background activity through optically-delivered background stimulus. EPSPs were recorded from CA1 cells while optically stimulating the CA3 region of CA3-cre mice injected with ChannlelRhodopsin2(ChR2)-lox virus. Using optical stimuli makes the STDP and background activity specific to the CA3-CA1 pathway. It further allows us to titrate the number of inputs by scaling spot size and intensity and to present rapidly varying patterned stimuli. We calibrated the optical patterns to deliver background activity to CA1 neurons that approximated *in vivo* observations. In this talk I will present our results on spike-timing and theta-burst stimuli in the presence of background activity. I will also indicate how the optical stimulus protocol can be adapted to obtain estimates of heterosynaptic plasticity for each of these protocols, across the target cell.

POSTER SESSION P1. AMYLOID-BETA

P1.1
THE MOLECULAR NETWORK BETWEEN AMYLOID BETA PEPTIDE, SPHINGOSINE KINASE AND SIRTUINS IN CELL SURVIVAL AND DEATH: IMPLICATION IN ALZHEIMER'S DISEASE
Joanna Strosznajder, Grzegorz Czapski, Magdalena Cieślik
Mossakowski Medical Research Centre, Polish Academy of Sciences, Warsaw, Poland

BACKGROUND AND AIMS: Deregulation of the sphingolipid metabolism plays an important role in the pathogenesis of Alzheimer's disease (AD). Mitochondrial function and mitochondrial deacetylases, i.e. sirtuins (Sirt3,-4,-5), are also affected in AD. The aim of this study was to analyse the interaction between amyloid-$\beta_{1\text{-}42}$ ($A\beta_{1\text{-}42}$), sphingosine kinases (SphKs) and mitochondrial sirtuins in cell survival/death.
METHODS: The spectrofluorometrical, immunochemical and QRT-PCR methods were applied.
RESULTS: PC12 cells were subjected to $A\beta_{1\text{-}42}$ oligomers and SphK inhibitor (SKI II) for 24–96 h. $A\beta_{1\text{-}42}$ enhanced SphK1 expression and activity after 24 h, but down-regulated them after 96 h and had no effect on SphK2. $A\beta_{1\text{-}42}$ and SKI II induced oxidative stress, disturbed the balance between pro- and anti-apoptotic proteins and evoked cell death. Simultaneously, up-regulation of anti-oxidative enzymes catalase and superoxide dismutase 2 occurred. Moreover, the total protein level of glycogen synthase kinase-3β (Gsk-3β) was reduced. $A\beta_{1\text{-}42}$ significantly increased the level of mitochondrial proteins: AIF and Sirt3, -4, -5. Additional analysis demonstrated a significant role of p53 protein at very early stages of $A\beta_{1\text{-}42}$ toxicity. However, during prolonged exposure to $A\beta_{1\text{-}42}$, the activation of caspases, MEK/ERK, and alterations in mitochondrial permeability transition pores were also involved in mechanism responsible for cell death. Moreover, SphK product, sphingosine-1-phosphate (S1P), and Sirt activators and antioxidants, effectively prevented toxicity of $A\beta_{1\text{-}42}$.
CONCLUSIONS: Our data indicated that p53 protein and SphKs may be involved at early stage of molecular mechanisms of Aβ toxicity. We suggest the important role of interactions between Aβ peptide, SphKs and Sirts in pathomechanism of AD. The activation of S1P-dependent signalling and Sirts may offer a promising cytoprotective strategy.
This study was supported by The National Science Centre Grant 2013/09/B/NZ3/01350 to J.B.S.

P1.2
GENE EXPRESSION PROFILES FOR MITOCHONDRIA SIRTUINS AND POLY(ADP-RIBOSE) POLYMERASES IN AMYLOID BETA TOXICITY
Robert Strosznajder, Przemysław Wencel

Laboratory of Preclinical Research and Environmental Agents, Department of Neurosurgery, Mossakowski Medical Research Centre, Polish Academy of Sciences, Warsaw, Poland

BACKGROUND AND AIMS: Sirtuins (SIRTs) and poly(ADP-ribose) polymerases (PARPs) are NAD dependent enzymes engaged in the regulation of energy metabolism, transcription, DNA replication and repair. SIRTs type III histone deacetylases (HDAC) target histone and many other proteins in nucleus, cytoplasm and mitochondria. PARP-1 is responsible for over 90% of poly (ADP-ribosylation) in the brain. However, the role of these NAD dependent enzymes in neurodegeneration /neuroprotection is till now not fully elucidated. In many neurodegenerative disorders metabolism of amyloid precursor protein (APP) is altered, amyloid beta (Aβ) is released and NAD dependent metabolic pathways are affected. This study focused on gene expression profiles for SIRTs and PARPs and on their functional relationship in cells survival/death under Aβ toxicity.

METHODS: PC-12 cells after exposition on exogenous $A\beta_{1-42}$ oligomers (ABO -1 mM, 24 h) were used. Moreover, the effect of endogenously liberated Aβ in PC12 cells transfected with human gene for APP wild type (APPwt) and bearing Swedish mutation (APPsw) was investigated. The both served as experimental models.

RESULTS: Our data indicated that ABO suppressed alpha secretase and enhanced gene expression for beta and gamma secretases. Moreover, ABO upregulated the gene expression for PARP-1, PARP-2 and SIRT4 which is responsible for monoADP-ribosylation of several mitochondrial proteins. The endogenously liberated Aβ in APPwt cells upregulated gene expression for PARP-1, -2 and decreased for SIRT5. In APPsw cells activation of genes for PARP -1,-2,-9 and for SIRT3 was observed. In our previous study we observed significant suppression of PARP activity in APPsw cells.

CONCLUSIONS: These results suggest that NAD is not used by PARPs in APPsw cells and it may be available for Sirt3 which is involved in regulation of antioxidative enzymes. The functional interactions between these NAD dependent enzymes may play crucial role in regulation of cell survival under Aβ peptide toxicity.

Supported by MRC.

P1.3

ALTERATIONS IN APOPTOTIC SIGNALING IN LYMPHOCYTES OF PATIENTS IN THE EARLY STAGE OF ALZHEIMER`S DISEASE PRECEDE MASSIVE NEURONAL LOSS IN THE BRAIN

Joanna Wojsiat[1], Katarzyna Laskowska-Kaszub[1], Monika Mandecka[2], Tomasz Gabryelewicz[2], Jacek Kuźnicki[3], Urszula Wojda[1]

[1]Nencki Institute of Experimental Biology, PAS, Warsaw, Poland; [2]Alzheimer's Disease Division, MSWiA Hospital in Warsaw, Warsaw, Poland; [3]International Institute of Molecular and Cell Biology, Warsaw, Poland

BACKGROUND AND AIMS: Alzheimer's disease (AD) is the most common age-related dementia worldwide of unclear early pathogenesis. Mild Cognitive Impairment (MCI) represents an early AD stage, preceding massive deposition of Aβ aggregates and associated neuronal loss in the brain. Recently we demonstrated that sporadic AD (SAD) lymphoblasts show increased levels of p21 protein, the key regulator of G1/S cell cycle checkpoint and apoptosis (Bialopiotrowicz et al. 2011, Neurobiol Aging). In the current study we aimed to elucidate if p21 levels are altered early in AD, in lymphocytes of MCI patients, and to investigate the effects of p21 on the apoptotic response of SAD and MCI lymphocytes

METHODS: We compared apoptotic response to 2 deoxy-D ribose (2dRib) in EBV-immortalized B-lymphoblasts from 16 patients with SAD, 17 patients with MCI and 10 age-matched healthy individuals without dementia. Apoptotic response was measured using flow cytometry assays: AnnexinV, mitochondrial membrane potential, and SubG1-phase.

RESULTS: Comparing to controls, under basal conditions p21 levels assessed by immunoblotting were significantly elevated in MCI lymphoblasts, similarly as in SAD cells. 24 h after 2dRib treatment, apoptosis was higher in SAD cells than in controls, and after stimulation p21 decreased significantly in SAD and MCI cells.

CONCLUSIONS: SAD lymphoblasts are significantly less resistant to oxidative apoptotic stimuli than controls. In MCI lymphoblasts there were similar tendencies but without statistical significance. These results suggest that changes in the p21 levels and in apoptotic response in lymphocytes appear early in AD and gradually increase with the disease progression. Furthermore, our data indicate that lymphocytes may be useful for the development of new early AD diagnostic markers based on apoptotic regulatory proteins such as p21.

This research was supported by the grant 2/BIOMARKAPD/JPND/2012 and by the Nencki Institute statutory funds.

P1.4

THE APOE GENOTYPE AND THE PLASMA EXPRESSION OF microRNA-107, -132 AND -138 IN PATIENTS WITH ALZHEIMER'S DISEASE – PRELIMINARY STUDY

Michał Prendecki[1], Jolanta Florczak-Wyspiańska[2], Urszula Łagan-Jędrzejczyk[1], Anna Płóciennik[1], Wojciech Kozubski[2], Jolanta Dorszewska[1]

[1]Laboratory of Neurobiology, Department of Neurology, [2]Chair and Department of Neurology, Poznań University of Medical Sciences, Poznań, Poland

BACKGROUND AND AIMS: Alzheimer's disease (AD) is a multifactorial disease conditioned by genes (70%) and environment (30%), characterized by brain deposition of amyloid-β and neurofibrillary tangles. An involvement of over 20 genes (including *APOE*) and dozens of microRNAs (e.g. miR-107) has been previously described in the pathogenesis of AD. *APOE* has 3 common variants: protective – ε2, neutral – ε3 and pathogenic – ε4. The miR-107, associated with amyloid cascade, seems to be a promising AD plasma biomarker, whose downregulation has been demonstrated in the early stage of the disease. Though miR-132 has been linked with apoptosis of neurons and miR-138 with tau hyperphosphorylation, their role in AD remain unclear, and neither of these miRNA has been previously associated with *APOE*. The aim of the study was the analysis of *APOE* genotypes and the expression of three miRNAs: miR-107, -132, -138 in patients with AD and in control subjects, both related and unrelated to AD cases.

METHODS: The DNA from 100 subjects (aged 47–83), including 42 patients with AD and 15 related and 43 unrelated controls of the same age was genotyped by "mismatch primer" qPCR. The subsequent expression analysis of miR-107, -132 and -138 in the plasma of ten subjects was performed by qPCR.

RESULTS: Our study have shown that the miR-107 was significantly downregulated in AD patients ($P<0.05$) comparing to related controls, but did not reach significance as compared to unrelated controls. The downregulation of miR-132 was not statistically significant as compared to related and unrelated subjects. Expression of miR-138 in AD was decreased only as compared to relatives. Subsequently, the presence of two *APOE* ε4 alleles in AD patient and at least one ε4 copy in the controls was associated with altered expression of the analyzed miRNAs.

CONCLUSIONS: It appears that in the course of AD the expression of miR-107, -132 and -138 depends on the *APOE* genotype.

P1.5

DIMETHYL FUMARATE ALLEVIATES REFERENCE MEMORY IMPAIRMENT AND MODULATES BRAIN-DERIVED NEUROTROPHIC FACTOR EXPRESSION IN STREPTOZOTOCIN-INDUCED RAT MODEL OF ALZHEIMER'S DISEASE

Ewelina Kurowska, Irena Majkutewicz, Dorota Myślińska, Beata Grembecka, Maria Grzybowska, Magdalena Podlacha, Jan Ruciński

Department of Animal and Human Physiology, University of Gdańsk, Gdańsk, Poland

BACKGROUND AND AIMS: Intracerebroventricular (icv) injection of streptozotocin (STZ) induces brain glucose hypometabolism, memory impairment, progressive cholinergic deficit, activation of microglia, oxidative stress and neurodegeneration. It is used as an animal model of sporadic form of Alzheimer's disease (AD). The aim of this study was to determine if dimethyl fumarate (DMF), oral anti-oxidative and immunosuppressive drug, alleviates spatial reference memory impairments in STZ-icv induced rat model of AD. Additionally, the expression of brain derived neurotrophic factor (BDNF) was measured.

METHODS: There were four experimental groups: STZ DMF (n=8) – STZ-icv infused and fed with 0.4% DMF fodder for three weeks until spatial memory test of Morris, STZ CTR (n=8) – STZ-icv infused and fed with standard fodder, and VEH DMF and VEH CTR groups (n=10) – vehicle-icv infused and fed with 0.4% DMF or standard fodder, respectively. A three-day Morris water maze test (four trials per day with unchanged platform location) and the probe test on the fourth day were performed. Rats were sacrificed and brain subjected to immunofluorescent BDNF labeling.

RESULTS: The latency to reach the platform in the second and third day of testing was significantly longer in the STZ CTR rats than in the remaining groups, which showed tendency to reduce the latency day by day. STZ DMF rats did not differ in the results of the spatial memory test of Morris from control VEH CTR and VEH DMF groups. All STZ rats showed reduced BDNF expression in the hippocampus, but in the hypothalamus STZ DMF showed more BDNF+ cells than STZ CTR rats.

CONCLUSION: Oral medication with DMF alleviates spatial reference memory impaired after STZ-icv infusion. The decrease of BDNF expression after STZ-icv infusion was prevented by DMF in the hypothalamus.

The study was financed by the National Science Centre Poland on the basis of decision DEC-2013/09/D/NZ4/01658.

P1.6

PHARMACOLOGICAL INHIBITION OF CYCLIN-DEPENDENT KINASE 5 MODIFIES GENE EXPRESSION IN MOUSE MODEL OF AMYLOID BETA TOXICITY

Grzegorz A. Czapski, Anna Wilkaniec, Magdalena Gąssowska, Agata Adamczyk

Department of Cellular Signalling, Mossakowski Medical Research Centre, Polish Academy of Sciences, Warsaw, Poland

BACKGROUND AND AIMS: The prominent features of Alzheimer's disease (AD) are accumulation of amyloid beta (Aβ) oligomers and neuroinflammatory processes. Previous data demonstrated that activation of cyclin-dependent kinase 5 (Cdk5) may be essential for pathology of AD and other neurodegenerative disorders. In this study we focused on the role of Cdk5 in controlling gene expression in the brain in experimental model of AD as well as during systemic inflammatory reaction (SIR).

METHODS: Alzheimer's Aβ toxicity and SIR were induced in mice by intracerebroventricular injection of $A\beta_{1-42}$ oligomers and intraperitoneal injection of lipopolysaccharide (LPS), respectively. Roscovitine, the inhibitor of Cdk5, was administered intraperitoneally.
RESULTS: Both Aβ and LPS induced an increase in Cdk5 activity in hippocampus, as evidenced by augmented formation of Cdk5 activator, p25, and enhanced phosphorylation of Cdk5. In concordance, Cdk5-related increase in phosphorylation of Gsk-3β (Ser9) and MAP tau (Ser396) was found. Moreover, we found Cdk5-dependent phosphorylation of ERK1/2 (Thr183/Tyr185) and MEF2A (Ser406), that may negatively modulate activity of transcription factors and in consequence the expression of various genes, i.a. those related to prosurvival pathways. Aβ and LPS injection evoked rapid activation of inflammation-related genes in hippocampus, *Nos2*, *Tnfa*, *Il1b*, *Il6*, *Il10*, whereas inhibition of Cdk5 with Roscovitine modified the level of micro RNA and augmented Aβ- and LPS-induced changes in mRNA level for several inflammation-related genes.
CONCLUSIONS: Cdk5 participates in regulation of gene expression in Aβ toxicity and in SIR. Our data suggest that modulation of Cdk5 activity may be prospective strategy for protection in neurodegenerative disorders.
This study was supported by The National Science Centre grant 2011/03/B/NZ3/04549.

P1.7

DIMETHYL FUMARATE PREVENTS SPATIAL WORKING MEMORY IMPAIRMENT AND DOES NOT AFFECT BRAIN IL-6 EXPRESSION IN STREPTOZOTOCIN-INDUCED RAT MODEL OF ALZHEIMER'S DISEASE

Irena Majkutewicz, Ewelina Kurowska, Dorota Myślińska, Beata Grembecka, Maria Grzybowska, Magdalena Podlacha, Jan Ruciński

Department of Animal and Human Physiology, University of Gdańsk, Poland

BACKGROUND AND AIMS: Animal model of sporadic form of Alzheimer Disease (AD) evoked by intracerebroventricular (icv) injection of betacytotoxic drug, streptozotocin (STZ), reflects memory impairments, brain hypometabolism, cholinergic deficit, activation of microglia and neurodegeneration found in AD patients. Brain inflammation is important factor contributing to exacerbation of AD symptoms, but some studies show neuroprotective properties of pro-inflammatory cytokine IL-6. The aim of this study was to determine if dimethyl fumarate (DMF), which has anti-oxidative and immunosuppressive properties, can alleviate spatial working memory impairments in STZ-icv induced rat model of AD and change the expression of IL-6 in the brains.
METHODS: Four experimental groups were separated: STZ DMF (n=8) – STZ-icv infused and fed with 0.4% DMF fodder for three weeks until spatial memory test of Morris, STZ CTR (n=8) – STZ-icv infused and fed with standard fodder, and VEH DMF and VEH CTR groups (n=10) – vehicle-icv infused and fed with 0.4% DMF or standard fodder, respectively. Morris water maze testing was performed for three days, with four trials per day with unchanged platform location, and rats were then sacrificed and brains subjected to immunofluorescent IL-6 labeling.
RESULTS: The latency to reach the platform in each trial was significantly longer in the STZ CTR rats than in the remaining groups. STZ CTR was the only group which did not decrease the latency and the distance swum to platform in the consecutive trials. STZ-icv infused rats (STZ CTR and STZ DMF groups) had also lower number of the IL-6 expressing cells in the hippocampus and the hypothalamus than control VEH CTR rats.
CONCLUSION: Oral medication with DMF prevents spatial working memory impairment evoked by STZ-icv infusion, but has no influence on the central expression of IL-6.
The study was financed by the National Science Centre Poland on the basis of decision DEC-2013/09/D/NZ4/01658.

P1.8

BEYOND AMYLOID: ALTERED CELL CYCLE REGULATION IN ALZHEIMER'S DISEASE: COMPARISON OF P21 SIGNALING IN PATIENTS' LYMPHOCYTES AND BRAIN NEURONS

Anna Mietelska-Porowska[1], Joanna Wojsiat[1], Katarzyna Laskowska-Kaszub[1], Tomasz Stępień[2], Teresa Wierzba-Bobrowicz[2], Urszula Wojda[1]

[1]Laboratory of Preclinical Studies of Higher Standard, Neurobiology Center, Nencki Institute of Experimental Biology, Polish Academy of Science, Warsaw, Poland; [2]Department of Neuropathology, Institute of Psychiatry and Neurology, Warsaw, Poland

BACKGROUND AND AIMS: Alzheimer's disease (AD) develops for decades, but the molecular mechanism of pathogenesis is poorly understood. In result, an effective AD cure is still missing. According to the cell cycle (CC) hypothesis, one of the AD causes is CC reactivation in mature neurons. We aimed at elucidation if similar CC alterations occur in AD brain neurons and in peripheral blood cells.
METHODS: As the study materials, we used 40 lines of immortalized lymphoblasts from sporadic AD (SAD) patients and 40 lines from healthy non-demented individuals (controls)1-4. CC in lymphocytes was analyzed by real-time PCR-arrays, immunoblotting, and flow cytometry. Human post mortem brain tissue from AD patients was prepared by paraffin embedding and microscopic tissue slides of hippocampus and enthorinal cortex was analyzed by anti-p21 immunohistochemical staining.

RESULTS: Our data demonstrated aberrant CC in SAD lymphoblasts that involved a prolongation of the G1 phase driven by a marked increase of levels of p21 protein (Walf1/Cip1/Sid1), the key regulator of the G1/S CC checkpoint and of apoptosis. Consistently, we also found differences in p21 levels and its signaling pathway in apoptotic response of SAD lymphoblasts to redox stess. The analysis of p21 protein levels and related signaling in AD brain neurons will also be presented.
CONCLUSIONS: In summary, these studies indicate that p21-related molecular changes underlie altered cell cycle and apoptosis in AD pathology and may represent novel therapeutic targets. Moreover, our data show that AD have a features of a systemic disease with CC alterations in peripheral lymphoblasts which thus have a potential diagnostic value.
Support: CEPT, Polish National Science Centre grant NN401 596840, and JPND grant 2/BIOMARKAPD/JPND/2012.

P1.9

BLOOD AND CEREBROSPINAL FLUID BIOMARKERS IN ALZHEIMER`S AND PARKINSON'S DISEASES

Martyna Siudak[1], Marta Ziętek[2], Anna Tober-Marczewska[3], Andrzej Borman[2], Artur H. Świergiel[2]

[1]Department of Animal and Human Physiology, [2]Department of Animal and Human Physiology, [3]Students' Scientific Society Homunculus, University of Gdańsk, Gdańsk, Poland

BACKGROUND AND AIMS: Biomarkers are biological indicators that permit qualitative study and quantitative evaluation of various conditions, phenomena or biological features. In modern medicine biomarkers are used for precise and relatively easy diagnosis of chronic diseases, and the assessment of likelihood of their occurrence. Nowadays, it is intensively explored research area for new therapies and biochemical, physiological, histological, morphological, or behavioral types of biomarkers are distinguished. The aim was to explain previously poorly explored correlation between biological markers of stress and neurodegenerative diseases such as Alzheimer's and Parkinson's disease and systematization of knowledge.
METHODS: PubMed search was used to find the available literature data, key words: biomarkers, Parkinson's disease, Alzheimer's disease, blood, cerebrospinal fluid.
RESULTS: Parkinson's disease (PD) is a degenerative disorder of the central nervous system that involves degeneration of dopaminergic neurons in the substantia nigra pars compacta resulting in impairing the motor skills, cognitive process and other function. However, it has been shown that noradrenergic (NAergic) cells from the locus coeruleus also degenerate in this disease. On the other hand, Alzheimer's disease (AD) is manifested by neocortical and hippocampal atrophy, the deposition of Aβ peptides and the formation of neurofibrillar tangles. AD is a progressive degeneration of cholinergic nuclei in the basal forebrain and of NAergic nuclei in the brainstem. It is considered that neuronal loss is greater in NAergic neurons than cholinergic neurons.
CONCLUSIONS: Presented is a succinct review of the role and designation of biological markers in neurodegenerative diseases.

POSTER SESSION P2. CALCIUM

P2.1

TETRABROMOBISPHENOL A DIRECTLY INTERFERES WITH ACTIVITY OF NMDA RECEPTORS IN CULTURED NEURONS AND ISOLATED CORTICAL MEMBRANES

Dominik Diamandakis, Elżbieta Ziemińska, Elżbieta Salińska, Jerzy W. Łazarewicz

Department of Neurochemistry, Mossakowski Medical Research Centre, Polish Academy of Sciences, Warsaw, Poland

BACKGROUND AND AIMS: The results of early studies suggested a role of glutamate receptors in the mechanism of increases in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) and cytotoxicity induced by the brominated flame retardant, tetrabromobisphenol-A (TBBPA). Although now interest has focused mainly on TBBPA-induced Ca^{2+} release from intracellular stores, here we revisited the former issue and tested the involvement of NMDA receptors (NMDARs) in Ca^{2+} imbalance in neurons induced by TBBPA.
METHODS: These effects were examined in primary cultures of rat cerebellar granule cells (CGC), and then, using isolated cortical membranes we checked whether TBBPA directly interacts with the agonist and modulatory sites of the NMDAR complex. On the 7th day *in vitro* CGC were treated with TBBPA at low μM concentrations. ^{45}Ca uptake was detected and changes in $[Ca^{2+}]_i$, and plasma membrane potential were measured using fluorescent probes fluo-3 and oxonol VI, respectively. Moreover effects of TBBPA on specific binding of [^{3}H]MK-801, [^{3}H]glutamate and [^{3}H]glycine to isolated fraction of the rat brain cortex membranes were studied.
RESULTS: The results demonstrated that TBBPA concentration-dependently increased ^{45}Ca uptake and $[Ca^{2+}]_i$ in CGC, and the increase was partially inhibited by NMDARs antagonist, MK-801. This effect was additive to glutamate-induced Ca^{2+} transients. TBBPA increased oxonol VI fluorescence in CGC reflecting depolarization of the cultured neurons. The binding assays demonstrated potentiation by TBBPA binding of [^{3}H]MK-801 in the presence of NMDA and glycine, with maximum at 20 μM TBBPA, which was inhibited by spermidine and antagonists of the polyamines' site; inhibition by TBBPA of [^{3}H]glutamate binding, and no significant effect on [^{3}H]glycine binding.

CONCLUSIONS: TBBPA directly enhances the activity of NMDARs in neuronal membranes by interfering with their modulatory sites, and by inducing depolarization of neurons.
Supported by the NCN grant 2012/05/B/NZ7/03225.

P2.2

TETRABROMOBISPHENOL A-INDUCED OXIDATIVE STRESS IN PRIMARY CULTURES OF RAT CEREBELLAR GRANULE CELLS: TRIGGERING ROLE OF CA^{2+} IMBALANCE

Elżbieta Ziemińska, Jacek Lenart, Jerzy W. Łazarewicz
Department of Neurochemistry, Mossakowski Medical Research Centre, Polish Academy of Sciences, Warsaw, Poland

BACKGROUND AND AIMS: Brominated flame retardant tetrabromobisphenol A (TBBPA) contaminates the environment and displays cytotoxic potential. The mechanism of its cytotoxicity in neurons encompasses interference with NMDA and ryanodine receptors (NMDARs and RyRs) resulting in increase in intracellular Ca^{2+} concentration (Ca^{2+}i), and induction of oxidative stress, which may be primary, or secondary to rises in Ca^{2+}i. The aim of this study was to assess the role of excitotoxicity and Ca^{2+} imbalance in TBBPA-evoked oxidative stress and cytotoxicity in neurons.
METHODS: Using the primary cultures of rat cerebellar granule cells (CGC) we evaluated oxidative stress induced by an acute challenge with 10 or 25 μM TBBPA by measuring reactive oxygen species (ROS) production, intracellular glutathione (GSH) level, SOD-1 and SOD-2 level, catalase activity, and the level of Zn^{2+} in CGC. Zn^{2+} and Ca^{2+}i were measured fluoromertically, moreover TBBPA toxicity 24 h after the exposure was evaluated using propidium iodide staining. The pharmacological tools included NMDARs antagonist MK-801, ryanodine with bastadin 12 inhibiting RyRs, and various free radical scavengers.
RESULTS: TBBPA induced concentration-dependent decrease in CGC viability, increase in Ca^{2+}i and Zn^{2+} level, in ROS production, decrease in GSH level, catalase activity and SOD-2 level. Co-application of NMDARs and RyRs inhibitors which completely prevented increase in Ca^{2+}i, resulted in a significant, but not complete cytoprotection, and only partially reduced the oxidative stress in the CGC. Also administration of ROS scavengers provided only partial cytoprotection and reduction of oxidative stress.
CONCLUSIONS: We conclude that the production of free radicals resulting secondarily from TBBPA-evoked excitotoxicity and increases in Ca^{2+} level together with oxidative stress possibly induced primarily by TBBPA, both participate in TBBPA cytotoxicity in cultured neurons.
Supported by the NCN grant 2012/05/B/NZ7/03225.

P2.3

SENSITIVITY OF STORE-OPERATED CALCIUM ENTRY TO ANTAGONISTS OF IONOTROPIC RECEPTORS

Joanna Gruszczyńska-Biegała[1], Maria Śladowska[1,2], Jacek Kuźnicki[1]
[1]International Institute of Molecular and Cell Biology, Warsaw, Poland; [2]Undergraduate student, Warsaw University of Life Sciences – SGGW, Warsaw, Poland

BACKGROUND AND AIMS: In non-excitable cells Ca^{2+} enters through Store-Operated Ca^{2+} Entry (SOCE) pathway, involving STIM1, STIM2 and Orai1 proteins. Upon activation of neurons [Ca^{2+}] is increased in the cytoplasm as a result of Ca^{2+} influx from the extracellular environment mainly through voltage-operated Ca^{2+} channels and ionotropic receptor-operated Ca^{2+} channels (IR). Recently, another possibility has been shown into neurons – Ca^{2+} influx *via* SOCE. Our earlier data indicated that both STIMs are involved in Ca^{2+} homeostasis in neurons, form complexes with endogenous ORAI1 (Gruszczynska-Biegala and Kuznicki 2013, J Neurochem), but played a distinct role in SOCE (Gruszczynska-Biegala et al. 2011, PLoS ONE). The aim of this study is to determine, which receptors react with STIM proteins and are involved in SOCE. The potential STIM partners in plasma membrane, belong to 3 types of IR (NMDAR, AMPAR and kainate receptors – KR).
METHODS: In cultured cortical neurons we recorded single-cell Ca^{2+} levels using the ratiometric Ca^{2+} indicator Fura-2AM. SOCE was measured after depletion of intracellular Ca^{2+} stores by thapsigargin (TG) and subsequent incubation of cells in 2 mM Ca^{2+} media. To investigate the involvement of IR in TG-induced Ca^{2+} entry, we applied antagonists of these receptors such as NS-102 (KR), CNQX (AMPAR/KR), NBQX (AMPAR), MK-801 (NMDAR), memantine (NMDAR), and D-AP5 (NMDAR).
RESULTS: We found that SOCE was decreased by CNQX, NBQX, D-AP5, memantine but insignificant changes were observed in the presence of MK801 and NS-102. The results showed that NMDA and AMPA receptors are involved in SOCE pathway. The interaction between endogenous STIM1/STIM2 with IR will be checked by co-immunoprecipitation.
CONCLUSIONS: The identification of new partners of STIMs will allow us to better understand the mechanisms of SOCE in healthy neurons and during Alzheimer's disease degeneration. Supported by funds from a National Science Centre (2011/01/D/NZ3/02051, JGB).

P2.4

TRP CHANNELS PARTICIPATE IN MEMORY FORMATION IN PASSIVE AVOIDANCE TASK IN ONE-DAY OLD CHICKS

Elżbieta Salińska, Aleksandra Stafiej, Marta Słomka
Department of Neurochemistry, Mossakowski Medical Research Centre, Polish Academy of Sciences, Warsaw, Poland

BACKGROUND AND AIMS: Influx of calcium ions (Ca^{2+}) into neurons after stimulation of glutamate receptors is a crucial step in intracellular cascade of memory formation. Recent findings showed the existence of additional mechanism involved in intracellular Ca^{2+} increase and triggered not by external signal but by internal signals like increase of Ca^{2+} within the cell and activation of G protein coupled receptors. We are talking here about transient receptor potential (TRP) channels. The aim of our study was to investigate the participation of TRP channels, especially TRPC and TRPV in intracellular mechanisms engaged in memory consolidation.
METHODS: The model of passive avoidance task on one day old chicks was used. Chicks were injected with TRP channels antagonist SKF96365 and three different concentrations of 2-APB, the inhibitor of IP3 receptors, which in small concentrations inhibits also TRP channels. The injections were made at different times before and after training, to find the most effective time of interference.
RESULTS: We found that injection of all antagonists immediately after training resulted in task amnesia when tested 24 h later. The amnesic effect of injection of SKF96365 or 2-APB immediately after training was tested at different times. It appeared that SKF96365 injection resulted in constant amnesia that manifested 1.5 h after training, whereas amnesia after injection of 2-APB was observed as early as 30 min after training. The effect of application of TRP channels antagonist SKF96365 on memorizing of the task in comparison with the effects of mGluR1 and mGlR5 antagonists showed similarities when memory was tested 2 h and 24 h training.
CONCLUSIONS: Our results show that inhibition of TRP channels results in disturbance in memory formation and that inhibition of both TRP channels and IP3 receptors using small concentrations of 2-APB has a strong impact on this process.

P2.5

ROLE OF HUNTINGTIN-ASSOCIATED PROTEIN 1 IN THE REGULATION OF SOCE IN MEDIUM SPINY NEURONS FROM TRANSGENIC YAC128 MICE, A MODEL OF HUNTINGTON'S DISEASE

Magdalena Czeredys[1], Filip Maciąg[1,2], Vladimir Vigont[3], Elena Kaznacheyeva[3], Axel Methner[4], Jacek Kuźnicki[1]

[1]International Institute of Molecular and Cell Biology, Warsaw, Poland; [2]Warsaw University of Technology, Warsaw, Poland; [3]Institute of Cytology of the Russian Academy of Sciences, St. Petersburg, Russia; [4]Johannes Gutenberg University Medical Center Mainz, Mainz, Germany

BACKGROUND AND AIMS: Huntington's disease (HD) is a hereditary neurodegenerative disease caused by the expansion of a polyglutamine stretch in the huntingtin (HTT) protein and characterized by deregulated Ca^{2+} homeostasis (Giacomello et al. 2013). One of the mechanisms that regulate Ca^{2+} homeostasis is store-operated Ca^{2+} entry (SOCE) (Majewski and Kuźnicki 2015), which is enhanced in HD (Wu et al. 2011). The mechanism by which mutated HTT affects SOCE is unknown. The changes in Ca^{2+} homeostasis could be explained by increased expression of huntingtin-associated protein 1 (*Hap1*) mRNA (3-fold) and HAP1 protein (about 2-fold) in the striatum of YAC128 mice, which we detected (Czeredys et al. 2013). If HAP1 influences ER Ca^{2+} release mediated by IP_3R in MSN from YAC128 mice, which was shown in other HD models by Tang et al. (2003, 2004), its increased level may explain changes in SOCE described by Wu et al. (2011). The aim of this study is to determine the role of HAP1 protein in the regulation of SOCE in MSN from transgenic mice YAC128, an HD model.
METHODS: Using prepared lentiviral constructs we overexpressed HAP1a or HAP1b in YAC128 neurons, and in HEK293 cells overexpressing mutant HTT. We imaged cells with Fura-2AM, a selective fluorescent Ca^{2+} probe. SOCE was measured after depletion of intracellular Ca^{2+} by mGluR1/5 receptor agonist, DHPG (3,5-dihydroxyglycine) and subsequent incubation of cells in 2 mM Ca^{2+} media.
RESULTS: In HD MSN we detected about 10% increase in basal Ca^{2+} level and found that the activity of SOCE was enhanced about 30%, thereby confirming results of Wu et al. (2011). The preliminary results showed that overexpressed Hap1 protein is involved in SOCE pathway in studied cells. Using electrophysiology we will also determine changes in the SOC currents in MSN and SK-N-SH cells, both transduced with HAP1 and mutant HTT.
CONCLUSION: The investigation of the role of HAP1 protein in deregulated.

P2.6

THE EFFECT OF TETRAHYDROCARBAZOLES ON THE ER CALCIUM RELEASE AND SOCE IN MEDIUM SPINY NEURONS FROM TRANSGENIC YAC128 MICE, A MODEL OF HUNTINGTON'S DISEASE

Filip Maciąg[1,2], Magdalena Czeredys[2], Jacek Kuźnicki[2]

[1]Warsaw University of Technology, Warsaw, Poland; [2]International Institute of Molecular and Cell Biology, Warsaw, Poland

BACKGROUND AND AIMS: Huntington's disease (HD) is a genetic neurodegenerative disorder caused by an extended polyglutamine tail in the huntingtin (HTT) protein and manifesting itself by neurodegeneration of medium spiny neurons (MSN) in the striatum. An early event in the pathology of HD is deregulated Ca^{2+} homeostasis (Giacomello et al. 2013). One of the mechanisms that regulate Ca^{2+} homeostasis is store-operated Ca^{2+} entry (SOCE), which was shown to be enhanced in HD (Wu et al. 2011). However, the mechanism by which mutated HTT affects SOCE is still unknown and there is no effective treatment of HD.

RESULTS: Therefore, we assessed the alterations of the Ca^{2+} signalosome in the striatum of transgenic YAC128 mice, a model of HD (Czeredys et al. 2013). In MSN isolated from these mice we detected an about 10% increase in the basal Ca^{2+} level. We found that the activity of SOCE was enhanced about 30%. Since the deregulation of Ca^{2+} homeostasis and signaling is considered to be the primary event in HD, the aim of this work was to investigate the effect of compounds called tetrahydrocarbazoles on the ER Ca^{2+} release induced by $mGluR_{1/5}$ receptor agonist, DHPG (3,5-dihydroxyglycine), as well as their influence on SOCE in MSN from YAC128 mice. It was previously shown that tetrahydrocarbazoles stabilize the ER Ca^{2+} release induced by carbachole in HEK293 cells overexpressing mutated presenilin 1, a cellular Alzheimer's disease model (Honarnejad et al. 2014). We have confirmed by immunostaining that in our *in vitro* model of HD, MSN culture from YAC128 mice, $mGluR_{1/5}$ receptors are specifically expressed. The MSN cells were treated with chosen tetrahydrocarbazoles and Ca^{2+} was imaged with Fura-2 AM.
CONCLUSIONS: Our preliminary data suggest that some tested compounds have a stabilizing effect on Ca^{2+} level in HD cellular model. Tetrahydrocarbazoles could be potentially used as drugs stabilizing the disturbed Ca^{2+} homeostasis in Huntington's disease.

P2.7

STIM1, STIM2 AND ORAI1 – OVEREXPRESSION OF KEY STORE OPERATED CALCIUM ENTRY IN TRANSGENIC MICE BRAIN

Łukasz Majewski[1], Grzegorz Wiera[2], Tomasz Wójtowicz[2], Paweł M. Boguszewski[3], Jerzy Mozrzymas[2], Jacek Kuźnicki[1]
[1]Laboratory of Neurodegeneration, International Institute of Molecular and Cell Biology, Warsaw, Poland; [2]Department of Biophysics, Laboratory of Neuroscience, Wroclaw Medical University, Wroclaw, Poland; [3]Laboratory of Limbic System, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland

BACKGROUND AND AIMS: Alzheimer's disease (AD) is the most common age-related neurodegenerative disorder. At least two types of AD can be distinguished: sporadic AD (SAD) of unknown etiology, which accounts for most cases, and genetically encoded familial AD (FAD), which affects up to 5% of all patients. Altered calcium homeostasis in neurons is proposed to be one of the early events responsible for AD. Disturbances in Ca^{2+} signaling are found in SAD patients before any obvious extracellular Aβ pathology; moreover, Ca^{2+} dysfunction augments Aβ formation and Tau hyperphosphorylation. One of the objectives of our present project is to understand how elevated basal Ca^{2+} level in neurons contributes to neurodegeneration.
METHODS: Generation of transgenic mice using DNA microinjection technique.
RESULTS: We have generated three transgenic mouse lines independently overexpressing, specifically in brain neurons, key proteins of SOCE – STIM1, STIM2 and Orai1. The phenotype of these mice is being analyzed by electrophysiology, behavior and Ca^{2+} imaging. Our group has shown that the cytoplasmic resting Ca^{2+} level in cultured neurons can be modulated by overexpression of STIM proteins, ER Ca^{2+} sensors involved in the Store Operated Calcium Entry (SOCE). We also detected the enhanced magnitude of Ca^{2+} influx during SOCE in human lymphocytes from SAD patients, and decreased level of STIM2 protein in human lymphocytes from FAD patients in parallel to an attenuation of SOCE.
CONCLUSIONS: The obtained lines can be a suitable model to verify the hypothesis that brain dysfunction during ageing is induced by changes in Ca^{2+} homeostasis.

P2.8

REGULATION OF NECTIN-3 PROCESSING IN THE HIPPOCAMPUS MEDIATED BY MMP-9 UNDER THE CHRONIC STRESS CONDITIONS

Emilia Rejmak-Kozicka, Katarzyna Kalita, Leszek Kaczmarek
Laboratory of Neurobiology, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland

BACKGROUND AND AIMS: Nectin-3 is Ca^{2+}-independent immunoglobulin (Ig)-like cell-cell adhesion molecule (CAMs) that is involved in the organization of various types of intercellular junctions, including interneuronal synapses. How cleavage of nectin-3 is regulated in neuronal cells is poorly understood. Emerging evidence suggest a role for CAMs and extracellular matrix remodeling in mechanisms that underlie the behavioral effects of stress. We tested the hypothesis that nectin-3 is involved in hippocampal region-specific effects induced by chronic stress.
METHODS: Adult male Spraque-Dawley rats were restrained 6 h/day for 21 days in wire mesh restrainers in their home cages. In the experiments that involved Western blot and gel zymography analyses, the rats were sacrificed by decapitation and CA1 or CA3 of the hippocampal formation were collected for synaptoneurosomes preparation.
RESULTS: The results show an increase in the nectin-3 cleavage in synaptoneurosomal fraction obtained from the hippocampal CA1 field stimulated with glutamate in the group of rats subjected to chronic stress procedure as compared to the control group. Proteolytic cleavage of the nectin-3 results in the appearance of a 20 kDa nectin-3 derived fragment. The cleavage was inhibited by the MMP-9 inhibitor (inhibitor I). Moreover, we shown that the increased nectin-3 proteolysis under chronic stress conditions cor-

relates with the elevated MMP-9 activity in the rat hippocampal CA1 fragment. In addition, taking into account the possibility of non-specific activity of the inhibitor we used we demonstrated experimentally its specific activity towards MMP-9. To test the connection between the nectin-3 shedding and activity of the MMP-9 *in vitro* experiments were performed on the hippocampal cultures.
CONCLUSION: Obtained results clearly confirm that this protease causes fragmentation of the nectin-3. Furthermore, it was shown that this process depends on the activation of NMDA receptor and the presence of Ca^{2+}/calmodulin.

POSTER SESSION P3. NEUROTRANSMITTER RECEPTORS AND SIGNALING PATHWAYS

P3.1
A_2-ADRENERGIC MEDIATION OF MEMBRANE POTENTIAL CHANGES IN MEDIAL PREFRONTAL CORTEX (mPFC) PYRAMIDAL NEURONS IN YOUNG RATS

Katarzyna Grzelka, Paweł Szulczyk
Medical University of Warsaw, Warsaw, Poland

BACKGROUD AND AIMS: Impairment of the signal transduction from adrenergic receptors to cellular effectors in prefrontal cortex (PFC) neurons occurs in many neuropsychiatric disorders (acute stress disorder, ADHD). Application of clonidine (α_2-adrenergic receptor agonist) evokes hyperpolarisation of the resting membrane potential in mPFC pyramidal neurons. The aim of the study was to define the cellular effectors and the exact signal transduction pathway from the receptor to the effector, which still remains unclear.
METHODS: The membrane potential was recorded in layer V mPFC pyramidal neurons in slices isolated from 3-week-old rats. Recordings were performed in perforated-patch configuration at a temperature of 34°C. Adrenergic antagonists, inhibitors of cellular effectors and intracellular signalling were applied to the bath medium before, during and after clonidine application (100 μM). Their effects on clonidine-dependent membrane potential changes were analysed.
RESULTS: The α_2-receptor antagonists yohimbine (60 μM, *n*=15) and atipamezole (20 μM, *n*=6) did not completely block clonidine-dependent hyperpolarisation. The effect of clonidine was attenuated by the blocker of hyperpolarisation-activated cyclic nucleotide-gated (HCN) channels (ZD7288, 50 μM, *n*=10) and by the selective Na^+/K^+-ATPase inhibitor (ouabain, 100 μM, *n*=7). The hyperpolarisation was affected neither by the adenylyl cyclase inhibitor (SQ22536, 100 μM, *n*=6), protein kinase A inhibitor (H-89, 40 μM, *n*=6), phospholipase C inhibitor (U73122, 10 μM, *n*=7) nor the protein kinase C inhibitor (chelerythrine, 5 μM, *n*=5), but it was attenuated by the G-protein βγ-subunit inhibitor (gallein, 20 μM, *n*=12).
CONCLUSIONS: α_2-Adrenergic receptor activation evokes hyperpolarisation due to HCN channel inhibition and modification of the Na^+/K^+-ATPase function. The transduction pathway occurs in a membrane-delimited fashion and involves the $G_{\beta\gamma}$ subunit released from the G-protein.
Supported by grants no: NN401584638 and NN301572940.

P3.2
GABAERGIC TRANSMISSION IN THE RAT DORSAL RAPHE NUCLEUS IS MODULATED BY THE 5-HT7 RECEPTOR

Joanna Sowa[1], Magdalena Kusek[1], Katarzyna Kamińska[2], Krystyna Gołembiowska[2], Krzysztof Tokarski[1], Grzegorz Hess[1]
[1]Department of Physiology, [2]Department of Pharmacology, Institute of Pharmacology, Polish Academy of Sciences, Kraków, Poland

BACKGROUND AND AIMS: The 5-HT7 receptor is one of the several 5-HT receptor subtypes which are expressed in DRN neurons. Some previous findings suggested that 5-HT7 receptors in the DRN are localized on local GABAergic interneurons, which modulate the activity of 5-HT projection neurons. The aims of this study were to determine how the 5-HT7 receptor activation and blockade influence the GABAergic synaptic input to presumed 5-HT DRN neurons and whether blockade of the 5-HT7 receptor would affect the release and metabolism of 5-HT in the prefrontal cortex *in vivo*.
METHODS: Male Wistar rats, with microdialysis probes implanted in the PFC, received ip injections of 5-HT7 receptor antagonist, SB 269970. 5-HT and 5-HIAA, were analyzed by HPLC. In another set of experiments whole-cell recordings were carried out from DRN slices. SB 269970 was used to block the 5-HT7 receptor. To activate the 5-HT7 receptor 5-CT was applied in the presence of WAY 100635.
RESULTS: Ip administration of SB269970 induced an increase in the level of 5-HT and 5-HIAA in PFC. SB 269970 application resulted in a depolarization of presumed DRN projection neurons and in an increase in the spontaneous firing frequency. A hyperpolarization of the cells and a decrease in the spontaneous firing frequency were observed after activation of the 5-HT7 receptor. Blockade of the 5-HT7 receptor caused a decrease in the mean frequency of sIPSCs, while its activation induced an increase.
CONCLUSIONS: These results show that blockade of the 5-HT7 receptor enhances the release and metabolism of 5-HT in the PFC. This effect appears to be mediated by depolarization and enhanced firing of DRN serotonergic neurons resulting from a decreased inhibitory synaptic input received by the projection cells. Activation of the 5-HT7 receptor caused opposite effects on activity and the inhibitory input to putative DRN projection neurons.
Support: National Science Centre Poland grant DEC-2013/11/B/NZ4/04743.

P3.3

SUBDIAPHRAGMATIC VAGOTOMY PREVENTS RESTRAINT STRESS-INDUCED ALTERATIONS IN GLUTAMATERGIC TRANSMISSION AND LTP IN THE RAT FRONTAL CORTEX

Bartosz Bobula[1], Paulina Rachwalska[1], Grzegorz Hess[1,2]

[1]Institute of Pharmacology, Polish Academy of Sciences, Kraków, Poland; [2]Institute of Zoology, Jagiellonian University, Kraków, Poland

BACKGROUND AND AIMS: Repeated exposure of experimental animals to stress induces a spectrum of depression-like symptoms including anorexia, weight loss, anhedonia, fatigue, impaired social interactions and memory dysfunctions. However, the mechanisms of the influence of stress on glutamatergic transmission and synaptic plasticity in the cerebral cortex remain poorly understood. The vagus nerve appears to be an important neural pathway for communicating immune signals originating in the periphery to the brain. Subdiaphragmatic vagotomy (SV) had earlier been shown to inhibit behavioral and neural effects of peripheral interleukin-1beta (IL-1β). We have previously demonstrated that peripherally produced IL-1β may mediate the influence of repeated restraint stress on the functions of the frontal cortex. The purpose of this study was to determine whether the vagus nerve mediates the effects of repeated restraint stress on excitatory synaptic transmission and long-term potentiation (LTP) in the rat frontal cortex.

METHODS: Subdiaphragmatic vagotomy or sham surgery was performed 10 days before restraint stress and electrophysiological measurements. The effects of 10 min restraint stress, repeated twice daily for 3 consecutive days were studied *ex vivo* in the rat frontal cortex slices prepared 24 h after the last stress session.

RESULTS: In slices originating from stressed animals, the amplitude of field potentials was increased, compared to control preparations. Consistent with the previous studies, restraint stress resulted in a reduced magnitude of LTP. Stress-induced modifications of the glutamatergic transmission and synaptic plasticity were prevented by SV procedure.

CONCLUSION: These data suggest that the vagus nerve may mediate the influence of repeated restraint stress on the rat frontal cortex.

