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Poland

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28–31 August, Warsaw

PTBUN 2017
Polish Neuroscience Society

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13TH INTERNATIONAL CONGRESS OF POLISH NEUROSCIENCE SOCIETY

PROGRAMME

AUGUST 26 (Saturday)

9:00–18:00	**Precongress Event** **CED Training Day (Cambridge Electronic Design Ltd)**

AUGUST 27 (Sunday)

13:00–17:30	**Precongress Event** **Workshop on Optogenetics – theoretical part**

AUGUST 28 (Monday)

9:00–18:00	**Precongress Event** **CED Training Day (Cambridge Electronic Design Ltd)**
10:30–13:30	**Precongress Event** **Workshop on Optogenetics – practical part (parallel sessions in small groups)**
10:30–13:30	**Special Symposium**
SS1	**Early and late neurotoxic (neurodegenerative) signals**
	(Sponsored by European Society for Neurochemistry)
Chairperson:	*Andrzej Szutowicz (Gdańsk, Poland)*
SS1.1	Membrane proteolysis in pathogenesis of Alzheimer's disease: friend or foe *Anthony Turner (Leeds, United Kingdom)*
SS1.2	LipiDiDiet – Nutritional Intervention in Pre-Dementia Alzheimer's Disease, from Molecular Mechanisms to Clinical Trial Results *Tobias Hartmann (Saarbrücken, Germany)*
SS1.3	Is glutamate dehydrogenase in astrocytes one of the keys to control brain glutamate homeostasis? *Helle Waagepetersen (Copenhagen, Denmark)*
SS1.4	Dysregulation of calcium homeostasis and oxidative stress: interrelated mediators of tetrabromobisphenol A toxicity in primary cultures of rat cerebellar granule cells *Jerzy Łazarewicz (Warszawa, Poland)*
SS1.5	Role of prenatal hypoxia in development of neurodegeneration-prone phenotype *Natalia Nalivaeva (Saint Petersburg, Russia)*

SS1.6	Interactions of early and late neurotoxic signals in cholinergic neurodegeneration *Andrzej Szutowicz (Gdańsk, Poland)*
14:00–15:45	**General PTBUN Assembly**
16:00–16:30	**Opening Ceremony**
16:30–17:25	**Plenary lecture** ***Presidential Lecture***
PL1	Organization and function of descending motor control circuits *Silvia Arber (Basel, Switzerland)*
17:35–18:30	**Plenary lecture** ***The Honorary PTBUN Membership Award presentation***
PL2	Fear learning and the barrel cortex *Małgorzata Kossut (Warszawa, Poland)*
19:00	**Welcome reception** Centre of New Technologies (CeNT) (Banacha 2C)

AUGUST 29 (Tuesday)

09:00–09:55	**Plenary lecture** ***Jerzy Konorski Lecture***
PL3	The functional logic of cortical circuits *Mriganka Sur (Cambridge, USA)*
09:55–10:20	**Coffee break**
10:20–12:20	**Symposia (morning session)**
S1	**Organization of inputs to spinal cord motoneurons in normal and pathological conditions**
Chairperson:	*Olga Gajewska-Woźniak and Julita Czarkowska-Bauch (Warszawa, Poland)*
S1.1	Activity-dependent synaptic plasticity in motoneurons during development *Sandrine Bertrand (Bordeaux, France)*
S1.2	The role of peripheral afferent inputs and their interactions with serotonin in the control of motoneuron activity *Urszula Sławińska (Warszawa, Poland)*
S1.3	Reduction in cholinergic and glutamatergic innervation of ankle extensor but not flexor motoneurons after spinalization calls for selective therapies *Olga Gajewska-Woźniak (Warszawa, Poland)*
S1.4	Modulation of mammalian motor control networks by glial-derived purines *Gareth B. Miles (St. Andrews, UK)*