P3.4

PROLONGED INCUBATION WITH DESIPRAMINE DIFFERENTIALLY MODULATE A1A- AND A1B-ADRENORECEPTOR SIGNALING IN PC12 CELLS

Piotr Chmielarz, Marta Kowalska, Katarzyna Rafa-Zabłocka, Irena Nalepa

Department of Brain Biochemistry, Institute of Pharmacology, Polish Academy of Sciences, Kraków, Poland

BACKGROUND AND AIMS: Action of tricyclic antidepressant drugs (TCA) involves inhibition of noradrenaline re-uptake, however, several of TCA also exhibit affinities for adrenergic receptors (α1-ARs). Furthermore there are reports in literature implying role of α1-AR in depression and antidepressant action. However due to scarcity of selective ligands discriminating the α1-ARs, the specific role of each of three subtypes remains elusive. Aim of current study was to investigate if and how prolonged incubation with desipramine (DMI), a TCA exhibiting affinity for α1-AR could affect the α1A- and α1B-adrenoreceptor reactiveness *in vitro*.

METHODS: Measurements were performed in PC12 cells stably transfected with human α1A- and α1B-AR. To assess receptor activity cells expressing either one of the receptors were incubated for 24 hours with 10 μM DMI or vehicle. After incubation receptor responsiveness to agonist was assayed by stimulation with serial dilutions of noradrenaline (form 10^{-9} to 10^{-4} M) and measurement of inositol phosphate generation with use of TR-FRET based assay.

RESULTS: The 24 hours preincubation with DMI shifted the noradrenaline dose response curve rightwards in case of both α1A-AR (EC50 = 0.9 μM and EC50 = 14.5 μM for VEH and DMI preincubated cells, respectively) and α1B-AR (EC50 = 0.5 μM and EC50 = 2.7 μM, for VEH and DMI preincubated cells, respectively). The effect of DMI was more pronounced for α1A-AR and difference between receptor subtypes was reflected in significant receptor×treatment interaction ($P<0.001$) in two-way ANOVA comparison.

CONCLUSIONS: Our data indicate that DMI can differentially modulate activity of α1A- and α1B-AR. The diverse susceptibility of α1A- and α1B-AR to DMI action may be interesting in the light of reports of different role of these receptors in depressive-like behaviors in mice.

This study was supported by statutory funds of Institute of Pharmacology, PAS.

P3.5

STUDY OF CHEMICAL CODING OF THE CSMG NEURONS SUPPLYING PREPYLORIC REGION OF THE PORCINE STOMACH FOLLOWING ACETYLSALICYLIC ACID SUPPLEMENTATION AND AFTER PARTIAL STOMACH RESECTION

Jarosław Całka, Katarzyna Palus

Department of Clinical Physiology, Faculty of Veterinary Medicine, University of Warmia and Mazury in Olsztyn, Olsztyn, Poland

BACKGROUND AND AIMS: The present study was designed to define localization and chemical coding of the sympathetic perikarya innervating the porcine stomach prepyloric area in physiological state, following prolonged aspirin supplementation and after partial stomach resection.

METHODS: The study was performed on 3 groups of 5 immature female pigs. The neuronal retrograde marker Fast Blue (FB) was

injected into the anterior prepyloric wall of the stomach of control, acetylsalicylic acid treated (ASA) and partial stomach resection (RES) groups. Animals in ASA group were given acetylsalicylic acid orally for 21 days. On 22nd day after injection, in animals of RES group partial stomach resection was performed. On 28th day all pigs were euthanized. Then cryostat sections were double immunolabeled using primary antisera directed towards tyrosine hydroxylase (TH), dopamine β-hydroxylase (DβH), neuropeptide Y (NPY), galanin (GAL), somatostatin (SOM), nitric oxide synthase (NOS), Leu 5-Enkephalin (LENK). As the secondary antibody AlexaFluor 546 and AlexaFluor 488 were applied. In the coeliac-superior mesenteric ganglion (CSMG) 1615±20.73 FB+ (gastric) neurons have been identified.

RESULTS: In the control group, gastric neurons expressed TH (94.85±1.01%), DβH (97.10±0.97%), NPY (46.88±2.53%), SOM (14.97±1.57%) and GAL (8.40±0.53%). In ASA and RES groups, TH- and DβH- positive nerve cells were reduced (ASA: 81.78±0.87% and 86.42±0.94%; RES: 84.56±2.56% and 88.30±1.62%). Moreover, in both groups increased expression of NPY (ASA: 76.59±3.02%, RES: 64.93±2.32%), SOM (ASA: 33.72±4.39%, RES: 39.02±3.65%), GAL (ASA: 26.45±2.75%, RES: 31.13±1.64%) as well as *de novo* synthesis of NOS (ASA: 6.13±1.11%, RES: 7.29±0.65%) and LENK (ASA: 4.77±0.42%, RES: 3.92±0.45%) in traced CSMG neurons were observed.

CONCLUSIONS: Our results indicate involvement of studied substances in the development and presumably counteraction of gastric inflammation and survival of damaged neurons.

P3.6

GLUTAMATERGIC AND GABAERGIC TRANSMISSION IN RAT PVN IS ALTERED AFTER ACUTE RESTRAINT STRESS

Magdalena Kusek[1], Anna Tokarska[2], Krzysztof Tokarski[1], Grzegorz Hess[1,2]

[1]Institute of Pharmacology Polish Academy of Sciences, Kraków, Poland; [2]Institute of Zoology, Jagiellonian University, Kraków, Poland

BACKGROUND AND AIMS: The hypothalamic paraventricular nucleus (PVN) plays a key role in the activation of the hypothalamic-pituitary-adrenal axis (HPA). In response to stress, corticotropin releasing hormone (CRH) is released from parvocellular PVN neurosecretory neurons into hypophysial portal vessels that access the anterior pituitary gland to stimulate the production of the adrenocorticotropic hormone (ACTH), which stimulates the adrenal cortex to produce glucocorticoid hormones. It is known that excitatory and inhibitory inputs that regulate the activity of parvocellular PVN neurosecretory neurons may undergo stress-related modifications. However, the influence of acute restraint stress on the function of glutamatergic and GABAergic synapses in PVN is not fully understood.

METHODS: Adolescent male Wistar rats were subjected to acute restraint lasting 10 min. Animals were decapitated either immediately after the stress session or 24 hours later. Whole-cell patch-clamp was used to record spontaneous and miniature excitatory and inhibitory postsynaptic currents (sEPSCs/mEPSCs, sIPSCs/mIPSCs) from parvocellular neuroendocrine neurons of the PVN *ex vivo*.

RESULTS: In animals decapitated immediately after the stress session, an increase in the mean frequency of sEPSCs/mEPSCs was observed. These effects were accompanied by a decrease in the mean frequency of sIPSCs/mIPSCs. The kinetics and amplitude of the currents remained unchanged. In slices prepared 24 h after the restraint there was no change in the frequency and amplitude of all recorded currents. Also the basal electrophysiological properties and the excitability of the neurosecretory parvocellular neurons remained unchanged in all tested slices.

CONCLUSIONS: Acute immobilization stress results in a transient (less than 24 h) enhancement of the glutamatergic and an attenuation of the GABAergic synaptic input to neurosecretory parvocellular neurons in the rat PVN.

Support: Grant 2012/07/N/NZ4/02687.

P3.7

THE ROLE OF TTYH1 PROTEIN IN REGULATION OF DENDRITIC TREE AND SPINE FORMATION

Małgorzata Górniak-Walas, Aleksandra Kaliszewska, Katarzyna Łukasiuk

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

BACKGROUND AND AIMS: Tweety homolog1 (Ttyh1) is a presumed volume-regulated Cl^- channel, regulated by cell swelling. Ttyh1 overexpression in dissociated cultures of hippocampal neurons leads to increase in neuritognesis implicating its role in regulation of neuronal morphology. However, the role of Ttyh1 protein in regulation of dendritic tree and spine formation is not well understood. The aim of the study was to examine the influence of Ttyh1 protein in regulation of dendritic tree complexity and formation of dendritic spines in organotypic hippocampal cultures with preserved three dimensional structure.

METHODS: Organotypic hippocampal slices were performed from 6 days old rats and transfected with plasmids coding RFP under β-actin promoter and Ttyh1-GFP under synapsin promoter or respective controls, using the Gene Gun. Pyramidal neurons in two areas of the hippocampus: CA1 and CA3 were analyzed using Neuromantic and Sholl software to quantify dendritic tree complexity and dendritic length. SpineMagick software was used to examine

dendritic spine morphology and spine density. Proximal and distal parts of apical secondary branches in the stratum radiatum and basal dendrites were analyzed. Dendritic parameters from different groups were compared using a Student's *t* test.

RESULTS: Sholl analyses did not reveal significant differences in arborization of CA1 and CA3 neurons overexpressing Ttyh1 in comparison to control neurons. Dendritic spine analysis revealed considerable reduction in number and size of dendritic spines. Ttyh1 transfected neurons had varicose swelling of dendrites and more stubby and mushroom spines.

CONCLUSIONS: On the basis of our results we conclude that Ttyh1 may participate in regulation of dendritic spine formation. Ttyh1 protein does not affect on dendritic complexity. Formation of dendritic beading may be dependent on Na^{2+} and Cl^- intracellular movement, followed by water movement to maintain osmolarity.

P3.8

DIFFERENT PHARMACOLOGICAL PROFILE IN ALPHA$_1$-GAMMA$_2$ AND ALPHA$_1$-BETA$_2$-GAMMA$_2$ GABA$_A$ RECEPTORS

Marek Brodzki[1,2], Marta M. Czyżewska[2], Radosław Rutkowski[2], Magdalena Kisiel[2], Magdalena Jatczak[1,2], Jerzy W. Mozrzymas[1,2]

[1]Laboratory of Cellular Neurobiology, Department of Animal Molecular Physiology, University of Wrocław, Wrocław, Poland; [2]Laboratory of Neuroscience, Department of Biophysics, Wrocław Medical University, Wrocław, Poland

BACKGROUND AND AIMS: $GABA_A$ receptors ($GABA_AR$) mediate the main component of ionotropic inhibitory transmission in adult mammalian brain. These receptors are heteropentamers and are strongly diversified throughout the CNS but the most frequent subunit composition is alpha$_1$-beta$_2$-gamma$_2$. It has been reported that alpha$_1$-gamma$_2$ receptors can be potently expressed in recombinant $GABA_AR$ model (Verdoorn et al. 1990). In this study we aimed to characterize the kinetic and pharmacological profile of these receptors in comparison to alpha$_1$-beta$_2$-gamma$_2$ ones.

METHODS: We used patch-clamp technique with ultrafast (~10e–4 s) solution exchange based on theta-glass capillaries driven by a piezoelectric translator. We used HEK293 cells which were transiently transfected by $GABA_AR$ subunit cDNA using standard calcium phosphate method. Modulators used were zinc ions, low extracellular pH, flurazepam and pentobarbital.

RESULTS: Zinc ions inhibited current responses more strongly for alpha$_1$-gamma$_2$ $GABA_ARs$, however, zinc effect on desensitization onset was observed only for alpha$_1$-beta$_2$-gamma$_2$ receptors. In the case of responses mediated by alpha$_1$-beta$_2$-gamma$_2$ receptors, lowering extracellular pH enhanced current amplitudes and prolonged deactivation time course of currents elicited by short and saturating GABA pulses to a much larger extent than for alpha$_1$-gamma$_2$ ones. Saturating GABA elicited responses mediated by alpha$_1$-beta$_2$-gamma$_2$ receptors were slightly inhibited by flurazepam, whereas in the case of alpha$_1$-gamma$_2$ receptors, currents were significantly potentiated. Activation by high concentrations of pentobarbital yielded similar rebound current amplitudes in both receptor subtypes.

CONCLUSIONS: We found that although alpha$_1$-gamma$_2$ receptors show a similar kinetic profile to alpha$_1$-beta$_2$-gamma$_2$ receptors they are characterized by pharmacological properties that are substantially different.

Supported by NCN grant DEC-2013/11/B/NZ3/00983.

P3.9

PROLONGED ELEVATION OF CORTICOSTERONE LEVEL ENHANCES SPONTANEOUS GLUTAMATE RELEASE IN THE RAT MOTOR CORTEX

Anna Czerw[1], Joanna Kula[1], Grzegorz Tylko[1], Anna Błasiak[1], Grzegorz Hess[1,2]

[1]Institute of Zoology, Jagiellonian University, Kraków, Poland; [2]Department of Physiology, Institute of Pharmacology, Polish Academy of Sciences, Kraków, Poland

BACKGROUND AND AIMS: Repeated and chronic forms of stress constitute a major risk factor in serious pathologies including depression, cognitive impairments and motor control dysfunctions. However, the mechanisms of the influence of prolonged elevation of the level of corticosteroid hormones on synaptic transmission in the cerebral cortex remain poorly understood.

METHODS: We studied the effects of the treatment of male Wistar rats with corticosterone for 7 days on excitatory and inhibitory synaptic inputs as well as on the excitability of layer II/III pyramidal neurons of the rat motor cortex. Spontaneous excitatory and inhibitory postsynaptic currents (sEPSCs and sIPSCs) were recorded from pyramidal cells in *ex vivo* slices of the rat frontal cortex, prepared 2 days after the last administration of the hormone.

RESULTS: Corticosterone treatment induced an increase in the frequency but not the amplitude of sEPSCs. Measurements with the use of tetrodotoxin (TTX) revealed that most of the recorded sEPSCs represented miniature EPSCs (mEPSCs). The frequency and amplitude of sIPSCs as well as the excitability of pyramidal cells remained unchanged. Corticosterone treatment modified neither the density of dendritic spines on pyramidal neurons nor the protein density levels of selected subunits of AMPA, NMDA and $GABA_A$ receptors.

CONCLUSIONS: Thus, prolonged administration of exogenous corticosterone selectively enhances glutamatergic transmission in the rat motor cortex, most likely *via* an enhancement of spontaneous glutamate release from presynaptic terminals.
Support: National Science Centre, Poland, grant UMO-2012/07/B/NZ4/01669.

P3.10

POSSIBLE NEURAL COMPENSATORY MECHANISMS FOR THE COMMUNICATION BETWEEN THE NERVOUS AND IMMUNE SYSTEMS AFTER CYCLOOXYGENASE INHIBITION

Anna Kobrzycka[1], Marek Wieczorek[1], Joanna Zubel[2], Ewa Oclon[2], Magdalena Hubner[1]

[1]Department of Neurobiology, Faculty of Biology and Environmental Protection, University of Łódź, Łódź, Poland; [2]Animals Physiology and Endocrinology, Agriculture University, Kraków, Poland

BACKGROUND AND AIMS: The phenomenon of communication between the nervous and immune systems is commonly accepted. There were postulated several pathways for information exchange between these two systems, among them: neuronal with the vagus nerve, and enzymatic exercised through cyclooxygenases. The fact of biological compensation of many processes in our organisms is well known. In our project we hypothesized the existence of such phenomenon is situation of affected pathway for transferring of immune signal from the periphery to the brain after inhibition of cyclooxygenases.
METHODS: To investigate the existence of these mechanisms, neurochemical changes occurring in the hypothalamus (the initial section of the stress axis – HPA) after intraperitoneal administration of LPS (10 mcg/animal) were analyzed. We studied the effect of long-term (10 days) administration of cyclooxygenases inhibitors on the neurochemical changes in the activity of the hypothalamus in response to LPS or saline ip administration. The rats were subcutaneously injected with selective cyclooxygenases inhibitors; Celecoxib (10 mg/kg) and SC-560 (3 mg/kg), respectively.
RESULTS: Future HPLC analysis shown an increased activity of the noradrenergic and serotonergic systems within the hypothalamus. These data are comparable with those obtained after saline injections.
CONCLUSIONS: These results suggest the presence of compensatory mechanisms responsible for the stimulation of the HPA axis after peripherally immune challenges. They also suggest that long-term intake of non-steroidal anti-inflammatory drugs may not affect, or in a very minor degree, the relationships between the immune and the nervous systems.
Supported by NCN: UMO-2012/07/B/NZ4/00205.

P3.11

SUBDIAPHRAGMATIC VAGOTOMY DOES NOT PROTECT AGAINST THE INCREASE OF NORADRENERGIC RESPONSE OF THE RAT

Marek Wieczorek[1], Anna Kobrzycka[1], Magdalena Hubner[1], Ewa Oclon[2], Joanna Zubel[2], Artur Świergiel[3]

[1]Department of Neurobiology, Faculty of Biology and Environmental Protection, University of Łódź, Łódź, Poland; [2]Animals Physiology and Endocrinology, Agriculture University, Kraków, Poland; [3]Faculty of Biology, University of Gdańsk, Gdańsk, Poland

BACKGROUND AND AIMS: Peripheral administration of gram-negative bacteria endotoxin – lipopolisaccharide (LPS) is known to activate the hypothalamo-pituitary adrenal axis (HPAA) and brain noradrenergic systems. We studied the vagotomized rats responses to peripherally administered LPS using the HPLC-ED to measure the concentration of noradrenaline and their metabolite MHPG in various brain regions.
METHODS: Rats were submitted to subdiaphragmatic vagotomy and after 30 days we used them for experiments. They were injected with saline (100 µl ip) and LPS (10 µg/100 µl ip) in random order, and two hours after injection they were euthanized. They brains where removed from the skull and we isolated the hypothalamus, amygdala, prefrontal medial cortex, hippocampus periaqueductal gray matter and the brainstem and submitted for chromatographic analysis.
RESULTS: Future chromatographic analysis indicates, that subdiaphragmatic vagotomy did not protect against increase of noradrenaline concentration in analyzed brain regions. In case of LPS injected animals we observed increased noradrenaline concentration *versus* saline injected ones. These results were comparable with those observed in sham operated rats.
CONCLUSIONS: There results suggest that there may be compensatory mechanisms, responsible for transferring of immune signal to the brain, and develop during such a long time of recovery after subdiaphragmatic vagotomy.
Supported by NCN, grant no. UMO-2012/07/B/NZ4/00205.

P3.12

PHOENIXIN IS PRESENT IN HYPOTHALAMIC NEUROENDOCRINE NUCLEI OF THE DOMESTIC PIG – IMMUNOHISTOCHEMICAL STUDY

Beata Hermanowicz[1], Krystyna Bogus-Nowakowska[1], Artur Pałasz[2], Ryszard Wiaderkiewicz[2], Anna Robak[1]

[1]Department of Comparative Anatomy, Faculty of Biology and Biotechnology, University of Warmia and Mazury in Olsztyn, Olsztyn, Poland; [2]Department of Histology, Medical University of Silesia, Katowice, Poland

BACKGROUND AND AIMS: Phoenixin (PNX) is one of the last revealed peptide in the rat hypothalamus. PNX so called a satiety

molecule, takes part in such processes as the regulation of energy metabolism and also in reproduction. The aim of the study was to examine PNX immunoreactive structures (PNX-ir) and their distribution in the neuroendocrine part of the pig (*Sus scrofa domestica*) hypothalamus, because PNX was examined only in rodent brains.
METHODS: Hypothalamic tissue was prepared by immunohistochemical techniques (immunofluorescence and DAB methods) with using Phoenixin-14 amide (H-079-01; Phoenix).
RESULTS: PNX was immunodetected in neurons of the paraventricular (PVN) and supraoptic (SON) nuclei and also in neighbouring areas. PNX-ir cells had oval or multipolar perikarya with 1 to 4 visible primary dendrites. PNX-ir cells in the PVN were situated loosely at dorsal and ventral parts close to the third ventricle, whereas between these parts PNX-ir cells were numbered and clustered. PNX-ir structures with morphology like dendrites and also single fibres covered with varicosities resembled axons were observed in the neuropil of the PVN. PNX-ir cells in the SON were clustered on the medial side of the SON from which narrow band of the PNX-ir perikarya was directed to the lateral side, along the optic tract. PNX-ir perikarya in the SON have similar shapes as in the PVN, but some of them possess short protoplasmic irregular processes, what gives them irregular shapes. In the SON there was not observed immunoreactive structures in neuropil, as above described in the PVN.
CONCLUSIONS: This is the first study which demonstrates the presence of PNX in axons. These results suggest that PNX in the PVN and SON may differ in signaling mechanism or acting as molecule-regulated neuroendocrine factor, but multidirectional functions of PNX complicate the understanding of the role played by this neuropeptide and further studies are needed.

P3.13

DISTRIBUTION AND IMMUNOHISTOCHEMICAL CHARACTERISTICS OF CEREBELLAR PROJECTING NEURONS IN THE LOCUS COERULEUS COMPLEX OF THE RABBIT

Dorota Bukowska[1], Leszek Zguczyński[2], Barbara Mierzejewska-Krzyżowska[2], Waldemar Sienkiewicz[3], Jerzy Kaleczyc[3]

[1]Department of Neurobiology, University School of Physical Education, Poznań, Poland; [2]Department of Anatomy, Biology and Health Sciences, University School of Physical Education, Gorzów Wlkp., Poland; [3]Department of Animal Anatomy, Faculty of Veterinary Medicine, University of Warmia and Mazury, Olsztyn, Poland

BACKGROUND AND AIMS: The locus coeruleus complex (LCx), subdivided into nucleus coeruleus proper (LC) and subcoeruleus (LCα) is the major noradrenergic nucleus of the brain. Only a few data concern LCx-cerebellar projection. The aim of study was to identify LCx neurons projecting to the caudal vermis and determine their immunohistochemical characteristics.
METHODS: The retrograde axonal transport method of fluorescent tracers Fast Blue (FB) and Diamidino Yellow injected respectively into lobules VIII and IX was used. In addition, double labeling immunofluorescence was applied to investigate the expression and coexistence of dopamine-β-hydroxylase (DBH) and choline acetyltransferase as well as neuropeptides including neuropeptide Y (NPY), somatostatin (SOM), leu-enkephalin (LENK) and substance P in LCx-cerebellar projecting neurons.
RESULTS: It was shown that only lobules VIII was supplied by afferents from LCx. The FB-labeled neurons were distributed bilaterally with ipsilateral predominance in both LC and LCα, and were much more frequent in LC. They occupied the lateral region of LC and dorsolateral region of LCα. These neurons were the most numerous in the caudal part of LC, but in LCα they clustered in its rostral part. Nearly all FB-labeled neurons displayed immunoreactivity to DBH and only the single cells stained simultaneously for DBH and SOM, and for DBH and NPY. However, the FB-labeled perikarya did not contain immunoreactivities to the remaining substances investigated. Moreover, some FB- or FB/DBH-positive somata were closely apposed by SOM-, NPY- or LENK-immunoreactive fibers.
CONCLUSIONS: This study has provided new data on the distribution of neurons projecting from LCx to the lobule VIII, and has revealed that they are noradrenergic (and non-cholinergic) in nature and that their activity can be modulated by some neuropeptides. The findings seem to be important considering the role of the lobule VIII in innervation of axial and proximal forelimb muscles.

P3.14

THE EFFECTS OF DOPAMINE D1 AND D2 RECEPTOR AGONISTS, GIVEN ALONE OR JOINTLY WITH A NITRIC OXIDE DONOR, ON EXPRESSION OF PROTEINS INVOLVED IN THE NITRERGIC SIGNALING AND ON CGMP PRODUCTION IN 6-OHDA-LESIONED RATS

Elżbieta Lorenc-Koci

Department of Neuropsychopharmacology, Institute of Pharmacology, Polish Academy of Sciences, Kraków, Poland

BACKGROUND AND AIMS: The aim of the present study was to examine the effects of selective dopamine (DA) D1 and D2 receptors agonists administered chronically, alone or in combination with the nitric oxide donor molsidomine on expression of proteins involved in the nitric oxide – soluble guanylyl cyclase – cGMP signaling pathway and on the cGMP levels in the striatum (STR) of 6-OHDA-lesioned rats.
METHODS: Two weeks after unilateral injection of 6-OHDA (8 μg/4 μl) into the medial forebrain bundle, rats were treated chronically (15

days) with DA D1 (SKF38393; 3 mg/kg sc) or DA D2 (quinpirole 0.2 mg/kg sc) receptor agonists and molsidomine (2 mg/kg ip), alone and in combination. The contents of neuronal nitric oxide synthase (nNOS), sGC and phosphodiesterase 1B (PDE-1B) proteins were determined in the STR by Western blot technique while cGMP level using competitive enzyme immunoassay cGMP kit.
RESULTS: In the ipsilateral STR chronic treatment with SKF38393 alone or jointly with molsidomine did not affect nNOS protein level. Quinpirole alone had no effect on nNOS level while given jointly with molsidomine decreased it markedly. Both SKF38393 and quinpirole alone did not change sGC protein level in the ipsilateral STR while their joint administration with molsidomine enhanced it markedly. SKF38393 and quinpirole alone decreased PDE-1B protein expression but only combined administration of quinpirole + molsidomine enhanced the content of this protein. As to cGMP level, chronic treatment with molsidomine alone increased cGMP concentration in the ipsi- and contralateral STR. SKF38393 administered chronically alone or jointly with molsidomine enhanced cGMP content only in the ipsilateral STR while quinpirole alone or in combination with molsidomine did not evoke such effects.
CONCLUSION: The obtained results suggest that the treatment with selective DA D1 and D2 agonists differently modulates the NO-sGC-cGMP signaling pathway in 6-OHDA-lesioned rats.

P3.15

ROLE OF SERUM RESPONSE FACTOR IN HOMEOSTATIC PLASTICITY

Anna Krysiak, Anna Suska, Leszek Kaczmarek, Katarzyna Kalita

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

BACKGROUND AND AIMS: Homeostatic plasticity is a regulatory mechanism which allows neurons to maintain a stable level of activity despite of the sustained alterations in neuronal network. Regulation of this form of plasticity is based on global changes of synaptic strength achieved by the alteration in AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) receptor levels on postsynaptic membrane. The process is called synaptic scaling and can be controlled on a transcriptional level. Arc (Activity-regulated cytoskeleton-association protein) accelerates endocytosis of AMPA-type glutamate receptors (AMPARs) and reduces their surface exposure during overexcitation. Our aim was to investigate if the transcription factor SRF (Serum Response Factor) may regulate homeostatic plasticity.
METHODS: To increase neuronal activity *in vitro* we applied widely used model of homeostatic plasticity where hippocampal neurons were treated with 20 μM gabazine ($GABA_A$ antagonist) for 24 or 48 hours.
RESULTS: Study revealed long-lasting stimulation with gabazine leads to the induction of SRF-driven transcription. We also detected upregulation of Arc protein 24 h after stimulation in control cells had abolished in neurons, in which the level of SRF has been decreased by a specific shRNA. Study revealed that SRF depleted neurons had lower level of surface GluR1 subunits comparing to control cells in basal condition. However, upon prolonged stimulation, loss of SRF results in increased incorporation of GluR1 to the synapses surface in contrast to control cells where decreased level of AMPARs was observed. Analysis of miniature excitatory postsynaptic currents (mEPSCs) confirmed decrease of AMPARs amplitude in control cells in response to stimulation. Conversely, loss of SRF led to increased AMPARs amplitude and abolished homeostatic scaling down of AMPARs.
CONCLUSION: Our preliminary data suggests lack of SRF inhibits neurons ability to decrease synaptic strength in response to overexcitation in hippocampal neurons *in vitro*.

P3.16

SENSITIZATION TO MORPHINE WITHDRAWAL SIGNS – A NEUROCHEMICAL BASIS IN DOPAMINERGIC RECEPTORS

Joanna Listos[1], Agnieszka Wąsik[2], Irena Bosiacka[3], Sylwia Talarek[1], Jolanta Orzelska[1], Małgorzata Łupina[1], Lucyna Antkiewicz-Michaluk[2], Sylwia Fidecka[1]

[1]Chair and Department of Pharmacology and Pharmacodynamics, Medical University of Lublin, Lublin, Poland; [2]Department of Neurochemistry, Institute of Pharmacology, Polish Academy of Sciences, Kraków, Poland; [3]Department of Biochemistry and Medical Chemistry, Pomeranian Medical University, Szczecin, Poland

BACKGROUND AND AIMS: In morphine addicted patients, chronic treatment with various abusers is interspersed with drug-free periods (periods of sleep or unsuccessful attempts to treat). These repeated withdrawal periods may intensify the withdrawal episode.
These data inspired us to examine if repeated withdrawal periods has the effect on the severity of naloxone-induced withdrawal signs in rats. We also investigated the effect of A1 and A2A receptors in observed withdrawal signs. Additionally, to elucidate the mechanisms underlying the effects of repeated morphine withdrawal signs, neurochemical experiments were performed.
METHODS: To obtain the state of dependence, the animals were treated with increasing doses of morphine, twice a day, for 8 days. To demonstrate the effect of sporadic treatment with morphine, we divided rats into two groups: continuously and sporadically treated with morphine. In sporadic group, morphine administration was modified by adding 3 morphine-free periods. On the 9th day of the study, the subsequent dose of morphine was injected. 1

hour later, the naloxone, was administrated for induction of morphine withdrawal signs in rats. Then, animals were placed into cylinders and jumpings were recorded for period 30 min. After decapitation, a neurochemical study, using HPLC-ED method was made. The concentration of dopamine and its metabolites was assessed in three brain structures (striatum, hippocampus, prefrontal cortex).
RESULTS: We confirmed that sporadic treatment with morphine induced the intensification of morphine withdrawal signs, and administration of both adenosine agonists reduced the severity of them. In neurochemical experiments we demonstrated significant differences in the release in dopamine and its metabolites in studied brain areas.
CONCLUSION: The recognition of neurochemical mechanisms, underlying the behavioral changes, may have an important role for further exploration of the various effects of repeated morphine withdrawal signs.

P3.17

EXPRESSION AND FUNCTION OF ANGIOMOTIN FAMILY PROTEINS IN THE BRAIN

Tomasz Prószyński[1], Katarzyna Rojek[1], Paweł Niewiadomski[1], Krzysztof Bernadzki[1], Hubert Dolężyczek[2], Marcin Rylski[3], Leszek Kaczmarek[2], Jacek Jaworski[4]

[1]Laboratory of Synaptogenesis, Department of Cell Biology, [2]Laboratory of Neurobiology, Department of Molecular and Cellular Neurobiology, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland; [3]The Medical Center of Postgraduate Education, Warsaw, Poland; [4]International Institute of Molecular and Cell Biology, Warsaw, Poland

BACKGROUND AND AIMS: Proper organization of synaptic connections is important for the transmission of information in the central and peripheral nervous systems (CNS and PNS). Synaptic remodeling is a process whereby synapses are rewired to form functional neuronal networks. The molecular mechanisms underlying this process are still poorly understood. We have recently identified the scaffold protein Amotl2 as a potential regulator of neuromuscular junction (NMJ) plasticity. Interestingly, many proteins involved in NMJ remodeling are also implicated in the plasticity of synapses in the brain. Therefore, we investigated the expression and function of Amotl2 and closely related proteins Amot and Amotl1, collectively called Angiomotins, in CNS neurons.
METHODS: Primary neuronal cultures were prepared from embryonic day 19 rat brains and transfected using Lipofectamine2000 or Amaxa nucleofection. Cells and mouse brain slices were immunostained to visualize Amot, Amotl1 and Amotl2. To assess the function of Amot in CNS, we knocked down its expression in hippocampal neurons using shRNA, and analyzed neuronal morphology using confocal microscopy.
RESULTS: All three angiomotins are widely expressed in the brain. In cultured rat hippocampal neurons and mouse brain slices Amotl2 and Amotl1 localize to the synaptic compartment, whereas Amot is distributed in neurites. Amot depletion in neurons leads to reduced dendritic tree arborization and malfunction of the axon initial segment as reflected by aberrant localization of its main components ankyrin G and neurofascin.
CONCLUSIONS: Our experiments identify a novel group of proteins that play a critical role in the organization of neurons and may regulate synaptogenesis both in the CNS and PNS. Amot may play a role in dendrite outgrowth and is critical for the establishment of the axon initial segment, suggesting a role in the maintenance of polarity in neurons.
This research was supported by the NCN grant 2012/05/E/NZ3/00487.

P3.18

EFFECTS OF BODY TEMPERATURE AND HYPERFERREMIA ON GLUTATHIONE AND VITAMIN E LEVELS IN THE BRAIN OF ASPHYXIATED NEONATAL RAT

Justyna Rogalska[1], Hanna Kletkiewicz[1], Anna Nowakowska[1], Alina Woźniak[2], Celestyna Mila-Kierzenkowska[2]

[1]Department of Animal Physiology, Faculty of Biology and Environment Protection, N. Copernicus University, Toruń, Poland; [2]The Chair of Medical Biology, Collegium Medicum in Bydgoszcz, Toruń, Poland

BACKGROUND AND AIMS: In asphyxiated newborns iron, deposited in the brain, contributes to formation of reactive oxygen species.
METHODS: In our study we found an increased iron accumulation in neurons of rats exposed to neonatal anoxia under hyperthermic conditions which was prevented with deferoxamine (DFO) injection. Key factors in the cellular protection against oxidative stress are glutathione and α-tocopherol. Therefore, we decided to study the influence of body temperature and chelation of iron with deferoxamine on antioxidant status in the brain of rats exposed to critical anoxia.
RESULTS: Two-day old newborn rats were exposed to anoxia in 100% nitrogen atmosphere. Rectal temperature was kept at 31°C (hypothermia), 33°C (physiological temperature in rat neonates), or at 37°C (hyperthermia) or at 39°C (extreme hyperthermia). Control rats were exposed to atmospheric air in respective thermal conditions. Half of the hyperthermic rats (39°C) exposed to anoxia were injected with DFO immediately after anoxia and 24 hours later. Cerebral concentrations of lipid peroxidation products (malondialdehyde – MDA) and the level of

glutathione and α-tocopherol were determined *post mortem*: (1) immediately, (2) 72 hours, (3) 1 week after anoxia and (4) 2 weeks after anoxia. We observed that there were no postanoxic changes in concentration of MDA and in the level of glutathione and α-tocopherol in newborn rats kept at their physiological body temperature of 33°C. In contrast, simulated perinatal anoxia at body temperature elevated to 37°C and 39°C as well as hypothermic conditions (31°C) intensified post-anoxic oxidative stress and depleted the antioxidant pool. The decrease of antioxidants under hyperthermic conditions was prevented with postanoxic DFO injection.

CONCLUSION: The data suggest that both elevated body temperature as well as cooling below the level of physiological body temperature of newborn rats may extend the perinatal anoxia-induced brain lesions.

Research was supported by NCN under grant 2011/03/B/NZ7/00682.

P3.19

$GABA_A$ RECEPTOR BINDING SITE RESIDUE BETA2 GLUTAMATE155: POSSIBLE ROLE IN CHANNEL PREACTIVATION

Magdalena Kisiel[1], Magdalena Jatczak[1,2], Marta M. Czyżewska[1], Marek Brodzki[1,2], Cynthia Czajkowski[3], Jerzy W. Mozrzymas[1,2]

[1]Laboratory of Neuroscience, Department of Biophysics, Wrocław Medical University, Wrocław, Poland; [2]Laboratory of Cellular Neurobiology, Department of Animal Molecular Physiology, Wrocław University, Wrocław, Poland; [3]Department of Neuroscience, School of Medicine & Public Health, University of Wisconsin-Madison, Madison, USA

BACKGROUND AND AIMS: The $GABA_A$ receptor is the main mediator responsible for inhibitory transmission in the brain. In our previous work (Szczot et al. 2014), we demonstrated for α1β2γ2 receptors that "classical" channel gating (opening/closing and desensitization) is preceded by a preactivation step, which is most likely initiated at the agonist-binding site. Here, we investigated the role of β2E155 residue in channel gating focusing on preactivation. Residue β2E155 is located in the GABA-binding site and may directly interact with agonist. Moreover, agonist induced local motions near this residue suggests it is an initial trigger that couples agonist binding to channel gating.

METHODS: In this study, we combined ultrafast solution exchange with patch-clamp electrophysiology to record macroscopic currents mediated by wild-type and mutant (β2E155C) α1β2γ2 and α1β2 receptors.

RESULTS: Cysteine substitution of β2E155 caused a large right-shift of the dose–response curves for GABA-elicited currents, which was independent of the presence of γ_2 subunit. Furthermore, especially for $\alpha1\beta_2\gamma_2$ receptors, β2E155C slowed down macroscopic desensitization kinetics. The mutant receptors also exhibited spontaneous channel activity. Taken together, the data suggest this mutation alters not only GABA binding but also GABA-mediated gating transitions. Non-stationary noise analysis of variance showed that for $\alpha1\beta_2\gamma_2$ receptors, the β2E155C mutation significantly decreased maximal open probability without affecting single channel conductance.

CONCLUSIONS: Model kinetic simulations of our data indicate that β_2E155 is likely involved in preactivation transitions that precede channel opening supporting its role as an initial trigger for coupling binding to gating.

This research has been financially supported by grant National Centre of Science grant: DEC-2013/11/B/NZ3/00983 and by ministry grant Pbmn135.

P3.20

BRAINSLICES – IMAGE SHARING FOR NEUROSCIENCE

Jakub Kowalski, Piotr Majka, Alicja Puścian, Daniel Wójcik

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

AIM: Images of brain tissue are common outcome of a great number of neuroscience experiments; however, their impact on scientific development is limited since they are usually not published directly. Internet technology facilitates making the images available online, nonetheless, raw image files of good quality are very large and therefore inconvenient to provide and analyze online directly. There is still no convenient way to share annotated images of neuroscientific specimens. To fill that gap we developed BrainSlices software – a user-friendly tool dedicated for that purpose.

METHODS: The software is built in the client-server model and consist of a web application at the client side, and server software running on a Linux system. Thanks to this design there is no need for installation and the only thing a BrainSlices user must do is to open a website. This approach also makes BrainSlices a cross-platform tool. Handling of large images is solved with image pyramid technique.

RESULTS: We used BrainSlices software to set up an online repository (http://brainslices.org) of annotated high quality images of brain tissue. The repository combines the power of the image pyramid technique with convenience of image upload. Every image can be easily annotated with exhaustive metadata which facilitate search. The user interface of the repository and the provided facilities were developed with typical neuroscience use in mind. Every image receives a unique identifier and a permalink which allows direct access and citing.

CONCLUSION: We provide the community with a user friendly tool for multiple image storage, viewing, sharing, and annotation. The tool may be used to store one's own collection of slice images,

to share high quality specimen images with collaborators, to share them with the whole community, or to provide the images online as supplementary material for publications.

POSTER SESSION P4. CHOLINERGIC SYSTEM

P4.1

THETA RHYTHM AND LOCAL CELL DISCHARGES RECORDED IN POSTERIOR HYPOTHALAMIC SLICES OF THE ADULT RAT

Bartosz Caban, Paulina Kłos-Wojtczak, Tomasz Kowalczyk, Renata Bocian, Paulina Kaźmierska, Jan Konopacki
Neurobiology Department, University of Łódź, Łódź, Poland

BACKGROUND AND AIMS: Hippocampal formation (HPC) theta rhythm is one of the best examples of neural synchrony in mammalian brain. HPC theta field potentials in rats consists of high-amplitude, almost sinusoidal waves in 3–13 Hz frequency range. It is well-known that the pathway of theta generation originates in the nucleus reticularis pontis oralis (RPO), then RPO projects to supramammillary nuclei (SuM), and finally through the medial septal area (MS) to HPC and other limbic structures. This tract is called the ascending brainstem-hippocampal synchronizing pathway. *In vivo*, HPC theta frequency is modulated at least partially by SuM which consists of neurons firing in the frequency of HPC theta. However, in our previous studies we have discovered for the first time that local theta activity can also be recorded in deafferented posterior hypothalamic preparations. The present *in vitro* study investigates theta-related neurons and their relation to local hypothalamic theta rhythm.
METHODS: 56 *in vitro* electrophysiology experiments were performed using brain slices taken from 56 Wistar rats. Each slice was perfused with 75 μM carbachol (cholinergic agonist) to induce rhythmic activity. The relation of firing neurons to local field theta rhythm was investigated according to an earlier developed classification.
RESULTS: This study resulted in recording 21 theta-related neurons and 35 neurons classified as non-related to theta.
CONCLUSION: Neuronal activity recorded in the posterior hypothalamic area *in vitro* resembles well-documented patterns of theta-related cell discharges in the hippocampal formation *in vitro* and *in vivo*.
Supported by NCN grant 2013/11/B/NZ4/04873.

P4.2

POSTERIOR HYPOTHALAMIC THETA RHYTHM AND LOCAL CELL DISCHARGES IN ADULT ANESTHETIZED RATS

Renata Bocian, Paulina Kłos-Wojtczak, Bartosz Caban, Tomasz Kowalczyk, Paulina Kaźmierska, Jan Konopacki
Department of Neurobiology, University of Łódź, Łódź, Poland

BACKGROUND AND AIMS: Theta rhythm is the best synchronized electroencephalographic activity that can be recorded in several brain regions, for example, in the cingulate cortex, entorhinal cortex, and hippocampal formation (HPC) that is considered to be the main structure involved in the generation of theta. Just recently Kowalczyk et al. (2014) have indicated that this pattern EEG could also be observed in the posterior hypothalamic area (HPa) in anesthetized rats. Specifically, in the supramammillary (SuM) and posterior hypothalamic (PH) nuclei theta field activity was produced independently of simultaneously occurring hippocampal theta. Previous experiments have demonstrated that the HPC theta field activity is accompanied by a characteristic pattern of local cell discharges. PHa cells discharges were classified in accordance to earlier developed classification as theta-related and theta-nonrelated. Theta-related cells were classified as theta-on and theta-off. Both theta-on and theta-off cells were subclassified as phasic and tonic.
The aim of the present studies was to describe firing cell repertoire during cholinergically induced theta rhythm in the PHa of rats.
METHODS: All the experiments were performed in urethanized adult male Wistar rats. PHa theta rhythm and single cell activity were recorded with use of tungsten and glass microelectrodes respectively.
RESULTS: One hundred fifty five out of 236 recorded in PHa cells were identified as theta non-related cells and 82 as theta-related cells: 48 theta-on, 29 theta-off, and 4 theta gating cells (the cells which activity is correlated with appearance of theta episodes).
CONCLUSION: The obtained data clearly demonstrates that cholinergically induced theta activity in PHa, similarly to the HPC theta, is accompanied by local cell discharges which can be successfully classified in accordance with earlier developed criteria for HPC theta.
The study was supported by National Science Centre (NCN, 2013/11/B/NZ4/04872).

P4.3

ENERGY METABOLISM IN NEURONS EXPOSED TO HYPOXIA-INDUCING CHEMICALS

Beata Gapys, Marlena Zyśk, Anna Ronowska, Hanna Bielarczyk
Chair of Clinical Biochemistry, Department of Laboratory Medicine, Medical University of Gdańsk, Gdańsk, Poland

BACKGROUND AND AIMS: Excess of zinc ions and intermittent hypoxia both cause neurodegeneration by disruption in energy metabolism and oxidative stress, resulting in diminished pyruvate dehydrogenase complex (PDHC) and aconitase activity and consequent shortages in acetyl-CoA. This deficiency may be particularly dangerous for cholinergic neurons as they consume acetyl-CoA in additional pathway of acetylcholine synthesis apart from energy production.

Aim of our study was to investigate effect of hypoxia on acetyl-CoA metabolism in neuronal cells under cytotoxic conditions.
METHODS: The SH-SY5Y neuroblastoma cells were recognized as an *in vitro* model of brain cholinergic neurons after differentiating with all-trans-retinoic acid and cAMP. Hypoxic conditions were induced by 24 h (chronic) exposition of SH-SY5Y cells to cobalt ions (Co). Zinc (Zn) ions were used to evoke cytotoxicity.
RESULTS: Chronic exposition of SH-SY5Y NC to 0.2 mM and 0.5 mM Co decreased cells number by 22% and 53%, respectively. The activities of PDHC and aconitase were reduced by 70% (0.2 mM Co) and over 75% (0.5 mM Co). IDH activity, in both concentrations, was decreased by 18%. The level of acetyl-CoA in SH-SY5Y NC was 28.4 pmol/mg of protein and chronic exposition of SH-SY5Y to 0.5 mM Co decreased acetyl-CoA level by about 60%. Chronic exposure of SH-SY5Y DC to 0.2 mM and 0.5 mM Co decreased number of cells by 28 and 60%, respectively, reduced activities of PDHC by 52% (0.2 mM) and 31% (0.5 mM Co). Aconitase activity was decreased by 77 and 90%, respectively. Activity of NADP-IDH and level of acetyl-CoA were also diminished regardless of the cobalt concentration by approximately 28% and 30%, respectively. In both groups addition of 0.1 mM Zn-aggravated cells mortality.
CONCLUSION: Presented data indicate that hypoxia enhance cytotoxic effects of Zn in highly differentiated cholinergic cells. Supported by Ministry of Research and Higher Education projects: MN 01-0058/08 and ST-57, IP 2011046071.

P4.4

CYTOTOXIC EFFECTS OF ZINC ON CHOLINERGIC SN56 NEUROBLASTOMA AND C6 ASTROCYTOMA CELLS

Aleksandra Dyś, Anna Ronowska, Sylwia Gul-Hinc, Joanna Klimaszewska-Łata, Dorota Bizon-Zygmańska, Andrzej Szutowicz, Hanna Bielarczyk

Chair of Clinical Biochemistry, Department of Laboratory Medicine, Medical University of Gdańsk, Gdańsk, Poland

BACKGROUND AND AIMS: The aim of this work was to find relationships between Zn accumulation and integrity of cholinergic and astroglial cells.
METHODS: Exposition of cAMP/RA-differentiated (DC) and nondifferentiated (NC) cells cholinergic SN56 neuroblastoma and astroglial C6 cells to Zn yielded its concentration dependent accumulation. The level of Zn was measured by fluorimetric method with TSQ.
RESULTS: After 24 h exposition of SN56 cells to 0.15 mM Zn their death rates were equal to 35 and 50% for NC and DC at cation levels equal to 4.0 and 5.5 nmol/mg protein, respectively. In the same conditions, the death rates of astroglial cells were close to 1–2% only, at intracellular Zn levels of 1.6 and 2.1 nmol/mg protein, respectively. Higher, about 0.25 mM Zn levels were required to evoke death rates of astroglial cells, similar to those seen in neuronal cells. In such conditions Zn levels in astroglia were about 6.4 and 27.0 nmol/mg protein, respectively. In this study we examined the effects of accumulation of Zn in cholinergic neurons and adjacent astrocytes on activity of enzymes involved in energy metabolism. It caused inhibition of PDHC, aconitase and IDH activities. The high susceptibility of cholinergic neurons and a relative high resistance of astrocytes induced by cytotoxic concentration of zinc. Higher levels of Zn may cause deeper inhibition of acetyl-CoA synthesis and the flow rate of the TCA cycle, which leads to a decrease in ATP synthesis and cell damage.
CONCLUSIONS: Chronic exposition to Zn apparently induced adaptative mechanisms eliminating excess of the metal from the cells. These changes may directly inhibit intramitochondrial acetyl-CoA synthesis and its transport to cytoplasmic compartment, yielding impairment of cell viability and suppression their transmitter functions. Chronic neurons are more susceptible to increase extracellular concentrations of zinc than astrocytes.
Supported by MNiSW projects MN 01-0108/08/248 and MUG fund ST-57.

P4.5

CARBACHOL-INDUCED PRESYNAPTIC MODULATION OF TRANSMISSION FROM CORTICAL LAYER 6 TO POSTEROMEDIAL THALAMIC NUCLEUS

Syune Nersisyan[1], Marek Bekisz[1], Ewa Kublik[1], Bjorn Granseth[2], Andrzej Wróbel[1]

[1]Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland; [2]Linkoping University, Linkoping, Sweden

BACKGROUND AND AIMS: Thalamic relay cells constitute important node of reciprocal sensory processing which is highly dependent on current behavioral demands realized by brainstem neuromodulatory systems. In these experiments, performed on rats' thalamic brain slices, we have investigated cholinergic influence on synaptic transmission from cortical layer 6 to the posteromedial thalamic nucleus (PoM).
METHODS: Neuronal membrane potentials and currents were recorded with whole-cell patch-clamp method and general cholinergic agonist carbachol was added to the bath in order to mimic cholinergic activation. Excitatory postsynaptic responses were evoked in PoM cells by repetitive trains of 5 electrical stimuli delivered at 20 Hz through bipolar electrode placed at the corticothalamic fibers in the internal capsule.
RESULTS: In all investigated cells, consecutive postsynaptic responses in the train showed pronounced frequency facilitation (i.e. increase in amplitude). Carbachol substantially decreased postsynaptic response amplitudes, but at the same time it enhanced the

magnitude of frequency facilitation. Moreover, the amplitudes of each consecutive postsynaptic potential in the train were characterized by much higher trial-to-trial coefficient of variation (SD/mean). These effects suggested presynaptic action of carbachol. To prove this, we measured the failure rate of excitatory postsynaptic currents in PoM cells in response to minimum stimulation of corticothalamic fibers.

CONCLUSION: The substantial increase of failure rate in the presence of carbachol supports the hypothesis that observed effects of cholinergic modulation relay on decreased probability of transmitter release from presynaptic site.

This research and SN were supported by the European Union Regional Development Fund through the Foundation for Polish Science within the frames of International PhD Program in Neurobiology.

P4.6

MUSCARINIC RECEPTOR REGULATION OF PYRAMIDAL NEURON MEMBRANE POTENTIAL IN THE MEDIAL PREFRONTAL CORTEX (mPFC)

Przemysław Norbert Kurowski, Maciej Gawlak, Paweł Szulczyk

Laboratory of Physiology and Pathophysiology, Centre for Preclinical Research and Technology, Medical University of Warsaw, Warsaw, Poland

BACKGROUND AND AIMS: Damage to the cholinergic input to the prefrontal cortex has been implicated in neuropsychiatric disorders. Cholinergic endings release acetylcholine, which activates nicotinic and/or muscarinic receptors and regulates the membrane potential in medial prefrontal cortex (mPFC) neurons. The aim of this study was to clarify the mechanism responsible for control of the medial prefrontal cortex (mPFC) pyramidal neurons by muscarinic receptors.

MATERIAL AND METHODS: Experiments were performed on mPFC pyramidal neurons in slices isolated from young (18–22-day-old) male rats. Recordings of membrane potential were performed with the gramicidin perforated-patch method in the absence of Ca^{2+} ions and in the presence of tetrodotoxin (TTX, 1 μM) in extracellular solution.

RESULTS: Cholinergic receptor stimulation by carbamoylcholine chloride (CCh; 100 μM) evoked depolarization (10.0±1.3 mV), which was blocked by the M1/M4 (pirenzepine dihydrochloride, 2 μM) and M1 (VU 0255035, 5 μM) muscarinic receptor antagonists and was not affected by a nicotinic receptor antagonist (mecamylamine hydrochloride, 10 μM). CCh-dependent depolarization was greatly attenuated in the presence of an inhibitor of the βγ-subunit-dependent transduction system (gallein, 20 μM). mPFC pyramidal neurons express Nav1.9 channels. CCh-dependent depolarization was abolished in the presence of antibodies against Nav1.9 channels in the intracellular solution and augmented by ProTx-I toxin (100 nM) in the extracellular solution.

CONCLUSION: Activation of M1 muscarinic receptors evokes depolarization of mPFC pyramidal neurons due to activation of Nav 1.9-like Na+ channels *via* G-protein βγ-subunits (in a membrane-delimited mode).

The study was supported by NCN grants no: NN401584638, NN301572940 and WUM grant no: FW5/PM31D/14.

P4.7

ACTIVATION OF ALPHA7 NICOTINIC ACETYLCHOLINE RECEPTORS AGAINST COGNITIVE AND SENSORIMOTOR GATING DEFICITS IN ANIMAL MODEL OF SCHIZOPHRENIA

Agnieszka Potasiewicz, Agnieszka Nikiforuk, Małgorzata Holuj, Piotr Popik

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

BACKGROUND AND AIMS: Alpha 7 nicotinic acetylcholine receptors (α7 nAChRs) are involved in the regulation of cognitive processes. Furthermore, it has been suggested that α7 nAChRs might be implicated in the pathophysiology of schizophrenia. Hence, selective activation of α7 nAChRs is considered to be a potential therapeutic strategy aimed at ameliorating cognitive dysfunctions associated with schizophrenia. Preclinical data indicated that orthosteric ligands, like the partial α7 nAChR agonist, A582941, produced procognitive effects, but little is known about efficacy of this compound in animal models of schizophrenia. The aim of present study was to evaluate the efficacy of A582941 against MK-801-induced schizophrenia-like deficits in rats. Prepulse inhibition of startle response test (PPI), discrete paired-trial delayed alternation task in a T-maze and five-choice serial reaction time task (5-CSRTT) were employed in this study.

RESULTS: A582941 reversed the sensorimotor gating and working memory impairment evoked by MK-801 as assessed in the PPI test and T-maze, respectively. However, this compound did not affect the rats' attentional performance in the 5-CSRTT.

CONCLUSIONS: The present study demonstrates the beneficial effects of α7 nAChRs agonist on sensorimotor gating and some aspects of cognition in rats tested in impaired conditions. Therefore, our results support the notion that α7 nAChRs may constitute a useful targets for procognitive therapy in schizophrenia.

This study was supported by the Polish National Science Centre grant NCN 2012/07/B/NZ/01150 and statutory funds from the Institute of Pharmacology, Polish Academy of Sciences (Krakow, Poland). Agnieszka Potasiewicz is a holder of scholarship from the

KNOW sponsored by Ministry of Science and Higher Education, Republic of Poland.

P4.8

TIMING CELLS AND CARBACHOL INDUCED THETA RHYTHM IN POSTERIOR HYPOTHALAMIC SLICE PREPARATIONS OF ADULT RATS

Renata Bocian, Bartosz Caban, Tomasz Kowalczyk, Paulina Kłos-Wojtczak, Paulina Kaźmierska, Jan Konopacki

Department of Neurobiology, University of Łódź, Łódź, Poland

BACKGROUND AND AIMS: Just recently we discovered a local theta rhythm in the posterior hypothalamic area (PHa) in *in vitro* and *in vivo* conditions. Theta rhythm which appeared in the PHa was produced independently of simultaneously occurring hippocampal formation theta. In the present study we analyse the correlation of local PHa cells discharges with carbachol induced theta field potentials. Specifically, we emphasise a novel type of theta related cells which we labelled "timing cells".

METHODS: Ninety experiments were conducted on the Wistar rat's PHa slice preparation. The local theta field potential was induced with 75 μM carbachol. Glass recording electrodes were positioned with use of micropositioner in different regions of PHa (mainly supramammillary nuclei). Single cell activity and local field potentials were recorded simultaneously with the same electrode with respect to the ground.

RESULTS: Sixty nonrelated and more that 50 theta-related cells were recorded. Twelve of theta-related cells were recognized and labelled as "timing cells". These cells discharge rhythmically in the frequency range of 5–8 Hz and can be phase locked with local field potentials which appeared in 1–3 s lasting theta epochs.

CONCLUSION: Posterior hypothalamic theta "timing cells" are probably involved in mechanisms responsible for programing the frequency of local theta field potentials and hippocampal formation theta rhythm.

P4.9

VOLTAGE-GATED CALCIUM CHANNELS AFFECTED ACETYL-CoA METABOLISM IN CHOLINERGIC NEURONS EXPOSED ON Zn IONS

Marlena Zyśk, Beata Gapys, Sylwia Gul-Hinc, Andrzej Szutowicz, Hanna Bielarczyk

Chair of Clinical Biochemistry, Department of Laboratory Medicine, Medical University of Gdansk, Gdansk, Poland

BACKGROUND AND AIMS: Cholinergic neurons produce acetyl-CoA, which is subsequently used as a fuel for energy production. Furthermore, exclusively those neurons produce acetylcholine from acetyl-CoA. As a results, extra utilization pathway may induce acetyl-CoA shortages and consequently impairment of brain energy metabolism. Disturbances in Ca-signaling could play regulatory role in neurons susceptibility to neurodegenerative conditions. The aim of our study was to investigate whether the Voltage-Gated Calcium Channels (VGCCs) could moderate the cholinergic neurons susceptibility on neurodegeneration.

METHODS: Selected blockers of VGCCs (10 μM nifedipine, 0.2 μM ω-conotoxin-MVIIC, 0.5 μM ω-conotoxin-GVIA) were used as a Ca-depletion factors in SN56 neuroblastoma cells.

RESULTS: Short-term SN56 cells exposition on 0.15 mM Zn increased the Zn level from 0.6 to 36 nmol/mg protein. However, in the presence of 10 μM nifedipine and ω-conotoxins, the Zn-accumulation were decreased by about 50%. Zn caused in SN56 about 49% increase of nonviable cells fraction. Whereas incubation cells with VGCCs blockers and Zn, led to 25% decline in the number of trypan blue positive cells the acetyl-CoA level in SN56 was 26.9 pmol/mg protein. However, the SN56 cells exposition on 0.15mM Zn decreased its level by 43%. In addition, acetyl-CoA level in VGCCs-blocked SN56 was as high as in control conditions.

CONCLUSIONS: Achieved results indicated that VGCCs regulated the Zn-evoked neurotoxic effects on acetyl-CoA metabolism in SN56 cholinergic cells. Moreover, VGCCs might play particular role in neurotoxicity of Zn and show that disturbance of Ca homeostasis in this condition can be one of the factors which moderate acetyl-CoA metabolism in cholinergic neurons.

Supported by MN0059/08 and ST-57 GUMed fund.

P5. CENTRAL CONTROL OF REPRODUCTION

P5.1

THE CENTRAL EFFECT OF β-ENDORPHIN AND NALOXONE ON KISSPEPTIN AND RFAMIDE-RELATED PEPTIDE-3 – THE HYPOTHALAMIC NETWORKS SIGNALLING GONADOTROPIN-RELEASING HORMONE NEURONS IN SHEEP

Edyta Paruszewska[1], Magdalena Ciechanowska[1], Magdalena Łapot[1], Krystyna Mateusiak[2], Małgorzata Nawrocka[1], Franciszek Przekop[2]

[1]Department of Pharmacology and Toxicology, The General Karol Kaczkowski Military Institute of Hygiene and Epidemiology, Warsaw, Poland; [2]Department of Neuroendocrinology, The Kielanowski Institute of Animal Physiology and Nutrition, Polish Academy of Sciences, Jabłonna, Poland

BACKGROUND AND AIMS: The aim of the present study was to investigate the effects of prolonged, intermittent infusion of β-endorphin or naloxone into the third cerebral ventricle of follicular phase ewes on kisspeptin (kiss 1) mRNA and RFamide-related peptide-3 (RFRP-3) mRNA levels in the hypothalamus. It was also examined the influence of β-endorphinergic stimulation or blockade

on the expression of gonadotropin releasing hormone (GnRH) and GnRH receptor (GnRHR) proteins in the hypothalamic-pituitary unit and on luteinizing hormone (LH) secretion from the anterior pituitary gland.

METHODS: The levels of GnRH and GnRHR proteins were analyzed using an enzyme-linked immunoabsorbent assay (ELISA) in selected tissue of the preoptic area-hypothalamic region: preoptic area (POA), anterior hypothalamus (AH), ventromedial hypothalamus (VM), stalk/median eminence (SME), and GnRHR in the anterior pituitary gland (AP). The Real-time PCR with SYBR Green dye was used for evaluation of kiss 1 mRNA in the POA and arcuate nucleus (ARC) and RFRP3 mRNA in the paraventricular nucleus (PVN) and in the dorsomedial hypothalamus (DMH).

RESULTS: Stimulation of β-endorphin receptors significantly decreased the levels of GnRH protein and kiss 1 transcript in all analyzed structures and usually led to similar responses in the expression of GnRHR. Precisely, β-endorphin decreased the level of GnRHR protein in the POA, MBH, SME and AP, but had no significant influence on the receptor quantity in the AH. In addition, β-endorphin decreased LH secretion. Naloxone had an opposite effect on proteins biosynthetic level.

CONCLUSIONS: The obtained results suggest that β-endorphin can modulate the biosynthesis and release of GnRH through complex changes in the expression of kiss 1 mRNA and GnRHR protein in the hypothalamus. It also appears, that in sheep β-endorphin influences GnRH/LH secretion by mechanism(s) excluding RFRP-3 neuronal system.

P5.2

THE INFLUENCE OF DOPAMINERGIC SYSTEM INHIBITION ON KISSPEPTIN AND RFAMIDE-RELATED PEPTIDE-3 TRANSCRIPTS AND GONADOTROPIN RELEASING HORMONE/RECEPTOR PROTEINS IN ANESTROUS EWES

Magdalena Łapot[1], Magdalena Ciechanowska[1], Marek Kowalczyk[2], Krystyna Mateusiak[3], Małgorzata Paluch[1], Bożena Antkowiak[1], Franciszek Przekop[3]

[1]Department of Pharmacology and Toxicology, The General Karol Kaczkowski Military Institute of Hygiene and Epidemiology, Warsaw, Poland; [2]Józef Piłsudski University of Physical Education, Warsaw, Poland; [3]Department of Neuroendocrinology, The Kielanowski Institute of Animal Physiology and Nutrition, Polish Academy of Sciences, Jabłonna, Poland

BACKGROUND AND AIMS: The aim of this study was to explain how prolonged inhibition of central dopaminergic activity affected the neuroendocrine processes controlling GnRH/gonadotropins secretion in the hypothalamus and in the anterior pituitary gland compartments of sheep during the non-breeding time. In this purpose, we investigated the effects of prolonged, intermittent infusions of small doses of sulpiride (dopaminergic D2 receptors antagonist) on transcript levels of the two central regulators of the hypothalamic-pituitary reproductive system – kiss 1 and RFRP-3. It was also examined the influence of sulpiride on the GnRH and GnRH receptor (GnRHR) biosynthesis in the hypothalamus-pituitary unit. Additionally, these analyses were complemented by the study of plasma LH concentration.

METHODS: The levels of kiss 1 and RFRP-3 transcripts and GnRH and GnRHR proteins were analyzed using Real-time PCR technique or an enzyme-linked immunoabsorbent assay (ELISA), respectively. Plasma LH concentration was measured by a double-antibody radioimmunoassay.

RESULTS: Pharmacological blockade of D2 receptors by sulpiride significantly increased kiss 1 mRNA levels in the preoptic area and in the arcuate nucleus/ventromedial hypothalamus and decreased the RFRP-3 mRNA in the paraventricular nucleus. The abolition of dopaminergic neurons activity also resulted in augmentation of the GnRH and GnRHR protein levels in the entire hypothalamus and led to similar changes in the expression of GnRHR in the anterior pituitary gland. The increase of kiss 1 transcription and GnRH biosynthesis has appeared with a concomitant increase of LH secretion.

CONCLUSIONS: Similar direction of changes of kiss 1 transcript and GnRH protein levels with LH secretion strongly suggest that the inhibition of D2 receptors has a stimulatory effect on kiss1/GnRH biosynthesis and consequently on LH release. It is likely, that dopamine inhibits gonadotropin synthesis and release in anestrous period through complex actions of kiss1/RFRP-3 neurons on GnRH cells.

P5.3

EFFECTS OF GONADECTOMY AND TESTOSTERONE REPLACEMENT ON NUMBER OF DYNORPHIN-IMMUNOREACTIVE (-IR) CELLS IN THE ARCUATE NUCLEUS OF THE HYPOTHALAMUS IN OBESE AND DIABETIC MALE RATS

Kamil Ziarniak[1], Monika Gawałek[1], Ewa Pruszyńska-Oszmałek[2], Paweł A. Kołodziejski[2], Przemysław Kaczmarek[2], Ewa Rodak[1], Joanna H. Śliwowska[1]

[1]Laboratory of Neurobiology, Institute of Zoology; [2]Department of Physiology and Biochemistry Poznań University of Life Sciences, Poznań, Poland

BACKGROUND AND AIMS: Dynorphins (Dyn) are involved in the regulation of feeding and kisspeptin together with dynorphin and neurokinin B play a role in reproductive functions. Besides primary metabolic health problems occurring in people with obesity and/or diabetes, there are disruptions of the reproductive system

(e.g. hypogonadism, infertility). Moreover, alterations in the hypothalamic dynorphin system in food deprived and diabetic animals were reported. However, there is no study looking at changes in number of Dyn-ir neurons in the arcuate nucleus of the hypothalamus (ARC), where integration of metabolism and reproduction may occur in obese and diabetic rats. There were 2 aims: (1) to assess if obesity induced by high fat diet and/or diabetes induced by injections of streptozotocin (STZ) alters the number of Dyn-ir neurons in the ARC in male rats; (2) to examine if gonadectomy (GDX) and testosterone (T) replacement differentially alters the number of Dyn-ir neurons in the ARC in male rats.

METHODS: Rats were fed with high fat diet (HFD) or control (C) diet for 5 weeks. Injections of STZ were performed to induce diabetes type 1 (C/STZ) or diabetes type 2 (HFD/STZ) The following groups were obtained: C, C/STZ, HFD, HFD/STZ. Next, animals were divided into 3 groups: gonadectomy (GDX); gonadectomy and T replacement (GDX+T) and control (Sham). Immunocytochemistry for the Dyn was performed.

RESULTS: Dynorphin-ir was found in: paraventricular nucleus of the hypothalamus, supraoptic nucleus, ventromedial nucleus, lateral hypothalamus, ARC and median eminence. Preliminary results indicate that there is a slight increase in number of Dyn-ir cells in C/STZ and a slight decrease in HFD/STZ group. Currently data from GDX and GDX+T animals is analyzed.

CONCLUSIONS: If data is confirmed on the bigger sample (currently analyzed), observed changes may contribute both to metabolic and reproductive deficits observed in these animals.

Supported by grant NCN 2011/01/B/NZ4/04992.

P5.4

EFFECTS OF GONADECTOMY AND TESTOSTERONE REPLACEMENT ON NKB-ir CELL NUMBER IN THE ARCUTE NUCLEUS OF THE HYPOTHALAMUS IN OBESE AND DIABETIC MALE RATS

Monika Gawałek[1], Paweł Kołodziejski[2], Ewa Pruszyńska-Oszmałek[2], Ewa Rodak[1], Kamil Ziarniak[1], Przemysław Kaczmarek[2], Joanna H. Śliwowska[1]

[1]Laboratory of Neurobiology, Institute of Zoology; [2]Department of Animal Physiology and Biochemistry Poznan University of Life Sciences, Poznań, Poland

BACKGROUND AND AIMS: Reproduction is governed by the hypothalamus-pituitary-gonadal (HPG) axis, with the gonadotropin releasing hormone being on the top of the axis. In the arcute nucleus (ARC) of hypothalamus, population of neurons expressing kisspeptin, neurokinin B (NKB) and dynorphin (KNDy neurons) is present. Those neurons are important in regulation of GnRH secretion. Beside metabolic problems obesity and diabetes are a major risk factors for reproductive dysfunctions (e.g. steroid imbalance and hypogonadism). Moreover, in hypogonadotropic hypogonadism patients mutation in NKB gene (TAC3) and its receptor – TAC3R was reported. In animals data on the role of gonadectomy (GDX) and sex steroids replacement in regulation of NKB expression is spare.

We hypothesized that: (1) diet-induced obese (DIO), and/or streptozotocin (STZ)-induced diabetic (type 1 and 2) male rats would have altered number of NKB-ir neurons in the ARC; (2) gonadectomy and testosterone (T) replacement would differentially altered number of NKB-ir neurons.

METHODS: Rats were fed with high fat diet (HFD) or control (C) diet for 5 weeks. Injections of STZ were performed to induce diabetes type 1 (C/STZ) or diabetes type 2 (HFD/STZ). The following groups were obtain: C, C/STZ, HFD, HFD/STZ. Next, animals were divided into 3 groups: gonadectomy (GDX); gonadectomy and T replacement (GDX+T) and (Sham). Immunocytochemistry for the NKB was performed.

RESULTS: We found that in C group there was no difference in number of NKB-ir neurons in the ARC between Sham and GDX. In contrast, in all experimental groups a decrease in NKB-ir cell number after GDX was shown. T replacement caused a decrease in NKB-ir cell number in C, HFD and HFD/STZ groups compare to Sham, respectively.

CONCLUSION: Obesity and diabetes type 1 and type 2 leads to alter response of NKB-ir cells in response to GDX.

Supported by NCN grant 2011/01/B/NZ4/04992.

P5.5

THE DIFFERENT EFFECTS OF KISSPEPTINS ON THE HYPOTHALAMIC-PITUITARY-GONADAL AXIS ARE DEPENDENT ON THEIR LENGTHS

Lea Bäcker, Maria E.K. Lie, Agnete Overgaard, Jens D. Mikkelsen

Neurobiology Research Unit, Copenhagen University Hospital Rigshospitalet, Copenhagen, Denmark

BACKGROUND AND AIMS: Kisspeptins (Kps) are structurally related peptides involved in the upstream regulation of the hypothalamic-pituitary-gonadal (HPG) axis. Their precursor is posttranslationally processed into peptides of various lengths. The forms considered to result from endogenous processing are the longest with 54 or 52 amino acids in humans or rodents, respectively. However, the shortest functional Kp consists of only the 10 amino acids at the C-terminal of the long forms. In this study, we used various mouse, rat and human Kps to assess the effect of peptide length and administration route on the activation of the HPG axis.

METHODS: Male Wistar rats (n=4–8) received an intraperitoneal (ip; 150 nmol/kg) injection of mouse (m) Kp-10, -13, -14, -16, -52, rat (r) Kp-16, human (h) Kp-16, -54 or vehicle, or an intracere-

broventricular (icv; 8 nmol/kg) injection of mKp10, mKp52, hKp54 or vehicle. After 45 min, we measured plasma concentrations of testosterone (T) and luteinizing hormone (LH) with radioimmunoassay and enzyme-linked immunosorbent assay, respectively, as well as expression of the immediate early gene c-Fos in gonadotropin-releasing hormone (GnRH) neurons with immunohistochemistry.
RESULTS: Ip injections of mKp10, -16, -52, rKp16, hKp16 and -54 significantly increased T levels. hKp54 also produced a significant increase in LH levels, whereas shorter forms had no significant effect. Similarly, only hKp54 increased c-Fos in GnRH neurons in the vicinity of the organum vasculosum of the lamina terminalis (OVLT) including the anteroventral periventricular nucleus (AVPV). Icv injections of mKp10, mKp52 and hKp54 caused T and LH increases for mKp10 only. All three peptides produced significant GnRH neuron activation in the OVLT, the medial septum and the AVPV.
CONCLUSIONS: This study demonstrated that the amino acid sequences of kisspeptins and their administration route are determining factors for the mechanisms by which they affect GnRH neurons and the HPG axis.

P5.6

NEUROPEPTIDE CO-EXPRESSION IN KISSPEPTIN NEURONS OF THE HUMAN HYPOTHALAMUS

Erik Hrabovszky[1], Katalin Skrapits[1], Beáta Á. Borsay[2], László Herczeg[2], Philippe Ciofi[3], Zsolt Liposits[1]

[1]Institute of Exprerimental Medicine, Hungarian Academy of Sciences, Budapest, Hungary; [2]Department of Forensic Medicine, Faculty of Medicine of the University of Debrecen, Debrecen, Hungary; [3]INSERM U862, Neurocentre Magendie, Bordeaux, France

BACKGROUND AND AIMS: Hypothalamic kisspeptin (KP) neurons use peptidergic signaling to regulate reproduction and puberty.
METHODS: Here we have carried out a series of immunofluorescent experiments on formalin-fixed post mortem histological samples to identify the neuropeptide co-transmitters of human KP cells.
RESULTS: In these colocalization studies using confocal microscopy, we found no evidence for neurotensin, cholecystokinin, proopiomelanocortin-derivatives, agouti-related protein, neuropeptide Y, somatostatin or tyrosine hydroxylase (dopamine) expression in KP neurons. In contrast, neurokinin B (NKB) was present in a large subset of these cells, as reported earlier in laboratory animals. Dynorphin, which has also been described in KP neurons of rodents and sheep, was observed rarely in human KP cells and their axons, and similarly, human KP neurons did not contain signal for galanin, a neuropeptide colocalized earlier with KP in mice. To the opposite, 30–50% of KP and NKB neurons in humans expressed immunoreactivity for substance P (SP) and cocaine- and amphetamine-regulated transcript (CART), unlike in laboratory species. In addition, we have provided evidence for a sexually dimorphic co-expression of proenkephalin (pENK) with KP and NKB. The pENK signal was detectable in 12.5±5.1% of NKB-IR and 1.9±1.0% of KP-IR neurons and in 5.7±2.5% of NKB-IR and 4.9±1.8% of KP-IR axon varicosities in human males. This colocalization was absent in postmenopausal women.
CONCLUSIONS: The presence of substance P, CART and pENK in human KP neurons, together with the absence of dynorphin and galanin in most of these cells, indicate that humans use considerably different neurotransmitter mechanisms than rodents, to regulate fertility.

P5.7

ANATOMICAL STUDIES OF HYPOTHALAMIC SITES WHERE NEURONAL NITRIC OXID SYNTHASE SIGNALING MAY REGULATE HUMAN FERTILITY

Erik Hrabovszky[1], Csilla Maurnyi[1], Katalin Skrapits[1], Vincent Prévot[2]

[1]Institute of Exprerimental Medicine, Hungarian Academy of Sciences, Budapest, Hungary; [2]Inserm U837, Lille, France

BACKGROUND AND AIMS: Recent anatomical and transgenic studies in mice provide evidence for the critical involvement of neuronal nitric oxide synthase (nNOS) neurons in metabolic- and sex steroid regulation of fertility. In humans, the nature and sites of putative interactions between nNOS neurons and the hypothalamic circuitry controlling reproduction is entirely unknown.
METHODS: To study anatomical sites where such interactions may occur, we have carried out immunohistochemical experiments on formalin-fixed post mortem histological samples of postmenopausal women.
RESULTS: The perikarya and fibers of nNOS-immunoreactive neurons were widely distributed in preoptic/hypothalamic tissue sections of the human. High labeling intensities were observed in the diagonal band of Broca, the medial preoptic area and the hypothalamic ventromedial, dorsomedial, infundibular (Inf), paraventricular and supraoptic nuclei. At many of these sites including the Inf, gonadotropin-releasing hormone (GnRH) neurons were often surrounded by nNOS-immunoreactive neurons. In addition, nNOS cells of the Inf received frequent axo-somatic and axo-dendritic neuronal contacts from the local kisspeptin neurons. Unlike in the mouse arcuate nucleus where nNOS and kisspeptin neurons are distinct, these two cell populations showed a partial overlap in the human; 1–20% of kisspeptin-immunoreactive cell bodies in three different individuals (12.98±3.7%) showed co-labeling for nNOS. The abundant kisspeptin input to nNOS cells, together with the

close anatomical relationship between nNOS and GnRH neurons, raise the possibility that kisspeptin neurons act on nNOS cells to influence GnRH neurons indirectly, in addition to activate GnRH neurons directly. Evidence for such indirect communication route has been reported recently in mice (Hanchate et al. 2012, J Neurosci 32: 932–945).

P5.8

KISSPEPTIN 1-10 AND RFRP-3 MODULATE SF-1/B-CATENIN/DAX-1 mRNAs STABILITY AND PROTEINS EXPRESSION IN THE ANTERIOR PITUITARY GLAND *IN VIVO*

Marlena Zielińska-Górska[1], Konrad Górski[2], Alina Gajewska[1]
[1]Neuroendocrinology Department, [2]Endocrinology Department, The Kielanowski Institute of Animal Physiology and Nutrition, Polish Academy of Sciences, Jabłonna, Poland

BACKGROUND AND AIMS: In gonadotrope cells, Wnt/β-catenin signaling regulates SF-1 transcription factor gene activity. SF-1 response elements are found in the promoter regions of four gonadotrope signature genes: LHβ, FSHβ, α-GSU and GnRH-R. β-catenin and DAX-1 act as SF-1 transcriptional co-activator/corepressor, respectively, to affect SF-1 mediated transcription of its target genes. The relationship between hypothalamic GnRH neurons steady-state level and posttranscriptional regulation of transcriptional factors genes activity in anterior pituitary gland remains unknown.
METHODS: The present study was to determine whether and how kisspeptin 1–10 and RFPR-3, both known as potent regulators of endogenous GnRH network, affect SF-1/β-catenin/DAX-1 mRNA stability and what is their impact on SF-1/β-catenin/DAX-1 proteins intracellular expression and localization. 32 female rats received intracerebroventricular pulsatile microinjections of 2 nM GnRH, 1 nM kisspeptin 1–10, 2 nM RFRP-3 and 0.9% NaCl (control). To estimate mRNAs stability, USB poly(A) tail-length Assay Kit (Affymetrics) was used. For protein analysis, double-label immunofluorescence technique was applied. Formalin fixed 4 μm paraffin sections were stained and visualized on a FluoView FV-1000 OLYMPUS confocal microscope.
RESULTS: Obtained results indicate that regulatory impact of kisspeptin 1–10 and RFRP-3 on pituitary SF-1/β-catenin/DAX-1 system activity can be exerted *via* modulation of mRNAs stability. Impact on poly(A) tail length is not related to effects exerted on mRNA expression level. Exogenous kisspeptin, ineffective in SF-1 as well as β-catenin genes transcription stimulation, promoted elongation of their poly(A) tails length. In contrast, exogenous RFRP-3 affected DAX-1 mRNA stability by promotion of poly(A) tail length shortening.

P5.9

LOW DOSE CHLORPYRIFOS EXPOSURE TO FEMALE RATS BEFORE AND DURING PREGNANCY AFFECTS THEIR BEHAVIOR

Volodymyr Rosalovskyi, Sofia V. Grabovska, Yuriy T. Salyha
Institute of Animal Biology, Lviv, Ukraine

BACKGROUND AND AIMS: Chlorpyrifos (CPF) is a common organophosphate pesticide that can be dangerous for non- target organisms, including humans, due to its neurotoxicity. Its main mechanism is cholinesterase inhibition, but CPF also leads to various alterations on physiological, biochemical and even cell levels, caused by other mechanisms. In the last few years, new data emerged, showing neurotoxic effects of even low doses of CPF, previously claimed as "safe". The aim of our study was to investigate if chronic exposure of female rats to low doses of chlorpyrifos, or a single dose at the beginning of the pregnancy (both causing no visible acute effects), does lead to any alterations in the emotional state and cognitive abilities of their offspring.
METHODS: We exposed three groups of female Wistar rats to 5, 10 and 15 mg/kg CPF daily for 30 days, 4 months before their pregnancy. One more group of females was exposed to a single dose of 30 mg/kg CPFat the 6th gestational day. Control females were intact. All rat pups were counted, measured and weighted for calculation of the survival ratio, weight gain temp, and Quetelet body mass index. The grown-up offspring of all groups was tested with behavioral tests: Open Field, Dark/Light Box, Extrapolation Escape Task.
RESULTS: The offspring of exposed females showed lower survival ratio and Quetelet index, comparing to controls. Also, the exploratory activity of rats whose mothers were exposed to 15 mg/kg CPF before pregnancy, abnormally increased in Open Field and Dark/Light Box tests, and the prenatally exposed rats showed poorer cognitive abilities in the Extrapolation Escape Task.
CONCLUSION: Chronic exposure of female rats to low doses of CPF before and during pregnancy caused an increase in exploratory activity and a decrease in cognitive abilities of the second generation.

P5.10

MECHANISMS OF CENTRAL NEURAL REGULATION OF GNRH/LH SECRETION UNDER SHORT AND PROLONGED STRESS IN THE HYPOTHALAMIC-PITUITARY UNIT OF EWES IN DIFFERENT STAGES OF REPRODUCTION

Magdalena Ciechanowska[1], Magdalena Łapot[1], Bożena Antkowiak[1], Krystyna Mateusiak[2], Edyta Paruszewska[1], Małgorzata Paluch[1], Franciszek Przekop[2]
[1]Department of Pharmacology and Toxicology, The General Karol Kaczkowski Military Institute of Hygiene and Epidemiology, Warsaw, Poland; [2]Department of Neuroendocrinology, The Kielanowski Institute of Animal Physiology and Nutrition, Polish Academy of Sciences, Jablonna, Poland

AIM: The aim of this study was (1) to analyze the effects of short and prolonged stress (footshock stimulation) on the biosynthesis of GnRH and GnRHR proteins in the hypothalamus-pituitary region and on luteinizing hormone (LH) secretion in anestrous and follicular phase ewes (2) to determine whether applied models of physical stress influence mRNA expression of kisspeptin (kiss1) and RFamide-related peptide-3 (RFRP-3), two central regulators of the mammalian reproductive axis.
METHODS: The levels of the GnRH and GnRHR proteins were analyzed in selected tissue of the hypothalamus-anterior pituitary unit using an enzyme-linked immunoabsorbent assay (ELISA). Plasma LH concentration was measured by a double-antibody radioimmunoassay. To determine the transcript levels of kiss 1 and RFRP-3 in the preoptic area-hypothalamus region, Real-time PCR with SYBR Green dye was applied.
RESULTS: Stress changed drastically the biosynthesis of GnRH and GnRHR, as well as the transcriptional activities of genes encoding kiss 1 and RFRP3 neuropeptides. The pattern of these changes was dependent upon physiological state of animal and on the time course of stressor application. The fluctuations of GnRH and GnRH-R protein levels under short or prolonged stress stimuli were associated with similar changes in LH secretion, thus suggesting the existence of a direct relationship between GnRH and GnRH-R biosynthesis and GnRH/LH release.
CONCLUSIONS: The results indicate that disturbances of gonadotropin secretion under stress condition in sheep may be due to dysfunction of the hypothalamus/pituitary GnRH/GnRHR system. It can not be excluded that interaction between kiss 1 and RFRP3 neuronal networks with GnRH cells may also play a critical role in the transduction of stress-induced changes in the activity of hypothalamic-pituitary-gonadal axis. However, a detailed explanation of this phenomenon requires further research.

POSTER SESSION P6. PARKINSON'S DISEASE

P6.1
CHANGES IN METABOLIC SUBSTRATES AFTER PROLONGED ASTROCYTES DYSFUNCTION AND DOPAMINERGIC NEURONS DEGENERATION IN SUBSTANTIA NIGRA

Katarzyna Kuter[1,2], Łukasz Olech[1], Urszula Głowacka[1]
[1]Department of Neuropsychopharmacology, Institute of Pharmacology, Polish Academy of Sciences, Kraków, Poland; [2]Physical Biochemistry, Department of Chemistry, Technische Universität Darmstadt, Germany

BACKGROUND AND AIMS: Underlying cause of movement disorder in Parkinson's disease is degeneration of dopaminergic neurons in substantia nigra (SN). Supportive role of astrocytes in neuronal energy metabolism was reported. Prolonged dysfunction of astrocytes could increase dopaminergic neurons vulnerability.
Our aim was to investigate if prolonged metabolic inhibition of astrocytes influences dopaminergic neurons metabolic substrates utilization.
METHODS: Rat model of selective nigrostriatal dopaminergic system degeneration was induced by injection of 6-hydroxydopamine (6-OHDA) into medial forebrain bundle. Astrocytes metabolic dysfunction was caused by 7-days infusion of fluorocitrate (FC) into SN.
RESULTS: Densytometric analysis of astrocytes marker – GFAP showed decreased staining after FC treatment. After 4 weeks this effect was diminished suggesting regrowth of astrocytes. 6-OHDA injection caused smaller decreases. FC infusion for 7 days decreased tissue levels of succinate and lactate in SN. Lesioning of dopaminergic neurons increased succinate but decreased lactate levels. Combined treatment neutralized effect on succinate but aggravated lactate decrease. 4 weeks after operation and FC withdrawal lactate levels increased while lesion effect was normalized. At this timepoint profile of the changes on succinate was the same as after one week. Beta-hydroxybutyrate levels significantly increased after FC and dopaminergic lesion and combined effect was enhanced at 7th day. After 4 weeks all groups still showed slightly elevated levels.
CONCLUSIONS: 7-day FC administration caused prolonged metabolic dysfunction of astrocytes and influenced dopaminergic neurons metabolism. We suggest that enhanced production of succinate and ketone bodies after dopaminergic neurons degeneration could be one of metabolic compensatory mechanisms.
Study supported by the Statutory Funds of the Institute of Pharmacology, PAS, Poland and NCN grant nr 2012/05/B/NZ4/02599.

P6.2
PERINATAL EXPOSURE TO LEAD (PB) AFFECTS THE FUNCTION OF TAU AND TAU-KINASES IN THE RAT BRAIN

Magdalena Gąssowska[1], Irena Baranowska-Bosiacka[2], Joanna Moczydłowska[1], Grzegorz Arkadiusz Czapski[1], Anna Falkowska[2], Lidia Strużyńska[3], Agata Adamczyk[1]
[1]Department of Cellular Signalling, Mossakowski Medical Research Centre, Polish Academy of Sciences, Warsaw, Poland; [2]Pomeranian Medical University, Department of Biochemistry and Medical Chemistry, Szczecin, Poland; [3]Department of Neurochemistry, Mossakowski Medical Research Centre, Polish Academy of Sciences, Warsaw, Poland

BACKGROUND AND AIMS: Hyperphosphorylation of Tau is involved in the pathomechanism of neurodegenerative disorders such as Alzheimer's and Parkinson's diseases. Epidemiological data suggest the significance of early life exposure to lead (Pb) in etiology

of disorders affecting brain function. However, the precise mechanisms by which Pb exerts neurotoxic effects are not fully elucidated. In this study, we investigated the effect of perinatal exposure to Pb on Tau pathology in selected rat brain structures: forebrain cortex (FC), cerebellum (C) and hippocampus (H). Concomitantly, we examined the ultrastructural alterations in these regions. Furthermore, the involvement of two major Tau-kinases: glycogen synthase kinase-3 beta (GSK-3β) and cyclin-dependent kinase 5 (CDK5) in Pb-induced Tau modification was analysed.

RESULTS: Our data revealed that pre- and neonatal exposure of rats to Pb (concentration in rat offspring's blood below a 'safe level') evoked significant increase in the phosphorylation of Tau at Ser396 with parallel rise in the level of total Tau protein in FC and C. Moreover, in these brain structures, GSK-3β activity was increased by phosphorylation of a tyrosine residue – Tyr-216. However, GSK-3β phosphorylation on serine residue, Ser-9 was unchanged. Parallel with GSK-3β activation we observed increase of total GSK-3β level in FC from rats subjected to Pb. In Pb-treated cerebellum we showed calpain-dependent cleavage of CDK5-activating protein p35 leading to formation of p25 and CDK5 overactivation. Molecular alterations were accompanied by pathological changes in ultrastructure of all examined brain structures from rats subjected to Pb.

CONCLUSION: Perinatal exposure to lead induces Tau modification in the rat cortex and cerebellum. We suggest that its neurotoxic effect might be mediated, at least in part, by GSK-3β and CDK5-catalysed Tau hyperphosphorylation, leading to impairment of cytoskeleton stability.

Supported from MMRC statutory theme 8.

P6.3

NEUROPROTECTIVE EFFECT OF FINGOLIMOD AND PRAMIPEXOLE IN PARKINSON'S DISEASE ANIMAL MODEL

Joanna Motyl[1], Łukasz Przykaza[2], Piotr Kosson[3], Paweł M. Boguszewski[4], Joanna B. Strosznajder[1]

[1]Department of Cellular Signalling, [2]Laboratory of Experimental Neurosurgery, Department of Neurosurgery, [3]Laboratory for Genetically Modified Animals, Mossakowski Medical Research Centre, Polish Academy of Sciences, Warsaw, Poland; [4]Laboratory of Limbic System, Department of Neurophysiology, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland

BACKGROUND AND AIMS: Recently sphingolipids alterations have been shown to play an important role in pathomechanism of neurodegenerative diseases. Our last study indicated suppression of gene expression and activity of sphingosine kinases (Sphk1/2)/sphingosine-1-phosphate (S1P) synthesis in cellular model of Parkinson's disease (PD). Moreover, the cytoprotective effect of S1P and its analog Fingolimod (P-FTY720) in this PD model was observed. The fundamental goal of current research was to determine the impact of FTY720 and dopamine D2/D3 receptors agonist – pramipexole (PPX) on death signalling and motor activity in mice PD model.

METHODS: Neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP, 40 mg/kg) was administrated ip to adult C57BL/6 mice. FTY720 (1 mg/kg) or PPX (1 mg/kg) was injected ip during 10 days. Then behavioral tests (open field, rota-rod, and pole test) were performed. Midbrain and striatum were used for further studies. The immunochemical, spectrofluorometrical, and QPCR methods were applied.

RESULTS: Our results indicated significant reduction in the number of dopaminergic cells in the midbrain of MPTP treated animals. Moreover, in this PD model alterations of Sphk1/2 and Akt kinase mediated signalling were found. It was also detected that gene expression of pro-apoptotic proteins in midbrain cells was activated. FTY720 and PPX protected dopaminergic cells against death as a result of Sphks up-regulation and apoptotic signalling suppression. In behavioural examination, MPTP mice exhibited impaired motor coordination in rota-rod test. Total time spent on the accelerating rota-rod was increased two-fold after FTY720 and PPX administration. We have also observed total distance elongation in animals treated with both above mentioned compounds during open-field test.

CONCLUSIONS: In conclusion, this study indicated that FTY720 and PPX contribute to improvement of mice motor activity and they offer opportunities for PD therapy.

Supported by NCN grant 2013/09/N/NZ4/02045.

P6.4

THE INFLUENCE OF PHOSPHODIESTERASE INHIBITORS ON DEGENERATION AND NEUROINFLAMMATION IN THE MOUSE MODEL OF PARKINSON'S DISEASE

Joanna Schwenkgrub[1], Małgorzata Zaremba[1], Ilona Joniec-Maciejak[1], Agnieszka Cudna[1], Dagmara Mirowska-Guzel[1], Iwona Kurkowska-Jastrzębska[2]

[1]Department of Experimental and Clinical Pharmacology, Centre for Preclinical Research and Technology, Medical University of Warsaw, Warsaw, Poland; [2]2nd Department of Neurology, Institute of Psychiatry and Neurology, Warsaw, Poland

BACKGROUND AND AIMS: A neuroprotective or disease modifying treatment of Parkinson's disease (PD) still remains an unmet need. The non-clinical and clinical studies have indicated that cyclic nucleotide phosphodiesterase (PDE) inhibitors represent a novel class of drugs which may be useful in treating neuroinflam-

mation disorders. The present study was designed to examine the efficacy of PDE inhibitors, ibudilast (IBD) and vinpocetine (VPC) in the mice model of PD induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP).

METHODS: 3 months-old male C57Bl/10Tar mice were treated with IBD (0, 20, 30, 40 or 50 mg/kg) BID for 9 days or VPC (0, 10, 20 or 30 mg/kg) once daily for 8 consecutive days (beginning 2 days or 1 day prior to MPTP (60 mg/kg) intoxication, respectively). Rotarod test was conducted on day 3 post MPTP injection. Mice were sacrificed 7 days after MPTP intoxication. IL-1β, IL-6, TNF-α and GDNF mRNA expression in the striatum was examined by the Real Time RT-PCR method. Western blot analysis was used to estimate tyrosine hydroxylase (TH) expression, micro- and astroglia activation markers (Iba1 and GFAP, respectively). Dopamine (DA) metabolism was evaluated by HPLC method.

RESULTS: Chronic administration of PDE inhibitors attenuated astroglial reactivity and increased glial cell-derived neurotrophic factor (GDNF) gene expression in the striatum. IBD reduced TNF-α, IL-6 and IL-1β expressions, whereas VPC had no impact on elevated levels of TNF-α. Moreover, mice receiving 40 mg/kg IBD showed significant improvement in the locomotor activity compared to control. However, PDE inhibitors did not change DA metabolism and TH expression in the striatum.

CONCLUSIONS: The findings provide evidence for the glia-derived protective properties of PDE inhibitors in the MPTP-induced model of PD. This response may be promising for the better outcome in the later stages of neurodegeneration. However, the further study is needed to confirm such possibility.

P6.5

OLFACTORY SENSITIVITY DEFICIT IN THE MPTP MODEL OF PD AS A CONSEQUENCE OF BIOCHEMICAL LATERALIZATION AND NORADRENERGIC DEPLETION IN THE OLFACTORY BULBS

Paweł M. Boguszewski[1], Małgorzata Zaremba[2,3], Ilona Joniec-Maciejak[2], Iwona Kurkowska-Jastrzębska[4]

[1]Laboratory of Limbic System, Department of Neurophysiology, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland; [2]Department of Experimental and Clinical Pharmacology, Centre for Preclinical Research and Technology, Medical University of Warsaw; [3]Environmental Laboratory of Magnetic Resonance Imaging of Small Animals, Mossakowski Medical Research Centre, Polish Academy of Sciences, Warsaw, Poland; [4]2nd Department of Neurology, Institute of Psychiatry and Neurology, Warsaw, Poland

BACKGROUND AND AIMS: Parkinson disease (PD) is a more general disease than thought previously and involves prominent nonmotor manifestations (e.g. anosmia, hyposmia). Olfactory dysfunction can precede onset of motor symptoms by up to 10 years and they might provide biomarkers of the pathogenetic process. The initiating neurobiological bases for such disturbance are still elusive. However, recent findings suggest a degree of independence of olfactory dysfunction from nigrostriatal dopamine (DA). The aim of this study was to assess the extent of influence of biochemical imbalance in the left and right olfactory bulbs (OBs) on olfaction in old (1 or 1.5 year) mice caused by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) intoxication.

METHODS: The olfactory capability was evaluated using a battery set of olfaction tests: The Buried Food Test (BFT), Olfactory Discrimination Test (ODT), Olfactory Sensitivity Test (OST). Noradrenaline (NE) and DA metabolism was evaluated by High-performance liquid chromatography (HPLC) method.

RESULTS: Intraperitoneal administration of MPTP caused significant reduction in NE and DA contents, observed mostly in the right OB at 7 and 180 days post intoxication. Interestingly, diminished noradrenergic projection and intensification of compensatory mechanism in the right OB exerted disturbing effect in some olfaction test (OST, BFT). Compared to controls, MPTP mice displayed insensitivity to low concentration odors in the OST and spend more time to find a buried food in the BFT.

CONCLUSIONS: Results of the study strong indicate on lateralization in the OBs. Reasons for this phenomenon are unknown, although they may reflect lateralized differences in the function of the two sides of OBs. Diminished NE projection may play more important role in odor sensitivity than DA and may be related to dysfunction in odor recognition (hyposmia).The present findings seem to justify future studies about possible role of NE for therapeutic manipulation in PD.

POSTER SESSION P7. GENETICS IN NEUROPATHOLOGY

P7.1

PHOTOTHROMBOTIC STROKE-INDUCED CHANGES IN EXPRESSION LEVEL OF GENES CODING FOR ENZYMES ASSOCIATED WITH HYALURONIC ACID METABOLISM IN THE MOUSE BRAIN

Anna Katarzyna Greda, Dorota Nowicka

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

BACKGROUND AND AIMS: Perineuronal nets (PNNs) are complex structures of the nervous system extracellular matrix, composed of hyaluronic acid and chondroitin sulfate proteoglycans. PNNs surround mostly the subset of parvalbumin-expressing interneurons by enwrapping their cell body and proximal dendrites. Their presence stabilizes existing synapses and limits neuronal plas-

ticity. Mechanisms that regulate PNNs expression remain unclear. Changes in PNNs expression are observed in perilesional area after photothrombosis and can be considered an attempt to create conditions favorable for synaptic remodeling. We hypothesize that they may result from the activity of endogenous glycolytic enzymes.
METHODS: In the present study, employing quantitative RT-qPCR, we analyzed the expression of genes coding for hyaluronic acid synthesizing and degrading enzymes in cortical region of the perilesional area at 1 h, 24 h and 7 days after photothrombotic stroke.
RESULTS: Analysis revealed substantial increase in hyaluronidase 1 (HYAL1) expression level in comparison to homotopic contralateral cortical region and control animals. The rise was recognized at 24 h after stroke and maintained up to 7 days after stroke. No change in expression level of HYAL2 was detected. While 24 h after photothrombosis the elevation in hyaluronic acid synthase 2 (HAS2) expression level was observed, the decrease in HAS3 expression level occurred. The expression level of HAS1 maintained unchanged after stroke.
CONCLUSIONS: The obtained data indicate photothrombotic stroke-evoked modification of hyaluronic acid component of PNNs in perilesional area. The results show parallel processes of degradation and synthesis at 24 h, which may exert different effects on neuronal plasticity. This work was supported by NCN grant 2012/05/B/NZ3/00851.