S2	**Motivational aspects of learning**
Chairperson:	*Ewelina Knapska, Ewa Kublik and Kacper Kondrakiewicz (Warszawa, Poland)*
S2.1	The role of the basal forebrain in associative learning *Balázs Hangya (Budapest, Hungary)*
S2.2	Cell-type specific thalamo-amygdalar interaction underlying associative learning *Ferenc Mátyás (Budapest, Hungary)*
S2.3	Central amygdala mediates social modulation of on-going behavior *Ewelina Knapska (Warszawa, Poland)*
S2.4	The role of glutamate receptor-dependent signaling in the dopamine system in reinforcement learning and adaptive decision-making *Przemysław Cieślak (Kraków, Poland)*
S3	**Novel biomarkers for early diagnosis of Alzheimer's disease**
Chairperson:	*Urszula Wojda (Warszawa, Poland)*
S3.1	Heparan sulfate sulfotransferases and pathological phosphorylation of TAU in Alzheimer's disease *Dulce Papy-Garcia (Paris, France)*
S3.2	CSF biomarkers of Alzheimer's disease *Barbara Mroczko (Białystok, Poland)*
S3.3	From blood-based redox profile to the validation of a lead biomarker for the timely diagnosis of Alzheimer's disease *Daniela Uberti (Brescia, Italy)*
S3.4	Circulating microRNAs in blood as biomarkers of early Alzheimer's disease *Urszula Wojda (Warszawa, Poland)*
12:25–13:10	**Special Lectures**
	Young Investigator Awards:
SL1.1	Harmonic discharge pattern reveals fundamental frequency synchronising elements of the rat subcortical visual system *Łukasz Chrobok (Kraków, Poland)*
SL1.2	Prenatal stress induces changes in excitatory and inhibitory synaptic transmission in the dorsal raphe nucleus of adolescent rats *Joanna Grażyna Sowa (Kraków, Poland)*
13:10–14:10	**Lunch** Centre of New Technologies (CeNT) (Banacha 2C)
14:10–16:10	**Symposia (afternoon session)**

S4	**The role of mTOR pathway in epileptogenesis**
Chairperson:	*Sergiusz Jóźwiak (Warszawa, Poland)*
S4.1	Role of the mTOR Pathway in Animal Models of Genetic and Acquired epilepsies *Michael Wong (St. Louis, USA)*
S4.2	Brain lesions in tuberous sclerosis complex: molecular pathogenesis and epileptogenesis *Eleonora Aronica (Amsterdam, The Netherlands)*
S4.3	Regulation and cellular functions of mTORC1 in animal models of epilepsy *Jacek Jaworski (Warszawa, Poland)*
S4.4	mTOR inhibitors in epilepsy management: from bench to bedside *Katarzyna Kotulska (Warszawa, Poland)*
S5	**Novel molecular and functional aspects of neuronal plasticity**
Chairperson:	*Jakub Włodarczyk (Warszawa, Poland) and Tomasz Wójtowicz (Wrocław, Poland)*
S5.1	Role of local palmitoylation machinery in the postsynaptic nanodomain organization *Masaki Fukata (Aichi, Japan)*
S5.2	The role of S-palmitoylation and S-nitrosylation interplay in the chronic stress disorders *Monika Zaręba-Kozioł (Warszawa, Poland)*
S5.3	Perisynaptic astrocyte morphology shapes hippocampal glutamate signaling *Christian Henneberger (Bonn, Germany)*
S5.4	Synaptic plasticity at basal and apical dendrites of hippocampal neurons engage unique intracellular cascades and matrix metalloproteases subtypes *Tomasz Wójtowicz (Wrocław, Poland)*
S6	**Recent advances in multielectrode arrays development and its impact on physiology: a breakthrough or a hoax?**
Chairperson:	*Daniel Wójcik (Warszawa, Poland)*
S6.1	Transducing neuronal activity at multiple scales: emerging opportunities from neuroelectronics and nano structures *Luca Berdonini (Genova, Italy)*
S6.2	Present and future of retinal implants *Paweł Hottowy (Kraków, Poland)*
S6.3	Network and behavioral dynamics of sensory integration in the rodent hippocampal system *Anton Sirota (Munich, Germany)*
S6.4	Conceptual and computational challenges in massive multielectrode data analysis *Daniel Wójcik (Warszawa, Poland)*

16:10–18:30	**Coffee break and Poster Session**
PS1	**THEMES:** Development and aging Disorders of the nervous system Sensory and motor systems Sleep, autonomic and neuroendocrine systems
18:00–18:30 **SE1**	**Special Event** ***Meet the Speakers***
18:30–19:25 PL4	**Plenary Lecture** Changing the fate – results coming from epileptogenesis in tuberous sclerosis *Sergiusz Jóźwiak (Warszawa, Poland)*

AUGUST 30 (Wednesday)

09:00–09:55 PL5	**Plenary Lecture** Gene silencing therapy for human neurodegenerative disease *Don W. Cleveland (San Diego, USA)*
09:55–10:20	**Coffee break**
10:20–12:20	**Symposia (morning session)**
S7	**Neuromuscular disorders**
Chairperson:	*Anna M. Kamińska (Warszawa, Poland)*
S7.1	The pathological consequences of desmin mutations: lessons from man and mice *Rolf Schroeder (Erlangen, Germany)*
S7.2	Duchenne muscular dystrophy – the slow death of a dogma *Dariusz Górecki (Portsmouth, UK)*
S7.3	Myotonic dystrophy: molecular pathomechanism and therapeutic strategies *Krzysztof Sobczak (Poznań, Poland)*
S7.4	Genetics of mitochondrial diseases – from mitochondrial DNA to whole exome studies *Katarzyna Tońska (Warszawa, Poland)*
S8	**GABAergic modulation of cortical function and plasticity**
Chairperson:	*Monika Liguz-Lęcznar (Warszawa, Poland)*
S8.1	Cortical inhibition: the gate-keeper of associative memories *Helen Barron (Oxford, United Kingdom)*
S8.2	Experience-dependent alterations in inhibition using high-throughput and input-specific fluorescence synapse imaging *Alison Barth (Pittsburgh, USA)*