P7.2

ACTIVITY OF MMP-3 PROTEASE AFFECTS EXCITATORY AND INHIBITORY SYNAPTIC TRANSMISSION IN HIPPOCAMPUS

Grzegorz Wiera[1], Enrica Petrini[2], Andrea Barberis[2], Jerzy W. Mozrzymas[1]

[1]Wrocław University; Wroclaw Medical University, Wrocław, Poland; [2]Italian Istitute of Technology, Genoa, Italy

BACKGROUND AND AIMS: Several neuronal processes are regulated by matrix metalloproteinases (MMPs), a family of zinc-dependent proteases. It is well established that long-term synaptic plasticity, learning and memory involve extracellular activity of MMPs but the role of some of them is not fully identified. The aim of this study was to address the functions of MMP-3 in synaptic plasticity of excitatory and inhibitory synaptic transmission.
METHODS: Field potentials recordings in acute mice hippocampal slices and single molecule-tracking of $GABA_A$ receptors in neuronal cultures.
RESULTS: We have shown that in MMP-3 knock-out mice late-LTP is impaired in CA3-CA1 projection (WT: 180±10% of baseline 2 h after induction, n=15; KO: 134±12%, n=16; P<0.01). Treatment of wild-type slices with SB3CT (a specific MMP-9 inhibitor) also blocked late-LTP, without affecting early phase of LTP. These observations suggest that both MMP-9 and MMP-3 together may regulate the maintenance of LTP. We next determined whether impaired late-LTP in MMP-3 KO slices is further weakened by MMP-9 inhibition with SB3CT. Knockout of MMP-3 together with MMP-9 blockade (SB-3CT) caused a strong reduction in both early and late LTP phases [WT: 170±8% of baseline 20 min after induction, n=16; MMP-3 KO: 157±8%, n=17; MMP-3 KO+SB-3-CT: 138±6%, n=8; P(WT vs. MMP-3 KO)=0.26, P(WT vs. MMP-3 KO+SB-3CT)=0.01]. Additionally, we studied the role of MMP-3 in inhibitory transmission using single molecule tracking of $GABA_A$ receptors (α1 subunit). We have found that 10 or 45 min treatment with active MMP-3 protein decreased the diffusion coefficient of both synaptic and extrasynaptic $GABA_A$ receptors (P<0.01).
CONCLUSIONS: Our observations indicate that activity of MMP-3 is necessary for LTP maintenance and suggest that MMP-3 regulates membrane mobility of GABA receptors. Future work is needed to elucidate the impact of MMP-3 on plasticity of inhibitory synapses.
Support: NCN grants NN401541540, 2013/08/T/NZ3/00999.

P7.3

EVALUATION OF TRANSGENIC MICE CONDITIONALLY LACKING CREB IN NORADRENERGIC NEURONS AS A NOVEL TOOL FOR STUDYING ITS ROLE IN THE ANTIDEPRESSANT DRUG ACTION

Katarzyna Rafa-Zabłocka, Grzegorz Kreiner, Monika Bagińska, Irena Nalepa

Institute of Pharmacology Polish Academy of Sciences, Kraków, Poland

BACKGROUND AND AIMS: Most of the current antidepressants modulate levels of monoamines just after administration, however, only after prolonged therapy the clinical effect may be observed. Myriad attempts tried to identify molecular factors responsible for such a delayed response, the prominent example being the cyclic AMP response element binding protein (CREB). Many research suggest that chronically given antidepressants enhance CREB levels and activity. On the other hand, CREB knock-out mice showed rather antidepressant-like behavior, however, the compensatory effects of cAMP response element modulator (CREM) in absence of CREB were not taken into account. In our study we evaluated transgenic mice with selective ablation of CREB in noradrenergic cells, maintained in CREM deficient background ($Creb^{DBHCre}$Crem-/-) for elucidation of the role of CREB in antidepressant treatment.
METHODS: mRNA levels of neurotrophins and α1-adrenoceptors in $Creb^{DBHCre}$Crem-/- mice were investigated in hippocampus and prefrontal cortex using qPCR method. Next mice were screened in behavioral tests like: open field (OF), tail suspension test (TST) and rotarod. Preliminary TST after acute desipramine (DMI) administration (20 mg/kg, ip) was executed.

RESULTS: Creb^{DBHCre}Crem-/- mutant mice did not show any abnormalities in their basal phenotype, moreover the mRNA levels of studied genes were not changed either. However, preliminary experiments revealed that Creb^{DBHCre}Crem-/- show a treatment-resistant phenotype after acute DMI administration in TST, (effect absent in single mutants).
CONCLUSIONS: Our results provide further evidence for the important role of CREM as a compensatory factor and indicate that these mice may represent an unique tool to dissect the role of CREB in the mechanism of antidepressants.
Supported by statutory funds of the Institute of Pharmacology and 2014/13/B/NZ7/02293 grant. K. Rafa-Zabłocka is a holder of scholarship from the KNOW sponsored by MSHE, Poland.

P7.4

EFFECT OF BODY TEMPERATURE ON THE LEVEL OF HYPOXIA INDUCIBLE FACTOR 1 AFTER NEONATAL ANOXIA

Hanna Kletkiewicz, Agnieszka Siejka, Justyna Rogalska
Department of Animal Physiology, Faculty of Biology and Environmental Protection, Nicolaus Copernicus University, Toruń, Poland

BACKGROUND AND AIMS: Complications after neonatal asphyxia are the most common cause of subsequent neurological disorders. The mechanism involved in brain damage is closely associated with abnormal iron metabolism that is a cofactor of free-radical reactions. There is a number of evidence that one of the endogenous processes that protect the brain from damage due to perinatal hypoxia is decreasing of body temperature. It is also known, that the transcriptional hypoxia-inducible factor-1α (HIF-1α) plays the fundamental role in adaptive process in response to hypoxia. HIF-1α upregulates several genes involved in glycolysis, erythropoiesis, and angiogenesis to promote survival. Our experiments aimed at checking the effects of body temperature during simulated perinatal anoxia on the subsequent changes of HIF-1α expression in brain. Considering the key role of iron as a cofactor of free radical reactions and it's contribution in proteasomal degradation of α subunit of HIF protein, the second goal of the project was to verify the influence of deferoxamine (iron chelating agent) on the level of expression of HIF-1α in a variety of thermal conditions.
METHODS: Two-day-old Wistar rats were divided into 4 temperature groups: (1) hypothermic (31°C), (2) normothermic (33°C), (3) hyperthermic typical to adult rats (37°C) and (4) hyperthermic typical to febrile adults rats (39°C). Within each group, infants were divided into two subgroups: animals with saline injection and animals with deferoxamine injection. The temperature was controlled starting 15 minutes before, and continuing during 10 minutes of anoxia (pure nitrogen atmosphere) as well as for 2 hours postanoxia. Levels of HIF-1α gene expression were analyzed post mortem: immediately, 3 and 7 days after anoxia using Western blot analysis.
RESULTS: The results showed, that the body temperature during neonatal anoxia affects the level of HIF-1α expression. Moreover, the use of deferoxamine increases the expression of this gene.

P7.5

ROLE OF ADAPTOR COMPLEX AP2 IN FORMATION OF DENDRITIC ARBORS OF HIPPOCAMPAL NEURONS

Alicja Kościelny, Anna Malik, Aleksandra Tempes, Jacek Jaworski
International Institute of Molecular and Cell Biology in Warsaw, Warsaw, Poland

BACKGROUND AND AIMS: The proper functioning of neurons is defined by many different aspects such as dendritic branching and receptor composition within synapses. Abnormalities in dendritic tree development lead to serious dysfunctions of the nervous system. Not surprisingly, the multi-step process of dendritic tree development is highly regulated. In shRNA library screen we have identified several proteins as modifiers of dendritic tree, also involved in various aspects of membrane trafficking. b-adaptin, a key component of AP2 adaptor complex, indispensable for clathrin-dependent endocytosis was one of them. Thus, the aim of this study was to find how exactly AP2 complex contributes to shaping dendritic arbor of hippocampal neurons.
METHODS: To study role of AP2 complex in dendritic arborization of hippocampal neurons we used primary hippocampal neurons expressing shRNA against subunits of AP2 complex alone or in combination of functional rescue constructs (e.g. shRNA-resistant AP2 subunits, mutants of GluA2 subunit).
RESULTS: We show that knockdown of a and b subunits of AP2 complex as well as clathrin lead to severe reduction in number of dendrites of developing hippocampal neurons. We also show that this negative effect of b-adaptin knockdown can be rescued by overexpression of GluA2, a prototypical AP2 cargo in neurons. It was, however, possible only when ion channel GluA2 was left intact. Our preliminary data from Western blot and immunofluorescence experiments, imply that in developing neurons knockdown of AP2 leads to abnormal processing and degradation of GluA2 what eventually may lead to dendritic arbor underdevelopment.
CONCLUSIONS: In developing hippocampal neurons b-adaptin is needed for proper growth of dendritic arbors, most likely by ensuring proper processing of surface receptors needed for this process.
This work has been financed by National Research Centre grant no. 2011/03/B/NZ3/01970.

P7.6

REGULATION OF ALTERNATIVE GENE EXPRESSION IN THE MOUSE STRIATUM IN RESPONSE TO COCAINE

Magdalena Zygmunt, Jan Rodriguez Parkitna, Sławomir Golda, Marcin Piechota, Joanna Ficek, Michał Korostyński

Department of Molecular Neuropharmacology, Institute of Pharmacology, Polish Academy of Sciences, Kraków, Poland

BACKGROUND AND AIMS: Cocaine is a potent psychostimulant that increases levels of striatal dopamine and activates neuronal circuits controlling motivation and reward-based learning. Transcriptional response to cocaine includes expression of alternative gene isoforms and splicing variants. Unraveling the regulatory mechanisms that are involved in selection of active transcription start and termination sites provides novel insight into molecular basis of drug-induced brain plasticity.

METHODS: We used next-generation sequencing (RNA-seq) to comprehensively map expression of genes in the mouse striatum. Total RNA and small RNA resequencing was performed in samples collected 1 h after acute treatment with 25 mg/kg cocaine. To identify transcripts responsive to drug treatment we used Tophat read-mapper and Cufflinks algorithm for FPKM quantification. The seqinterpreter online tool was used to search for key regulatory factors that control alternative gene transcription in the brain (http://seqinterpreter.cremag.org).

RESULTS: In addition to increased expression of activity-regulated genes, different types of cocaine-inducible splicing variants and transcript biotypes were identified. Examples of different modalities of gene expression include alternative first exon (e.g. *Stxbp1*), alternative last exon (*Hsph1*), intron retention (*Dnajb5*), long noncoding RNA (*Gm13889*) and small RNA (*Mir92b* and *Mir130a*). In order to investigate neuron-type specificity of gene expression we have used fluorescence-activated cell sorting to isolate genetically labeled dopamine receptor 1 expressing neurons.

CONCLUSIONS: Our results provide a comprehensive assessment of neuronal activity-induced gene expression at the level of individual transcriptional units rather than whole genes. Further experiments will explore differences in activity-regulated gene expression in discrete neuron types, i.e. the D1 or D2 expressing medium spiny neurons of the striatum.

This work was supported by NCN grant SONATA 2011/03/D/NZ3/01686.

P7.7

TRANSCRIPTOMIC CHANGES IN FETAL BRAIN AND PLACENTA ASSOCIATED WITH ADVANCED PATERNAL AGE AND BEHAVIORAL ABNORMALITIES

Silvestre Sampino[1], Adrian M. Stankiewicz[1], Joanna Gościk[2], Artur H. Świergiel[3], Pasqualino Loi[4], Jacek A. Modliński[1], Grażyna E. Ptak[1]

[1]Institute of Genetics and Animal Breeding, Jastrzębiec, Poland; [2]Medical University of Białystok, Białystok, Poland; [3]University of Gdańsk, Gdańsk, Poland; [4]University of Teramo, Teramo, Italy

BACKGROUND AND AIMS: Advanced Paternal Age (APA) has been shown to be a significant risk factor for neurodevelopmental psychiatric disorders, such as autism. We have recently shown that mice conceived by old fathers display behavioral abnormalities which resemble key diagnostic symptoms of human autism, including social deficits, communicative defects and repetitive behaviors. The aim of the present study was to evaluate the transcriptomic profiles of brains and placentas collected from fetuses conceived by young (CTR) or old (APA) fathers, in order to reveal molecular mechanisms acting early in life leading to postnatal autistic behaviors.

METHODS: Tissues were subjected to genome-wide mRNA expression analysis using Agilent microarray technology and subsequently to Real time qPCR validation.

RESULTS: Comparison of fetal brains transcriptomic profiles of CTR and APA offspring revealed that paternal age affected the expression of 1060 genes in males and 857 genes in females. Comparisons of placentas revealed alteration of 3383 genes in males and 711 genes in females. Gene set enrichment analysis performed using i.e. the KEGG pathway database, identified significant functional clusters involved in axonal growth, extracellular matrix receptors, neuroactive ligand receptors and cytokine receptors, in fetal brains. Intriguingly, placental group of deregulated genes represented functional networks involved in neuronal and metabolic pathways. qPCR confirmed that expression of genes involved in axonal growth (Neurod2, Neurod6, Epha5) were deregulated in brains of male fetuses, and that neuronal-related genes (Nrxn3, Hif3a) were expressed by the placenta and deregulated in APA mice.

CONCLUSIONS: Overall, these results indicate that early events of brain development could be altered in fetuses conceived by old males, consistent with an indirect influences of the placenta on early neurodevelopment programming, which could underlie the subsequent onset of behavioral alterations.

P7.8

TRANSLATIONAL REGULATION OF MATRIX METALLOPROTEINASE 9 mRNA AT THE SYNAPSE

Magdalena Dziembowska[1,2], Jacek Miłek[1,2], Magdalena Jasińska[1,2], Szymon Łęski[3], Leszek Kaczmarek[1]

[1] Laboratory of Molecular Neurobiology, [3]Laboratory of Neuroinformatics, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland; [2]Laboratory of Molecular Basis of Synaptic Plasticity, Center of New Technologies, Warsaw University, Warsaw, Poland

BACKGROUND AND AIMS: Matrix metalloproteinase 9 (MMP-9) is locally translated in dendrites in response to synaptic stimulation. Its enzymatic activity at the synapse is involved in the reorganization of spine architecture and was shown to regulate spine morphology in Fragile X syndrome (FXS) which is caused by the loss of mental retardation protein (FMRP). Application of MMP-9 on neurons in culture induces formation of filopodia-like immature dendritic spines that resembles these in FXS. Furthermore, inhibiting MMP-9 activity by application of minocycline, the tetracycline analogue, to Fmr1 KO mice can rescue the abnormal spine phenotype both *in vivo* and in cultured neurons. Deregulation of local protein synthesis at the synapse contributes to spine dysmorphogenesis and synaptic dysfunction in patients with the Fragile X Syndrome.
RESULTS: Here we show that MMP-9 mRNA is a specific target of FMRP and that FMRP regulates its transport and translation at the synapse. In the absence of FMRP MMP-9 mRNA translation is increased and this causes an excess of active MMP-9 protein at synapses leading to the abnormal spine morphology. Moreover our results indicate that synaptic translation of MMP-9 can be regulated by microRNAs.
CONCLUSIONS: Our data support a model in which synaptic MMP-9 mRNA is translationally regulated by FMRP and microRNAs. We propose that the regulation of synaptic MMP-9 mRNA translation can contribute to the aberrant spine morphology observed in patients with FXS.

P7.9

EXPRESSION OF SP, CGRP, NOS, VIP AND GAL IN THE PORCINE NG NEURONS SUPPLYING PREPYLORIC STOMACH REGION FOLLOWING ACETYLSALICYLIC ACID SUPPLEMENTATION AND PARTIAL STOMACH RESECTION

Jarosław Całka, Liliana Rytel

Department of Clinical Physiology, Faculty of Veterinary Medicine, University of Warmia and Mazury, Olsztyn, Poland

BACKGROUND AND AIMS: We analyzed expression of SP, CGRP, NOS, VIP and GAL in the porcine nodose ganglion (NG) sensory neurons innervating prepyloric stomach region in physiological state, following gastritis evoked by acetylsalicylic acid supplementation and after partial stomach resection.
METHODS: The study was performed on 12 immature gilts divided into 3 groups (*n*=4 each). All animals were injected retrograde marker Fast Blue (FB) into the anterior prepyloric stomach wall. Since 7th day after FB injection animals of the ASA group were given acetylsalicylic acid (100 mg/kg BW) orally for 21 days, while animals of RES group underwent partial stomach resection on 22nd day following FB injection. On 28th day all pigs were euthanized and bilateral right (rNG) and left (lNG) were collected. Cryostat sections were double immunolabeled using antibodies against SP, CGRP, NOS, VIP and GAL.
RESULTS: Microscopic analysis of the control group revealed expression of SP (45.9±3.4% in rNG and 60.4±1.7% in lNG), CGRP (32.5±4.3% in rNG and 42.6±9.5% in lNG), nNOS (34.9±6.8% in rNG and 49.9±9.3% in lNG), VIP (17.9±2.7% in rNG and 31.5±5.1% in lNG) and GAL (21.9±3.3% in rNG and 35.5±6.8% in lNG) in FB+ perykaria. In RES group increased expression of SP (61.3±3.3% in rNG and 55.2±5.6% in lNG), CGRP (60.6±2.7% in rNG and 52.8±3.7% in lNG), nNOS (49.3±4.5% in rNG and 52.8±9.0% in lNG), VIP (61.3±2.5% in rNG and 67.1±6.8% in lNG) and GAL (35.1±7.3% in rNG and 37.1±5.8% in lNG) in FB+ perykaria was found. In ASA group increased expression of SP (56.5±3.4% in rNG and 67.2±3.9% in lNG), CGRP (52.8±6.9% in rNG and 64.8±6.2% in lNG), nNOS (47.9±9.0% in rNG and 59.3±7.1% in lNG), VIP (51.9±6.0% in rNG and 60.3±5.6% in lNG), GAL (47.2±7.2% in rNG and 55.8±4.2% in lNG) in FB+ perykaria was observed.
CONCLUSIONS: Our data suggest that SP, CGRP, NOS, VIP and GAL play a role in sensory transduction, posttraumatic dendrites regeneration, and development of the stomach.

POSTER SESSION P8 GLIAL CELLS

P8.1

DOES RESTRICTION OF JUGULAR VENOUS OUTFLOW INDUCE MULTIPLE SCLEROSIS-LIKE SIGNS IN RAT BRAINS?

Halina Jędrzejowska-Szypułka[1], Katarzyna Kotulska[2], Bartosz Kapustka[1], Mariusz Łączyński[1], Magdalena Larysz-Brysz[1], Joanna Lewin-Kowalik[1]

[1]Department of Physiology, School of Medicine in Katowice,, Medical University of Silesia, Katowice, Poland; [2]Department of Neurology and Epileptology, Children Memorial Hospital, Warsaw, Poland

BACKGROUND AND AIMS: Hypothesis that multiple sclerosis (MS) may be caused by chronic cerebrospinal venous insufficiency (CCVI) has gained public interest from both patients and physicians. However, there's still lack of evidence for it. We have investigated presence of neuronal demyelination and degeneration, similar to these found in MS, in rat model of CCVI created by occlusion of jugular veins (JVs).
METHODS: Twenty-five young female Wistar C rats were used. Complete ligation of both JVs (BO group), left JV (UO), or partial ligation (stenosis resulting in ~70% reduction of blood flow) of both JVs were performed. Blood flow in JVs was measured with Laser Doppler Flow Assessment. Neurological assessment using

Neurologic Deficit Scale (NDS), 5-point EAE staging protocol, and gait analysis with CatWalk was performed. After 12 weeks, MRI for detecting demyelinating plaques as well as signs of blood-brain barrier (BBB) disruption was performed. Histologic analysis of brain specimens was focused on markers of inflammation and demyelination.
RESULTS: No neurologic deficits were found in all experimental animals. Both NDS, EAE and gait analysis did not differ from normal. MRI T2- and T1- weighted imaging as well as FLAIR sequence did not reveal any abnormalities in the brains of experimental rats. Histological analysis did not show any signs of inflammation or demyelination.
CONCLUSIONS: Twelve-week CCVI in rats, both complete and partial, did not induce any changes resembling pathologies observed in MS. Therefore, linking CCVI with origin of MS remains controversial.

P8.2

ANTIPROLIFERATIVE EFFECTS OF CK2 INHIBITORS ON CELL LINES OF HUMAN GLIAL TUMOR

Emanuela Pucko[1], Zygmunt Kazimierczuk[2], Robert Ostrowski[1], Katarzyna Stępień[1], Ewa Matyja[1]

[1]Department of Experimental and Clinical Neuropathology, Mossakowski Medical Research Centre, Polish Academy of Sciences, Warsaw, Poland; [2]Department of Chemistry, Warsaw University of Life Sciences, Warsaw, Poland

BACKGROUND AND AIMS: Gliomas are the most common primary brain tumours. The malignant gliomas is characterized by infiltrative growth related with poor prognosis. The therapy of astroglial tumours remains still challenging. Protein kinase (CK2) inhibitors have been suggested as promising drugs for antitumour therapy. CK2 (known as caseine kinase II) is an ubiquitous Ser/Thr protein kinase present in both the nucleus and the cytoplasm of neoplastic cells. CK2 has been frequently found to be deregulated (mostly hyperactivated) in malignancies. It plays a role in cell survival, proliferation and can exert an anti-apoptotic role by protecting regulatory proteins from caspase-mediated degradation. The aim of this study was to evaluate the cytostatic effect of novel CK2 inhibitors on cell lines of human glial tumours.
METHODS: The study was performed on human glioblastoma cell line (T98G) and cell lines derived from subependymal giant cell astrocytoma (SEGA) – a low-grade pediatric brain tumour. The tested compound included selected CK2 inhibitors: TBIAEA, DMAT, TBI and TBB. We analyzed the cell viability (MTT metabolism assay) and cell proliferation (Multisizer3 Beckman Coulter cell counter).
RESULTS: The marked decrease of a total number of neoplastic cells was observed in all experimental groups, especially after 24 and 48 hours of treatment. TBIAEA appeared to be the most effective compounds that exhibit a strong anti-proliferative effect on neoplastic astroglial cells in gliomas of low and high grade malignancy *in vitro*. It most effectively inhibited the viability of cultured glioma cells after 24 hours at concentration 25–100 μM.
CONCLUSIONS: The results show that selected CK2 inhibitors have a potent antiproliferative efficacy against human malignant glioma cells. It may offer a promising anti-tumour therapy, including treatment of glioma-derived primary brain tumours.
The research was supported by the Foundation for the Development of Diagnostic and Therapy, Warsaw.

P8.3

EFFECT OF HYPOXIC AND HYPERBARIC OXYGEN CONDITIONS ON CYTOTOXIC ACTION OF MODIFIED ISOTHIOUREA DERIVATIVE (ZKK-3) AGAINST T98G GLIOBLASTOMA CELL LINE

Katarzyna Stępień[1], Robert P. Ostrowski[1], Emanuela B. Pucko[1], Zygmunt Kazimierczuk[2], Ewa Matyja[1]

[1]Department of Experimental and Clinical Neuropathology, Mossakowski Medical Research Centre, Polish Academy of Sciences, Warsaw, Poland; [2]Department of Chemistry, Warsaw University of Life Sciences, Warsaw, Poland

BACKGROUND AND AIMS: Gliomas, derived from astroglial cells, constitute about 50% of all brain tumours. The hypoxic state of neoplastic tissue is regarded to be an important reason of treatment failure with chemo- and radiotherapy. The aim of this study was to examine if administration of gas mixture with increased and decreased oxygen content will result in changes of cytotoxic properties of modified isothiourea derivative (ZKK-3) against human glioblastoma tumour *in vitro*.
METHODS: The experiment was performed on human glioblastoma T98G cell line, which were cultured in hypoxic (for 24 hours) or hyperbaric oxygen conditions (HBO, 1 hour of hyperbaric oxygen under the pressure of 3 ATA, then 23 hours of normoxia) in a medium containing selected modified isothiourea derivative: N,N'-dimethyl-S-(2,3,4,5,6-pentabromobenzyl)-isothiouronium bromide (ZKK-3). Control cultures were preserved in standard conditions. The viability of the cells was evaluated after 24 and 48 hours using CellTiter 96® AQ_{ueous} One Solution Cell Proliferation Assay (Promega). Cell proliferation was assessed after 24 hours by Multisizer 3 Beckman Coulter.
RESULTS: Glioma cells subjected to hypoxia conditions were significantly more resistant to cytotoxic action of ZKK-3. The proliferative rate of neoplastic glioma cells appeared not to be affected when the cultures were exposed to low oxygen partial pressure. The HBO treatment resulted in enhancement of anti-tumour properties of tested isothiourea derivative, evidenced by a decrease in T98G cells viability and growth.

CONCLUSIONS: Hypoxia culture conditions reduce the therapeutic effect of ZKK-3 and promote glioma cells survivability, which is possibly associated with the induction of drug resistance. Increase of sensitivity of glioma cells to the tested agent after administration of hyperbaric oxygen allows to consider HBO as a promising therapeutic strategy for patients with gliomas.
The research was supported by the KNOW-MMRC project.

P8.4

INFLUENCE OF DALBK – AN ANTAGONIST OF KININ RECEPTOR B1R – ON THE COURSE OF EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS IN RATS

Karolina Podsiadło, Tomasz Grygorowicz, Lidia Strużyńska
Mossakowski Medical Research Centre, Polish Academy of Sciences, Warsaw, Poland

BACKGROUND AND AIMS: Experimental autoimmune encephalomyelitis (EAE) has been used for several decades as an animal model of multiple sclerosis (MS), an inflammatory demyelinating disease. According to the previous studies mammalian central nervous system presents all components of the kallikrein-kinin system. Biological activity of kinin is mediated by two types of G protein-bound receptors – B1 and B2. Therefore, there are reasons to investigate the participation of B1 receptor in enhancement of the BBB permeability during development of EAE.
METHODS: One group of female Lewis rats was immunized by intradermal injection. The second group was injected ip with DALBK (B1R antagonist) after immunization. Control group was not immunized. Rats were monitored daily for clinical signs and loss of weight. Animals were sacrificed in different stages of the disease. Parts of brains were used for Western blotting analysis. Immunohistochemical study was also performed.
RESULTS: We noticed the increased level of B1R protein in rat brain in the symptomatic phase of EAE. Animals treated with DALKB exhibited improvement of neurological symptoms and decreased level of B1R protein in most cases. Using a confocal microscope we assessed immunoreactivity of B1R and markers of individual components of glio-vascular unit (astrocytes, endothelial cells and pericytes). We also noticed changes in the level of astroglial markers GFAP and AQP4 during EAE, so as decreased expression of thight jounctions proteins (ZO-1, Occludin, Claudin 5), which were partially abolished by DALBK.
CONCLUSIONS: Administration of kinin B1 receptor antagonist (DALBK) significantly improved the condition of animals by reducing the proinflammatory effects of kinins. The results show that the kinin receptor B1 may play a role in pathomechanisms operating during the course of EAE and appears to be a potential therapeutic target.
This work was supported by funds from KNOW 2013-2017 project.

P8.5

REMYELINATION PROMOTES AXON REGENERATION AFTER SPINAL CORD INJURY

Marine Yeghiazaryan[1], Anna Cabaj[2], Henryk Majczyński[1], Urszula Sławińska[1], Małgorzata Zawadzka[3]
[1]Laboratory of Neuromuscular Plasticity, [3]Laboratory of Molecular Neurobiology, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland; [2]Laboratory of Neuromuscular Plasticity, Institute of Biocybernetics and Biomedical Engineering Polish Academy of Sciences, Warsaw, Poland

BACKGROUND AND AIMS: Demyelination in the experimental models is followed by successful remyelination. However, remyelination in post-spinal cord injuries is failed and often incomplete. The purpose of the present study is to determine if induction of remyelination could promote axon growth in the total spinal cord injury model of the adult rat.
METHODS: Demyelination within the dorsal and ventral funiculi were induced with an intraspinal injection of ethidium bromide (EB) either immediately after transection (single injected group) or twice, immediately after and at 5 days post-transection (double injected group).
RESULTS: We observed decrease in total area covered by Iba1-positive macrophage and GFAP-positive astrocyte at the injury epicenter in both single and double EB injected rats at 14 dpl. The number of oligodendrocyte precursor cells (OPCs) significantly increased in adjacent to the transection area at 14 days after injury in both experimental groups and remained elevated for 2 months after injury in double injected rats. The highest area of neurofilament-positive axons at the injury epicenter and lesion adjacent area was observed in double EB injected animals. Neurofilament-positive axons in these animals were frequently found associated with periaxin, which presumably expressed by myelinating Schwann cells. Remyelination improved the recovery of locomotion and supported serotonergic 5HT fiber growth through the injury site.
CONCLUSIONS: Our findings suggest that focal demyelination reduces glial scar formation, induces OPCs recruitment and differentiation, and creates a unique environment, which is permissive for spontaneous axon growth which could be promising in order to achieve functional improvement.

P8.6

THE ROLE OF P2X7R IN ACTIVATION OF GLIAL CELLS DURING THE EARLY PHASE OF EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS

Tomasz Grygorowicz[1], Marlena Wełniak-Kamińska[2], Aleksandra Lenkiewicz[1], Lidia Strużyńska[1]
[1]Laboratory of Pathoneurochemistry, Department of Neurochemistry, [2]Department of Experimental Pharmacology, Mossakowski Medical Research Centre, Polish Academy of Sciences, Warsaw, Poland

BACKGROUND AND AIMS: Multiple sclerosis (MS) is an autoimmune, neuroinflammatory disease resulting in progressive demyelinization. Glial cells are important players during development of inflammation within central nervous system. Under inflammatory signals astroglia and microglia change their morphology and up-regulate expression of GFAP and Iba1 proteins, respectively. Both of these cell populations express purinergic receptor P2X7. The aim of the study was to investigate the effect of P2X7R antagonist (Brilliant Blue G, BBG) on the course of experimental autoimmune encephalomyelitis (EAE) with the focus on the influence of BBG-treatment on the reactive gliosis.
METHODS: EAE, an animal model of MS, was evoked by the immunization of Lewis rats with guinea pigs' spinal cord homogenate with complete Freund's adjuvant. BBG (50 mg/kg) was administered daily from day 0 to day 6 post immunization through the jugular catheter.
RESULTS: Immunofluorescent analysis of brain slices showed the activation of astroglial and microglial cells in EAE rats, but not in EAE rats treated with BBG. The expression of GFAP was 5,5 times higher in EAE than in EAE+BBG group at the asymptomatic phase and 3.1 times higher at symptomatic phase of the disease. These results were confirmed by western blot analysis. Expression of Iba1 was 5.4 times and 3.6 times higher in the EAE than in EAE+BBG group at the asymptomatic and symptomatic phases, respectively. The effects of BBG treatment on the expression of proinflammatory cytokines such as IL-1β, IL-6, TNF-α were determined by western blot method. The level of these cytokines was significantly lower in EAE+BBG group than in EAE animals and remained similar to that observed in control rats.
CONCLUSIONS: Blockade of P2X7R during the course of EAE results in: (1) improved condition of Animals; (2) decreased activation of astro- and microglia; (3) decreased level of proinflamatory cytokines
Supported by Polish National Science Centre, grant nr: DEC-2012-/05/N/NZ4/02191.

P8.7

ST8SIA2 GENE DEFICIENCY LEADS TO AGED-RELATED AXONAL DEGENERATION AND WEAKENING OF MYELIN SHEATH

Łukasz Szewczyk[1,2], Nikola Brozko[2], Andrzej Nagalski[1], Ewa Liszewska[3], Małgorzata Zawadzka[3], Herbert Hildebrandt[4], Jacek Kuźnicki[1], Marta Wiśniewska[1,2]

[1]International Institute of Molecular and Cell Biology, Warsaw, Poland; [2]Center of New Technologies, University of Warsaw, Warsaw, Poland; [3]Nencki Insitute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland; [4]Hannover Medical School, Hannover, Germany

BACKGROUND AND AIMS: This study was to investigate the involvement of ST8SIA2 in myelination of the brain. ST8SIA2 and its paralog ST8SIA4 synthesize polysialic acid chains (PSA) to NCAMs. Synthesis of PSA and its downregulation during brain development are crucial for a proper myelin formation. However, myelin forms normally in St8sia4-/- mice. So far, myelin-related phenotype of St8sia2-/- mice has not been investigated.
METHODS: Mass-spectrometry, westernblot, myelin staining, immunostaining, electron microscopy
RESULTS: Quantitative mass-spectrometry showed that the levels of myelin proteins MBP and PLP1 in the hippocampus are lower in adult St8sia2-/- mice than in control. Westernblot confirmed this result and revealed the same changes in cortical areas. Then, in order to determine the onset of the myelin impairment in the knockout mice, we labeled white matter in brain sections from mice at postnatal ages (P15 to P240) with Black Gold II, and showed that this phenotype develops with age. In agreement with this result, western blot analysis of major myelin proteins: PLP1, MBP, MOBP, MOG and CNPase, in the brain of mice from P15 to P90 revealed their lower levels in the knockouts, especially in the older mice. Electron microscopy revealed thinning of myelin sheath in the adult knockouts at P90 and P240, as well as abnormalities in axonal morphology and their degeneration at P240. Western blot revealed twofold lower levels of neurofilament proteins also suggesting axonopathy.
CONCLUSIONS: ST8SIAII-mediated deficiency of polysialylation leads to axonal pathologies and their degeneration accompanied by myelin weakening. A decrease in polysialylated NCAM has been observed in postmortem schizophrenics brains, and the mice lacking the St8sia2 gene display schizophrenia-related behavior and anatomical abnormalities. We propose that myelin and axonal pathologies of schizophrenics might be a consequence of unsufficient level of polysialylation during development and in early adulthood.

P8.8

EARLY BIOCHEMICAL ALTERNATIONS AND GLIAL CELL DYSFUNCTION IN THE HIPPOCAMPUS IN THE MPTP MODEL OF PD

Malgorzata Zaremba[1], Jarosław Orzeł[2-3], Maciej Świątkiewicz[2], Ilona Joniec-Maciejak[1], Michał Fiedorowicz[2], Paweł Grieb[4]

[1]Department of Experimental and Clinical Pharmacology, Centre for Preclinical Research and Technology, Medical University of Warsaw, Warsaw, Poland; [2]Laboratory of Magnetic Resonance Imaging of Small Animals, Mossakowski Medical Research Centre, Polish Academy of Sciences, Warsaw, Poland; [3]Faculty of Electronics, Warsaw University of Technology, Warsaw, Poland; [4]Department of Experimental Pharmacology, Mossakowski Medical Research Centre, Polish Academy of Sciences, Warsaw, Poland

BACKGROUND AND AIMS: Parkinson's disease (PD) patients show a wide and variable spectrum of cognitive deficits involving

multiple domains like memory and visuo-spatial learning. Thus, there is strong evidence that striatal and cortical dopamine cannot be the only major factors in the cognitive profile presented by PD. The initiating neurobiological bases for such disturbances are still uncertain but current hypothesis suggest possible role of diminished projection into hippocampus (Hpc). The present study was designed to determine the impact of MPTP-induced nigrostriatal neurodegeneration on the biochemical profile and glial cell reaction in the Hpc in mice.

METHODS: One year old male C57Bl/10Tar mice were injected ip with MPTP (40 mg/kg) or equivalent vehicle volume. One week later brains of the control and MPTP-injected mice (n=10/group) were scanned with Bruker BioSpec 70/30 Avance III system equipped with 7T magnet. Metabolite concentrations were estimated by proton magnetic resonance spectroscopy (^{1}HNMR) with LC model and expressed as ratios to total creatine concentration. In addition, contents of DA, NE, 5-HT, Glu, Glx and corresponding metabolites were estimated by HPLC with electrochemical detection. The astrocytic and microglia reactions was assessed by western blot analysis of GFAP and Iba1 proteins expression.

RESULTS: Compared to controls, MPTP mice displayed significantly lower glutamate (Glu) and combined glutamate+glutamine (Glx) resonance signals and slightly lower glycerophosphocholine+phosphocholine (GPC+PCh) signals in the right Hpc. However, no significant differences were observed in the left Hpc. Western blot analysis showed altered astroglial protein expression in the right Hpc.

CONCLUSIONS: Reduced Glu and GPC+PCh in the right Hpc and altered astroglial protein expression may signify deficits in neuronal metabolism and/or firing rate and possible astroglia shrinkage toward the right hippocampus.

P8.9

LPS CHANGES ENERGY METABOLISM IN MICROGLIAL N9 AND CHOLINERGIC SN56 NEURONAL CELLS

Joanna Klimaszewska-Łata, Sylwia Gul-Hinc, Hanna Bielarczyk, Anna Ronowska, Dorota Bizon-Zygmańska, Aleksandra Dyś, Andrzej Szutowicz

Chair of Clinical Biochemistry, Department of Laboratory Medicine, Medical University of Gdańsk, Gdańsk, Poland

BACKGROUND AND AIMS: Inhibition of brain energy metabolism, accompanied by inflammatory activation of microglial cells is a characteristic feature of several neurodegenerative brain diseases, including Alzheimer's, aluminum or vascular encephalopathies. Microglial inflammatory response to neurotoxic signals may contribute to neuronal degeneration through excessive production of nitric oxide (NO) and a vast range of pro-inflammatory cytokines. The aim of this work was to investigate whether and how lipopolysaccharide (LPS), and its key mediator NO, may differentially affect energy and acetyl-CoA metabolism of microglial N9 and cholinergic SN56 neuroblastoma cells.

METHODS: In experimential model cell cultures were used: N9 murine microglial cells and SN56.B5.G4 cholinergic murine neuroblastoma cells.

RESULTS: Exposition of murine microglial N9 cells to LPS caused concentration-dependent several-fold increases of nitrogen oxide synthesis, accompanied by inhibition of pyruvate dehydrogenase complex (PDHC), aconitase and α-ketoglutarate dehydrogenase complex (KDHC) activities, and by depletion of acetyl-CoA, but by small losses in ATP content and cell viability. On the other hands, SN56 cells were insensitivity to LPS, which was probably caused by lower than in N9, expression of TLR4. However, exogenous NO caused inhibition of PDHC and aconitase activities, depletion of acetyl-CoA and loss of SN56 cells viability. Microglial cells appeared to be more resistant than neuronal cells to acetyl-CoA and ATP depletion evoked by these neurodegenerative condition.

CONCLUSIONS: These data indicate that preferential susceptibility of cholinergic neurons to neurodegenerative insults may results from competition for acetyl-CoA between mitochondrial energy-producing and cytoplasmic acetylocholine synthesizing pathways. One of the reasons for greater resistance of microglial cells to cytotoxic inputs could be their lower energy demand.

Supported by MNSW project MN 01-0067/08 and GUMed fund ST-57.

POSTER P9. MOTOR CONTROL

P9.1

PRAMIPEXOLE AT A LOW DOSE INDUCES BENEFICIAL EFFECTS IN THE HARMALINE-INDUCED MODEL OF ESSENTIAL TREMOR IN RATS

Barbara Kosmowska[1], Jadwiga Wardas[1], Urszula Głowacka[1], Subramaniam Ananthan[2]

[1]Department of Neuro-Psychopharmacology, Institute of Pharmacology, Polish Academy of Sciences, Kraków, Poland; [2]Department of Chemistry, Southern Research Institute, Birmingham, AL, USA

BACKGROUND AND AIMS: Harmaline-induced tremor is a model of essential tremor (ET). Tremor induced by harmaline has been suggested to result from activation of the olivo-cerebellar projection. Recent studies have indicated that cerebellum functions may be modulated by dopamine.

METHODS: We examined the effects of preferential agonists of dopamine D_3 receptors: pramipexole and 7-OH-DPAT on the harmaline-induced tremor in rats. In order to study receptor mecha-

nisms of these drugs rats were pretreated with dopamine D_3 receptor antagonists [SB-277011-A (10 mg/kg ip) and SR-21502 (15 mg/kg ip)], an antagonist of presynaptic D_2/D_3 receptors [amisulpride (1 mg/kg ip)], or a non-selective antagonist of postsynaptic dopamine receptors [haloperidol (0.5 mg/kg ip)].
RESULTS: The tremor was measured using fully automated Force Plate Actimeters. The following parameters were calculated: power within 0–8 Hz band (AP1) and 9–15 Hz band (AP2), and tremor index (a difference in power between AP2 and AP1). Harmaline-triggered (15 mg/kg ip) tremor was manifested by an increase in AP2 and tremor index. Pramipexole at a low (0.1 mg/kg sc), but not higher doses (0.3 and 1 mg/kg sc) lowered the harmaline-increased AP2. 7-OH-DPAT (0.1 mg/kg sc) reduced AP2 in the harmaline-treated rats. None of the examined dopamine antagonists influenced the above effect of dopamine agonists. In contrast, SB-277011-A administered alone lowered the harmaline-increased AP2.
CONCLUSIONS: The present study indicates that pramipexole reduces the harmaline-induced tremor. However, mechanisms underlying its action are still unclear and require further examination.
Financial support: Statutory Funds of the Department of Neuro-Psychopharmacology, Institute of Pharmacology, PAS; National Science Center (grant OPUS 6, 2013/11/B/NZ4/04565); Southern Research Institute, Birmingham, AL, USA (S. Ananthan) - SR-21502 donation. Barbara Kosmowska is a holder of scholarship from the KNOW sponsored by Ministry of Science and Higher Education, Poland.

P9.2

SEX DIFFERENCES IN THE DECOMPOSITION OF MOTOR UNIT TETANIC CONTRACTIONS OF RAT SOLEUS MUSCLE

Hanna Drzymała-Celichowska[1], Rositsa Raikova[2], Piotr Krutki[3], Jan Celichowski[3]
[1]Department of Neurobiology, Division of Biochemistry, University School of Physical Education, Poznań, Poland; [2]Institute of Biophysics and Biomedical Engineering, Bulgarian Academy of Sciences, Sofia, Bulgaria; [3]Department of Neurobiology, University School of Physical Education, Poznań, Poland

BACKGROUND AND AIMS: The aim of this study was to reveal variability of twitch-shape decomposed components of motor unit tetanic contractions of rat soleus muscle, which is almost exclusively composed of slow motor units (MUs). Moreover, sex differences in ranges of the force amplitude or time parameters of these decomposed twitches were analyzed.
METHODS: Experiments were performed on adult Wistar rats (three males and three females) under general anesthesia. Functional isolation of a MU was achieved by electrical stimulation of single axons from the ventral roots of L4–L5 spinal nerves. Unfused tetanic contractions were evoked by stimulation at variable interpulse intervals for 10 MUs of males and 10 MUs of females.
RESULTS: Significantly higher variability between parameters of the decomposed responses was observed for male than female soleus MUs; the mean ratio of forces of the strongest decomposed twitch to the first (the weakest) decomposed twitch amounted to 3.8 for males and 2.8 for females. The ratios of the contraction times of the longest decomposed to the first twitch were less different, and amounted to 2.6 for male and 2.9 for female MUs. Consequently, the mean ratio of the force-time area for the strongest decomposed to the first twitch was considerably higher for male than for female MUs (7.35 vs. 5.07, respectively). The comparison to the data for slow or fast MUs in rat medial gastrocnemius indicates that high variability of responses to successive stimuli is a general property of slow MUs, but the mechanisms of summation of individual twitches into tetanic contractions of MUs are sex-related.
CONCLUSIONS: A method of mathematical decomposition of tetanic contractions appears to be a useful and an effective tool to study differences in mechanisms of MU force development between different MU types, the same MU types in different muscles or the same muscles in different sexes.

P9.3

CHANGES IN ELECTROPHYSIOLOGICAL PROPERTIES OF RAT MOTONEURONS EVOKED BY A 5-WEEK STRENGTH TRAINING

Włodzimierz Mrówczyński[1], Dawid Łochyński[2], Dominik Kaczmarek[3], Marcin Bączyk[1], Jan Celichowski[1], Piotr Krutki[1]
[1]Department of Neurobiology, [2]Department of Motor Rehabilitation, Department of Neurobiology, [3]Division of Biochemistry, Department of Neurobiology, University School of Physical Education, Poznań, Poland

BACKGROUND AND AIMS: Different forms of chronic physical activity evoke adaptive changes in the neuromuscular system. Long-lasting strength training, with repeated short-term and high-intensity exercises, is responsible for an increase of muscle mass and generation of larger forces. However, adaptations in properties of motoneurons innervating muscles subjected to such training have been unknown so far. The aim of this study in the rat was to determine whether the strength training induces changes of passive and threshold membrane properties, and rhythmic firing of motoneurons.
METHODS: The study was performed on eight adult Wistar rats, randomly assigned to the training or the control groups. Animals from the training group were nutritionally conditioned in order to make weightlifting put on their shoulders in a special apparatus with progressively increasing load, for 5 weeks. Acute electrophys-

iological experiments were performed on deeply anesthetized animals from both groups, using microelectrode intracellular recordings from motoneurons innervating hind limb muscles.
RESULTS: It was demonstrated that 5-week strength training evoked adaptive changes in both fast and slow types of motoneurons: a shortening of the rise time of action potentials, an increase of the maximum frequencies of rhythmic firing, and an increase in the slope of the frequency-current relationship.
CONCLUSIONS: Obtained data suggest higher susceptibility of motoneurons to an increased or decreased intensity of stimulation. Moreover, a decrease in rheobase currents, and a decrease in the minimum currents required to evoke rhythmic firing was observed in fast-type motoneurons only, suggesting their higher excitability.
Supported by the National Science Center grant No. 2013/11/B/NZ7/01518.

P9.4

IA MONOSYNAPTIC PATHWAY IN SOD1 MOUSE MODEL OF AMYOTROPHIC LATERAL SCLEROSIS

Marcin Bączyk, Clémence Martinot, Nicolas Delestrée, Marin Manuel, Daniel Zytnicki

Laboratory of Neuropysics and Physiology, UMR CNRS 8119, University Paris Descartes, Paris, France

BACKGROUND AND AIMS: Glutamate excitotoxicity has long been suggested to contribute to the degeneration of motoneurons in Amyotrophic Lateral Sclerosis (ALS). However, it has recently been shown that spinal motoneurons do not display an intrinsic hyperexcitability just prior to their degeneration in SOD1 G93A mice, the standard model of ALS. Furthermore average densities of excitatory (VGLUT1 and VGLUT2) and inhibitory (VGAT) boutons on the dendritic tree and the soma of affected motoneurons were unchanged. However glutamate excitotoxicity can still take place if excitatory pathways are more active. Therefore the aim of this study was to investigate the excitability of the monosynaptic Ia pathway in SOD1 mice.
METHODS: Eight SOD1 G93A and six SOD WT control mice were used in this study. Intracellularly penetrated motoneurons were identified as a medial gastrocnemius (MG) or lateral gastrocnemius (LG) motoneurons by their threshold for antidromic activation. Ia monosynaptic EPSPs were recorded from these motoneurons after stimulation of their homonymous nerve. In parallel the afferent Ia volley was recorded from the cord dorsum. Increasing stimulation intensities were used to obtain minimum and maximum Ia Volleys and EPSPs.
RESULTS: The average amplitudes of monosynaptic Ia EPSPs in SOD1 motoneurons were significantly smaller when compared to SOD WT controls (1.40 mV and 2.24 mV, respectively). Moreover the EPSP difference was mainly visible for the group of motoneurons characterized by high input resistance (above 3 MΩ). On the other hand, the recruitment curves (normalized amplitude vs. stim intensity) of Ia volleys and EPSPs were unchanged, suggesting no change in excitability of Ia fibers but alterations in Ia monosynaptic synapses.
CONCLUSIONS: Results indicate alterations in Ia monosynaptic pathway in presymptomatic SOD1 ALS mouse model. This can stem from several reasons, e.g. increased presynaptic inhibition of Ia terminals or reduced efficacy of post-synaptic receptors.

P9.5

MOTOR UNIT CONTRACTILE PROPERTIES AND MYOSIN HEAVY CHAIN PROTEIN EXPRESSION AFTER RESISTANCE EXERCISE

Dawid Łochyński[1], Dominik Kaczmarek[2], Włodzimierz Mrówczyński[3], Joanna Majerczak[4], Janusz Karasiński[5], Jerzy Żołądź[4], Jan Celichowski[3]

[1]Department of Neurobiology and Department of Motor Rehabilitation, [2]Department of Biochemistry and Department of Neurobiology, University School of Physical Education, Poznań, Poland; [3]Department of Muscle Physiology, Chair of Physiology and Biochemistry, [4]Faculty of Rehabilitation, University School of Physical Education, Kraków, Poland; [5]Department of Cell Biology and Imaging, Institute of Zoology, Jagiellonian University, Kraków, Poland

BACKGROUND AND AIMS: Expression of distinct types of myosin heavy chain (MHC) isoforms in skeletal muscle is accepted as one of critical factors providing the molecular basis of muscle fibre functional diversity and plasticity. However, chronic nerve stimulation experiments have provided a strong evidence that modifications in the contractile regulatory and the energy metabolism systems occur much earlier than transition of myosin isoforms.
METHODS: In this study we investigated expression of MHC isoforms, contractile kinetics (twitch time characteristics), force regulation (force-frequency relationship), and fatigability of motor units in rat fast-type skeletal muscle at the early stage of voluntary progressive resistance exercise program. Motor units classified into slow (S), fast resistant to fatigue (FR) and fast fatigable (FF) were functionally isolated from medial gastrocnemius muscles of rats subjected to weight lifting (WL) exercises or control, sedentary animals.
RESULTS: MHC isoform expression was not changed. Shortening of the twitch contraction was observed in both types of fast MUs. The twitch half-relaxation time of FF units was prolonged in WL animals. The contraction-to-half-relaxation time ratio (twitch shape) was significantly decreased in fast MUs of WL rats. Force-frequency curve was shifted towards higher stimulation rates in FR but not FF units. In FF MUs of WL rats force declined less in time

during initial 15 seconds and then was better maintained during the next 75 seconds of performed 180-second fatigue test. In FR units of WL rats force declined more in time during the last 120 seconds of the test. S MUs contractile parameters were not changed.
CONCLUSIONS: At the early stage of resistance training modifications in the contractile regulatory and energy metabolism systems of fast MUs, as acknowledged by observed alterations in their contractile kinetics and fatigability, occur before any transition in muscle MHC isoforms is observed.

P9.6

GRAFTED SEROTONERGIC NEURONS CAN REVERSE CHANGES IN GENE EXPRESSION IN MOTONEURONS PRODUCED BY SPINAL CORD INJURY IN RATS

Krzysztof Miazga[1], Ewa Joachimiak[2], Hanna Fabczak[2], Urszula Sławińska[1]

[1]Interinstitute Laboratory of Neuromuscular Plasticity, [2]Laboratory of Physiology of Cell Movements, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland

BACKGROUND AND AIMS: Serotonin, which is supplied to the spinal cord by serotoninergic cells localized in the raphe nuclei and parapiramidal areas of the medulla, plays a very important role in control of the spinal locomotor central pattern generator (CPG). In our previous study we showed that intraperitoneal application of: 8-OH-DPAT (5-HT_{1A} and 5-HT_7 serotonin receptor agonist) and quipazine (mainly 5-HT_{2A} serotonin receptor agonist), or intraspinal transplantation of serotonergic cells isolated from 14-day old rat embryo brain stem, facilitates locomotor-like hindlimb movements in spinal rats (spinal cord total transection between Th9 and Th10). 5-HT_7 and 5-HT_2 serotonin receptor antagonists blocked the locomotor-like hindlimb movements that had been restored in spinal rats grafted with embryonic serotoninergic cells. The aim of the present study was to examine the influence of spinal cord total transection and transplantation of serotonin neurons isolated from the 14-day old rat embryo brain stem on changes in expression of genes encoding 5-HT_{2A}, 5-HT_{2C} and 5-HT_7 serotonin receptors in populations of motoneurons innervating tibialis anterior, gastrocnemius lateralis, and extensor caudae medialis muscles.
METHODS: For motoneurons labeling a method of retrograde staining using intra muscle injection with cholera toxin B subunit conjugated with Alexa Fluor 555 was used. Motoneurons were then collected by using the laser capture micro-dissection method, and changes in expression of genes encoding serotonin receptors were analyzed by Real-time PCR.
RESULTS: The results show that total spinal cord transection changed expression of genes encoding 5-HT_{2A}, 5-HT_{2C} and 5-HT_7 serotonin receptors in ankle flexor and ankle and tail extensor muscles. Grafting of serotonin neurons reverses the effects of spinal cord injury on expression of these genes.
CONCLUSION: This is the first demonstration that grafts of serotonergic neurons can reverse changes in gene expression in motoneurons produced by spinal cord injury.

P9.7

CHANGES IN CONTRACTILE PROPERTIES OF MOTOR UNITS IN RATS WITH DECREASED MUSCLE CARNOSINE CONTENT AFTER 14 DAYS OF HISTIDINE DEPRIVATION

Dominik Kaczmarek[1], Dawid Łochyński[2], Maciej Pawlak[3], Inge Everaert[4], Laura Blancquaert[4], Wim Derave[4], Jan Celichowski[5]

[1]Department of Biochemistry, Department of Neurobiology, [2]Department of Motor Rehabilitation, Department of Neurobiology, [3]Department of Biochemistry, [5]Department of Neurobiology, University School of Physical Education, Poznań, Poland; [4]Department of Movement and Sports Sciences, Ghent University, Ghent, Belgium

BACKGROUND AND AIMS: Motor unit (MU) force is regulated by neural and muscular mechanisms. Recently, effects of carnosine on skeletal muscle contractility have been broadly studied. We found that increased muscle carnosine content by beta-alanine supplementation improved MU contractility. Several studies showed that histidine-free diet decreases carnosine content and causes weight loss, anaemia or hypoproteinaemia but its effect on muscle contractility is unknown. Here we studied MU contractile properties in histidine-deprived rats with reduced muscle carnosine content.
METHODS: Ten 6 mo male Wistar rats were randomly assigned to experimental (HFD) or control (CON) group fed histidine-free or standard diet for 14 days, respectively. In order to maintain the same level of body mass loss food intake was controlled and balanced between both groups. Body mass decreased by 11.7 and 10.6% in HFD and CON groups, respectively.
RESULTS: In HFD group carnosine levels were lower than in CON group in white and red portion of MG muscle by 26% and 34%, respectively. Histidine deprivation did not result in lower muscle mass or muscle-to-body mass ratio. In electrophysiological experiments contractions of MUs in medial gastrocnemius (MG) muscle were evoked by electrical stimulation of ventral root filaments. MUs were classified into fast fatigable (FF), fast resistant (FR) and slow (S). Maximum tetanic force (TetF) and force profile during the two separate fatigue tests were analysed. The TetF did not differ between groups either in fast or slow MUs. During the first fatigue test in FR MU force was initially higher but from 40 to 120 s it was lower in HFD animals. Unexpectedly, in the second fatigue test the force of FR and S MU was better maintained in HFD than CON rats.

CONCLUSIONS: The results indicate that short-term histidine deprivation and the carnosine decrease do not attenuate force of MUs. Moreover, compensatory mechanisms may be involved in the regulation of MU force in this condition.
Support: grant 2013/09/B/NZ7/02554.

P9.8

THE TRANSITORY FORCE DECREASE FOLLOWING HIGH-FREQUENCY STIMULATION BURST IN UNFUSED TETANI OF MOTOR UNITS

Joanna Grzesiak, Hanna Drzymała-Celichowska, Jan Celichowski

Department of Neurobiology, University School of Physical Education, Poznań, Poland

BACKGROUND AND AIMS: The changes in force of motor units (MUs) following changes in activation pattern are still not well understood, especially in relation to the relaxation course at decreasing rate of stimuli. It is known that at linearly decreasing stimulation rate the force decrease is slower than expected when comparing to the constant stimulation frequency. The present study aimed to verify a hypothesis that at a sudden decrease of stimulation frequency the force decrease is also lower than expected.
METHODS: The research was conducted on 4 adult female Wistar rats under pentobarbital anesthesia. 8 slow (S), 16 fast fatigable (FF) and 26 fast resistant (FR) MUs were isolated. Studied MUs were stimulated with the several trains of stimuli composed of three phases: first, 500 ms at low frequency, second, 300 ms at high frequency and third, 500 ms at the same low frequency for fast motor units and 1000 ms at low frequency, second, 300 ms at high frequency and third, 1000 ms at the same low frequency for slow motor units. The tested low frequencies for fast MUs were 10, 20, 30, 40 and 50 Hz, and high frequency amounted to 75 Hz, whereas for slow motor units low frequencies were 10, 12.5, 15, 20 and 25 Hz and high frequency amounted to 50 Hz.
RESULTS: Surprisingly, for MU of the three types at the middle-fused tetanic contractions (the fusion index 0.15–0.80) the sudden switch from high to low frequency evoked the transitory force decrease below the force level at initial low-frequency stimulation. On the average the decrease amounted to 15.99% and the highest noted decrease amounted to 40%. Among the three MU types the force decrease was most frequent and the strongest for FR MUs.
CONCLUSIONS: The phenomenon most probably is related to low-force generating state of cross-bridges in muscle fibers at a new overlapping following the force decrease and/or to a slow adaptation of stretched collagen fibers to the lower force level in the contracting muscle.

P9.9

ADAPTIVE CHANGES IN MOTOR UNIT CONTRACTILE PROPERTIES TO ENDURANCE TRAINING

Katarzyna Kryściak[1], Jakub Kryściak[2], Dawid Łochyński[3], Dominik Kaczmarek[4], Hanna Drzymała-Celichowska[4], Piotr Krutki[1], Jan Celichowski[1]

[1]Department of Neurobiology; [2]Department of Physiology, [3]Department of Neurobiology and Department of Motor Rehabilitation; [4]Department of Neurobiology and Division of Biochemistry University School of Physical Education, Poznań, Poland

BACKGROUND AND AIMS: Endurance training is based on a repeated, prolonged activation of a large number of muscles. It causes morphological, biochemical and metabolic changes in the muscles and the nervous system. However, there are no data concerning changes in motor unit (MU) contractile properties following endurance training.
METHODS: 61 male rats were assigned to 4 groups, untrained – control (C), and 3 groups trained on a treadmill, 5 days a week for 2 weeks (2W), 4 weeks (4W) or 8 weeks (8W). The special protocol determined duration and speed of locomotion in the consecutive days of the training. Finally, rats of the 2W group covered average distance of 5.5 km, 4W of 21 km, and 8W of 56 km. Afterwards, functionally isolated MUs of the medial gastrocnemius muscle were electrophysiologically investigated.
RESULTS: The mean body mass of rats of all trained groups was lower in comparison to the C group, but no differences between 2W, 4W and 8W groups were noted. The muscle mass and the muscle-to-body mass ratio were not different between groups. The proportion of fast resistant (FR) MUs was higher, while of fast fatigable (FF) lower in all trained groups, in comparison to the C group. The relative number of slow (S) MUs increased only in the 8W group. MU contractile properties were changed mainly in FR MUs and included: lower contraction and half-relaxation times, lower twitch forces, higher tetanus forces and lower twitch-to-tetanus ratio. Few adaptive changes were noted also for S MUs of trained animals: lower twitch and tetanus forces as well as lower twitch-to-tetanus ratio. For FR and S MUs of all trained groups the force decrease within four minutes of the fatigue test was considerably slower or even completely abolished, which was reflected in the increased fatigue indexes.
CONCLUSION: The main adaptive changes appeared early and then slowly increased within the endurance training.
Supported by the National Science Center grant 2013/09/B/NZ7/02555.

P9.10

ENRICHMENT IN GLUTAMATERGIC AND CHOLINERGIC BOUTONS OF ANKLE EXTENSOR A-MOTONEURONS AFTER LOW-THRESHOLD STIMULATION OF PROPRIOCEPTIVE FIBERS IN THE ADULT RAT

Olga Gajewska-Woźniak, Małgorzata Skup, Julita Czarkowska-Bauch

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

BACKGROUND AND AIMS: The effects of low-threshold stimulation of muscle afferents (Ia) on glutamatergic and cholinergic innervation of α-motoneurons (Mns) were tested. Two types of synaptic terminals were analyzed: (1) glutamatergic Ia carrying VGLUT1, contacting monosynaptically Mns; (2) cholinergic C-terminals, carrying VAChT, originating from V0c-interneurons of lamina X, which might receive indirect input from sensory afferents of unknown origin. Our aim was to clarify whether enhancement of proprioceptive input to ankle extensor Mns, *via* direct electrical stimulation of Ia afferents in the tibial nerve of awake rats, will affect excitatory innervation of lateral gastrocnemius (LG) Mns.

METHODS: LG Mns were identified with True Blue (TB) tracer injected intramuscularly. Tibial nerve was stimulated for 7 days with bursts of 3 pulses (pulse width 200 μs, 4 ms of inter-pulse interval, 25 ms inter-burst interval) in four 20 min sessions daily. The Hoffmann reflex recorded from the soleus muscle, LG synergist, allowed controlling low-threshold stimulation. Proprioceptive Ia glutamatergic and cholinergic C-terminals abutting TB-labeled Mns were detected immunohistochemically on transverse spinal sections, using input-specific anti- VGLUT1 and anti-VAChT antibodies.

RESULTS: Confocal analysis revealed that the number of both VGLUT1 and VAChT immunoreactive terminals, contacting the soma and proximal dendrites of LG Mns, increased after stimulation by about 35% and 20%, respectively, comparing to sham-stimulated side (P<0.03, Wilcoxon test).

CONCLUSIONS: One week of repetitive low-threshold stimulation of proprioceptive fibers in the tibial nerve enriched glutamatergic and cholinergic innervation of LG Mns indicating that this method might be useful for enhancing an activity of selected group of Mns which are the most vulnerable to the spinal cord injuries (Skup et al. 2012, EJN 36: 2679).

Supported by grants: NCN 2013/09/B/NZ4/03306, statutory for the Nencki Institute.

P9.11

NOVEL ALPHA-DYSTROBREVIN INTERACTORS REGULATE NEUROMUSCULAR JUNCTION POSTSYNAPTIC MACHINERY

Marta Gawor[1], Jacinthe Gingras[2], Krzysztof Bernadzki[1], Paweł Niewiadomski[1], Joshua R. Sanes[2,3], Tomasz J. Prószyński[1,2]

[1]Laboratory of Synaptogenesis, Department of Cell Biology, Nencki Institute of Experimental Biology, PAS, Warsaw, Poland; [2]Department of Molecular and Cellular Biology and Center for Brain Science, Harvard University, Cambridge, USA; Department of Neuroscience, Amgen Inc., Cambridge, USA; [3]Department of Molecular and Cellular Biology and Center for Brain Science, Harvard University, Cambridge, USA; [4]Department of Anatomy and Neurobiology, Washington University School of Medicine, St. Louis, USA

BACKGROUND AND AIMS: Alpha-Dystrobrevin-1 (aDB1) is a component of the dystrophin-associated glycoprotein complex (DGC), which stabilizes the neuromuscular junction (NMJ) postsynaptic machinery. The goal of our research is to gain insight into the mechanism of aDB-1 function at the NMJ, with special focus on the role of aDB1 phosphorylation, which was previously shown to be critical for proper synaptic organization.

METHODS: To determine the importance of each tyrosine phosphorylation site, we expressed mutant proteins lacking individual sites in myotubes. Next, we developed phospho-specific antibodies and used them to analyze the level of aDB1 phosphorylation during NMJ remodeling. We also performed a biochemical screen to identify general and phospho-specific aDB1-binding proteins. Finally, we performed RNAi experiments on cultured myotubes to demonstrate the importance of these proteins in the organization of AChR clusters.

RESULTS: Our experiments identified Liprin-α1, α-Catulin and Usp9x as novel aDB1-interacting proteins. We have demonstrated that aDB1 phosphorylation is dynamically regulated during NMJ remodeling in development and upon denervation and that the phosphorylation at the most critical tyrosine Y713 triggers recruitment of aDB1 phospho-specific interacting proteins, including Grb2, SH3BP2, Arhgef5 and PI3K. Subsequently, we demonstrated that Liprin-α1, α-Catulin and Grb2 are associated with the postsynaptic NMJ machinery and are indispensable for proper AChR organization.

CONCLUSIONS: Our research highlights the importance of aDB1 phosphorylation in the remodeling of neuromuscular synapses. We identify several novel aDB1-interacting components of the postsynaptic machinery, which play important roles in its organization.

This research was supported by the grant 2013/09/B/NZ3/03524 from the National Science Centre (NCN).

P9.12

ROLE OF AMOTL2, RASSF8 AND HOMER1 IN THE ORGANIZATION OF POSTSYNAPTIC MACHINERY

Marta Gawor[1], Paweł Niewiadomski[1], Krzysztof Bernadzki[1], Jolanta Jóźwiak[2], Katarzyna Rojek[1], Maria Jolanta Rędowicz[2], Tomasz J. Prószyński[1]

[1]Laboratory of Synaptogenesis, Department of Cell Biology, [2]Laboratory of Cell Motility, Department of Biochemistry, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland

BACKGROUND AND AIMS: Vertebrate neuromuscular junction (NMJ) undergoes a series of topological rearrangements in order to achieve its mature complex shape resembling pretzels. We have previously reported that podosomes, actin-rich dynamic adhesive organelles are implicated in the NMJ developmental remodeling. The main aim of this study was to understand molecular mechanisms regulating formation of podosomes and/or remodeling of the postsynaptic machinery with a special focus on the role of Amotl2 scaffold protein in these processes.

METHODS: To identify Amotl2-binding proteins we used TAP-tag affinity purification and mass spectrometry. Localization of proteins to NMJ subsynaptic compartments was performed using standard cytochemical procedures and confocal microscopy. We performed RNAi-based knockdown experiments on cultured C21C12 myotubes to assess the importance of proteins in the organization of postsynaptic AChR clusters.

RESULTS: We identified several novel Amotl2-binding proteins and subsequently focused our experiments on two of them, Rassf8 and Homer1, that remain uncharacterized at the NMJ. Amotl2, Rassf8 and Homer1 are concentrated at postsynaptic areas of NMJs in the indentations between the AChR-rich branches. Our results suggest that Rassf8 and Homer1 may be involved in AChR organization and development of the neuromuscular synapses.

CONCLUSIONS: We identified novel components of the muscle postsynaptic machinery that specifically localize to the sites of NMJ remodeling. Our results suggest that Amotl2 may be involved in the developmental remodeling of the postsynaptic machinery through the interactions with Rassf8 and Homer1.

This research was supported by the NCN grant 2012/05/E/NZ3/00487.

P9.13

LOCOMOTOR TRAINING OF SPINAL RATS DECREASES ABUNDANCE OF GlyR- ANCHORING GEPHYRIN IN THE ANKLE EXTENSOR AND FLEXOR MOTONEURONS MILDLY REDUCING PERINEURONAL NETS ENCAPSULATING THEM

Małgorzata Skup, Anna Głowacka, Olga Gajewska-Woźniak, Julita Czarkowska-Bauch

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

BACKGROUND AND AIMS: Complete spinal cord transection (SCT) causes reorganization of spinal networks involving changes of synaptic terminals abutting on α-motoneurons (MNs). We showed that SCT impoverishes excitatory cholinergic input to ankle extensor but not to flexor MNs and locomotor training leads to its enrichment on both MNs groups (Skup et al. 2012). The opposite effect of training after SCT was found on inhibitory glycinergic (Gly) inputs to MNs. To disclose the impact of SCT and training on postsynaptic components of Gly transmission and on MNs perineuronal nets (PNN), which are inhibitory to synaptic plasticity, and to verify if they respond differently in ankle extensor and flexor MNs.

METHODS: GlyR and gephyrin (Geph) were detected immunohistochemically and PNN were visualized with Wisteria floribunda agglutinin (WFA) on sections of L3–6 spinal segments in adult rats 5 weeks after SCT (Th9–10) and after 4 weeks of treadmill training of spinal rats. Extensor and flexor MNs were identified with Diamidine Yellow and Fast Blue respectively, injected intramuscularly. Images acquired in confocal microscope were deconvolved and analyzed with Image-Pro Plus Software. WFA and Geph were quantified in a 3 μm rim around MNs.

RESULTS: When all groups of motoneurons were analyzed, no effect of SCT on GlyR and Geph MNs membrane occupancy was detected, but the training decreased GlyR (P<0.05) and Geph (P<0.01) membrane expression to approximately 50% of control. Extensor but not flexor MNs tended to respond to SCT with Geph increase by 22% whereas training decreased it in both to 75%. PNN staining intensity increased after SCT by 75% in extensor and by 44% in flexor MNs (P<0.02) and the training tended to decrease it.

CONCLUSIONS: Locomotor training after CST may facilitate reorganization of MN inputs by reducing PNN-encapsulation of MNs and alter MN properties by decreased glycinergic signaling.

Supported by grants: NCN 2013/09/B/NZ4/03306, statutory for the Nencki Institute.

P9.14

AAV5-MEDIATED OVERPRODUCTION OF L1CAM DOWN-REGULATES PERINEURONAL NETS ENCAPSULATING MOTONEURONS AND THEIR PHOSPHACAN COMPONENT AFTER COMPLETE SPINAL CORD TRANSECTION IN THE RAT

Kamil Grycz, Rafał Płatek, Julita Czarkowska-Bauch, Małgorzata Skup

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

BACKGROUND AND AIMS: The motoneurons (MNs) of the spinal cord are surrounded by perineuronal nets (PNNs) that restrict plasticity, maintain synapses and compartmentalize the neuronal surface. One of the PNN components, inhibitory to axonal growth

in the injured spinal cord, is phosphacan (Pho), a chondroitin sulfate proteoglycan which binds to cell surface adhesion molecules such as L1CAM. Because L1CAM overexpression was found to promote recovery of spinal networks after injury we hypothesized that the mechanism may be through providing signals to down-regulate Pho and PNNs. To evaluate the expression of Pho and response of PNNs encapsulating MNs to the injury and L1CAM overexpression in a chronic phase (5 weeks) after complete spinal cord transection.
METHODS: Two groups of spinal rats (transected at Th9–10), injected with AAV5 vector carrying L1CAM or EGFP reference transgene and intact group were compared at the transcript (RT-PCR) and protein (Pho immunofluorescence) level. PNNs were visualized with Wisteria floribunda agglutinin (WFA). Image analysis was performed on the longitudinal sections from the L1–2 segments acquired in confocal microscope. When analyzing PNN and Pho thickness, the perimeter of the net was taken as the point at which the most intense staining around the MN ended. Next, to focus on area of nerve terminals abutting on MNs, staining intensity of both markers was quantified in a rim around MNs limited to 2.1 mm.
RESULTS: Pho around MNs formed the inner rim of PNN, occupying <50% of PNN thickness. Spinalization led to up-regulation of pho mRNA (2-fold, $P<0.05$) in L1–2 segments, and increased Pho protein (3-fold, $P<0.05$) in a rim. AAV-L1 injection decreased Pho towards controls ($P<0.05$) and reduced PNN thickness (by 45%), not modifying lesion-upregulated pho mRNA.
CONCLUSION: L1 overexpression in spinal rats may promote MN reinnervation reducing PNNs involving Pho down-regulation.
Support: NCN grants: 2013/09/B/NZ4/03306, Preludium12/05/N/NZ4/02241.

P9.15

BRAIN-DERIVED NEUROTROPHIC FACTOR (BDNF) EXPRESSION AND BEHAVIORAL RESPONSE DURING STIMULATION OF BED NUCLEUS OF THE STRIA TERMINALIS (BST) IN RATS

Dorota Myślińska[1], Irena Majkutewicz[1], Magdalena Podlacha[1], Ziemowit Ciepielewski[1], Mateusz Karnia[2], Agnieszka Wądołowska[1], Jan Ruciński[1], Martyna Siudak[1], Stanisław Zajączkowski[3], Danuta Wrona[1]

[1]Department of Animal and Human Physiology, University of Gdańsk, Gdańsk, Poland; [2]Department of Biochemistry, Academy of Physical Education and Sport, Gdańsk, Poland;[3]Department of Physiology, Medical University of Gdańsk, Gdańsk, Poland

BACKGROUND AND AIMS: The bed nucleus of the stria terminalis (BST) is a part of "the extended amygdala", a formation responsible for emotional aspects of behavior. The BST is considered as a site of convergence of information from brain regions associated with the control of emotional, cognitive, autonomic and behavioral responses to stress and noxious stimuli. On the basis of our previous study we assumed that the BST also influenced the primary antitumor immune response. In the present study we investigated the influence of 14-day electrical stimulation of the BST on the brain-derived neurotrophic factor (BDNF) expression and behavioral response.
METHODS: Male Wistar rats implanted with electrodes into the BST were divided into groups: BST stimulated and BST sham. The intensity of stimulation current (120–160 μA; 50 Hz) was determined individually for each stimulated rat to induce a behavioral response such a locomotor reaction. The current intensity was raised incrementally in 30 s trials (30 trials/day, 20 s rest between the trials). Behavioral reaction was measured in the Opto Varimex Minor actometer during stimulation procedure. BDNF was detected during immunofluorescence procedure.
RESULTS: The stimulation of the BST caused induction of BDNF expression in brain cortical and subcortical motor structures: the frontal primary motor cortex (areas FR1 and FR2), prefrontal cortex, ventral tagmental area, as well as in the central amygdala nucleus and in the hypothalamus: the paraventricular and the supraoptic nuclei, the medial preoptic and the lateral areas positively correlated with the augmentation of the behavioral activity which appeared as locomotor activity (increase in the average number of movements in horizontal and vertical plane).
CONCLUSION: This suggests that the behavioral outcome of the BST stimulation, imitating physical exercise, could be responsible for brain BDNF synthesis observed in the study.
Supported by: NN303819040.

P9.16

MUSCLE ACTIVITY CAN FAKE THE EFFECT OF HIGH-FREQUENCY EEG-NEUROFEEDBACK

Katarzyna Jurewicz[1], Katarzyna Paluch[1], Jacek Rogala[1], Mirosław Mikicin[2], Rafał Krauz[3], Ewa Kublik[1], Andrzej Wróbel[1]

[1]Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland; [2]University of Physical Education, Warsaw, Poland; [3]Military University of Technology, Warsaw, Poland

AIM: EEG-based neurofeedback trainings (EEG-NFB) belongs to a broader category of biofeedback techniques aimed to alter various physiological parameters such as heart rate (ECG-feedback), muscle tension (EMG-feedback) etc., with help of continuous feedback provided in a form of sensory information about the current value of a particular parameter. During EEG-NFB training EEG is recorded and the power of chosen frequency bands is fed back to a trainee in a form of sensory information. The trainings are founded on the assumption that one can learn to

change the content of his/her EEG spectrum. In our experiment we aimed to verify the effectiveness of such training and study its mechanisms.
METHODS: We chose to train healthy young participants to voluntarily up-regulate (*n*=6) and down-regulate (*n*=6) their beta band (15–22 Hz) amplitude recorded from the scalp electrodes placed at frontal and parietal positions (F3, F4, P3, P4) during visually guided monitor-play. As a control we used sham group (*n*=7), which feedback signal wasn't related to recorded EEG but generated by the algorithm.
RESULTS: We found that NFB training directed to this frequency band was inefficient – we did not observe any modification of the EEG beta band amplitudes neither within nor across the sessions of EEG-NFB. Instead, we observed training-induced increase of high-frequency activity of muscle origin (recorded by the same EEG electrodes) in three participants from group trained to up-regulate beta.
CONCLUSIONS: In these cases EMG-driven effects were perceived as the true positive effect of EEG-NFB by trainers and trainees. Importantly, if the data were analyzed and presented according to the standards prevailing in the current EEG-NFB literature, the result of the study could be presented as positive, i.e. up-regulation of the beta band would be claimed successful.
Supported by the Polish National Science Centre grant 2012/07/B/NZ7.

POSTER SESSION P10. EPILEPSY

P10.1
SELECTED microRNAs REGULATED DURING EPILEPTOGENESIS AND EPILEPSY IN A RAT MODEL OF TEMPORAL LOBE EPILEPSY

Anna Bot, Konrad Dębski, Katarzyna Łukasiuk
Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland

BACKGROUND AND AIMS: To verify expression level of selected microRNAs regulated during epileptogenesis and epilepsy in a rat model of temporal lobe epilepsy according to global analysis of miRNA expression and to characterize their cellular localization.
METHODS: Epilepsy was induced by status epilepticus triggered by electrical stimulation of the lateral nucleus of the amygdala. Animals were video-EEG monitored to detect spontaneous seizures. Rats were sacrificed at 7, 14, 30 and 90 days after stimulation. The expression levels of selected miRNAs were verified using qPCR and cellular localization was determined using in situ hybridization combined with immunohistochemistry. Bioinformatics analysis was performed using R packages and Ingenuity Pathway Analysis Software.
RESULTS: We have previously shown alterations in miRNA expression in the epileptic dentate gyrus. For qPCR analysis we selected 6 candidates and confirmed upregulation of miR-21, miR-132, miR-212, miR-370 and downregulation of miR-187 and miR-551b. At 90 days after stimulation, number and frequency of spontaneous seizures was positively correlated with expression level of upregulated miRNAs and negatively correlated with downregulated miRNAs. Hierarchical clustering of Spearman correlation between selected miRNAs and their target mRNAs expression levels revealed distinctive pattern of correlation. Functional analysis of target mRNAs revealed their involvement in inflammatory response, axonal guidance and changes in extracellular matrix. In situ hybridization of miR-132 revealed ubiquitous, neuronal expression not only in the dentate gyrus but also in other brain structures.
CONCLUSIONS: QPCR confirmed alterations in miRNAs expression obtained using global profiling method. In chronic epilepsy, changes in expression level of selected miRNA correlate with disease phenotype. Potential mRNAs targets of selected miRNAs may play a role in processes accompanying epilepsy. Expression of miR-132 is cell specific.

P10.2
SEIZURES, SLEEP-WAKE STATES AND JET LAG: DOES THE MELATONERGIC ANTIDEPRESSANT AGOMELATINE HELP TO RESTORE INTERNAL SYNCHRONY?

Magdalena Smyk[1], Gilles van Luijtelaar[2], Heidi Huysmans[3], Wilhelmus Drinkenburg[3]
[1]Department of Neurophysiology and Chronobiology, Chair of Animal Physiology, Institute of Zoology, Jagiellonian University in Kraków, Kraków, Poland; [2]Donders Centre for Cognition, Donders Institute for Brain, Cognition and Behaviour, Radboud University Nijmegen, Nijmegen, the Netherlands; [3]Department. of Neuroscience, Janssen Research & Development, a Division of Janssen Pharmaceutica NV, Beerse, Belgium

BACKGROUND AND AIMS: Spontaneous, rhythmic occurrence of absence seizures in WAG/Rij rats, a validated animal model of childhood absence epilepsy, is determined by the circadian timing system. Stable phase-relationship between seizures and sleep-wake states is maintained in the 12:12 light-dark cycle. In man, rapid changes in the photoperiod, e.g. caused by long-distance air travels, result in loss of the synchrony. Melatonin and its agonists were found to accelerate re-synchronization. The aims of the present study were to investigate in WAG/Rij rats the re-adaptation of the various sleep-wake states and absence seizures to an 8 h phase delay and to assess the effect of the melatonergic antidepressant agomelatine on the speed of re-synchronization of the different electroencephalographic (EEG) states.

METHODS: Simultaneous EEG and electromyographic (EMG) recordings were made in adult, male rats to assess the effect of various doses of agomelatine on sleep-wake states and absence seizures (acute study, 3 days) and to investigate the effect of the compound on the process of re-synchronization after the phase delay (chronic study, 11 days).
RESULTS: Agomelatine showed neither an effect on sleep-wake parameters in the acute study, nor affected the SWDs and the re-synchronization process in the chronic study. Internal desynchronization between various rhythms was observed, however, some rhythms remained coupled (active wakefulness and deep slow-wave sleep, SWDs and light slow-wave sleep). A post-shift increase in passive wakefulness and a reduction in deep slow-wave sleep resulted in an aggravation of epileptic activity (light phase).
CONCLUSIONS: Different speed of re-entrainment and coupling between various rhythms suggests that SWDs and light slow-wave sleep are controlled by common circadian mechanism distinct from that for active wakefulness and deep slow-wave sleep. The increase in the number of seizures after the phase shift may be of significant importance for people with epilepsy planning a transmeridian flight.

P10.3

KESI –A NOVEL METHOD FOR SPATIAL EPILEPTIC SOURCE LOCALIZATION IN HUMANS

Chaitanya Chintaluri, Daniel K. Wójcik
Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland

BACKGROUND AND AIMS: Develop a novel method to assist presurgical evaluation in epileptic patients with pharmacologically intractable epileptic seizures, by spatial source localization of epileptic epicenters using stereoencephalography (SEEG) and electrocorticography (EcoG) recordings.
METHODS: We developed kernel Electrical Source Imaging (kESI), which takes into account realistic brain morphology and spatial variations in brain conductivity. This method is parameter free, can localize multiple sources, and is flexible to allow arbitrary electrode positions. To account for the patient specific brain morphology, a patient's MR scan can be used to evaluate the measured potential in a forward model using Finite Element Method in FEniCS software. The inhomogeneous electrical conductivity of the gray and white matter, skin and skull etc. can also be included. kESI is an inverse method, which relies on the construction of kernel functions requiring computation of the potentials generated in the brain by numerous basis functions covering the probed volume. This approach is based on our previous approach of kernel Current Source Density in 3 dimensions, while utilizing the patient specific forward modeling scheme above.
RESULTS: To show the proof-of-concept we generated dipolar ground truth data in a simplified spherical brain model with uniform conductivity. We assumed the electrodes on the surface of the sphere and inside the spherical volume emulating ECoG and SEEG style recordings respectively. We show that the proposed method works, and can help in deciding how different distributions of electrodes affect the quality of reconstruction.
CONCLUSIONS: kESI method facilitates accurate localization of the seizure onset zones, and a possible procedure for prescribing optimal distributions of electrodes depending on available prior knowledge (e.g. dysfunction of specific brain structures) and clinical resources (availability of specific electrodes, etc.).

P10.4

THE ROLE OF SERUM RESPONSE FACTOR IN EPILEPTOGENESIS

Karolina Nader, Leszek Kaczmarek, Katarzyna Kalita
Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland

BACKGROUND AND AIMS: Serum Response Factor is a transcription factor that plays a prominent role in various programs of gene expression in the brain, including neuronal plasticity. Recently, we have shown the inducible-conditional deletion of SRF in the adult mouse hippocampus increased the epileptic phenotype in the kainic acid model of epilepsy, reflected by more severe and frequent seizures. Yet the molecular mechanism underlying these changes remains unknown. In this study we aimed to investigate mechanism of increased epileptogenesis in SRF KO animals using pentylenetetrazol (PTZ) kindling model of epileptogenesis.
RESULTS: We showed that SRF knockout animals developed more severe seizures in response to subconvulsive doses of PTZ over time compared to WT littermates. There was no difference in convulsive behavior upon the first PTZ injections in WT and SRF KO animals. To check whether sensitivity to PTZ kindling is related to neuronal sprouting we performed staining for zinc transporter ZnT3 to visualize infrapyramidal mossy fibers. There were no difference in length of mossy fibers between kindled animals from both groups. To further investigate changes in neuronal morphology we analyzed dendritic spines morphology using DiI protocol. Analysis showed no initial differences in dendritic spines density between WT and SRF KO animals in Cornu Ammonis (CA1) and dentate gyrus (DG) field of hippocampus. Next we plan to examine spines morphology in WT and SRF KO animals upon PTZ kindling. To further investigate mechanism of increased SRF KO susceptibility to seizures the level of PSD-95 was analyzed. KO and WT had the same level of PSD-95 under basal condition. In WT mice level of PSD-95

decreased upon PTZ kindling presumably due to homeostatic plasticity mechanisms. However, in SRF KO animals the level of PSD-95 remained at the stable level.
CONCLUSION: Taken together these results suggest that SRF transcription factor may play a role in the aberrant plasticity observed in epilepsy.

P10.5

THE EFFECTS OF NMDA RECEPTOR ANTAGONISTS ON THE DEVELOPMENT OF SENSITIZATION TO DIAZEPAM WITHDRAWAL SIGNS IN MICE

Sylwia Talarek, Joanna Listos, Jolanta Orzelska, Małgorzata Łupina, Sylwia Fidecka
Chair and Department of Pharmacology and Pharmacodynamics, Medical University of Lublin, Lublin, Poland

BACKGROUND AND AIMS: Repeated administration of benzodiazepines can alter $GABA_A$ receptors, which contributes to the development of dependence and often limits their clinical use. Although multiple chemical mediators are now hypothesized to be involved in the addictive effect of benzodiazepines, the mechanisms involved in the benzodiazepine dependence are not fully understood. The aim of the present study was to investigate the effects of two uncompetitive N-methyl-D-aspartate (NMDA) receptor antagonists, ketamine and memantine, on the development of sensitization to diazepam withdrawal signs in mice.
METHODS: In order to show the sensitization to benzodiazepine withdrawal signs the animals were divided into groups: the animals continuously (for 21 days) treated with diazepam (15 mg/kg/day sc) and the animals receiving diazepam during three 7-day periods interspersed with 3 day diazepam-free period in which the animals were treated with vehicle injections. Ketamine (2.5, 5 mg/kg ip) or memantine (2.5, 5 mg/kg ip) were administrated in sporadic diazepam treated mice during the diazepam-free periods (three, daily injections in each of the periods). In all animals, the intensity of diazepam withdrawal signs, observed as the increase in seizure activity (in pentylenetetrazole (PTZ)-induced seizures model) was assessed 48 h after the last injection of diazepam or vehicle. The animals, after concomitant administration of subthreshold dose of PTZ (55 mg/kg sc) with flumazenil (5 mg/kg ip), were placed in glass cylinders and were observed for 60 min.
RESULTS: The present experiments showed that administration of ketamine (2.5, 5 mg/kg) or memantine (2.5, 5 mg/kg) during two diazepam drug-free periods in sensitized mice, significantly attenuated their seizures activity.
CONCLUSION: These findings support the hypothesis that glutamatergic system is involved in the mechanisms of sensitization to withdrawal signs precipitated after sporadic treatment with diazepam.

P10.6

SRF REGULATES THE EXPRESSION OF GENES THAT MAY CONTROL EPILEPSY

Katarzyna Kalita[1], Bożena Kuźniewska[1], Karolina Nader[1], Michał Dąbrowski[2], Leszek Kaczmarek[1]
[1]Laboratory of Neurobiology, [2]Laboratory of Bioinformatics, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland

BACKGROUND AND AIMS: Although the transcription factor serum response factor (SRF) has been suggested to play a role in activity-dependent gene expression and mediate plasticity-associated structural changes in the hippocampus, no solid evidence has been provided for its role in brain pathology. A genome-wide program of activity-induced genes that are regulated by SRF also remains unknown.
RESULTS: In the present study, we showed that the inducible-conditional deletion of SRF in the adult mouse hippocampus increased the epileptic phenotype in the kainic acid model of epilepsy, reflected by increase in the susceptibility to spontaneous seizure development and more severe seizures. Moreover, we observed a robust decrease in activity-induced gene transcription in SRF knockout mice at 6 hours after kainic acid injections. We characterized the genetic program controlled by SRF in neurons and found that SRF target genes are associated with synaptic plasticity and epilepsy. Several of these SRF targets function as regulators of inhibitory/excitatory balance and the structural plasticity of neurons. We also identified novel direct SRF targets in neurons: Npas4, Gadd45g, and Zfp36.
CONCLUSIONS: Altogether, our data indicate that proteins that are highly upregulated by neuronal stimulation, identified in the present study as SRF targets, function as endogenous protectors against overactivation by increasing the level of inhibition or modulating dendritic spine number and morphology. Thus, the lack of these effector proteins in SRF knockout animals may lead to uncontrolled excitation and eventually epilepsy.

POSTER SESSION P11. STRESS RELATED BEHAVIOURS

P11.1

BASOLATERAL AMYGDALA AND HIPPOCAMPAL INPUTS TO PREFRONTAL CORTEX NEURONS, ACTIVATED BY HIGH AND LOW LEVELS OF FEAR

Jerzy Bukowczan, Weronika Szadzińska, Ewelina Knapska
Department of Neurophysiology, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland

BACKGROUND AND AIMS: Fear extinction is a useful model for exposure-based therapies for the treatment of human anxiety disorders, such as phobias and posttraumatic stress disorder. Extinction

of conditioned fear leads to formation of a new memory trace. Extinction memory is susceptible to the change of environment (context) in which conditioned stimulus (CS) is presented (promoting fear renewal), and to the passage of time (leading to spontaneous recovery of fear). Though the return of fear after extinction is a considerable challenge for the efficacy of exposure-based therapies, the neuronal basis of this phenomenon is not fully understood.
METHODS: To understand better the neuronal bases of extinction memory, we characterize the amygdalar and hippocampal active projections to prefrontal cortex during retrieval of extinction memory. We use anterograde tracing in a transgenic rat in which neurons express a dendritically-targeted PSD-95:Venus fusion protein under the control of a c-fos promoter. Rats were subjected to auditory fear conditioning, followed by fear extinction and then presented to the extinguished CS in the extinction or fear conditioning context.
RESULTS: Rats showed low levels of freezing when tested in the extinction context 24 hours after extinction and high levels of fear when tested in the conditioning context (either 24 hour or 28 days after extinction) or in the extinction context after 28 days (spontaneous recovery). The analysis of active projections shows that prefrontal cortex receives equal number of inputs from both basolateral amygdala and ventral hippocampus. However, the basolateral projections are dominant in neurons activated by high levels of fear.
CONCLUSION: The obtained results suggest that basolateral inputs to the prefrontal cortex may drive retrieval of fear memory, as opposed to hippocampal inputs.

P11.2

THE LACK OF GLUCOCORTICOID RECEPTOR ON NORADRENERGIC CELLS DOES NOT INFLUENCE AN INFLAMMATORY RESPONSE AFTER CHRONIC STRESS

Justyna Kuśmierczyk, Piotr Chmielarz, Ewa Trojan, Adam Roman, Irena Nalepa

Department of Brain Biochemistry, Institute of Pharmacology, Polish Academy of Sciences, Kraków, Poland

BACKGROUND AND AIMS: Noradrenergic neurons with terminals in the hypothalamus are known to regulate activity of the hypothalamic pituitary adrenal (HPA) axis. The aim of the study was to evaluate whether glucocorticoid receptor (GR) ablation in noradrenergic cells affects the inflammatory response in the central nervous system (CNS) and functioning of immune organs under chronic restraint stress (CIS) conditions.
METHODS: Selective ablation of GRs in the noradrenergic system (GRDBHCre mice) was achieved using the Cre/loxP approach. The male mice were kept in standard laboratory conditions. The CIS procedure was performed by placing animals, for 2 hours daily, in 50 ml disposable centrifuge tubes and was repeated for 14 days. The expression of cytokines in selected brain structure was analyzed with TaqMan RT-PCR assay. The relative thymus and spleen mass were calculated as well as peritoneal cell ability to production of selected cytokines after stimulation.
RESULTS: We found that CIS procedure caused the decrease in body and relative thymus weights in both wt and mutant mice. The mRNA expression of interferon gamma and interleukin-6 genes was elevated in the hypothalamus, prefrontal cortex and hippocampus in mutation independent manner. We also found the increase in production by peritoneal macrophage cells on tumor necrosis factor alpha and interleukin 1beta after pro-inflammatory stimulation and increase in interleukin 4 productions in anti-inflammatory stimulation in both wt and mutant mice.
CONCLUSIONS: The regulation of inflammatory process is a complex process in which a number of cells and molecules play different roles in a coordinated and well-controlled manner. Therefore, the lack of GR in noradrenergic cells might be too subtle and insufficient modification to cause disturbances in inflammatory responses after chronic stress.
Supported by grant no. POIG.01.01.02-12-004/09 co-financed by the ERDF and by statutory funds of Institute of Pharmacology PAS.

P11.3

THE EFFECT OF CHRONIC MILD STRESS ON THE REGULATION OF RECOGNITION MEMORY BY THE LIMBIC D_1, D_2 AND D_3 RECEPTORS IN RATS

Mariusz Papp, Piotr Gruca, Magdalena Lasoń-Tyburkiewicz, Katarzyna Tota

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

BACKGROUND AND AIMS: Stress plays a crucial role in the development of neuropsychiatric disorders, such as depression and schizophrenia. In animals, chronic stress impairs several brain systems, including dopaminergic (DA) neurotransmission. DA, apart from its involvement in the brain mechanisms of reward, regulates cognitive functions that are likewise disrupted in depression and schizophrenia. The impact of chronic stress on this regulation is, however, much less recognized.
METHODS: One of the many models used in preclinical studies on the interaction between stress and human diseases is a chronic mild stress (CMS) procedure. In this model animals, subjected to a variety of mild stressors for a period of several weeks, develop impairments, which show striking similarity to symptoms of human depression and schizophrenia. The purpose of this project was to study the effect of intracranial injections of agonists and antagonists of D_1, D_2 and D_3 on behaviour of control animals and stressed animals in the Novel Object Recognition (NOR) test.

RESULTS: It was found that in the control animals a significant and dose-dependent improvement of the NOR behaviour was caused by D_1 agonist SKF 81297 (0.05–0.75 μg) injected into prefrontal cortex (PFX), hippocampus (HPC) and nucleus accumbens septi (NAS), D_2 agonist Quinpirole (0.1–5 μg) injected into PFX and HPC, and D_3 antagonist SB 277-011 (0.1–1 μg) injected into PFX and HPC. The enhancement of novel object exploration induced by Quinpirole (1 and 5 μg) and SB 277-011 (0.5 and 1 μg), but not by SKF 81297 (0.2 and 0.5 μg), was substantially attenuated in animals subjected to the CMS procedure.
CONCLUSIONS: These data provide evidence that the regulation of recognition memory by the limbic D_2 and D_3 receptors is under a potent influence of prolonged stress, and are discussed in terms of their implications for understanding the neurobiological mechanisms underlying pathology of affective and psychotic diseases.

P11.4

EFFECTS OF B1-ADRENERGIC RECEPTOR BLOCKADE DURING CHRONIC RESTRAINT STRESS ON THE EXPRESSION OF SELECTED PROTEINS OF THE GLUTAMATERGIC TRANSMISSION IN THE RAT PREFRONTAL CORTEX

Agnieszka Zelek-Molik, Marta Kowalska, Adam Roman, Justyna Kuśmierczyk, Adam Bielawski, Irena Nalepa
Department of Brain Biochemistry, Institute of Pharmacology, Polish Academy of Sciences, Kraków, Poland

BACKGROUND AND AIMS: The stress impaired the structure and activity of the prefrontal cortical (PFC) neurons has been postulated to underlie the pathology of stress related psychiatric disorders. NMDA and AMPA glutamate receptors of PFC were shown to be affected by stress. High level of the noradrenaline release during stress is known to stimulate the β adrenergic receptors (βAR), densely expressed in PFC. The βAR can directly or indirectly, by means of Fyn kinase, regulate glutamatergic receptors activation. Also, evidence have shown that pharmacological blockade of β1AR alleviated anxiety in stress models. The aim of the study was to evaluate the stress induced changes in the expression of total- and phospho-(Y1472)GluN2B, (Ser845)GluA1, and (Y530)Fyn proteins in rat dorso-medial (dm) PFC and to assess whether β1AR blockade can modulate it.
METHODS: Male Wistar rats underwent the chronic restraint stress procedure applied for 3 hours daily, for 14 days. During the last 7 days rats were treated with betaxolol (1 or 5 mg/kg/po) given immediately after daily stress. Next day after a completion of stress procedure, the rats were decapitated, their dmPFC was dissected and subjected to standard Western blot analysis.
RESULTS: Neither stress nor betaxolol treatment changed the expression of studied NMDA and AMPA subunits. Repeated stress increased phosphorylation level of (Y530)Fyn kinase (by 25% vs. non stressed groups) and betaxolol treatment did not influence this effect.
CONCLUSIONS: The Fyn kinase is a known regulator of NMDA receptors' membrane stability, on the other hand phosphorylation at Tyr 530 inactivates the kinase. Our results showing the increased phosphorylation of Fyn suggest the inhibition of Fyn activity which can be responsible for disturbed glutamatergic transmission observed in PFC after prolonged stress. This mechanism seems to be independent on β1AR activity in PFC.
Supported by statutory funds of the Institute of Pharmacology PAS.

P11.5

EARLY LIFE STRESS-INDUCED CHANGES IN SYNAPTIC MODIFICATION RANGE IN THE RAT LATERAL AMYGDALA ARE PARTIALLY REVERSED BY IMIPRAMINE TREATMENT

Joanna Danielewicz[1], Jakub Bagiński[1], Grzegorz Hess[1,2]
[1]Institute of Zoology, Jagiellonian University, Kraków, Poland; [2]Institute of Pharmacology, Polish Academy of Sciences, Kraków, Poland

BACKGROUND AND AIMS: Maternal separation (MS) of rat pups has been widely used to study the mechanisms underlying the effects of early life stress on the adult organism. Such stress has been shown to induce alterations in the functions of the amygdala, a structure which plays a key role in the acquisition, consolidation and retrieval of fear-related memories. Our previous study has revealed that in the cortical input (CoI) to the lateral amygdala (LA) MS has shifted the potential for bidirectional synaptic modification towards LTD, but it shrank the synaptic modification range in the thalamic input (ThI) to the LA. Since imipramine (IMI) has been reported to reverse some effects of stress on the cerebral cortex, here we studied whether the effects of MS on the LA could be reversed by IMI.
METHODS: Rat pups were subjected to MS (3 h/day) on postnatal days (PND) 1–21 and weaned at PND 28. On PND 29–42 males previously subjected to MS were administered IMI (10 mg/kg/2 ml). Control rats received saline. On PND 43–60 brain slices containing the LA were prepared and field potentials were recorded. Saturating levels of LTP or LTD were induced using repeated sequences of theta-burst stimulation or low frequency stimulation.
RESULTS: Both LTP and LTD were reduced in ThI in slices obtained from MS-subjected rats receiving saline when compared to controls. However, in slices prepared from MS-subjected rats receiving IMI the magnitude of LTP and LTD was similar to control preparations obtained from non-stressed rats. In CoI the magnitude of LTP in slices prepared from stressed rats administered IMI was still smaller, when compared to control rats receiving saline or IMI, but the magnitude of LTD was similar to controls. These results confirm that MS alters the synaptic modification range both in ThI and CoI to LA.

CONCLUSIONS: Treatment with IMI fully reversed the effects of MS in the thalamic input. In the cortical input the reversal was only partial.
Support: National Science Center, Poland, grant no. 2011/03/N/NZ4/02176.

P11.6

CHANGED EXPRESSION OF APOPTOTIC SIGNALING-RELATED GENES, Pmaip1 AND Rock1, IN THE PREFRONTAL CORTEX OF RATS TREATED WITH IMIPRAMINE IN CHRONIC MILD STRESS MODEL OF DEPRESSION

Adam Bielawski[1], Katarzyna Rafa-Zabłocka[1], Agnieszka Zelek-Molik[1], Mariusz Papp[2], Irena Nalepa[1]

[1]Department of Brain Biochemistry, [2]Laboratory of Behavioral Pharmacology, Department of Pharmacology, Institute of Pharmacology, Polish Academy of Sciences, Kraków, Poland

BACKGROUND AND AIMS: The chronic mild stress (CMS) procedure induces depression-like symptoms in animals. In this model, rats subjected for a prolonged time to mild stressors gradually decrease their responsiveness to rewarding stimuli. This deficit can be effectively reversed by chronic antidepressant treatment. Apoptotic changes in the prefrontal cortex were shown in animal stress models, as well as degenerative changes, which are reversed by antidepressant treatment in depressed patients. We aimed to study the apoptotic signaling-related genes in the prefrontal cortex (PFC) of rats treated with imipramine (IMI) in CMS model.
METHODS: First, we used the TaqMan Low Density Arrays to indentify genes in the three groups of Wistar rats: sham-saline; stress-IMI-responders and stress-IMI-nonresponders (the sucrose intake score did not return to the control level). Then, these groups of rats and two additional ones (stress-saline and sham-IMI) were assessed in the PCR reactions with one TaqMan probe for detailed mRNA analysis of the identified genes. Finally, the levels of these proteins were assessed by Western Blot in all experimental groups.
RESULTS: We found that CMS decreased the expression of Pmaip1 mRNA (by 18%) and the effect remained unchanged in rats nonresponding behaviorally to IMI treatment. Furthermore, in rats nonresponding to IMI treatment, the Rock1 mRNA was decreased by 40% vs. sham and IMI responding rats. However, at the Rock1 protein level its decreased expression was observed in both groups, the IMI nonresponding and IMI responding animals (by 22% and 29%, respectively).
CONCLUSION: Our results suggest the involvement of apoptotic Pmaip1 and Rock1 genes in the process of response to IMI treatment in CMS model of depression in rats.
Supported by statutory funds of the Institute of Pharmacology PAS and POIG.01.01.02-12-004/09-00 grant financed by European Regional Development Fund.

P11.7

THE INFLUENCE OF MATERNAL SEPARATION STRESS ON LTP IN THE CA1 AREA OF THE RAT HIPPOCAMPUS

Aleksandra Trenk[1], Joanna Danielewicz[1], Grzegorz Hess[1,2]

[1]Department of Neurophysiology and Chronobiology, Institute of Zoology, Jagiellonian University, Kraków, Poland; [2]Institute of Pharmacology, Polish Academy of Sciences, Kraków, Poland

BACKGROUND AND AIMS: The maternal separation (MS) of rat pups is a widely used paradigm to study the influence of early life adversities on the functions of the adult organism. MS stress exerts deleterious effects during the development of the brain. It has been shown that MS decreases the number of nerve cells in hippocampus and profoundly affects behavior of adult animals. However, the influence of MS on hippocampal synaptic plasticity is still poorly understood. Here we examined the effects of MS on long-term potentiation (LTP) in the CA1 area of the rat hippocampus.
METHODS: Rat pups were subjected to MS (3 h/day) on postnatal days (PND) 1–21. They were weaned at PND 28 and between PND 42–60 the electrophysiological experiments were conducted. Coronal brain slices containing the hippocampus were incubated in ACSF containing either 1 mM or 0.1 mM Mg^{2+}. Field excitatory postsynaptic potentials (fEPSP) were recorded from the CA1 region of the hippocampus and LTP was induced by high frequency stimulation (HFS).
RESULTS: In the present study we observed an impairment of LTP in slices obtained from MS-subjected animals, compared to control, when the slices were incubated in ACSF containing a low concentration of Mg^{2+} ions. In contrast, in ACSF containing 1 mM Mg^{2+} the magnitude of LTP induced in control slices and in the slices isolated from MS rats was similar.
CONCLUSION: The effect of MS on hippocampal LTP appears to depend on different conditions of the NMDA receptor activation.
Support: National Science Center, Poland, grant no. 2011/03/N/NZ4/02176.

P11.8

BIOMARKERS IN FIREFIGHTERS FOR BEHAVIORAL AND PSYCHOPHYSICAL

Martyna Siudak[1,2], Anna Tober-Marczewska[2], Marta Ziętek[2], Andrzej Borman[2], Marek Wieczorek[2], Artur H. Świergiel[1]

[1]Department of Animal and Human Physiology, University of Gdańsk, Gdańsk, Poland; [2]Students' Scientific Society Homunculus, University of Gdańsk, Gdańsk, Poland; [3]Department of Neurobiology, University of Łódź, Łódź, Poland

BACKGROUND AND AIMS: This interdisciplinary project, linking neurophysiology, neuroendocrinology and psychology, aims to explore biomarkers of stress among rescue personnel under emergency situations (catastrophe) and correlating the results of

psychophysical features. We expect that an catastrophe produces significant changes in the concentration of biological indicators of stress that may be related to differences in the style of coping with stress. Our hypothesis is that the differences in the concentrations of biomarkers of stress depend on the temperament and ability to learn, assessed by appropriately selected psychological tests. Furthermore, the released hormones affect behavior and, therefore, performance. The expected results will explain and systematize knowledge about so far poorly explored correlation between the biological markers of stress and psychological parameters in selected professional group.

METHODS: The harmful effect of the stressor interpreted as a threat to the individual can affect a variety of intellectual functions. According to Janis (1982) stress is the cause of disturbances in the evaluation and decision-making because it replaces creative ways of responding with rigid and stereotyped thinking (Zimbardo 1999). Activity of the endocrine system will be measured using high performance liquid chromatography (HPLC), while ability to learn, style of coping with stress and temperament would be measured using carefully selected questionnaires and psychological tests under psychologist supervision.

RESULTS: In our preliminary research we found that stress associated with start of the practical driving course caused a rise in salivary cortisol that depended only on the time of sampling.

CONCLUSIONS: The results may be important by contributing to the development of biological tests to assist in determining the effectiveness of performance under stress conditions. The approach is discussed in the context of current research in the world.

P11.9

CO-OCCURRENCE OF DEPRESSIVE AND ANXIETY-LIKE BEHAVIORS FOLLOWING REPEATED NECK RESTRAINT STRESS IN MICE

Jadwiga Spyrka[1], Kamil Szumielewicz[1], Marcin Tynek[1], Grzegorz Hess[1,2]

[1]Department of Neurophysiology and Chronobiology, Institute of Zoology, Jagiellonian University, Kraków, Poland; [2]Institute of Pharmacology Polish Academy of Sciences, Kraków, Poland

BACKGROUND AND AIMS: Affective spectrum disorders are a group of affective-, anxiety-, and stress-related syndromes which present a number of overlapping features. These relatively common syndromes are potentially life threatening and are associated with a high morbidity and mortality of individuals in the developed countries. Stress has been shown to be an important factor in the pathophysiology of affective spectrum disorders. The aim of the present study was to determine how the repeated neck restraint stress influences the behavior of the mice. We tested whether effects of the repeated stress depend on the number of daily restraint sessions. We also measured blood levels of ACTH and corticosterone.

METHODS: C57BL/6 male mice were exposed to 1, 3, 7, 14 or 21 daily neck restraint sessions lasting 10 minutes. Control animals were subjected to manual handling. On the next day after the last restraint or handling depressive-like symptoms were evaluated using the tail suspension test (TST) and anxiety-like behavior was examined using the elevated plus maze (EPM) and open field test (OF). Plasma levels of corticosterone and ACTH were measured using the immunoassay kits.

RESULTS: The data obtained so far suggest that mice subjected to 3 neck restraint stress sessions display a significant increase in the immobility time in the TST and enhanced behavioral signatures of anxiety in EPM and OF. After 7, 14 and 21 restraint sessions we observed a gradual decline of the behavioral response to stress, however depressive and anxiety signs were still present. Behavioral consequences of restraint stress were correlated with alterations in the ACTH and corticosterone level.

CONCLUSION: These results shed a light on physiological mechanisms of the stress response and may lead in future to new therapies for depressed and/or anxious patients.

Support: Jagiellonian University (project SET), Polish National Science Center grant no. 2013/09/D/NZ4/00592

P11.10

ELECTRICAL ACTIVITY OF LIMBIC STRUCTURES DURING CLASSICAL FEAR CONDITIONING AFTER TEMPORAL BLOCKADE OF BASOLATERAL COMPLEX OF AMYGDALE IN RATS

Karolina Rejniak, Maciej Olszewski, Stefan Kasicki

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

BACKGROUND AND AIMS: The basolateral complex of amygdala (BLA) and nucleus accumbens (NAc) are involved in acquisition and extinction of the conditioned fear. The aim of our study was to verify if the electrical activity of these structures is correlated with the behavior of rats during fear conditioning after tetrodotoxin (TTX) blockade of BLA. The disturbances of cognitive function (learning) were estimated on the basis of the conditioned fear response.

METHODS: Male Wistar rats were implanted with electrodes in right NAc and BLA, guides bilaterally in BLA and divided in 2 groups: A-TTX group (TTX infused before acquisition sessions), E-TTX group (TTX before the first 3 extinction sessions). As a CS we used a tone associated at the end with electric shock (acquisition) or a tone alone (extinction). During each session we recorded local field potentials (LFPs) in NAc and BLA. The number of boli and rat behavior (video) were also recorded.

RESULTS: Infusion of TTX resulted in longest freezing duration both in A-TTX and E-TTX groups. In the A-TTX group the number of boli in acquisition sessions was smaller than in extinction sessions, while in the E-TTX group it was greater than in extinction sessions. The number of boli in all sessions was higher in the E-TTX group. Analysis of LFPs showed that in the A-TTX group there were practically no differences in frequency bands power between all sessions. The same analysis for the E-TTX group showed many differences between all sessions.
CONCLUSIONS: Our results are in line with previous data that blockade of BLA during acquisition sessions prevents conditioning, and at LFP level the activity was not different between sessions. Results of the group E-TTX show that the activity in BLA and NAc is changing parallel to changes in behavior what may reflect neural processes involved in acquisition and extinction of conditioned response.
Project funded by the National Center of Science DEC-2011/03/B/NZ4/03768.

P11.11

EFFECTS OF LESIONS OF NORADRENERGIC NEURONS ON ANXIETY-LIKE BEHAVIOR AND CELLS PROLIFERATION IN HIPPOCAMPUS OF ADULT MICE

Marta Ziętek[1], Martyna Siudak[1], Marek Wieczorek[2], Artur H. Świergiel[1]

[1]Department of Animal and Human Physiology, University of Gdańsk, Gdańsk, Poland; [2]Department of Neurobiology, University of Łódź, Łódź, Poland

BACKGROUND AND AIMS: We investigated the number of proliferating cells in the dentate gyrus of the hippocampus and anxiety-like behavior of mice after damage to the projections from the locus coeruleus as a result of a single administration (50 mg/kg) of DSP-4 that is a selective neurotoxin of central and peripheral noradrenergic neurons.
METHODS: Adult male Swiss mice were divided into 2 groups whose behavior was examined for 3 and 15 days. Brain sections were examined immunohistochemically (IMH) 4 and 16 after the injection of DSP-4. I group was tested in open field (OF) and elevated plus maze (EPM) 3 days after injection of DSP-4 (n=4) or saline (n=8). 4 days after the injection the brains were dissected and investigated by immunohistochemistry. In II group the same behavioral and IMH procedures were performed 15 and 16 days after injection of DSP-4 (n=4) or saline (n=8). Cell proliferation was determined using 5-bromo-2'-deoxyuridine.
RESULTS: In I group there were no significant changes in mice behavior in OF and EPM. In II group a significant effect of administration of DSP-4 on behavior of mice was observed only in the OF, no differences were observed in the EPM. The immunohistochemical studies in group I revealed significant decrease in number of proliferating cells in all examined section as compared to the control group. In group II an increased number of proliferating cells was found in all experimental groups.
CONCLUSIONS: The results suggest that the reduced level of noradrenaline in brain that could have been produced by administration of DSP-4 may increase anxiety-like behavior in mice. The lesions of noradrenergic neurons caused by DSP-4 inhibit cell proliferation in the dentate gyrus but do not damage the progenitor cells which produce granule neurons in the hippocampus.

P11.12

EFFECTS OF ELECTRICAL STIMULATION OF THE CENTRAL NUCLEUS OF THE AMYGDALA ON STRESS-RELATED BEHAVIORS AND PLASMA CORTICOSTERONE LEVEL IN RATS

Jan Ruciński, Katarzyna Czerwiec, Agnieszka Wądołowska, Ewelina Kurowska, Magdalena Podlacha, Dorota Myślińska, Edyta Jurkowlaniec

Department of Animal and Human Physiology, University of Gdańsk, Gdańsk, Poland

BACKGROUND AND AIMS: The central nucleus of the amygdala (CeA) has efferent projections to regions involved in autonomic, endocrine, and behavioral responses to emotional stimuli. Due to its role in the regulation of the activity of corticosterone releasing factor (CRF) neurons in the paraventricular nucleus of the hypothalamus (PVN), the CeA is considered a key modulator of behavioral responses to stress. Examine how electrical stimulation of the CeA, which is known to participate in the integration of sensory and emotional informations with neuroendocrine responses, influences stress-related behaviors and plasma corticosterone level in rats.
METHODS: Male Wistar rats implanted under standard stereotaxic surgery with stimulating electrodes into the CeA were divided into the following groups: CeA 14–day electrical stimulation (n=12) and CeA sham (n=10) – control, no stimulation. Behavioral activity during the 5-min testing period in the elevated plus maze (EPM) was recorded and analyzed (e.g. number of entries into closed arms, time spent in open arms). Additionally episodes of grooming were measured. Blood samples were collected by heart puncture (isoflurane anesthesia) seven days before (basal), one hour and seven days after the last stimulation. The plasma corticosterone level (CORT) was determined by radioimmunoassay.
RESULTS: Stress-related activity in the EPM and grooming episodes were increased in stimulated rats after last stimulation.

Whereas the control group showed gradual adaptation to the testing conditions which was manifested by decreased behavioral activity. Effects of CeA stimulation were also confirmed by an increase in plasma corticosterone level.
CONCLUSIONS: Electrical stimulation of the CeA induces stress-related behavior which is reflected by active avoidance of stressful situation (escape behavior) and adaptive activities to stressful conditions (grooming).
Research was supported by The National Science Centre, grant number: NN303819040.

P11.13

MEDIAL SEPTAL NMDA RECEPTOR INHIBITION INCREASES TIME SPENT IN OPEN ARMS IN HIGH AND LOW RESPONDER RATS SUBMITTED TO THE ELEVATED PLUS-MAZE MODEL OF ANXIETY

Magdalena Podlacha, Maria Grzybowska, Karolina Plucińska, Danuta Wrona
Department of Animal and Human Physiology, Faculty of Biology, University of Gdańsk, Gdańsk, Poland

BACKGROUND AND AIMS: The limbic glutamatergic neurotransmission may be involved in the biological mechanisms underlying anxiety-related disorders. The purpose of the present study was to determine the influence of NMDA glutamatergic receptor antagonist – D-AP7 infusions into the medial septum (MS) on time spent in open arms in rats differing in behavioral characteristics, stress susceptibility and anxiety level measured by their locomotor response to novelty: high (HRs) or low (LRs) responders under the elevated plus maze (EPM) test paradigm which is a rodent model of anxiety.
METHODS: Male Wistar rats prior categorized as HRs or LRs in the novelty test (2 h) were exposed to the EPM test (5 min) in the baseline and 15 min after injection of D-AP7 (DL-2-amino-7-phosphoheptanoate, receptor antagonist; 0.1 µg/rat in 0.5 µl saline solution; n=15) or saline (0.5 µl/rat; n=12) *via* implanted cannulae into the MS. Data are presented as mean±SD.
RESULTS: Following the D-AP7 injection, a significant increase in time spent in the open arms in both HRs (46±6 s) and LRs (102±6 s) within the D-AP7 group, in comparison with the baseline value (HR: 19±9 s, LR: 11±5 s; P≤0.001) and the SAL control group (HR: 24±9 s, LR: 19±9 s; P≤0.001), was observed. In the LRs, time in the open arms was significantly longer (LR: 102±6 s), as compared to the HRs (HR: 46±6 s; P≤0.001).
CONCLUSIONS: The obtained results indicate that blocking of MS NMDA glutamate receptors decreases the expression of anxiety – like behavior, indicating by increased time spent in open arms in rats. This effect is more pronounced in rats with higher anxiety level but lower behavioral activity and stress susceptibility, which are attributed to the low responders.
This study was supported by Gdańsk University grant for young scientists from Poland: 538-L124-B597-14.

P11.14

THE EFFECTS OF HIGH FREQUENCY SUBTHALAMIC STIMULATION ON PLASMA CORTICOSTERONE AND PRO-INFLAMMATORY CYTOKINE CONCENTRATIONS IN HEMIPARKINSONIAN RATS

Beata Grembecka, Magdalena Listowska, Wojciech Glac, Karolina Plucińska, Grażyna Jerzemowska, Danuta Wrona
Department of Animal and Human Physiology, Faculty of Biology, University of Gdańsk, Poland

BACKGROUND AND AIMS: The subthalamic nucleus (STN) is the best target for deep brain stimulation (DBS) elevating motor symptoms in Parkinson's disease (PD) patients. Although DBS is beneficial for patients with PD, it can also cause psychiatric and autonomic side effects of unknown etiology. Since the depression-like behavior is described after high frequency subthalamic stimulation (HFS-STN) in rats, it is important to examine impact of HFS-STN on hypothalamic-adrenal axis (HPA) activation and plasma pro-inflammatory cytokine levels. The aim of this study was to investigate the plasma corticosterone (CORT), TNF-α, IFN-γ and IL-6 levels following HFS-STN in hemiparkinsonian rats.
METHODS: Unilateral, continuous HFS-STN (pulse width: 60 µs, frequency: 130 Hz, stimulation intensity: 30–115 µA, during 1 h. stimulation period) was provide in freely moving hemiparkinsonian rats. The model of PD was obtained by stereotactic microinjection of 6-hydroxydopamine into the right substantia nigra pars compacta. The blood samples were collected by heart puncture after the electrical or sham stimulation. The CORT level in plasma was measured by radioimmunoassay method, while concentrations of cytokines were quantified using ELISA method.
RESULTS: We found that HFS-STN applied in hemiparkinsonian rats significantly increase plasma CORT level (t_{27}=2.31, P≤0.05) in comparison to SHAM control group). We also observed an increase in IFN–γ (t_{27}=2.89, P≤0.01) and TNF-α (t_{27}=2.88, P≤0.01) concentrations, while the IL-6 level decrease (t_{27}=3.38, P≤0.01) following HFS-STN.
CONCLUSIONS: These data shows that HFS-STN influence endocrine and immune parameters in peripheral blood in hemiparkinsonian rats. One hour, continuous HFS-STN activated HPA axis (measured by plasma corticosterone level) and elevated concentrations of pro-inflammatory cytokines. Our results suggest that HFS-STN provokes peripheral immune and endocrine effects similar to observed in behavioral depression.

POSTER SESSION P12.
MEMORY AND BEHAVIOUR

P12.1
HIPPOCAMPAL LESIONS IMPAIRED SPATIAL WORKING MEMORY SYSTEM IN RATS

Weronika Duda[1], Paweł Ostaszewski[2], Joanna Sadowska[1], Małgorzata Węsierska[1]

[1]Department of Neurophysiology, Nencki Institute of Experimental Biology Polish Academy of Sciences,, Warsaw, Poland; [2]Department of Psychology, University of Social Sciences and Humanities, Warsaw, Poland

BACKGROUND AND AIMS: Working memory is based on multi-component system, which comprises short term memory, long term memory and Cognitive Skill Learning (CSL) that allows to perform task based on newly acquired information. In our study rats with bilateral hippocampal lesions were tested in the Allothetic Place Avoidance Alternation Test (APAAT) to verify functional and anatomical substrates of spatial working memory.

METHODS: Lesions were made using ibotenic acid injections into the hippocampus. The task of rats in the APAAT was to remember and to avoid entering onto a 45° sector (described in the room frame coordinates) on the rotating arena, where shocks were given. It requires segregation of useful, room and misleading, arena frame information. The APAAT consists of four sessions (D1, 2, 3 and D21), which comprised of habituation, training 1, training 2 and test. Each session condition lasted 5 min. Effect of hippocampal lesion on working memory was evaluated by the maximum time spent on avoiding the shock sector (T_{max}). Longer T_{max} during training 2 than training 1 and habituation shows on effective memory functioning. CSL was determined by number of shocks/number of entrances ratio (S/E). Low value of S/E indicate that rats well know rules in the APAAT. Non-cognitive activity was determined by the total path length (TPL).

RESULTS: Rats with hippocampal lesions presented shorter T_{max} during training 2 in comparison with other session conditions ($F_{3,33}$=9.55; P<0.0001). Moreover, S/E ratio was on higher level in operated rats than in control ($F_{1,12}$=10.37; P<0.007). All rats presented TPL on similar level ($F_{1,11}$=0.15; P=0.7). Both groups walked the shortest distance on day 21. Hippocampal rats contrary to control had impaired ability to learn rules of the test (high S/E), to segregate information and to maintain the goal (short T_{max}).

CONCLUSION: The results indicate that short term memory and cognitive skill learning, components of spatial working memory system, strongly depend on hippocampus.

P12.2
THE EFFECT OF CO-TREATMENT WITH ARIPIPRAZOLE AND ANTIDEPRESSANTS ON THE MK-801-INDUCED DEFICITS IN THE SOCIAL INTERACTION TEST IN RATS

Zofia Rogóż, Katarzyna Kamińska, Elżbieta Lorenc-Koci

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

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

METHODS: The experiments were conducted on male Wistar rats (185–200 g). The social interaction was measured 4 h after the subcutaneously administration of MK-801 (0.1 mg/kg), and 60 or 30 min after injection of the antidepressant and ARI, respectively.

RESULTS: The present results showed that MK-801 (0.1 mg/kg) induced deficits in both parameters studied, i.e. the number of episodes and the time of interactions. ARI at a higher dose (0.3 mg/kg) reversed that effect. Co-treatment with an ineffective dose of ARI (0.03 mg/kg) and MIR or ESC (5 mg/kg) abolished the deficits evoked by MK-801, and this effect was partly blocked by a 5-HT1A receptor antagonist (WAY 100635, 0.1 mg/kg) or a dopamine D1 receptor antagonist (SCH23390, 0.5 mg/kg).

CONCLUSIONS: The obtained results suggest that the enhancement of antipsychotic-like effect of ARI by antidepressants on the MK-801-induced deficits in the social interaction test may be associated with serotonin 5-HT1A and dopamine D1 receptors.

P12.3
MATERNAL IMMUNE ACTIVATION DYSREGULATES THE SYNAPTIC ProSAP/Shank EXPRESSION AND MIGHT CONTRIBUTE TO AUTISM SPECTRUM DISORDERS

Agata Adamczyk[1], Magdalena Cieślik[1], Henryk Jęśko[1], Krzysztof Jaroń[2], Agnieszka Dominiak[3], Urszula Śmietanka[1], Paweł M. Boguszewski[4]

[1]Department of Cellular Signalling, Mossakowski Medical Research Centre, Polish Academy of Sciences, Warsaw, Poland; [2]Department of Pharmaceutical Biology and Medicinal Plant Biotechnology, [3]Department of Drug Bioanalysis and Analysis, Medical University of Warsaw, Warsaw, Poland; [4]Laboratory of Limbic System, Department of Neurophysiology, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland

BACKGROUND AND AIMS: Autism spectrum disorders (ASD) are neurodevelopmental diseases impairing social behaviour and cognition. Shank proteins that are involved in the maturation and maintenance of synaptic function have been implicated in the pathogenesis of ASD. Prenatal maternal immune activation (MIA) is a risk factor for ASD and is commonly used as animal model of ASD.
METHODS: We investigated the effect of MIA on the expression of Shank1, 2, 3 in male offspring of Wistar rats ip injected with 0.1 mg/kg lipopolysaccharide at gestational day 9.5. Moreover, redox potential and pro-oxidative/pro-inflammatory proteins were analysed along with the autism-associated behaviour.
Gene expression and protein levels were analysed using Real-time PCR and Western blot methods, respectively. Glutathione was measured spectrophotometrically. Behavioural tests were conducted to assess social communication, motions and anxiety, play behaviours as well as learning and memory.
RESULTS: The data showed MIA-induced down-regulation of Shank1, 2 and 3 in the cerebral cortex, without changes in other brain structures. The GSH/GSSG ratio has been used as an indicator of oxidative stress. MIA slightly decreased the reduced GSH level but significantly elevated the GSSG level, which led to reduction of the GSH/GSSG ratio in brain cortex. Furthermore, we analysed the expression of cyclooxygenase-2 (COX-2) as well as 12-lipoxygenase (LOX-12) that may be engaged in oxidative stress depending on cellular redox state and found up-regulation of both COX-2 and LOX-12 in the cerebral cortex. Along with the biochemical changes MIA evoked a tendency towards impaired pup communication (ultrasonic vocalization) and learning and memory (T-maze) in adult animals.
CONCLUSIONS: Our findings indicate MIA-induced down-regulation of Shank family and reduced antioxidative capacity. These changes may disturb synaptic function and social/cognitive behaviour.
Supported from MMRC statutory theme 8.

P12.4

THE EFFECT OF CHRONIC TREATMENT WITH THE SELECTED SSRIS AND L-DOPA ON ROTATIONAL BEHAVIOR AND MONOAMINE METABOLISM IN THE MOTOR AND LIMBIC BRAIN STRUCTURES OF 6-OHDA-LESIONED RATS

Kinga Kamińska[1], Tomasz Lenda[1], Jolanta Konieczny[1], Anna Czarnecka[1], Zofia Rogóż[2], Elżbieta Lorenc-Koci[1]

[1]Department of Neuro-Psychopharmacology; [2]Department of Pharmacology, Institute of Pharmacology, Polish Academy of Sciences, Kraków, Poland

BACKGROUND AND AIMS: The objective of the present study was to examine rotational behavior and monoamine metabolism in motor (striatum, STR; substantia nigra, SN) and limbic (prefrontal cortex, PFC; hippocampus, HIP) brain structures of unilaterally 6-OHDA-lesioned rats treated chronically with the chosen selective serotonin reuptake inhibitors (SSRIs): fluoxetine or paroxetine alone or jointly with L-DOPA.
METHODS: The experiment was performed on male Wistar rats injected unilaterally with 6-OHDA (16 μg/4 μl) into the medial forebrain bundle. Two weeks later, the animals were tested for the rotational behavior induced by apomorphine. Rats exhibiting more than 100 contralateral turns/1 h were administered fluoxetine (5 mg/kg) or paroxetine (5 mg/kg) and L-dopa (12 mg/kg), alone or in combination, once daily for 21 consecutive days. Rotational behavior was recorded after the first and the penultimate doses of the examined drugs. The levels of dopamine (DA), serotonin (5-HT), and their metabolites were determined in the tissue homogenates of motor and limbic brain structures 1h after the last drug injections using HPLC method.
RESULTS: Chronic combined administration of fluoxetine+L-DOPA significantly increased while paroxetine+L-DOPA decreased the number of contralateral rotations compared to the group receiving L-DOPA alone. L-DOPA given alone or jointly with SSRIs increased DA levels on both sides of all the examined structures. Fluoxetine intensified L-DOPA effect in the HIP while paroxetine in the SN. Joint administration of fluoxetine+L-DOPA decreased 5-HT level in the ipsilateral SN more distinctly than each of these drugs alone. Paroxetine+L-DOPA evoked similar decreases in tissue 5-HT content in the ipsilateral STR, HIP and PFC.
CONCLUSION: The obtained data are discussed in the context of motor and psychiatric disturbances observed in Parkinson's disease.
The study was supported by the Project "Depression-Mechanisms-Therapy" – POIG.01.01.02-12-004/09-00.

P12.5

THE INFLUENCE OF ELECTRICAL STIMULATION OF THE RAPHE MAGNUS ON RAT BEHAVIOURS

Kacper Ptaszek, Karolina Plucińska, Paweł Polasik, Edyta Jurkowlaniec

Department of Animal and Human Physiology, University of Gdańsk, Gdańsk, Poland

BACKGROUND AND AIMS: The activity of serotonin (5-HT) in the brain is strictly connected with the raphe nuclei. They are con-

nected mainly with and influence the prefrontal cortex and limbic structures. Clinical studies indicate that 30% of people with autism spectrum disorder (ASD) have elevated platelet 5-HT level. The aim of the study was to investigate whether chronic (16-days) electrical stimulation of the raphe magnus (RMg) in rats can evoke behaviours comparable with the behaviours present in people with ASD.

METHODS: Male Wistar rats were implanted with electrodes into the RMg under isoflurane anaesthesia. After 10-days convalescence chronic electrical stimulation began. Rats were divided into stimulated (n=6) and non-stimulated (sham/n=7) groups. Every day 25 stimulation trials were carried out, consisting of 30 s stimulation followed by 20 s interval. In sham group no current was passed through the electrode. During stimulations the locomotor activity was measured. Furthermore, the rats' anxiety level and social responses were analyzed respectively on the 3rd and 8th day after the first stimulation.

RESULTS: Locomotor activity was significantly higher in the experimental groups. At the intensity of 60–90 μA we observed rapid breathing, sniffing, and cage exploration, and at 110–140 μA – cage exploration and circular body movements. The anxiety level, analyzed as the time spent in closed arms in the elevated plus maze test, was comparable; nevertheless, social activity, measured in the three chamber test as a preference to a social stimulus, was reduced in the stimulated rats.

CONCLUSIONS: Electrical stimulation of the RMg induced hyperlocomotor and reduced social behaviours, which are the symptoms often present in the course of ASD. The obtained results suggest that hyperactivity of the serotonergic system may play a role in the development of ASD.

The research was funded by Young Researcher grant, 538-L124-B598-14.

P12.6

REPRESENTATION OF SPACE AND OBJECTS IN RAT ANTERIOR CLAUSTRUM

Maciej M. Jankowski, Shane M. O'Mara

Trinity College Institute of Neuroscience, Trinity College Dublin, Dublin, Ireland

BACKGROUND AND AIMS: The claustrum of the mammalian brain is an anatomically-substantial but largely unexplored and uninvestigated structure. The claustrum has been the subject of a limited degree of speculation regarding its potential functions, however, its physiological role still remains unknown. In the present study, we investigated the spatial and temporal properties of neurons located in the anterior claustrum in freely-moving rats.

METHODS: Extracellular recordings were performed using 32-channel drivable microelectrode arrays. The spiking activity of neurons was simultaneously coupled with the animal's position in the environment and direction of the head in horizontal plane. Recordings were performed in different environmental conditions including presentation of objects.

RESULTS: Our data suggest, unexpectedly, the presence of cells in anterior claustrum that are responsive to the position in space of the animal, to boundaries enclosing the environment and finally to the presence of objects in the environment.

CONCLUSIONS: This novel claustral signal potentially directly modulates a wide variety of anterior cortical regions. We hypothesise that a key function of the claustrum is to provide dynamic information about body position, boundaries, landmark information and content of the environment, enabling dynamic control of behaviour.

P12.7

THE NEW DERIVATE OF KISSPEPTIN-54 – KISSORPHIN (KSO) REDUCES THE EXPRESSION OF MORPHINE- AND ETHANOL-INDUCED CONDITIONED PLACE PREFERENCE IN RATS

Ewa Gibuła-Bruzda[1], Jolanta H. Kotlińska[1], Marta Marszałek--Grabska[1], Róża Trzcińska [2], Jerzy Silberring[2,3]

[1]Department of Pharmacology and Pharmacodynamics, Medical University , Lublin, Poland; [2]Centre of Polymer and Carbon Materials, Polish Academy of Sciences, Zabrze, Poland; [3]Faculty of Materials Science and Ceramics, AGH University of Science and Technology, Kraków, Poland

BACKGROUND AND AIMS: The kisspeptins (KP) are a family of peptide hormones, which in recent years have been shown to play a crucial role in the regulation of the hypothalamic–pituitary–gonadal axis, thus in turn influence fertility and reproduction. One of the fragments of KP-54 termed Kissorphin (KSO) shares amino acid similarities with the biologically active sequence of NPFF therefore it is possible that KSO could exhibit NPFF-like activity (Simonin et al. 2006) without influence on concentration of gonadotropin-releasing hormone (Roseweir et al. 2009, Milton 2012). This is particularly important since the NPFF may participate to the control of the mesocorticolimbic dopamine system activity by counteracting the effect of many psychostimulants (Marco et al. 1995, Cador et al. 2002). Therefore the aim of the present study was to investigate whether a KSO (Tyr-Asn-Trp-Asn-Ser-Phe-NH2) influence the expression of morphine- and ethanol-induced CPP.

METHODS: Morphine- and ethanol-induced CPP were induced according to the previously established methods (Kotlinska et al. 2007).

RESULTS: Our experiments showed that KSO, given intravenously (iv) at the doses of 1, 3 and 10 nmol/300 μl, inhibited the expression of both morphine and ethanol-induced CPP. KSO gave itself, neither induced place preference nor aversion.

CONCLUSIONS: These results suggest that KSO is involved in the expression of morphine and ethanol reward. Moreover, our study supports an anti-opioid character of this peptide.

P12.8

NEURONAL CIRCUITS IN THE CENTRAL AMYGDALA UNDERLYING EMOTIONAL CONTAGION

Karolina Rokosz, Ewelina Knapska

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

BACKGROUND AND AIMS: Human empathy emerges over phylogeny from various behavioral precursors. One of the simplest is emotional contagion, i.e. sharing emotional states between individuals, which can be modelled in rodents. In our model of socially transferred fear we showed that a brief social interaction with a fearful cage mate (demonstrator) promotes aversive learning in an otherwise naïve rat (observer) and activates the amygdala of the observers, especially its central part (CeA).

METHODS: To elucidate the role of neuronal circuits in the central amygdala of the observers, we used two methods of functional mapping: transgenic rats expressing in behaviorally activated neurons a PSD-95:Venus fusion protein and injected with anterograde tracer and a combination of retrograde tracing with c-Fos ISH.

RESULTS: We have identified several afferent and efferent CeA projections active during socially transferred fear. We discovered strong activation especially in the periaqueductal gray (PAG) and dorsal raphe nuclei (DRN), structures receiving dense projections from the CeA and implicated in fear and anxiety disorders. Moreover, we observed that most of the activated cells are GABA-ergic neurons. To test whether the activated circuits are similar for the socially and non-socially induced emotions, we used double immunodetection for a PSD-95:Venus construct and endogenous c-Fos. About 70% of neurons was activated by both social interaction with fear conditioned partner and subsequent fear conditioning. Moreover, using optogenetics, we showed that specific activation of CeA neurons involved in socially transferred fear results in increased anxiety.

CONCLUSIONS: These findings suggest that there exists a group of neurons in the central amygdala that is involved in integrating information about a threat, activated during socially transferred fear and subsequently recruited by learning of fear responses. Part of these cells is probably specifically involved in socially induced anxiety.

P12.9

THE INFLUENCE OF DMPX ON THE ACTIVITY OF COMMON ANTIDEPRESSANTS

Ewa Poleszak[1], Anna Serefko[1], Aleksandra Szopa[1], Sylwia Wośko[1], Karolina Bogatko[1], Katarzyna Świąder[1], Piotr Wlaź[2]

[1]Chair and Department of Applied Pharmacy, Medical University of Lublin, Lublin, Poland; [2]Department of Animal Physiology, Institute of Biology and Biochemistry, Faculty of Biology and Biotechnology, Maria Curie-Skłodowska University, Lublin, Poland

BACKGROUND AND AIMS: Though depression is known as one of the most frequent chronic health problems in the world, the optimal treatment of the depressed patients remains an important challenge. Due to serious adverse reactions and common ineffectiveness, the conventional antidepressant therapy is usually not sufficient. The identification of the best treatment strategies and development of new, safer, and more effective ones are crucial. Literature data confirm participation of adenosine neurotransmission in the development of depression. Adenosine, which is responsible for the suppressed release of serotonin, noradrenalin, and dopamine, contributes to a decrease of neuronal excitability. The main objective of our study was to investigate an antidepressant activity of a joint administration of adenosine A_{2A} receptor antagonist DMPX (3,7-dimethyl-1-propargylxanthine) and the common antidepressant drugs: imipramine, reboxetine, escitalopram, tianeptine, venlafaxine, moclobemide, and agomelatine.

METHODS: The experiments were carried out on male Albino Swiss mice. The antidepressant-like effect was assessed by the forced swim test. In order to avoid the risk of obtaining the false positive/negative effects, the spontaneous locomotor activity was measured as well.

RESULTS: The obtained results demonstrated that DMPX at the dose of 3 mg/kg significantly enhanced the antidepressant-like effect of imipramine (15 mg/kg), reboxetine (2.5 mg/kg), escitalpram (2 mg/kg), tianeptine (15 mg/kg), venlafaxine (1 mg/kg), moclobemide (1.5 mg/kg), and agomelatine (20 mg/kg). None of the used combinations changed the overall spontaneous locomotor activity of the animals.

CONCLUSION: Forced swim test outcomes indicated a synergistic action of adenosine A_{2A} receptor antagonist in combination with the tested antidepressants.

P12.10

THE ROLE OF NMDA RECEPTOR-DEPENDENT NEURONAL PLASTICITY IN THE DOPAMINE SYSTEM IN REWARD-DRIVEN LEARNING

Jan Rodriguez Parkitna, Kamila Łopata, Przemysław Cieślak, Łukasz Szumiec, Magdalena Zygmunt, Magdalena Sikora

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

BACKGROUND AND AIMS: Reinforcement-based learning drives behavior towards actions with highest perceived outcome

value. It's essential features are the ability to associate actions or stimuli with rewards, discounting of the delay or probability of the rewards and balance between exploitation of known rewarded actions against exploration of new possibilities. Here we investigate how disrupting NMDA receptor-dependent signaling in the brain's dopamine systems affects reinforcement learning.
METHODS: Genetically modified mice with selective inactivation of NMDA receptors on dopaminergic or dopaminoceptive neurons were generated using the CreERT2/loxP system. Behavior of control and mutant mice was assessed in tasks involving instrumental or Pavlovian learning as well as discounting of reward probability and delay.
RESULTS: Inactivation of NMDA receptors on dopaminergic neurons impaired the acquisition of conditioned reinforcement, even though it had no general effect on associative learning. Conversely, in mice with inactivation of NMDA receptors in dopaminoceptive neurons, an opposite phenotype was observed: deficits in associative learning but normal conditioned reinforcement. Interestingly, the effects of the mutations on performance in probabilistic reversal or discounting was limited.
CONCLUSIONS: These results show discrete functions of dopamine signaling in control of reinforcement learning. Mutations in either dopaminergic or dopaminoceptive neurons selectively affected conditioned reinforcement or associative learning, respectively.

P12.11

A NEW MODEL TO STUDY DELAY DISCOUNTING IN GROUP HOUSED MICE

Łukasz Szumiec, Jan Rodriguez Parkitna

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

BACKGROUND AND AIMS: The immediacy or delay of a reward affect its perceived value. Here we present a new model of assessing delay discounting in group housed mice.
METHODS: A group of animals was implanted subcutaneously with radiofrequency identification chips and placed in a cage equipped with sensors for automatic tracking (IntelliCage Plus). Each of the cage's corners had 2 drinking bottles accessed through a small compartment that allows only one mouse inside. The effect of the delay was assessed by first allowing mice with free access to water or 0.1% saccharin and then progressively increasing the delay till gate blocking the saccharin bottle raised.
RESULTS: In line with expectations, while mice initially showed a 95.4% preference for saccharine it decreased to 50% at 17 s delay and finally to 12% at 55 s delay. In the delay discounting model with 3 types of rewards (saccharin 0.01% or 0.1% and water), initial preference of 0.1% saccharine was 92.4%, an increase in delay to 55 s of access decreased preference to 7.8% and caused an increase of preference of the 0.01% saccharine solution from 3.2% to 64.7%. We tested the effects of tranylcypromine, a monoamine oxygenase inhibitor (3 mg/kg, 3 injections in 48 h intervals), cloccinamox, an opioid receptor antagonist (10 mg/kg, single injection) and ketamine, an NMDA receptor partial antagonist (20 mg/kg, single injection) on delay discounting. Treatment with tranylcypromine led to increase in discounting of the delay, at 17 s the saccharin preference was 51% in the control group but only 4% in drug-treated mice. A similar trend towards increased discounting was observed in case of ketamine, while clocinnamox had no effect.
CONCLUSIONS: The main advantages of the new model are the ability to test behaviour in the home cage, during natural activity cycles, no interaction with the experimenter and without food deprivation. Further development of the model may permit testing of social effects on discounting.

P12.12

LONG-TERM MORPHINE SELF-ADMINISTRATION SCHEDULE IN INTELLICAGES AS A PRECLINICAL MODEL OF OPIOID ABUSE IN MICE

Urszula Skupio, Magdalena Sikora, Mateusz Turbasa, Ryszard Przewłocki

Department of Molecular Neuropharmacology, Institute of Pharmacology, Polish Academy of Sciences, Kraków, Poland

BACKGROUND AND AIMS: Chronic exposure to opiates induces various alterations in brain physiology that may lead to formation of dependence, tolerance and addiction. Commonly used approaches for modeling morphine dependence involve the use of conditioned place preference, which lacks voluntary intake of the drug, and morphine self-administration, which requires isolating the animals. Here, we describe a novel model of long-term morphine self-administration in C57BL/6J mice.
METHODS: We have used IntelliCage (New Behavior, Switzerland) system to observe the animals without unnecessary experimental intervention. The animals in two separate cages were allowed access to sweetened morphine (0.5 mg/ml) or saccharin solutions for 3 executive months with saccharin concentration being gradually lowered (from 0.2% to 0.02%). We behaviorally challenged animals to test for symptoms of compulsive morphine drinking, using paradigms like saccharin reduction, progressive ratio schedule and intermittent access to rewarding substance.
RESULTS: We have observed stable preference to both saccharin and morphine throughout the drinking schedule. The animals performed significantly increased number of instrumental responses to obtain access to the bottle with morphine (progressive ratio schedule) and significantly more nose pokes in attempt to obtain rewarding substance during intermittent access procedure, when compared

to saccharin group. What is more, morphine dependent animals exhibited a variety of spontaneous withdrawal symptoms that lasted up to 32 hours.
CONCLUSIONS: This study demonstrate that our model reliably leads to stable morphine drinking while avoiding the limitations associated with testing isolated animals. Mice drinking morphine exhibit many of the symptoms of dependence and craving compared to control animals. Therefore, this model may be well suited to screening for the effects of genetic mutations or pharmacological treatments on morphine-induced behaviors.

P12.13

CAN THE AMYGDALA CODE THE SUBJECTIVE IMPORTANCE OF HUMAN SOCIAL VALUES?

Emilia Leszkowicz[1], George Zacharopoulos[2], David Linden[2], Gregory R Maio[2], Niklas Ihssen[2]

[1]Department of Animal and Human Physiology, Faculty of Biology, University of Gdańsk, Poland; [2]School of Psychology, Cardiff University, Cardiff, UK

BACKGROUND AND AIMS: The amygdala has been proposed to act as a "relevance detector" involved in the processing of behaviorally relevant or significant stimuli. We investigated that putative function of the amygdala in the context of human social values, such as achievement, honesty or stimulation.
METHODS: We used functional magnetic resonance imaging (fMRI) during a behavioral task of social values rating. During the task, participants were presented with different social values and asked to rate their importance as guiding principles in their lives. We used the conjunction analysis of values and their ratings to localize brain regions where value ratings modulated activity evoked by value processing.
RESULTS: The conjunction analysis with the amygdala mask revealed that amygdala activity during value processing correlated with value rating scores. Moreover, the rating scores modified activity in the visual cortex during the response phase, when participants were pressing response buttons, so that higher scores were associated with stronger activation.
CONCLUSIONS: The results suggest that the amygdala can code the subjective importance of social values. The increased activity in the visual cortex with higher rating scores may reflect amplified processing within the ventral visual stream, mediated by the amygdala. An intriguing possibility is that excitatory feedback from the amygdala in response to more important/significant stimuli during task performance could enhance functional connectivity between the amygdala and visual cortex. To investigate if that connectivity differed according to the ratings of value importance, a psychophysiological interaction analysis will be performed in further analysis.

P12.14

INFLUENCE OF GLUTAMATE INJECTION INTO UNILATERAL NUCLEUS ACCUMBENS SHELL ON BEHAVIORAL RESPONSE ELICITED BY IPSILATERAL STIMULATION OF THE MESOLIMBIC SYSTEM

Karolina Plucińska, Grażyna Jerzemowska, Magdalena Podlacha

Department of Animal and Human Physiology, Faculty of Biology, University of Gdańsk, Gdańsk, Poland

BACKGROUND AND AIMS: Food intake is regulated not only by homeostatic requirements but also by emotional factors. The nucleus accumbens, particularly its shell region (AcbSh) is a part of the mesolimbic dopaminergic system which is responsible for a positive emotional aspect of various homeostasis-relevant stimuli. In the present work, we tested the AcbSh involvement in feeding behavior using an experimental paradigm specifically designed to assess motivational vs. motor aspect of food ingestion.
METHODS: In rats (n=4), feeding was evoked by electrical stimulation of the midbrain ventral tegmental area (a somatodendritic region of mesolimbic system) and assessed quantitatively with the use of the latency to feed/stimulation frequency curve shift paradigm before and after ipsilateral glutamate injection (dose 2.0 µg dissolved in 0.5 µl of distillated water) into AcbSh (distillated water injection as a control, volume: 0.5 µl).
RESULTS: Effect of ipsilateral glutamate injection into the AcbSh on behavioral response following the VTA stimulation was varied. In three rats the percentage reaction threshold did not change significantly and was approximated the baseline (not exceed ±10%). We observed an increase/decrease in the reaction threshold by only +0.31%, +2.85% and −1.90% in comparison to the water injection. In one rat the feeding threshold reaction was changed by significantly more than 10%. This significant increase was about +19.40% as compared to the baseline (water injection).
CONCLUSIONS: Glutamate AcbSh activation – the major terminal area of the mesolimbic system does not affect the behavior induced by stimulation of the dopaminergic cells at the level of VTA. Presumably a different effect observed in one rat is dependent on the injected place within the AcbSh area.
Research was financed by the Polish National Science Centre (NCN); decision no: DEC-2013/09/N/NZ4/02195.

P12.15

BEHAVIORAL AND NEURAL CORRELATES OF ATTENTION NETWORK TEST IN ADHD CHILDREN AND TEENAGERS: AN EVENT RELATED POTENTIAL STUDY

Katarzyna Anna Giertuga[1], Marta Zakrzewska[2], Ewa Racicka[3], Maksymilian Bielecki[4], Małgorzata Kossut[1,4], Anita Cybulska-Kłosowicz[1]

[1]*Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland;* [2]*Gösta Ekman Laboratory, Department of Psychology, Stockholm University, Stockholm, Sweden;* [3]*Department of Child Psychiatry, Medical University of Warsaw, Warsaw, Poland;* [4]*University of Social Sciences and Humanities, Warsaw, Poland*

BACKGROUND AND AIMS: Attention Deficit/Hyperactivity Disorder (ADHD) is one of the most common neurodevelopmental disorders affecting approximately 5% of children and teenagers population. ADHD is characterized by developmentally inappropriate levels of inattention, impulsivity and overactivity. The aim of this study was to investigate attentional processes and their electrophysiological correlates in children and teenagers with ADHD diagnosis.
METHODS: Fifty ADHD children and teenagers and 50 age-, and sex-matched healthy controls participated in this study. Participants performed Attention Network Test (ANT, Fan et al. 2002) – paradigm combining cue detection (Posner 1980) with flanker-type paradigm (Eriksen and Eriksen 1974). ANT allows for the behavioral assessment of different attention functions – alerting, orienting, and executive functions. During task performance EEG data were collected using 64 channel EGI Geodesic System. Measures of attention evaluated in attentional test were analyzed in terms of relation to EEG recording results.
RESULTS: The results obtained on behavioral level revealed significant differences in ANT performance in above mentioned groups. ADHD participants, when compared with healthy controls, were less accurate and had longer reaction times in the condition evaluating executive attention. We have also found different patterns of Event Related Potentials (ERPs) related to ANT performance in these groups. Results of ERPs calculated for parietal electrodes cluster revealed that P300 amplitude was lower in ADHD group in comparison with controls.
CONCLUSIONS: The results of EEG recordings suggest worse allocation of attention resources which may result in deficits in behavioral performance, especially in executive function of attention. These results are discussed in context of current views and theories on attentional networks and deficits observed in ADHD patients.
National Science Centre Grant 2011/01/D/NZ4/04958.

P12.16

INVOLVMENT OF INHIBITORY SKILLS IN BEHAVIOUR OF MICE SUBJECTED TO DETOUR TEST

Grzegorz Juszczak, Michał Miller

Department of Animal Behavior, Institute of Genetics and Animal Breeding, Magdalenka, Poland

BACKGROUND AND AIMS: A common test of intelligence is the detour task, where various barriers are applied to prevent animals from reaching a goal. However, there is no information available about the effect of goal visibility on rodent behavior and about the involvement of inhibitory skills and physical cognition in performance of rodents faced with the detour problem. Therefore, we tested the effect of target visibility both in naïve mice and in subjects that already had an experience in detouring obstacles.
METHODS: The subjects were F1 hybrid (C57BL10×CBA/H) male mice. The water escape paradigm was used to motivate mice to detour the barrier. The apparatus consisted of circular tank and visible platform. Transparent, semitransparent and opaque barriers were used to prevent animals from reaching their goal.
RESULTS: We have found that naïve mice tested with transparent barrier displayed high level of perseveration. In contrast, mice that were initially trained with opaque or semitransparent barriers displayed no deficits during tasks applying transparent barrier. Improvement in both transparent and semitransparent groups was associated with changes in path direction. We have also found that mice displayed consistent lateralization during inward and outward detour trials.
CONCLUSIONS: We have found that both inhibitory skills and physical cognition affects performance of mice subjected to the detour task. The difficulty to inhibit proponent responses interfered with the ability of mice to find the proper solution. We have also found that mice followed their respective left or right side instead of using landmarks to navigate during the test. Obtained results show for the first time that behavior of mice subjected to the detour task is comparable with the behavior of other species including human infants and monkeys.

P12.17

AMPHETAMINE INJECTION INTO CONTRALATERAL NUCLEUS ACCUMBENS SHELL ALTERS FEEDING EVOKED BY STIMULATION OF THE MESOLIMBIC SYSTEM

Grażyna Jerzemowska, Karolina Plucińska, Kacper Ptaszek

Department of Animal and Human Physiology, Faculty of Biology, University of Gdańsk, Gdańsk, Poland

BACKGROUND AND AIMS: Dopamine projections from the ventral tegmental area (VTA) – a somatodendritic region of mesolimbic system to the nucleus accumbens (particularly its shell region, AcbSh) – which constitutes its main terminal structure may play a central role in modulating affective states. In the present work, we used model of the VTA electrical stimulation-induced feeding reaction to research the role of AcbSh dopamine transmission in motivated behaviors.
METHODS: In 5 rats (n=5) latency to eat was measured as a function of stimulation frequency before and after contralateral intra-AcbSh injection of dopamine agonist D-amphetamine (dose 5.0 µg

dissolved in 0.5 µl of water). This experimental method allowed us to distinguish between motivational vs. motor aspects of tested reaction.

RESULTS: Inactivation of the AcbSh caused of its intra injection of dopamine agonist D-amphetamine on the contralateral side (in relation to the hemisphere with VTA stimulation) affects motivational processes assessed by changes in frequency threshold for stimulation-induced feeding response. We observed increase, by more than 10% (about 13.37%), in the reaction threshold as compared with water control. Increase of the frequency threshold was accompanied by a parallel rightward shift of the function relating latency to feed to stimulation frequency, but statistical analysis of latency at the specific frequencies showed no significance (from 17.71 Hz to 81.38 Hz, $P>0.05$). Distilled water injected into the contralateral AcbSh (volume of the 0.5 µl), as a control group ($n=5$), did not cause any effect in comparison with the preinjection baseline.

CONCLUSION: We conclude that the contralateral inactivation at the level of AcbSh does not impair of the behavior induced by activity of the dopaminergic cells at the level of VTA.

Research was financed by the Polish National Science Centre (NCN); decision no: DEC-2013/09/D/NZ4/02499.

P12.18

BEHAVIOURAL AND NEURAL CORRELATES OF ACTION SELECTION: PROBING INDECISIVENESS IN OBSESSIVE-COMPULSIVE DISORDER

Bartosz Żurowski[1], Antonie Arnoldussen[1], Andreas Kordon[1], Thilo van Eimeren[2], Fritz Hohagen[1]

[1]Center for Integrative Psychiatry, University of Lübeck, Lübeck, Germany; [2]Department of Neurology, University of Köln, Köln, Germany

BACKGROUND AND AIMS: The observation of every-day problems in decision making in patients with obsessive-compulsive disorder (OCD) is reflected by their inclusion in psychometric scales for OCD. Experimental studies in OCD patients did not consistently find deficits in DM, however. Our aim was to minimize the hypothesized influence of working memory capacity, reward/punishment processing upon feedback, planning and strategy on action selection performance and neural correlates thereof using fMRI.

METHODS: We conducted an event-related 3T-fMRI study in 12 unmedicated OCD patients and 10 healthy controls. We used a forced-choice reaction time (RT) paradigm with parametrically increasing number of choice alternatives to be indicated by button press (0–4). Choices were based on abstract stimuli and rules, with no feedback provided.

RESULTS: As expected both groups showed nearly errorless task performance and increasing RTs with increasing number of choice alternatives (0–4). This effect was paralleled by a parametrically increasing recruitment of a bilateral parieto-premotor-prefrontal cortical network and the cerebellum in all subjects. However, we did not observe any group differences applying a $P=0.05$ threshold with correction for multiple comparisons in SPM8. Analoguously, patients with OCD did not differ from controls regarding error rate or RT.

CONCLUSIONS: Using a "purified" action selection paradigm we suggest that forming and executing simple decisions without switching contingencies and without delivering reward or punishment is unimpaired in OCD, both in behavioural and neural terms. Further studies will need to determine which additional task requirements associated with action selection may be responsible for observable deficits.

P12.19

AGE-RELATED EFFECTS OF $5HT_{1A}$ RECEPTORS ACTIVATION ON SEROTON

Maria Krotewicz[1], Magdalena Strzelczuk[1], Maria K. Krotewicz[2]

[1]Department of Neurobiology, University of Łódź, Łódź, Poland; [2]Independent Public Healthcare Centre in Garwolin, Garwolin, Poland

BACKGROUND AND AIMS: Serotonin (5-HT) and its receptors are engaged in the regulation of the different brain functions. Pharmacological agents modifying central serotonin activity are effective in the treatment of brain dysfunctions. It has been also postulated that 5-HT is involved in the process of brain aging. The present study was designed to examine age-related effects of peripheral administration of 5-HT_{1A} agonist, 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT), on serotonergic system activity in the brain of rats exposed to stressogenic conditions of transition test.

METHODS: Regional brain concentration of 5-HT and 5-hydroxyindoleacetic acid of mature (12 months old) and aged (24 months old) rats exposed to transition test were examined using high performance liquid chromatography with electrochemical detection after ip administration of 8-OH-DPAT. Serotonin turnover was measured by 5-HIAA/5-HT ratio.

RESULTS: We observed anxiolytic influence of 8-OH-DPAT administration in rats of both age (mature and aged). Peripheral administration of 8-OH-DPAT significantly decreased serotonin concentration in the brain regions of emotional-defensive system in mature rats. The serotonin turnover index was lower in the all studied brain regions of 12 months old animals. The results obtained for 24 months old rats were different. The administration of 8-OH-DPAT induced elevation of serotonin concentration while the analyzed turnover index was lower in the most studied brain regions of aged rats.

CONCLUSIONS: The 5-HT_{1A} receptors are postulated to be engaged in the emotional mechanisms, and above all in the fear/

anxiety mechanisms. The present study confirmed that hypothesis. Comparison of the behavioral data showed similar, independent of age, anxiolytic effect caused by administration of 5-HT_{1A} receptor agonist. Simultaneously, the obtained data revealed age-related neurochemical differences after 5-HT_{1A} receptors activation.

P12.20

CHRONIC FLUOTEXINE TREATMENT DISRUPTS APETITIVELY MOTIVATED LEARNING AND CENTRAL AMYGDALA STRUCTURAL PLASTICITY

Alicja Puścian, Szymon Łęski, Maciej Winiarski, Łukasz Charzewski, Evgeni Nikolaev, Magdalena Dziembowska, Ewelina Knapska

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

AIMS: Fluoxetine, a selective serotonine reuptake inhibitor, is commonly used to treat psychiatric disorders. Available data show that fluoxetine has limited side effects and, more importantly, may improve patient's cognitive abilities. However, little is known about the mechanisms by which fluoxetine affects learning, especially appetitively motivated one. Thus, in the present project we investigated the effects of a long-term fluoxetine treatment on appetitively motivated discrimination learning.
METHODS: We used fully automated behavioral assessment of discrimination learning in group-housed subjects, DI-staining for determining changes in morphology of dendritic spines and gel zymography for measurement of activity of MMP-9 (matrix metaloproteinase 9, an enzyme involved in synaptic plasticity).
RESULTS: We showed that above-described learning is severely impaired in mice subjected to the long-term fluoxetine treatment. Since we have previously shown that such learning depends on MMP-9 activity in the central amygdala (CeA), we examined MMP-9 activity in the CeA of the fluoxetine treated mice. We found decreased MMP-9 level. Further, we tested fluoxetine influence on dendritic spine morphology in the CeA and observed that behavioral performance of the control wild type mice was highly correlated with a size and of mature, mushroom-shaped dendritic spines. No such correlation was found in MMP-9 knock out mice. Applied treatment abolished this correlation in wild type mice and did not reinstated it to a significant level in MMP-9 knock outs.
CONCLUSIONS: Obtained results show that chronic fluoxetine treatment impairs appetitive discrimination learning in healthy controls, decreases MMP-9 activity and disrupts correlation between subjects' performance in appetitive learning and structural synaptic plasticity in the CeA. The data shed light on dendritic spines' dependent learning mechanisms, that may be disarrayed in the CeA by commonly applied fluoxetine treatment in patients.

POSTER SESSION P13. VISUAL SYSTEM

P13.1

SPECVIS: FREE AND OPEN-SOURCE SOFTWARE FOR VISUAL FIELD EXAMINATION

Piotr Dzwiniel[1], Mateusz Gola[2], Anna Wójcik-Gryciuk[3], Wioletta Waleszczyk[1]

[1]Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland; [2]Institute of Psychology, Polish Academy of Sciences, Warsaw, Poland; [3]Central Clinical Hospital MSW, Warsaw, Poland

BACKGROUND AND AIMS: The main purpose of our work is to create a reliable, free and open-source software for visual field diagnosis, which is mainly designed for the use with personal computers. Our goal is argued by the fact, that professional clinical equipment used for the visual field estimation is very expensive and mostly occur only in specialized medical centers. This, in turn, affects its availability, giving rise to a problem not only for patients requiring frequent and regular testing of the visual field, but also for those, who would like to examine their sight purely preventive.
METHODS: To date, the initial version of the application called Specvis was established. It has been satisfactorily tested so far in a brief study on sample of 20 healthy volunteers, where half of them had simulated deficits of the left visual field. The group with „deficits" used specially prepared glasses with covered left and partially right eye, where in contrast „control" group had covered only left eye. Experimental procedure consisted of responding to stimuli appearing in different parts of the screen with simultaneous monitoring of fixation by responding to changes of the centrally located fixation point.
RESULTS: The use of specially prepared glasses resulted in the observation of deficits in the left part of visual field in the „deficits" group. Statistical analyzes revealed the existence of differences between both groups in the left part of the visual field.
CONCLUSIONS: The brief validation study had shown, that Specvis has the potential worth developing further. Actually our software is being validated in clinical conditions with automated perimetry in the study of visual field deficits of glaucomatous patients. It is worth mentioning, that thanks to wide configuration settings of the stimulus characteristics Specvis is also useful in animal studies.
Our work is supported by ERA-NET Neuron grant REVIS.

P13.2

CHARACTERIZATION OF LIGHT-SENSITIVE NEURONS WITHIN THE DORSAL LATERAL GENICULATE NUCLEUS (DLGN) OF URETHANE-ANAESTHETIZED LONG EVANS RATS – *IN VIVO* STUDY

Jagoda Jęczmień[1], Patrycja Orłowska-Feuer[1,2], Marian Henryk Lewandowski [1]

[1]Department of Neurophysiology and Chronobiology, Institute of Zoology, Jagiellonian University, Kraków, Poland; [2]The Malopolska Centre of Biotechnology, Jagiellonian University, Kraków, Poland

BACKGROUND AND AIMS: The dorsal lateral geniculate nucleus (dLGN) is a relay station for the transmission of visual information to the cerebral cortex. It receives input from all three types of photoreceptors: rods, cones and melanopsin cells. Electrophysiological studies on mice have shown that eye illumination induces three types of neuronal responses within the dLGN: sustained, transient ON and OFF. The purpose of the present study was to verify, if similar types of light-induced responses occur in the dLGN of pigmented rat and whether they are associated with the specific firing pattern of neurons (bursting, tonic, oscillatory). The second goal was to determine if these neurons are modulated by sleep-like cycle (cortical activation/deactivation) visible in the EEG signal.
METHODS: We performed *in vivo* extracellular single-unit neuronal recordings from the dLGN of urethane-anaesthetized Long Evans rats in combination with EEG recordings and light stimulations with different intensities.
RESULTS: We observed excitation or suppression of dLGN cells upon the eye illumination. The most common response (63%) was a transient ON, and only sustained cells support irradiance-dependent increases in the firing rate of dLGN neurons. Furthermore, the type of light-induced responses was independent of the neuronal firing pattern. The activity of 44% of recorded neurons was reflected by fluctuations of the EEG signal (reduced activity during NREM-like sleep).
CONCLUSIONS: All three types of light-induced neuronal responses were present within the dLGN of pigmented rat. Only sustained cells were able to code light intensity, what may suggest, that they received input from melanopsin cells, like it was observed in mice. Moreover, the obtained results showed that rhythmic changes in the EEG signal reflects alterations in the mean firing rate of recorded cells, what indicating the influence of state-dependent changes in CNS activity on sensitive to light dLGN neurons.
This study was supported by grant 2013/08/W/N23/00700.

P13.3

UNCOUPLING OF RETINAL GAP JUNCTIONS DEPRESSES LIGHT SIGNAL TRANSDUCTION TO THE RAT OLIVARY PRETECTAL NUCLEUS (OPN)

Patrycja Orłowska-Feuer[1,2], Jagoda Jęczmień[2], Marian Henryk Lewandowski[2]

[1]The Malopolska Centre of Biotechnology, Jagiellonian University, [2]Department of Neurophysiology and Chronobiology, Institute of Zoology, Jagiellonian University, Kraków, Poland

BACKGROUND AND AIMS: The olivary pretectal nucleus (OPN) is a midbrain structure well-known for the pupillary light reflex regulation. It receives strong retinal innervation from all photoreceptors types, however the main projection is from melanopsin cells. A subpopulation of OPN neurons discharges action potentials in an oscillatory manner with the period of minutes. This rhythmic firing pattern depends on the retinal input which deactivation results in neuronal rhythm abolition. Interestingly, all photoreceptors are required for the generation of oscillations in the OPN, but their engagement in driving the rhythm is determined by the lighting conditions, thus their selective activation. The aim of the present study was to verify the role of retinal gap junctions in the generation of oscillations in the OPN and in light signal transduction between the retina and the OPN.
METHODS: We performed extracellular single-unit recordings in anaesthetized rats combined with the intravitreal injection of non-specific gap junctions blocker – carbenoxolone (CBX; 5 µL; 1, 5, 20 mM) and light stimulations (10 and 160 lux) presented before and after the injection.
RESULTS: Dose-dependent effects of intravitreal injections of CBX on the oscillatory rhythm in the OPN were observed, with the highest dose (20 mM) being the most effective in abolishing the rhythm. The effect was temporary, and partial recovery of oscillations was observed after 41.45±6.84 min. Moreover, such retinal desynchronization transiently depresses the sensitivity of OPN neurons to weak light stimulation (10 lux) – the responses decreased up to 70% just after the injection and gradually recovered to reach 50% of the baseline response 70 min after the injection.
CONCLUSIONS: It implies that affecting retina gap junctions coupling influences rhythmic pattern of spikes generation in the OPN and at least partially disrupts light signal transmission, so one of five existing photoreceptors pathways to the higher visual brain centers.

P13.4

SENSORY EXPERIENCE INFLUENCES MAGNITUDE OF RESPONSES IN THE RAT VISUAL SYSTEM

Katarzyna Żeber, Andrzej Foik, Paulina Urban, Agnieszka Porowska, Wioletta J. Waleszczyk

Neurobiology of Vision Laboratory, Department of Neurophysiology, Nencki Institute of Experimental Biology, PAS, Warsaw, Poland

BACKGROUND AND AIMS: The aim of this study was to evoke plasticity in chosen structures of the rat visual system by visual training (sensory experience).
METHODS: Local field potentials (LFPs) were recorded from the rat superior colliculus (SC) and primary visual cortex (VCx) of contralateral hemisphere to stimulated eye. LFPs were collected before, during and after visual training. The training consisted of series of 300 repetitions of light flashes separated by 2–3 s inter-

vals. The series of stimuli were presented to the one eye every 15 minutes through three hours.
RESULTS: Our data show that visual stimulation significantly enhanced magnitude of visual responses in both recorded structures. A significant increase of visual responses occurred after first hour of training (four stimulating series) both in SC and VCx. The largest increase of VEP amplitudes in the SC was observed after the third hour of stimulation and that was significantly different compared to the first and the second hour of training. Regarding the VCx, advanced alterations of VEPs were observed already after the first hour and then the amplitudes of cortical VEPs remained at a similar level to the end of training. To examine whether the above changes did not result from the changes in the level of anesthesia and global brain state, we considered the VCx LFP power ratio in delta (1–4 Hz) and beta (13–30 Hz) frequency range of the signal recorded for 30 s before each series of stimulation. Changes in the course of delta/beta ratio were similar for all channels in VCx during three hours of visual training and didn't correlate with increase of VEPs.
CONCLUSIONS: Repetitive visual stimulation enhance responses in the visual system, both at cortical and subcortical level, independently of the global brain state, thus may constitute a fundamental approach to improve visual functions.
Supported by ERA-NET Neuron project REVIS.

P13.5

FOCAL CORTICAL STROKE IN THE CAT VISUAL CORTEX

Marcelina Szczerba[1], Paulina Urban[2], Andrzej Foik[3], Ewa Kublik[3], Anaida Ghazaryan[3], Joanna Borowska[4], Maria Sadowska[4], Jan Jabłonka[5], Wioletta Waleszczyk[3]
[1]Faculty of Biology, [2]Faculty of Physics, University of Warsaw, Warsaw, Poland; [3]Nencki Institute of Experimental Biology, Polish Academy of Sciences Warsaw, Poland, Warsaw, Poland; [4]College of Inter-Faculty Individual Studies in Mathematics and Natural Sciences, [5]Laboratory of Animal Physiology, Faculty of Biology, University of Warsaw, Warsaw, Poland

BACKGROUNS AND AIMS: The study develops methodology of photothrombotic stroke (PtS) induction in cats visual cortex for future studies of post-stroke visual recovery. Considering its well known organisation and similarity to the human, cat visual system is a good model for spontaneous and supported brain reorganisation after ischemia.
METHODS: Photothrombosis was used as a model of focal ischemic cortical stroke. Standardisation of the methodology of cortical PtS was performed on four experimental cats with parallel local field potential (LFP) recordings in and around the stroke core – before, during and after the infarct. Intravenously injected Bengal Rose was locally irradiated by cold light *via* an optic bundle placed on the skull, thinned skull or directly on dura surface. Different light source parameters and irradiation time were tested. Postoperatively isolated brains were preserved and frozen, cut and stained. Final position of the electrodes was monitored on dried 50 μm slices. The cytochrome oxidase (CO) activity and Nissl staining were used to monitor the state of the tissue injury.
RESULTS: The aimed unilateral stroke was performed in the dorsal zone of the left marginal gyrus over the visual cortex on a border of the cortical areas 17 and 18. CO visualizes the areas of lower mitochondrial activity in the illuminated tissue of the irradiated cortex in comparison to the contralateral intact homotopic areas. The spontaneous LFP dynamic decreased for at least three hours within the irradiated cortex but not in opposite hemisphere or surrounding tissue.
CONCLUSIONS: The 25 minutes of the unilateral irradiation directly to the dura surface with the light temperature 2750K resulted in the most accurate lesion covering all the width of the marginal gyrus and partially the sulcus area not spreading on adjacent gyri or further blood vessels. The infarct reaches the white matter without its pronounced injury.
Supported by:ERA-NET:REVIS&MNiSW0292/IP1/2013/72.

P13.6

THE INFLUENCE OF ASSOCIATIVE PAIRING OF VISUAL STIMULATION AND TAIL SHOCK ON SOMATOSTATIN EXPRESSION IN MOUSE PRIMARY VISUAL CORTEX

Ida Raciborska, Jan Popiołkiewicz, Wioletta J. Waleszczyk
Nencki Institute of Experimetal Biology, Polish Academy of Sciences, Warsaw, Poland

BACKGROUND AND AIMS: Previous study showed that associative pairing involving monocular visual stimulation resulted in a significant increase of the density of cells expressing glutamic acid decarboxylase (GAD), γ-aminobutyric acid (GABA) synthesizing enzyme, in mouse primary visual cortex contralateral to the stimulated eye. The effect was attributed to the group of parvalbumin-(PV)-negative interneurons, since the density of PV-positive cells remained unaffected. The aim of the present study is to identify the group(s) of PV-negative GABA-ergic cells in mouse visual cortex that are mobilized by visual training combined with tail shock.
METHODS: The same method of associative pairing (classical conditioning) involving monocular visual stimulation was used. We used nine young adult male mice, which were divided into two experimental groups: trained (5 mice) and naïve (4 mice) that served as a control. During three days lasting training, monocularly presented visual stimulus, drifting gratings of optimal spatial and temporal frequency (conditioned stimulus, CS), was coupled with electric shock applied to the tail (unconditioned stimulus, UCS) at

the end of visual stimulation. Twenty four hours after the training (CS+UCS), mouse brains were subject to immunohistochemistry. The brain slices were photographed through fluorescent microscope and analyzed quantitatively to determine the amount of fluorescence using Fiji ImageJ software.
RESULTS: Monocular visual training combined with tail shock resulted in a significant increase of GAD- and SOM-positive puncta in the contralateral primary visual cortex of (CS+UCS) mice in comparison to naive group.
CONCLUSIONS: Our results confirm earlier finding of the upregulation of cortical GABA-ergic system by classical conditioning involving sensory stimulation and indicate on engagement of SOM-positive interneurons.

P13.7

FREQUENCY SPECIFIC CHANGES IN SIGNAL POWER AND FUNCTIONAL CONNECTIVITY FOLLOWING STROKE IN THE CAT VISUAL CORTEX

Paulina Urban[1], Andrzej Foik[1], Anaida Ghazaryan[1], Anna Popek[1], Jan Jabłonka[3], Ewa Kublik[2], Maciek Kamiński[4], Rafał Kuś[4], Jarosław Żygierewicz[4], Wioletta Waleszczyk[1]
[1]Neurobiology of Vision Laboratory, [2]Laboratory of Visual System, Department of Neuropgysiology, Nencki Institute of Experimetal Biology, PAS, Warsaw, Poland; [3]Laboratory of Animal Physiology, Faculty of Biology, University of Warsaw, [4]Laboratory of Medical Physics, Institute of Experimental Physics, University of Warsaw, Warsaw, Poland

AIM: The aim of the study was to characterize early reorganization of cortical electrical activity within and around the stroke-affected area.
METHODS: Photothrombotic stroke was induced in the visual cortex during the acute experiments in anaesthetized cats. The activity of neuronal populations (local field potential, LFP) were continuously monitored in the central region of the stroke, at the stroke border, and in the healthy tissue, up to three hours after stroke. In the off-line analysis, using Welch and autoregressive parametric methods, we evaluated the changes in the frequency spectrum spanning from delta to gamma. Functional connectivity between cortical locations within and outside the stroke region was determined with Directed Transfer Function (DTF). Indirect and direct interactions in different frequency bands were determined by DTF and direct DTF, respectively.
RESULTS: The stroke resulted in an overall decrease of the power within full frequency spectrum in the stroke affected region, but not outside this region, where an increase in the spectral power was observed. The most pronounced changes were observed three hours after the stroke. In one cat, we observed increase of the power in the stroke area in low frequency bands while the power in beta-gamma band was diminished. DTF and direct DTF revealed weakening of neuronal connections between the healthy tissue and the stroke region and a transient strengthening of local connections outside the stroke region. The earliest decrease in the strength of connections in stroke affected region was observed in high frequencies (beta and gamma).
CONCLUSION: Stroke induce diverse effects in different frequency bands in both the LFP power spectrum and in the functional connectivity indicating complex influence on the neuronal activity within the stroke and in the vicinity of ischemic region.
Supported by ERA-NET Neuron project REVIS

POSTER SESSION P14. NEURODEGENERATION AND PROTECTION

P14.1

MEMANTINE AND MEMANTINE COMBINED WITH HH OR HBO DECREASES APOPTOSIS AND AFFECTS EXPRESSION OF BCL-2, BAX AND HIF1A IN BRAINS OF 7 DAYS OLD RATS IN EXPERIMENTAL HYPOXIA-ISCHEMIA

Marcin Gamdzyk, Apolonia Ziembowicz, Elżbieta Salińska
Department of Neurochemistry, Mossakowski Medical Research Centre, Polish Academy of Sciences, Warsaw, Poland, Warsaw, Poland

BACKGROUND AND AIMS: Perinatal hypoxia ischemia (HI) is a frequent cause of neonatal brain injury. The aim of present study was to investigate the effect of combining HBO or HH with memantine on HI evoked apoptosis and on Bcl-2, Bax and HIF-1α expression in hippocampus and cerebral cortex of the brains of neonatal rats.
METHODS: HI on 7-day old rats was induced by ligation of ipsilateral common carotid artery, followed by 75 min hypoxia. HBO (2.5 ATA) or HH (0.5 ATA) were applied 1 or 6 h after H-I for 60 min. Memantine in dose of 20 mg/kg of body weight was applied 15 minutes before HBO or HH. These treatments were repeated for 3 following days. Expression of Bcl-2, Bax and HIF-1α was examined using western blotting.
RESULTS: In our study we showed that memantine and combined therapies reduced number of apoptotic cells in brains of treated animals. Memantine applied 1 or 6 h after HI increased Bcl-2 expression in the ipsilateral hemispheres by, respectively, 41 and 9%. Memantine combined with HBO 1 or 6 h after HI upregulated Bcl-2 by 13 and 21%, respectively, and memantine combined with HH postconditioning upregulated it by 42 and 29%. The preliminary results show that both memantine alone and combined therapies changed Bax expression in ipsilateral brain hemisphere comparing to untreated HI. Our study showed also that memantine and memantine combined with HBO or HH affected expression of HIF-1α which controls several genes that play a key role in neuroprotective processes.
CONCLUSIONS: Our results show that application of memantine alone and in combination with HBO or HH reduce apoptotic pro-

cesses initiated by HI in developing brain. However, the neuroprotection achieved by combined therapies is not significantly bigger than that resulted from memantine alone.
This project was funded by the National Science Centre based on decision nr DEC 2012/07/N/NZ4/02072.

P14.2

THE NEW TOOL FOR PRECISE TRANSECTION OF PERIPHERAL NERVES AND SOFT TISSUES – BLADE WITH GRADED BIOACTIVE SURFACE

Joanna Lewin-Kowalik[1], Wiesław Marcol[1], Jan Miodoński[2], Bogusław Rajchel[3], Elżbieta Pyza[4], Adam Własczczuk[1], Magdalena Larysz-Brysz[1]

[1]Department of Physiology, Medical University of Silesia, School of Medicine in Katowice, Katowice, Poland; [2]Specialist Hospital No. 2, Department of Neurosurgery, Jastrzębie-Zdrój, Poland; [3]Department of Material Science, Institute of Nuclear Physics, Polish Academy of Sciences, Kraków, Poland; [4]Department of Biology and Cell Imaging Jagiellonian University, Kraków, Poland

BACKGROUND AND AIMS: Current methods of peripheral nerve cutting are highly unsatisfactory. Imperfect cutting plane directly influences the effect of nerve anastomosis. For precise rejoining of cut nerve stumps directly, or to bridge large gap with autologous nerve graft, the surfaces of nerve stumps must be even and perfectly matching. Actual methods still do not provide such undisturbed pathway for regenerating fibers. In this study, we examined the *in situ* morphological properties of peripheral nerves cut with an innovative device.
METHODS: Rat and rabbit sciatic nerves were cut with the carbon-metal coated blade with bioactive surface mounted on the high-speed dental drill. Transected nerves were subjected to immediate analysis of the plane surface. It was performed using the scanning electron microscope, and standard H-E as well as Masson-trichrome stainings of the serial transverse sections. Analysis was performed by two experienced certified histopathologists.
RESULTS: The procedure provided a section area devoid of unevenness or shreds of epineurium or perineurium. Moreover, individual axons seemed to be cut smoothly. The scanning electron microscope images showed the transected axons arranged paralelly to the main axis as well as other undisturbed components of the nerve (i.e. blood vessels and Schwann cells). Also, there was no thermal damage of the cutting plane.
CONCLUSION: This technique holds a promise for the development of a minimally invasive alternative approach that utilizes already available technology and equipment, with cutting plane allowing for perfect matching of nerve stumps subjected for rejoining.

P14.3

THE INVOLVEMENT OF PURINERGIC SIGNALING IN MITOCHONDRIA DYSFUNCTION INDUCED BY ALPHA-SYNUCLEIN

Anna M. Lenkiewicz, Anna Wilkaniec, Agata Adamczyk

Mossakowski Medical Research Centre, Polish Academy of Sciences, Warsaw, Poland

BACKGROUND AND AIMS: The purinergic P2 receptors for adenosine 5'-triphosphate (ATP) have been shown to be involved in neurodegenerative disorders including Parkinson's (PD) and Alzheimer's diseases (AD). However, the mechanisms underlying the disturbances in ATP-mediated neurotransmission is not clear. Our previous studies support the idea that α-Synuclein (ASN) oligomerisation and its intercellular spreading play a pivotal role in progressive development of these neurodegenerative disorders. Therefore, the aim of our study is to examine the role of purinergic P2 receptors in extracellular ASN evoked mitochondria dysfunction and cell death.
METHODS: The experiments were performed in human SH-SY5Y neuroblastoma cells differentiated with the all-trans retinoic acid (ATRA) using immunochemical, spectrophotometrical, radiochemical and spectrofluorometrical methods.
RESULTS: Our study showed that exogenously added ASN (10 μM) induces release of ATP from SH-SY5Y cells leading to activation of P2 receptors and extracellular Ca^{2+} influx. Moreover, ASN treatment results in mitochondria dysfunction manifested by decrease of intracellular ATP level and dysregulation of mitochondria enzymes expression. All mentioned dysfunctions lead to SH-SY5Y cell death. It was demonstrated hat selective purinergic P2 receptors antagonist, PPADS (200 μM), significantly prevented ASN-evoked Ca^{2+} influx, decrease of intracellular ATP level and SH-SY5Y cell death.
CONCLUSIONS: Summarizing, ASN may exerts its toxic effect *via* purinergic P2 receptors activation leading to impairment of calcium homeostasis, mitochondria dysfunction and cell death. We suggest that the P2 signaling pathway could be a therapeutic target for ASN toxicity in neurodegenerative disorders.
Supported by NCN grants: 2013/09/D/NZ3/01359 to A.W. and 2012/05/B/NZ3/02047 to A.A.

P14.4

VALPROIC ACID BUT NOT MINOCYCLINE ALLEVIATES STRIATAL NEURON DEGENERATION IN THE RAT MODEL OF INTRACEREBRAL HEMATOMA

Katarzyna Majak, Przemysław Kowiański, Jerzy Dziewiątkowski, Sławomir Wójcik, Janusz Moryś

Department of Anatomy and Neurobiology, Medical University of Gdańsk, Gdańsk, Poland

BACKGROUND AND AIMS: The study evaluates the effect of experimentally induced intracerebral haematoma (ICH) on density of neurodegenerating neurons and volume of the rat striatum. In addition, we studied how administration of valproic acid and minocycline, the two drugs generally believed to be neuroprotective agents, influences the density of these neurons.
METHODS: 80 adult male Wistar rats were assign to one of 4 experimental groups: control, (CON), haematoma (HAEM), haematoma and minocycline (MINO) and haematoma and valproic acid (VAL). Five animals from each experimental group were sacrificed at four time points: 2 weeks, 4 weeks, 24 weeks and 48 weeks after ICH. To assess the neurodegeneration the sections were stained with FluoroJadeB (FJB). The CellSense Dimension 1.5 (Olympus, Japan) image analysis system was employed to measure the densities of FJB-labelled neurons as well as volume of the striatum. Statistical analyses were performed using two way ANOVA with replications. To find differences between groups *post-hoc* Tukey test was applied. A P-value <0.05 was considered significant.
RESULTS: Our study revealed that valproic acid treatment decreased the density of FJB-labeled degenerating neurons 4 weeks after ICH induction in the ipsilateral striatum. Minocycline did not have such effect. None of the two drugs influenced the volume of the striatum.
CONCLUSIONS: Our study revealed that valproic acid alleviates striatal neuron degeneration in the rat model of ICH. Because striatum has been implicated to play role in motor control as well as cognition and affective control, the valproic acid treatment might have beneficial effects on these processes after ICH.

P14.5

OXIDATIVE STRESS AND INFLAMMATORY RESPONSE IN RAT BRAIN AND LIVER FOLLOWING ORAL ADMINISTRATION OF SILVER NANOPARTICLES

Joanna Skalska[1], Małgorzata Frontczak-Baniewicz[2], Aleksandra Lenkiewicz[1], Lidia Strużyńska[1]

[1]Laboratory of Pathoneurochemistry, Department of Neurochemistry, [2]Electron Microscopy Platform, Mossakowski Medical Research Centre, Polish Academy of Sciences, Warsaw, Poland

BACKGROUND AND AIMS: Silver nanoparticles (AgNPs) are one of the most important class of nanomaterials used in a wide range of medical and industrial applications. However, the information about their toxicity to mammals is limited. The aim of this study was to investigate the effect of oral exposure to AgNPs on brain and liver of rats. The deposition of silver nanoparticles in these organs has been shown to induce hepatotoxicity and neurotoxicity. These toxic effects may include oxidative stress with subsequent inflammatory response.
METHODS: Wistar rats were exposed orally to AgNPs (10±4 nm in diameter) or silver ions at a dose of 0.2 mg AgNPs or Ag+/ kg bw for 14 days. Then all animals were sacrificed 24 h after last exposure and tissues were collected for further studies.
RESULTS: The presence of AgNPs in brain tissue was confirmed by using TEM technique. The level of free radicals and end-products of lipid peroxidation increased in both organs after exposure to both, silver nanoparticles or silver ions. However, the changes in inflammatory markers (based on IL-1β, IL-6 and TNF-α relative protein levels) were not statistically significant.
CONCLUSIONS: These results show that following AgNPs or silver ions administration, oxidative stress is induced in brain and liver tissues. However, it seems that the time of exposure to both of silver forms was too short to cause the inflammatory response.

P14.6

SELOL PROTECTS PC12 CELLS AGAINST SODIUM NITROPRUSSIDE-INDUCED APOPTOSIS THROUGH ACTIVATION OF SE-DEPENDENT ANTIOXIDATIVE ENZYMES

Agnieszka Dominiak[1], Anna Wilkaniec[2], Piotr Wroczyński[1], Agata Adamczyk[2]

[1]Department of Drug Bioanalysis and Analysis, Medical University of Warsaw, Warsaw, Poland; [2]Department of Cellular Signaling, Mossakowski Medical Research Centre, Polish Academy of Sciences, Warsaw, Poland

BACKGROUND AND AIMS: It has been hypothesized that Se depletion followed by decreased activity of Se-dependent enzymes may be responsible for the development of oxidative stress observed in various neurodegenerative diseases. Thus, Se-dependent protection strategy to reduce neuronal oxidative injuries, can contribute to attenuation of neurodegeneration. The compound Selol is an organic mixture of selenitetriglycerides, which was previously shown to exhibit antitumor properties. The aim of the present study was to investigate the cytoprotective effect of Selol against sodium nitroprusside (SNP) induced oxidative stress and PC12 cells death.
METHODS: The study was carried out using spectrophotometric and spectrofluorometric methods as well as real-time PCR analysis.
RESULTS: We found that treatment with SNP (0.5 mM) induced significant elevation of free radicals, decreased the reduced glutathione (GSH) level as well as increased the formation of oxidized glutathione (GSSG). In addition, the significant alteration in the activities of antioxidative enzymes, such as glutathione peroxidase (GPx), glutathione reductase (GR) and thioredoxin reductase (TrxR) were observed after SNP treatment. Selol, at low molecular range neutralized SNP-induced free radicals generation and modulated GSH homeostasis. Moreover, Selol significantly elevated the activi-

ties of GPx, GR and TrxR, thus preventing cell death evoked by SNP. The involvement of Selol on the intracellular antioxidative defence system was further confirmed by using a GPx and TrxR inhibitor, sodium aurothiomalate, that abolished its cytoprotective effect.
CONCLUSIONS: Taken together, these findings suggest that treatment with Selol protects cells from oxidative stress *via* enhancement of the intracellular antioxidant potential. The Selol's efficacy in combating free radical damage suggests that it can be a valuable therapeutic agent in the treatment of neurodegenerative disorders.
Supported from MUW FW27/PM34D/14 and MMRC statutory theme 8.

P14.7

STREPTOZOTOCIN AND DIMETHYL FUMARATE DECREASES PLASMA TUMOR NECROSIS FACTOR ALPHA CONCENTRATION IN RATS

Maria Grzybowska, Magdalena Podlacha, Irena Majkutewicz, Ewelina Kurowska, Dorota Myślińska, Beata Grembecka, Danuta Wrona

Department of Animal and Human Physiology, University of Gdańsk, Gdańsk, Poland

BACKGROUND AND AIMS: This study aims to determine if dimethyl fumarate (DMF), antioxidant having immunosuppressive properties, taken orally for three weeks will affect plasma tumor necrosis factor alpha (TNF-α) concentration in an animal model of sporadic form of Alzheimer's disease (sAD) induced by intracerebroventricular (icv) injection of betacytotoxic drug, streptozotocin (STZ).
METHODS: Blood samples from young, male Wistar rats divided into four groups: STZ DMF (subjected to icv injection of STZ, fed with 0.4% DMF fodder), VEH DMF (subjected to icv injection of vehicle, fed with 0.4% DMF fodder), STZ CTR (subjected to icv injection of STZ, fed with standard fodder), VEH CTR (subjected to icv injection of vehicle, fed with standard fodder) were taken one hour after Morris water maze test finishing. Then, TNF-α concentration was determined with ELISA method using a Rat TNF-α ELISA Kit.
RESULTS: Injections of STZ in rats being on the control feed (STZ/CTR) significantly decreased ($P \leq 0.05$) the plasma concentration of TNF-α (22±2 pg/ml; mean±SE) as compared to the controls (VEH/CTR: 33±3 pg/ml). Moreover, within the STZ/DMF group, a significant ($P \leq 0.01$) decrease in the concentration of TNF-α (22±0.8 pg/ml) as compared to the controls (VEH/DMF: 30±2 pg/ml), was observed.
CONCLUSION: The obtained results indicate that streptozotocin injection and feeding with dimethyl fumarate of the streptozotocin-induced sAD rats reduce such a peripheral blood pro-inflammatory cytokine level as TNF-α

P14.8

METABOTROPIC GLUTAMATE RECEPTORS GROUP II (MGLUR2/3) AGONISTS EXERT NEUROPROTECTION BY REDUCING APOPTOSIS AFTER HYPOXIC-ISCHEMIC PRECONDITIONING

Ewelina Bratek, Apolonia Ziembowicz, Elżbieta Salińska

Department of Neurochemistry, Mossakowski Medical Research Centre, Polish Academy of Sciences, Warsaw, Poland

BACKGROUND AND AIMS: Perinatal asphyxia is characterized by clinical and laboratory evidence of acute brain injury due to asphyxia. It was shown that mGluR2/3 activation before or after ischemic insult results in neuroprotection but the exact mechanism of this effect is not clear. The aim of present study was to investigate whether mGluR2/3 activation after hypoxia-ischemia (HI) reduces brain damage and if the activation of antioxidant enzymes and decrease of oxidative stress.
METHODS: We used an animal model of HI on 7-day old rat pups. Animals were anesthetized and the left common carotid artery was isolated and double-ligated and then cut between the ligatures. After completion of the surgical procedure the pups were subjected to hypoxia (7.4% oxygen in nitrogen for 75 min at 35°C). Animals were injected intraperitoneal with specific mGluR2 (LY379268) and mGluR3 (NAAG) agonists 24 h or 1 h after HI. First weight deficit of HI brain hemisphere were measured and examined the expression of Bax. Next in our investigation we were used TUNEL assay and TTC1% staining.
RESULTS: Our results show a neuroprotective effect of mGluR2/3 agonists.Both agonists applied decreased brain tissue weight loss in ischemic hemisphere independently on the time of application (from 40% in HI to 15–20% in treated). In our study we show the relative changes in the expression of Bax protein in ipsilateral and contralateral hemisphere. Our results show that both mGluR2/3 antagonists applied 24 h and 1 h after HI reduced number of TUNEL-positive cells in ipsilateral hemispheres. We observed more number of TUNEL- positive cells in HI. Both mGluR2/3 agonists decreased area of ipsilateral hemisphere infraction.
CONCLUSIONS: This study is the demonstration of the neuroprotective effect of mGluR2/3 agonist on neonatal HI brain injury. These data suggest the possibility that preconditioning reduces irreversible ischemic injury in part by decreasing apoptosis.

P14.9

RAPID ACTIVATION OF CB1 RECEPTORS IN MOUSE BARREL CORTEX AFTER WHISKER-SHOCK FEAR CONDITIONING

Ewa Siucińska, Wojciech Brutkowski, Tytus Bernas

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

BACKGROUND AND AIMS: We have previously reported that whisker-shock fear conditioning produced expansion of the cortical representation of the activated vibrissae ("trained row"), this was demonstrated by labeling with 2-deoxyglucose in layer IV of the barrel cortex. Functional reorganization of the primary somatosensory cortex was accompanied by an increase in the density of small GABAergic neurons, GAT67 boutons and GAT-1 puncta in the hollows of barrels representing the trained row.

The goal of this study was to investigate how whisker-shock fear conditioning affects the expression of puncta of the cannabinoid receptor 1 (CB1), in the hollows of the trained row barrels in of the primary somatosensory cortex evaluated by immunocytochemistry 24 h after associative learning paradigm.

METHODS: The present study estimates the CB1+ puncta (Abcam 1:500) mean numerical density (Nv) in hollow of all rows barrels of the barrel cortex. In a whisker-shock and control groups precise location of layer IV cells were identified using Hoechst 33258 staining of tangential sections. A confocal microscopy stereological technique, was used in the CB1+ puncta analyses.

RESULTS: Our present data revealed increased CB1+ puncta density by approximately 58% in the hollows of barrels representing the trained row compared to the hollows in the barrel field of the opposite hemisphere in the same mouse. In contrast, density of CB1+ puncta was unchanged in the control groups, which received shock alone and naïve animals. We also observed very low density of CB1+ puncta concentrated in the hollows of the all rows barrels of the barrel cortex belonging to pseudoconditioned group of animals.

CONCLUSION: The findings suggest that CB1 receptors plays a selective active role in fear conditioning-dependent plasticity.

Funding: Scientific Research Grant 6420/B/P01/2011/40 to ES.

P14.10

THE EFFECTS OF TREHALOSE ADMINISTRATION ON AUTOPHAGY ENHANCEMENT IN MICE WITH CONDITIONAL AND PROGRESSIVE DEGENERATION OF MEDIAL SPINY NEURONS

Grzegorz Kreiner, Katarzyna Rafa-Zabłocka, Monika Bagińska, Irena Nalepa

Department of Brain Biochemistry, Institute of Pharmacology, Polish Academy of Sciences, Kraków, Poland

BACKGROUND AND AIMS: In neural cells, autophagy is proposed to serve as a surveillance mechanism which helps to clear protein aggregates, and loss of autophagy leads to neurodegeneration in mice. Our previous experiments, performed on genetically engineered mice with conditional and progressive neurodegeneration of medial spiny neurons (TIF-IAD1RCre mice) mimicking the typical progression of Huntington's disease (HD), showed that the delayed onset of neurodegeneration observed in these mutants might be associated with temporary increased autophagy. The aim of current study was to evaluate a new strategy proposed recently for the anti-HD treatment, based on enhancing autophagy by administration of trehalose, a natural alpha-linked disaccharide.

METHODS: Trehalose (2%) was dissolved in water and presented to the mice as a replacement for their water bottles for 1 or 2 months prior the experiments. The effects of trehalose were compared with groups receiving maltose (2%) as well as water (vehicle). The autophagy was determined by Western blot (WB) and immunohistochemistry (IHC) with use of anti-LC3B antibody. The animals were screened for their motor coordination by accelerated rotarod, and post-mortem for selected neurodegenerative markers by WB and IHC.

RESULTS: Both control and mutant mice showed enhanced autophagy after trehalose administration as revealed by WB and IHC staining. Nevertheless, further analysis of quantitative assessment of several neurodegenerative markers by WB did not reveal any significant effects in attenuating the neurodegenerative process. There have been also no differences in behavioral phenotype.

CONCLUSIONS: Our results provide additional evidence for stimulation of autophagy evoked by chronic administration of trehalose. However further study is needed, the enhancement of autophagy has not yet been proved to be neuroprotective in investigated model.

Supported by 2011/03/B/NZ7/05949 grant financed by National Science Center (NCN).

P14.11

INITIATION OF NEURODEGENERATIVE PROCESS DURING IMPAIRED BIOSYNTHESIS OF COA AND EXCESS INTAKE OF CARBONYL IRON

Nina Kanunnikova[1], Valery Gurinovich[2], Dzmitry Semenovich[1], Tatyana Pekhovskaya[2], Sofya Omelyanchik[2], Andrey Moiseenok[2]

[1]State University of Grodno, Grodno, Belarus; [2]Institute of Biochemistry of Biologically Active Compounds, National Academy of Sciences of Belarus, Grodno, Belarus

BACKGROUND AND AIMS: The syndrome of neuroacantocytosis, characterized by degeneration of basal ganglia, accumulation of iron in CNS structures and the presence of acantocytes in the blood, is combined with defect of the key CoA biosynthetic enzyme, pantothenate kinase (PANK). Administration of D-hopanthenic acid (HPA), a competitive inhibitor of PANK, can simulate this form of neurodegeneration when it is initiated by inducer of systemic inflammation.

METHODS: Adult male Wistar rats treated intragastrically with carbonyl iron (225 mg/kg daily over one month) were administered with HPA intraperitoneally (200 mg/kg daily over the last week) and with *E. coli* lipopolysaccharide (LPS) (500 μg/kg for 24 h).

RESULTS: It was found that the LPS caused a 3-fold decrease of blood plasma total iron in the HPA-treated group, oxidative stress was pronounced and redox-buffer erythrocyte capacity decreased. The levels of CoASH and acyl-CoA were reduced in the basal ganglia and hippocampus on intake of carbonyl iron and LPS, which was not potentiated by HPA. The LPS administration caused an increase in the level of GSH in the brain hemispheres due to activation of the glutathione reductase. In this situation, the Eh value diminished after HPA treatment. This was also observed in the hippocampus with an increase in reduced glutathione fraction. The neuroblastoma SN56 cell culture was used to show (in collaboration with the Depart. Labor Med., MU, Gdansk) that activation of CoASH biosynthesis and (or) its enhanced transport in the presence of succinate increased cell viability and biomembrane stability after exposure to rotenone due to intramitochondrial production of acetyl-CoA.
CONCLUSIONS: The CoA-dependent mechanism of production of the acetyl-CoA can be a key one in formation of intracellular redox-potential (Fisher-Wellman et al. 2015, Biochem J) that can potentiate development of neurodegeneration *via* the erythron signal function and iron deposition.

P14.12

DYNAMICS OF DEVELOPMENT AND MORPHOLOGY OF REACTIVE ASTROGLIOSIS IN RESPONSE TO ONE HOUR TRANSIENT CEREBRAL ISCHEMIA IN THE RAT – IMMUNOHISTOCHEMICAL STUDIES

Aleksandra Steliga[1], Grażyna Lietzau[2], Monika Waśkow[1], Zbigniew Karwacki[3], Sławomir Wójcik[2], Waldemar Sienkiewicz[4], Janusz Moryś[2], Przemysław Kowiański[1,2]
[1]Department of Health Sciences, Pomeranian University in Słupsk, Poland; [2]Department of Anatomy and Neurobiology, [3]Department of Neuroanesthesiology, Medical University of Gdańsk, Poland; [4]Department of Animal Anatomy, Faculty of Veterinary Medicine, University of Warmia and Mazury, Olsztyn, Poland

BACKGROUND AND AIM: Transient focal cerebral ischemia is stimulus triggering reactive astroglial response characterized as the cellular proliferation and restoring of differentiation abilities necessary for reduction of the consequences of primary brain lesion and separation of ischemia. Growing evidence of data point to specific, context-dependent character of this response dependent from the pathological stimuli, intensity of lesion and time-lapse from the beginning of the pathological process. In our study we aimed to assess the potential for proliferation and differentiation of reactive astroglia after transient focal brain ischemia in the rat.
METHODS: Transient focal cerebral ischemia was evoked in 20 adult Wistar rats by placement of the surgical thread into the carotid artery and occlusion of the middle cerebral artery for 1 h. The postoperative survival period was up to 6 weeks. Immunocytochemical double-stainings for glial fibrillary acidic protein (GFAP), with proliferative (Ki-67 and Pax6,) and characteristic for various stages of gliogenesis (nestin and S100beta protein) markers were performed, with subsequent confocal microscopic study.
RESULTS: The apparent differences in the pattern of colocalization within GFAP-immunorecative (ir) astrocytes were observed in the survival period. The intensity of double-labeling for the studied markers increased gradually after 24 h after initiating the ischemia. Intense hypertrophic reaction and increased concentration of GFAP-ir fibers was observed especially after two weeks of reperfusion.
CONCLUSION: The astroglial reaction in the course of reperfusion indicate regaining of the morphological features, characteristic for the earlier developmental stages, as well as the development of proliferative capacities. The considerable potential of astroglial proliferative response even after short-lasting transient cerebral ischemia must be taken into account in the future studies of cerebral infarcts.

P14.13

MINOCYCLINE ADMINISTRATION PROTECTS TIGHT JUNCTION PROTEINS FROM DEGRADATION AFTER PRE-CHIASMATIC SUBARACHNOID HEMORRHAGE IN RATS

Daria Gendosz, Kamila Dębska, Halina Jędrzejowska-Szypułka, Joanna Lewin-Kowalik
Medical University of Silesia, School of Medicine, Chair and Department of Physiology, Katowice, Poland

BACKGROUND AND AIMS: Subarachnoid hemorrhage has complex, multisystem and multifaceted pathogenesis that involves several ongoing pathological processes, including BBB degradation. Our aim was to survey potential protective properties of minocycline on Tight Junctions (TJ) proteins in rat brain, after experimentally induced pre-chiasmatic SAH (pSAH).
METHODS: pSAH was induced by injection of 200 μL of fresh autologous arterial blood into pre-chiasmatic cistern in rat brain. Minocycline was administrated ip twice, at 1st and 10th h after surgery (dose: 30 mg/kg). 24 h following the surgery, animals were perfused transcardialy and whole brains were collected. In order to investigate immuno-localization of TJ proteins, coronal sections were immuno-stained against Zonulin-1, Occludin and Claudin-5. ZO-1, OCN, CLN-5 proteins levels were examined by WB reactions.
RESULTS: We observed numerous blood vessels around the site of blood application as well in more distant areas, where we found essential alterations in immunostaining patterns of ZO-1, OCN and CLN-5, comparing to controls. Minocycline administration preserves physiological ZO-1 and OCN cellular localization comparing to SAH group. Subsequently we provided WB reactions to de-

fine SAH impact on TJ protein level. We observed that SAH leads to significant decrease in both OCN and ZO-1 protein level in first 24 h after ictus. Important message comes from the minocycline experiment. We found out significant increase of ZO-1 level comparing to SAH group. Though OCN doesn't reach significance, we can observe some positive trend in minocycline group.
CONCLUSIONS: Administration of arterial blood directly to prechiasmatic cistern leads to serious affections of TJ integrity during first 24 h after pSAH. Minocycline protects TJ proteins from degradation and also preserves TJ unit from morphological alterations at the level of brain vessel endothelium.

POSTER SESSION P15. ELECTROPHYSIOLOGY

P15.1
ANOMALOUS DECAY OF POWER OF HIGH FREQUENCY OSCILLATIONS (HFO) WITH DISTANCE FROM THE SOURCE

Tomasz Górski, Mark Hunt, Stefan Kasicki, Daniel Wójcik
Nencki Institute of Experimental Biology, Polish Academy of Science, Warsaw, Poland

BACKGROUND AND AIMS: Brain electric potentials recorded extracellularly are generated by transmembrane currents leaving and entering active neurons. Electric charge conservation requires that these currents are balanced in every cell separately, which implies a dipolar structure of sources. We previously reported results suggesting a monopolar structure of high-frequency oscillations (135–165 Hz) generated in nucleus accumbens (NAc) which seems to be inconsistent with known physics. The goal of this study is to find out through numerical simulations if the specific morphology and spatial distribution of accumbal neurons can shed light on this paradox.
METHODS: Multi-compartment models of medium spiny neuron (MSN) from NAc (based on ModelDB, accession nr 112834) were used for simulations in the NEURON simulator. We computed transmembrane activity of a population of MSNs in response to different oscillating stimuli. The sum of extracellular potentials generated by individual cells in selected points in space simulated the local field potential. Potentials were filtered in different bands to study the decay of the power with distance from the source.
RESULTS: We show that the observed increased power in the HFO band can be explained by coherent spiking of population of MSNs. In all the studied frequency bands and in all directions we observe asymptotic decay with distance close to what is expected from dipolar sources (1/r^2). However, over short spatial scales, the scaling does get closer to monopolar decay (1/r). These results are compared with reevaluated decay data from Hunt et al. (2010).
CONCLUSIONS: Since the numerical results are consistent with the experimental results it seems that the specific morphology and distribution of MSNs within NAc are sufficient to explain the observed anomalous decay of HFO power over intermediate distances. There is no need for consideration of additional mechanisms, for example, capacitive effects of the extracellular space.

P15.2
MODELLING STIMULATION ARTIFACT ON LOCAL FIELD POTENTIAL RECORDINGS FROM MULTI-ELECTRODE ARRAYS

Michał Czerwiński[1], Marinka Brouwer[2], Dirk Schubert[2], Daniel K. Wójcik[1]
[1]Department of Neurophysiology, Laboratory of Neuroinformatics, Nencki Institute of Experimental Biology Polish Academy of Sciences, Warsaw, Poland; [2]Donders Institute for Brain, Cognition and Behaviour, Centre for Neuroscience, Department of Cognitive Neuroscience, Radboud University Medical Centre, Nijmegen, The Netherlands

BACKGROUND AND AIMS: A common paradigm in electrophysiology is a study of responses of neural tissue to voltage or current stimulation. Even a short stimulation can elicit artifacts lasting for tens of milliseconds after stimulation, of amplitude comparable to the responses to be measured. The ability to automatically detect and remove stimulation artifacts from physiological recordings would improve the reliability of biological conclusions obtained in the experiments. In this work we show how to assess and subtract such artifacts from multi-electrode array (MEA) recordings.
METHODS: In acute brain slice preparations of the rat somatosensory cortex we investigated *in-vitro* evoked extracellular responses using 60-channel MEAs (Multichannel Systems). We applied voltage stimulations at different locations under artificial cerebro spinal fluid (ACSF) (a) without tissue, (b) with tissue and (c) with tissue after application of sodium channel blocker (TTX).
RESULTS: We have considered several models of artifact dependence on the distance from the stimulating electrode and on time from the onset of stimulation. We found that the best model for prediction of the artifact on every electrode for physiological recordings is its value recorded on slices after TTX application. The slice need not be the same. A proxy from recordings with just the ACSF can be used to construct a still acceptable model. Using Independent Component Analysis and Current Source Density reconstruction we investigate the structure of the artifact and example physiological responses.
CONCLUSION: With the help of extracellular recordings of slices after application of TTX it is possible to reliably estimate stimulation artifacts interfering with physiological responses and thus improving the quality and precision of data obtained in such experiments.

P15.3

CORRELATION BETWEEN ACTIVITY PATTERN OF MIDBRAIN DOPAMINERGIC NEURONS AND SPONTANEOUS BRAIN STATE ALTERNATIONS IN URETHANE ANAESTHETISED RATS

Magdalena Walczak, Tomasz Błasiak

Department of Neurophysiology and Chronobiology, Institute of Zoology, Jagiellonian University, Kraków, Poland

BACKGROUND AND AIMS: Dopaminergic midbrain neurons are able to generate two distinct patterns of electrical activity: tonic and bursting. The latter one is suggested to be essential for phasic dopamine release in target structures. It has been previously found that DA-like neurons change their pattern of activity during sleep, with prominent bursting during REM and tonic firing during nonREM phase. Since urethane anaesthesia is postulated to be a model of cyclic sleep-like alternations of the brain state, we have performed experiments aimed to correlate changes in the firing pattern of midbrain DA neurons with changes of the brain state.

METHODS: We have performed extracellular *in vivo* recordings of midbrain DA neurons activity and simultaneous electrocorticographic monitoring of the brain state in urethane anesthetised Wistar rats.

RESULTS: Obtained results showed that the activity pattern of putatively DA neurons in the ventral tegmental area subregions (VTA) and substantia nigra pars compacta (SNc) strongly correlates with the cyclic changes in the brain state. This relationship was opposite to the one observed during natural sleep. Tonic firing pattern was dominating during cortical activation (REM-like state) whereas bursting was observed mainly during cortical deactivation (NonREM-like state). Magnitude of this phenomenon was strongly correlated with the anatomical localisation of the recorded neurons within the VTA subregions (PBP, PIF, PN).

CONCLUSIONS: Our results confirm that activity of midbrain DA neurons is correlated with alternating states of the brain and shows opposite correlation to the one observed in freely moving animals. They emphasize that the influence of anaesthetic drugs should be taken under consideration during the experiments on dopaminergic midbrain neurons.

P15.4

BLOCKADE OF NEURONAL ACTIVITY IN THE RAT PREFRONTAL CORTEX AFFECTS LOCAL FIELD POTENTIAL OSCILLATIONS RECORDED IN THE NUCLEUS ACCUMBENS

Karolina Nowak-Stańczyk[1], Stefan Kasicki[2]

[1]Institute of Applied Psychology, The Maria Grzegorzewska Academy of Special Education, Warsaw, Poland; [2]Laboratory of Limbic System, Nencki Institute of Experimental Biology Polish Academy of Sciences, Warsaw, Poland

BACKGROUND AND AIMS: Non-competitive NMDA receptor (NMDAR) antagonists such as dizocilpine (MK801) are widely used to model schizophrenia in animals. The disease is thought to be associated with abnormal activity of several brain areas including the medial prefrontal cortex (mPFC), hippocampus, amygdala and nucleus accumbens (NAc). Of these, the mPFC is one of the most important structures, as its efferent pathways are considered to exert a top-down regulatory control of other limbic structures. NMDAR antagonists induce abnormal neuronal activity, e.g. they enhance high frequency oscillations (HFO, 130–180 Hz) in the NAc. It is unclear whether HFO are independently generated in NAc or are modulated by an afferent excitatory transmission from e.g. the mPFC. To address this issue, we used local infusion of tetrodotoxin (TTX) or picrotoxin (PIC) to mPFC to determine whether changes in the prefrontal neurons activity influence the power and frequency of HFO recorded in the NAc.

METHODS: Male Wistar rats were bilaterally implanted with guide cannulas and electrodes in the mPFC and electrodes in the right NAc. MK801, PIC and TTX (preceded by ip injection of MK801) were infused into mPFC. In freely moving rats local field potentials were recorded in NAc and mPFC. The effect of infusions on power and frequency of HFO in the NAc was evaluated.

RESULTS: Bilateral (mPFC) administration of MK-801 produced a substantial increase in the power of HFO in the NAc. No change, in relation to control group, in the power of HFO was observed in the NAc after infusion of TTX or picrotoxin into the mPFC.

CONCLUSIONS: Generation of HFO in NAc does not depend on the activity in the mPFC. However, MK801-induced disturbance in the neuronal activity in the mPFC may cause changes in accumbal HFO, suggesting that generation of HFO may be modulated by afferent signal from mPFC.

P15.5

INTERACTION BETWEEN VOLTAGE-DEPENDENT SODIUM CHANNEL AND ITS SITE-3 LIGAND MODIFIED BY EXPOSURE TO 50 HZ ELECTROMAGNETIC FIELD

Milena Jankowska[1], Agnieszka Pawłowska-Mainville[2], Maria Stankiewicz[1], Justyna Rogalska[1], Joanna Wyszkowska[1]

[1]Faculty of Biology and Environmental Protection, Nicolaus Copernicus University, Toruń, Poland; [2]Department of First Nations Studies, University of Northern British Columbia, Prince George, Canada

BACKGROUND AND AIMS: Extremely low-frequency (50 Hz) electromagnetic field (ELF-EMF) is produced by electrical appliances and electric power transmission lines. It affects organism functions and induces among others: the discrete changes in neuronal membrane potential, the increase of calcium channel activity and intracellular concentration of Ca^{2+}. In insects, ELF-EMF causes

an increase in a level of stress hormone – the octopamine. Site-3 ligands of voltage-dependent sodium channel inhibit fast inactivation of the channel. Receptor site-3 is located in the immediate vicinity of voltage sensor of the sodium channel. The LqhαIT toxin from the scorpion *Leiurus quinquestriatus* venom is an example of a site-3 ligand. The aim of our study was to investigate whether ELF-EMF exposure affects the action of the LqhαIT toxin on an axonal membrane.

METHODS: We have observed an action of 0.7 mT ELF-EMF and LqhaIT (5×10^{-8} M) on the bioelectrical activity of an isolated nerve cord of the cockroach – *Periplaneta americana*. The tests have been conducted using extracellular electrodes, which allowed to record the total bioelectric activity from one connective and one cercal nerve. Additionally, we have performed *in-vivo* tests, during which we have estimated the impact of 0.7 mT and 7 mT ELF-EMF on the degree of the insect paralysis caused by the LqhαIT (10^{-8} M and 10^{-7} M).

RESULTS: ELF-EMF exposure induced important changes in the LqhαIT activity – much higher during synaptic transmission than on a level of "cable transmission". *In-vivo*, electromagnetic field probably accelerates the insect detoxification process because paralysis induced by the toxin was much lower after the exposure than in control.

CONCLUSION: Results of our experiments can suggest the changes in organism sensitivity to neurotoxic ligands.

P15.6

CLOZAPINE, GLYCINE AND NMDA ALL REDUCE THE FREQUENCY OF HIGH FREQUENCY OSCILLATIONS IN THE NUCLEUS ACCUMBENS OF FREELY MOVING MICE

Maciej Olszewski[1], Joanna Piasecka[1], Miles A. Whittington[2], Stefan Kasicki[1], Mark J. Hunt[1]

[1]Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland; [2]The Hull York Medical School, University of York, York, UK

BACKGROUND AND AIMS: To examine the effect of NMDA receptor antagonists and antipsychotics on high frequency oscillations (HFO, 130–180 Hz) recorded in local field potentials from the nucleus accumbens (NAc) of freely moving mice. To identify the receptors that may underlie clozapine-induced reductions in HFO frequency.

METHODS: Freely moving mice, with electrodes implanted in the NAc, received systemic injection of NMDAR antagonists (ketamine and MK801); antipsychotic compounds (clozapine and haloperidol) were administered to MK801-pretreated mice. We attempted to identify the receptors mediating clozapine-induced reductions in HFO frequency using a pharmacological agents targeting $5HT_{1A}$, $5HT_{2A}$, histamine H_3 and NMDA receptors.

RESULTS: Ketamine and MK801 dose dependently increased the power of HFO and produced small increases in their frequency. Clozapine, dose dependently reduced the frequency of HFO whereas haloperidol had little effect on HFO. Systemic injection of glycine, which has antipsychotic properties, and allosterically modulates NMDAR, reduced the frequency of HFO to values comparable after injection of clozapine. Systemic administration of NMDA produced a short-lasting reduction in MK801-enhanced HFO frequency. Other receptors known to be targets for clozapine, namely $5\text{-}HT_{2A}$, $5\text{-}HT_7$ and histamine H_3 receptors had no effect on MK801-enhanced HFO, although we did find a reduction in HFO frequency after injection of $5HT_{1A}$ agonist.

CONCLUSIONS: These results show that NMDAR antagonists and antipsychotics produce broadly similar fundamental effects on HFO in mice and rats. Stimulation of NMDAR (directly, or through the glycine site) as well as activation of $5HT_{1A}$ receptors, reduces the frequency of MK801-enhanced HFO suggesting that atypical antipsychotic drugs may alter HFO by interacting with NMDA and $5HT_{1A}$ receptors.

Project funded by the National Center of Science DEC-2011/03/B/NZ4/03053.

P15.7

INVOLVEMENT OF THE VENTRAL TEGMENTAL AREA IN THE GENERATION OF HIGH FREQUENCY OSCILLATIONS IN THE NMDAR HYPOFUNCTION MODEL OF SCHIZOPHRENIA

Joanna Piasecka[1], Maciej Olszewski[1], Miles A. Whittington[2], Stefan Kasicki[1], Mark J. Hunt[1]

[1]Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland; [2]The Hull York Medical School, University of York, York, UK

BACKGROUND AND AIMS: NMDA receptor hypofunction is widely considered to contribute to the symptoms of schizophrenia. Although the pathophysiology of this disease remains unclear the nucleus accumbens (NAc) is one of the brain regions that has been widely implicated. Abnormal high frequency oscillations (HFO, 130–180 Hz) can be recorded in the rat NAc after injection of NMDA receptor antagonists. We have shown previously that reversible inhibition of the NAc by local infusion of tetrodotoxin reduces the amplitude of MK801-enhanced HFO indicating that this oscillation is generated by the intrinsic NAc network. Afferent regions powerfully modulate the activity of NAc neurons. However, it is not known to what extent HFO in the NAc may be driven by its afferent projections (ventral hippocampus, basolateral amygdala, prefrontal cortex and the vental tegmental area).

METHODS: To address this issue, rats were implanted with electrodes in the NAc and guides targeted at these afferent sites.

RESULTS: We found that infusion of TTX to the ventral tegmental area reduced the power of MK801-enhanced HFO on the ipsilateral but not contralateral side. In contrast infusion of TTX to the prefrontal cortex or ventral hippocampus had negligible effect MK801-enhanced HFO, although TTX infusion to the amygdala was found to produce a much weaker reduction in HFO power.
CONCLUSIONS: These findings indicate that projections from the ventral tegmental area are capable of driving abnormal HFO in the NAc after injection of NMDA receptor antagonist. Further it suggests a loop involving these regions are required for the generation of HFO in rodent NAc.
Project funded by the National Center of Science DEC-2011/03/B/NZ4/03053.

P15.8

SOMATOSENSORY RESPONSES IN POSTERIOR MEDIAL NUCLEUS OF NON-ANESTHETIZED RATS

Zuzanna Borzymowska, Aleksandra Składowska, Andrzej Wróbel, Ewa Kublik

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

BACKGROUND AND AIMS: Medial sector of posterior complex (PoM) of the thalamus receives two driving somatosensory inputs – from the periphery and from the cortical layer 5b and their functional significance was proposed to depend on arousal level. In anesthetized rats sensory evoked potentials in PoM revealed only late latency, cortex-dependent responses while in wakefulness they contained also fast latency components. In aroused animals this early activity is effectively transmitted to the sensory cortex. The current experiments were set up to record activity of single PoM neurons from conscious rats in order to confirm the field recording data and characterize the role of PoM in fast transmission of somatosensory information.
METHODS: Rats were habituated to head fixation and body restrain, then implanted with chronic electrodes located in primary and higher order cortical somatosensory and motor areas. For extracellular recordings from PoM, microelectrodes were implanted on movable microdrives or the cranial window was opened for semichronic recording with silicon probe multichannel electrodes. Continuous signal containing field potentials and unitary activity was recorded for offline analysis. Single and multi-unit activity was extracted with template matching and clustering methods by Spike 2 software. The average evoked potentials and peristimulus time histograms were calculated to analyze the responses to whisker stimulations.
RESULTS: Our preliminary results indicate that in awake rats PoM neurons respond to whisker stimulation with short-latency (5–6 ms) discharges followed by later, more dispersed activity.
CONCLUSIONS: Short-latency action potentials generated by PoM cells after vibrissae stimulation suggest that this nucleus participate in fast detection of tactile stimuli. Further research should elaborate the role of early response of this mixed-order somatosensory thalamic nucleus in more detail.
Supported by National Science Centre grant DEC-2013/08/W/NZ4/00691.

P15.9

RELAXIN-3 IN THE HYPOTHALAMIC PARAVENTRICULAR NUCLEUS (PVN) OF RAT: ELECTROPHYSIOLOGICAL APPROACH TO UNDERSTAND THE SEX-DEPENDENT RELATIONS BETWEEN STRESS AND FEEDING

Alan Kania[1], Marian H. Lewandowski[1], Grzegorz Hess[1], M. Akhter Hossain[2], Andrew L. Gundlach[2], Anna Blasiak[1]

[1]Department of Neurophysiology and Chronobiology, Jagiellonian University, Kraków, Poland; [2]The Florey Institute of Neuroscience and Mental Health, The University of Melbourne, Melbourne, Australia

BACKGROUND AND AIMS: Relaxin-3 (RLN3) is recently discovered orexigenic peptide expressed in the brainstem. Neurons synthesizing RLN3 are highly responsive to stress factors, which makes RLN3 and its receptor (RXFP3) excellent candidates at the interface of stress- and feeding-related signaling. Hypothalamic paraventricular nucleus (PVN) is considered a main site of action for RXFP3-mediated food intake and weight gain; which appeared linked to inhibition of PVN oxytocin neurons. RLN3 role in appetite control is considered sexually differentiated since increased expression of RLN3 in the NI (associated with reduced c-fos expression in the PVN) was observed only in female binge eating rats. To characterize the RLN3 influence on PVN neurons we conducted *in vitro* patch clamp recordings.
METHODS: Male Wistar rats and Sprague-Dawley rats of both sexes (4–6-week old) were used. Rats were anesthetized and the brains collected for whole cell patch clamp experiment on hypothalamic slices. All drugs were applied *via* bath perfusion. Immunofluorescent staining was carried out to further characterize recorded neurons.
RESULTS: RXFP3-A2 (600 nM) – a selective RXFP3 agonist, inhibited the majority of recorded PVN neurons [the effect persisted in the presence of 0.5 μM TTX, glutamate and GABA receptor blockers (10 μM)]. Moreover, studies on Sprague Dawley PVN neurons indicate discrepancy in proportion of cells responsive to RXFP3 selective agonist with more neurons affected in female than male rats. Importantly, among PVN neurons sensitive to RXFP3 agonist oxytocin-positive cells were present.
CONCLUSIONS: Our data support the hypothesis that the relaxin-3/RXFP3 network is associated with feeding control in both male

and female rats, indicating higher sensitivity of female rats PVN neurons to RXFP3 activation. Currently we are exploring sexual differences in behavioral effects of hypothalamic RXFP3 activation under different stress and dietary conditions. Funding: NSC, Poland DEC-2012/05D/NZ4/02984 and MSHE, Poland 0020/DIA/2014/43.

P15.10

MECHANISM THAT FORMS BETA BAND ATTENTIONAL DE/SYNCHRONIZATION IN THE VISUAL CORTEX

Elżbieta Gajewska-Dendek[1], Wioletta Waleszczyk[2], Marek Bekisz[2], Andrzej Wróbel[2], Piotr Suffczyński[1]

[1]Department of Biomedical Physics, Institute of Experimental Physics, University of Warsaw, Warsaw, Poland; [2]Department of Neurophysiology, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland

BACKGROUND AND AIMS: Enhanced beta frequency activity (16–24 Hz) serves as a carrier for distributing attentional activation across the visual system. This work aims to characterize beta activity and its generators in the primary visual cortices (V1/2) by measuring the correlations between signals from different cortical locations. In general, the degree of synchronization between two neuronal sites results from interplay of driving sensory inputs, neuronal connectivity and the arousal state. In order to test the mechanisms that influence the high amplitude synchronized beta activity we compared cortical recordings of cats performing visual attentional task and the relevant computational model.

METHODS: We recorded local field potentials from several sites of the cats' V1/2 during stimulus-driven attentional task and measured their correlation strengths. We hypothesized that higher correlation indicated closer functional relation between given signal pair. In parallel we used network model comprising 16 domains representing cortical patches that included mutual lateral inhibitory connections. The model consisted of single compartment excitatory and inhibitory cells with extended Hodgkin-Huxley dynamics. These cells received two kinds of Poisson inputs, representing the bottom-up sensory input and top-down cortical modulation.

RESULTS: The physiological recordings showed that correlation strength mostly decreased with higher amplitude beta signals except of few recording pairs, which increased their correlation coefficients close to one. Similar results could be obtained with the modeled network of cortical neurons receiving common sensory input *via* lateral inhibitory interneurons.

CONCLUSIONS: Our modeling study explains the appearance of heterogeneous organization of cortical beta activity obtained in physiological experiments. The synchronized signals activated by common sensory input form patches of cortical mosaic, which are spatially contrasted by lateral inhibitory connections.

P15.11

INTERHEMISPHERIC ACTIVITY EQUILIBRIUM CHANGES AFTER THE SPREADING DEPRESSION WAVES

Maciej Winiarski, Jan Jabłonka

Department of Animal Physiology, University of Warsaw, Warsaw, Poland

BACKGROUND AND AIMS: The study examined in a healthy rat brain the metabolic correlates of cortical activity one month after the spreading depressions (SD) episodes and its potential influence on prolonged experience dependent plasticity in the somatosensory cortex distant from the SDs focus. The SD is silenced spontaneous cortical activity propagating as a wave across one hemisphere, which follows spreading depolarisations. It is generated by disturbed ion distribution leading to increased extracellular K^+ concentration. SDs are known to participate in the migraine episodes and in peri-infarct depolarisations (PiD). It was shown that after single SD episode the cortical sensory responses were altered and induced reduction of the dynamics of cortical activity rearrangement.

METHODS: In our experiment six rats were subjected to unilateral SDs by administration of 3M KCl to the dura of occipital cortex. Sham and control animals underwent the same procedures, but instead of KCl received 3M NaCl or aCSF, respectively. Starting on the same day the animals had experience-dependent plasticity induced by 28 days deprivation of all but row B contralateral whiskers. After the deprivation cortical activity was mapped by 14 C-2-deoxy-D-glucose (2DG) brain mapping with bilateral rows B stimulation.

RESULTS: The autoradiography revealed that the overall 2DG incorporation was higher in post-SD hemisphere than in the intact one in comparison to the control rats. The interhemispheric differences were also greater in the representations of the stimulated rows of whiskers likewise in the simultaneously stimulated auditory cortex.

CONCLUSIONS: The findings suggests that SDs have no influence on experience dependent cortical map reorganisation, but may influence interhemispheric activity equilibrium increasing the excitability in the post SD hemisphere.

P15.12

PROTONS AFFECT $GABA_A$ RECEPTOR GATING BY ALTERING BOTH PREACTIVATION AND DESENSITIZATION TRANSITIONS

Magdalena Kisiel[1], Magdalena Jatczak[1,2], Jerzy W. Mozrzymas[1,2]

[1]Laboratory of Neuroscience, Department of Biophysics, Wrocław Medical University, Wrocław, Poland; [2]Laboratory of Cellular Neurobiology, Department of Animal Molecular Physiology, Wrocław University, Wrocław, Poland

BACKGROUND AND AIMS: $GABA_A$ receptors are essential for inhibitory transmission in the adult central nervous system. It has

been demonstrated that protons are potent modulators of $GABA_A$Rs. It is known that α1F64 residue – which plays a role in the receptor preactivation (Szczot et al. 2014) – is also involved in pH sensitivity (Huang et al. 2004). For this reason, we decided to examine whether preactivation transitions are affected by protons.
METHODS: To this end we used patch-clamp technique with rapid exchange system, and tested the impact of pH changes on macroscopic and single-channel currents evoked by saturating concentration of full (GABA) or partial (P4S) agonist and mediated by wild type ($\alpha1\beta2\gamma2$) receptors or by α1F64 leucine and cysteine mutants.
RESULTS: Acidification (from pH 8.0 to 6.0) caused a significant increase in current amplitude for all used combinations of receptors and agonists. This effect was accompanied by slowing down of desensitization kinetics (especially for currents elicited by GABA in non-mutated). Surprisingly, protons differently influenced deactivation kinetics in WT and mutated receptors. Kinetic simulations suggest that the mechanism of $GABA_A$Rs modulation by pH changes includes both modifications in preactivation and in one of the classical gating components (opening or desensitization). Single-channel recordings for non-mutated receptors and for cysteine mutants indicated no effect of pH changes on closing/opening transitions suggesting thus the lack of protons impact on channel efficacy. Moreover, we observed that acidification caused prolongation of bursts in WT receptors and the longest component of closure dwell times in cysteine mutants.
CONCLUSION: We conclude that protons modulate $GABA_A$Rs by the impact on gating transitions involving both preactivation and microscopic desensitization.
This work has been supported by grant DEC-2013/11/B/NZ3/00983 of National Centre of Science and ministry grant Pbmn135.

P15.13

OREXINS EXCITES THE NEURONS OF THE RAT VENTRAL LATERAL GENICULATE NUCLEUS PREDOMINANTLY *VIA* OX2 RECEPTORS

Katarzyna Palus, Łukasz Chrobok, Marian Henryk Lewandowski
Department of Neurophysiology and Chronobiology, Jagiellonian University, Kraków, Poland

BACKGROUND AND AIMS: Lateral geniculate nucleus (LGN) is the relay center for visual information received from the retina and consists of the dorsal (dLGN) and ventral lateral geniculate nucleus (vLGN) which are divided by intergeniculate leaflet (IGL) – the important element of the biological clock. The vLGN can be easily distinguished from the IGL based on the neuropeptide Y (NPY) immunoreactivity present only in the IGL. The vLGN is believed to control of the visuomotor functions being among the neuronal structures creating non-image forming visual system. One of the nonspecific brain projection that transmit non-photic information is orexinergic system. Orexins are involved in many behavioral states such as arousal, sleep, stress, and feeding. The aim of this study was to examine the direct effect of orexins on the spontaneous activity of vLGN neurons and the type of the orexin receptor present in the investigated structure.
METHODS: Whole-cell patch clamp *in vitro* technique was used to record spontaneous activity of the vLGN neurons. All of the experiments were performed on the acute brain slices (250 μm) from 13–18 days old male Wistar rats. Orexins, TCS OX2 29 and SB 334867 were applied by bath perfusion. Immunofluorescent staining was performed to verify location of the neuron after each electrophysiological recording.
RESULTS: Our data shows that orexins have a direct postsynaptic effect on the vLGN neurons. Orexin A and orexin B depolarized (6.26±1.07 mV, 6.75±1.69 mV, respectively).
CONCLUSIONS: Our experiments with OX1 and OX2 receptors antagonists indicate predominance of OX2 receptor in the vLGN. To our best knowledge, there are the first data which shows depolarizing effect of orexins in the vLGN. Based on our data we can suppose that orexin system modulates this structure of non-image forming visual system. Further study is needed to evaluate the complex effect of orexins in the lateral geniculate complex.
This work is supported by NSC grant OPUS V: 2013/09/B/NZ4/00541.

P15.14

OREXINS EXCITE THE DORSAL LATERAL GENICULATE NUCLEUS NEURONS IN PIGMENTED AND ALBINO RATS

Łukasz Chrobok, Katarzyna Palus, Marian Henryk Lewandowski
Department of Neurophysiology and Chronobiology, Jagiellonian University, Kraków, Poland, Kraków, Poland

BACKGROUND AND AIMS: The dorsal lateral geniculate nucleus (dLGN) is the relay thalamic nucleus for the visual pathway. It consists of the thalamo-cortical neurons that convey visual information from the retina to the occipital cortex. Orexins (OXA and OXB) are two hypothalamic neuropeptides involved in many physiological processes like the control of the sleep/wake state, feeding or arousal. Orexinergic system of the lateral hypothalamus is considered to be the major non-specific system and its fibers can be found throughout the whole brain. Moreover, orexin receptors are known to be located on the dLGN neurons. The aim of our study was to evaluate effects of orexins on the neuronal activity and membrane potential of the dLGN in three different rat strains: pigmented Long Evans rats (LE), albino Wistar (WIS) and Sprague Dawley rats (SD).

METHODS: The whole-cell *in vitro* patch clamp technique was used on the acute (250 µm thick) brain slices derived from 13–18 days old male rats. Majority of the registrations was performed in the presence of tetrodotoxin (0.5 µM), to isolate the investigated neuron. OXA and OXB (200 nM) were applied by bath perfusion. All the tested cells were filled with biocytin and visualised under confocal microscope.
RESULTS: Our results indicate that the most of dLGN neurons was affected by orexins. We observed the increase in the firing rate or the direct depolarisation after the peptide application and this result was repetitive in all three rat strains (LE: 8.3±2.4 mV and 14.1±3.3 mV; WIS: 9.4±1.9 mV and 8.0±1.5 mV; SD: 8.4±1.3 mV and 8.0±1.7 mV; for OXA and OXB, respectively). However, our results are on the contrary to other studies claiming dLGN insensitivity to orexins.
CONCLUSIONS: This study sheds new light on the role of orexins in the mammalian visual pathway. To our knowledge, we are the first to suggest the orexinergic modulation of the primary sensory ("first order") thalamic nuclei.
This work is supported by NSC grant OPUS V: 2013/09/B/NZ4/00541.

P15.15

SEMI-AUTOMATIC MICRODRIVE SYSTEM FOR POSITIONING ELECTRODES DURING ELECTRO-PHYSIOLOGICAL RECORDINGS FROM RAT BRAIN

Ewa Kublik[1], Piotr Dąbrowski[2], Jakub Możaryn[2]
[1]Dept. of Neurophysiology, Nencki Institute of Experimental Biology, Warsaw, Poland; [2]Institute of Automatic Control and Robotics, Faculty of Mechatronics, Warsaw University of Technology, Warsaw, Poland

BACKGROUND AND AIMS: Recording of local field potentials and spiking activity offers comprehensive insight into the functioning of neuronal populations. However, spikes are not easy to record in behaving rats implanted with chronic electrodes since glial wound isolates electrode's tip from spiking neurons. It is thus essential to advance the electrode to recover spike recordings. This requires portable micro-manipulators that can be mounted on a head of experimental animal. Our aim is to develop a microdrive system for independent, precise positioning of up to 8 electrodes (tetrodes, optogenetical fibres), which will be semi-automatically controlled from the computer with a single step of ~12 µm.
METHODS: Chronic micro-manipulators which can be purchased or hand-made consist of small screws with movable plastic elements. Their minimal step is limited by the screw thread (e.g. with M1,4 screw ¼ of a turn advances the electrode by ~90 µm) and a precision and repeatability highly depends on a quality of an assembly. Therefore, our prototype micro-drive system was created with a mechanism based on stepper micro-motor with halfstep controller to improve the overall performance of such system.
RESULTS: The accuracy of the prototype was tested in experiments during which multiwire electrode was advanced in a boiled egg white, and in rat brain. LabView environment was used for remote control of electrode position and acquisition of control data. Prototype met initial requirements, showing small positioning errors (0.58–1.63 µm depending on stepper mode and a thickness of penetrated material) and ~12 µm repeatability.
CONCLUSIONS: Further tests will include recording of electrophysiological signals from the brain of anaesthetized rat. Development of final version of the device will include its miniaturization to obtain total mass of less than 20 g, so that the device will be small enough to be mounted on a rat's head.
Supported by National Science Centre grant DEC-2013/08/W/NZ4/00691.

P16. PERIPHERAL NERVOUS SYSTEM

P16.1

SYMPATHETIC NERVOUS SYSTEM MEDIATES AMPHETAMINE-INDUCED STIMULATION OF BLOOD AND SPLENIC NATURAL KILLER CELL CYTOTOXICITY IN RATS

Wojciech Glac, Piotr Badtke, Aleksandra Orlikowska, Arkadiusz Działoszewski, Grzegorz Kłoss
Chair of Animal Physiology, Department of Biology, University of Gdańsk, Gdańsk, Poland

BACKGROUND AND AIMS: Amphetamine, besides its well known psychological and behavioral effects, was found to influence the immune functions. However, the mechanism of amphetamine-induced changes in the immune system remains unknown. In search of a possible mechanism of immunomodulating effect of amphetamine, in the present study we tested the involvement of sympathetic nervous system in that effect.
RESULTS: After pretreatment with 6-hydroxydopamine (3×75 mg/kg, ip), we evaluated the effect of acute amphetamine (1 mg/kg, ip) administration on natural killer cell cytotoxicity (NKCC; Cr-51 release assay) and the number of NK (LGL) cells in the peripheral blood and spleen in male Wistar rats. Amphetamine-induced stimulation of blood and splenic NKCC was completely blocked by chemical sympathectomy. Blood NKCC in amphetamine-injected rats was 260% higher in comparison to a control group. Rats pretreated with 6-hydroxydopamine before amphetamine administration showed over 70% lower NKCC then rats which received amphetamine without chemical sympathectomy. Similarly to the peripheral blood, over 190% increase in NKCC

in rats injected with amphetamine was observed in the spleen. Splenic NKCC in rats pretreated with 6-hydroxydopamine was about 60% lower after amphetamine in comparison with rats without chemical sympathectomy. The similar effects were observed in the case of LGL number.
CONCLUSIONS: The data clearly show that AMPH-induced stimulation of NK cells numbers and function both in the peripheral blood and spleen are mediated by peripheral sympathetic nervous system.

P16.2
PERIPHERAL INFLAMMATION AFFECTS FUNCTION OF TRIGEMINAL GANGLION NEURONS
Olga Kuzawińska, Krzysztof Lis, Tomasz Grygorowicz, Agnieszka Cudna, Marta Dąbrowska, Dagmara Mirowska-Guzel, Ewa Bałkowiec-Iskra
Department of Experimental and Clinical Pharmacology, Medical University of Warsaw, Warsaw, Poland

BACKGROUND AND AIMS: Our previous studies showed that inflammatory reaction in the area of trigeminal ganglion (TG) nociceptive endings affects neurochemical properties of TG, causing increase in concentration of both brain-derived neurotrophic factor (BDNF) and calcitonin gene-related peptide (CGRP). In the present study we have employed CFA-induced TG inflammation model to begun investigating the underlying molecular mechanisms of the inflammation-induced changes in trigeminal neurons properties.
METHODS: A model of orofacial inflammation was obtained by local injection of CFA to the mice whisker pad. Pain reaction was assessed every day by von-Frey filaments. 7 and 14 days after CFA injection mice were euthanized. Both TG were removed for ELISA and quantitative PCR analysis of differences in concentration and expression of BDNF, CGRP and selected proinflammatory cytokines, both in male and female mice. After perfusion with 4% paraformaldehyde, TG were removed from the sculls, crioprotected and cut on the 20 μm sections. Tissue was immunostained using primary antibodies against BDNF and TRPV1 and then with secondary antibodies conjugated with Alexa Fluor.
RESULTS: Expression of proinflammatory cytokines, BDNF and CGRP was different in male and female mice. Our results indicate that response of TG to peripheral inflammatory reaction is gender-dependent, what may explain differences in frequency and severity of trigeminal nerve-associated disorders observed between women and men.
CONCLUSIONS: TG neurons in female mice showed increased expression of CGRPa, this neuropeptide may act as the main mediator of trigeminal signaling during migraine.

This project has been supported by the Polish National Science Center, based on the Decision No. DEC-2012/05/B/NZ4/02385. Research subject implemented with CePT infrastructure financed by the European Union – the European Regional Development Fund within the Operational Programme "Innovative economy" for 2007-2013"

P16.3
CHANGES IN EXPRESSION OF SOMATOSTATIN IN THE CSMG NEURONS SUPPLYING PREPYLORIC AREA OF THE PORCINE STOMACH INDUCED BY INTRAGASTRIC INFUSION OF HYDROCHLORIC ACID
Katarzyna Palus, Jarosław Całka
Department of Clinical Physiology, Faculty of Veterinary Medicine, University of Warmia and Mazury, Olsztyn, Poland

BACKGROUND AND AIMS: Gastric hyperacidity is frequent gastric disorders. However, little is known about the changes in the expression of somatostatin (SOM) in the the coeliac-superior mesenteric ganglion (CSMG) neurons supplying prepyloric area of the porcine stomach during inflammation induced by intragastric infusion of hydrochloric acid. The present study was designed to define localization and chemical expression of somatostatin in the sympathetic perikarya supplying the porcine stomach prepyloric area in physiological state and during gastritis induced by intragastric infusion of hydrochloric acid.
METHODS: Ten juvenile female pigs of the Large White Polish breed were used. The animals were divided into two groups: control and animals with hydrochloric acid infusion (HCl). The neuronal retrograde marker Fast Blue (FB) was injected into the anterior prepyloric wall of the stomach of all animals. 23 days after FB injection, the animals of HCI group were introduced into a state of general anesthesia and given intragastrically 5 ml/kg of 0.25 M hydrochloric acid solution. 28 days after FB injections all animals were deeply anaesthetized, transcardially perfused with buffered paraformaldehyde and tissue samples were collected. The CSMG cryostat sections were stained immunocytochemically for SOM and TH (tyrosine hydroxylase).
RESULTS: In the control group 14.97±1.57% out of 200 FB-positive CSMG neurons contained SOM. Inflammation induced by intragastric infusion of hydrochloric acid resulted in upregulation of the SOM-IR neurons to 29.63±0.85%. All SOM-IR neurons in both groups showed the simultaneously TH immunoreactivity.
CONCLUSIONS: Increase in the expression of SOM in FB-possitive neurons of the HCL group may suggest its participation in the protective mechanisms of neurons in different pathological processes, such as gastric hyperacidity.

P16.4

ANALYSIS OF EXPRESSION OF SP AND NOS IN THE PORCINE NODOSE GANGLION (NG) SENSORY NEURONS SUPPLYING PREPYLORIC STOMACH REGION AFTER INTRAGASTRIC HYDROCHLORIC ACID INFUSION

Liliana Rytel, Jarosław Całka

Department of Clinical Physiology, Faculty of Veterinary Medicine, University of Warmia and Mazury, Olsztyn, Poland

BACKGROUND AND AIMS: We anylazed expression of substance P (SP) and nitric oxide synthase (nNOS) in the porcine nodose ganglion sensory neurons innervating prepyloric stomach region in physiological state and following intragastric hydrochloric acid infusion.

METHODS: The study was performed on 8 immature gilts of the Large White Polish breed. All animals were injected retrograde neuronal marker Fast Blue (FB) into the anterior prepyloric stomach wall and then divided into 2 groups (n=4 each). On 23rd day after FB injection gilts of the HCL group received single infusion of hydrochloric acid into the stomach. On 28th day all control and HCL pigs were euthanized and bilateral right (rNG) and left (lNG) were collected. Cryostat sections were processed for double immunofluorescence using antibodies against SP and NOS.

RESULTS: Immunofluorescence staining of the nodose ganglia in control group showed the presence of FB-positive (gastric) neurons expressing SP (45.9±3.38% in rNG and 60.4±1.71% in lNG) and NOS (34.9±6.83% in rNG and 49.9±9.32% in lNG). The SP-positive neurons revealed granular immunoreaction product evenly distributed throughout the cytoplasm. The NOS immunostaining appeared as smooth immunoprecipitate observed throughout the cytoplasm. In HCL group increased expression of SP in the rNG (54.8±5.34%) and decreased in lNG (56.9±3.28%) was found in gastric neurons. While number of FB+/NOS-immunoreactive perikarya increased in both rNG (54.9±4.45%) and lNG (52.5±2.17%) respectively. Both appearance as well as distribution of immunoreaction products resembled that in control group.

CONCLUSIONS: The acquired results suggest that SP and NOS function as neurotransmitters/neuromodulators in the vagal sensory transduction from prepyloric region of the porcine stomach. Additionally, they are possibly involved in pathological changes related to hyperacidity induced gastritis.

AUTHOR INDEX

Table of Contents