S8.3	Modulation of excitatory synaptic input to somatostatin-expressing neurons *Joanna Urban-Ciećko (Warszawa, Poland)*
S8.4	Recent advances in pharmacology of plasma membrane GABA transporters *Kinga Sałat (Kraków, Poland)*
S9	**Brain ageing and rejuvenation**
Chairperson:	*Ewa Sikora (Warszawa, Poland)*
S9.1	Aging of a brain neural stem cell niche *Joanne Conover (Connecticut, USA)*
S9.2	The complex role of regular exercise on brain function *Zsolt Radak (Budapest, Hungary)*
S9.3	Exercise, blood-brain barrier integrity, and hippocampal neurogenesis *Michał Toborek (Katowice, Poland)*
S9.4	Inflammation in aging and neurodegeneration disorders: friend or foe? *Jerzy Leszek (Wrocław, Poland)*
12:25–13:10	**Special Lecture**
SL2	Adolf Beck Award Beta frequency attentional activation of the visual system *Andrzej Wróbel (Warszawa, Poland)*
13:10–14:10	**Lunch** Centre of New Technologies (CeNT) (Banacha 2C)
14:10–16:10	**Symposia (afternoon session)**
S10	**Human stem/progenitor cells from different sources and their CNS disease targets**
Chairperson:	*Leonora Bużańska (Warszawa, Poland)*
S10.1	Human neural stem cells for neurodegenerative diseases treatment *Angelo Luigi Vescovi (Milano, Italy)*
S10.2.	Defining recovery neurobiology of injured spinal cord by stem cell-based multimodal approaches *Yang D. Teng (Boston, USA)*
S10.3	Stem cells migrate from the brain to the periphery using lymphatic vessels to sequester stroke-induced inflammation *Cesar V. Borlongan (Tampa, USA)*
S10.4	Preclinical characteristics and regenerative potential of adipose – derived MSC *Anna Sarnowska (Warszawa, Poland)*

S11	**Activity driven gene expression in neurons: new vistas**
Chairperson:	*Leszek Kaczmarek and Katarzyna Kalita (Warszawa, Poland)*
S11.1	Molecular mechanisms of retinal ganglion cells degeneration and neuroprotection *Dorota Skowronska-Krawczyk (San Diego, USA)*
S11.2	CtBP1: a new presynapse-to-nucleus messenger linking the cellular metabolic state with the activity-dependent gene expression *Anna Fejtová (Erlangen, Germany)*
S11.3	Complex regulation of Matrix Metalloproteinase-9 (MMP-9) expression in the normal and epileptic hippocampus *Marcin Rylski (Warszawa, Poland)*
S11.4	Transcription factor SRF controls neuronal plasticity Katarzyna Kalita *(Warszawa, Poland)*
S12	**Neuronal control of metabolic functions**
Chairperson:	*Joanna Śliwowska (Poznań, Poland)*
S12.1	Brain mechanisms for the integral control of metabolism, puberty and fertility *Manuel Tena-Sempere (Cordoba, Spain; Turku, Finland)*
S12.2	Seasonal plasticity of the brain: the use of sheep model to study leptin resistance and obesity *Dorota A. Zięba (Kraków, Poland)*
S12.3	Mapping synaptic inputs to Kisspeptin neurons using a conditional transneuronal viral tracer *Wiliam.H. Colledge and Shel-Hwa Yeo (Cambridge, UK)*
S12.4	KNDy neurons and reproductive dysfunctions in animal models of obesity and diabetes *Joanna H. Śliwowska (Poznań, Poland)*
16:10–18:30	**Coffee break and Poster Session**
PS2	**THEMES:** Cognition and behaviour Computational neuroscience Excitability, synaptic transmission, network functions Neuroimmunology Neurons and glia: cellular mechanisms Novel methods and technology development
16:30–18:30	**Special Symposium (patronage by Faculty of Physics University of Warsaw)**
SS2	**Bridging the gaps: from neural networks to behaviour**
Chairperson:	*Jarosław Żygierewicz (Warszawa, Poland)*

SS2.1	Complex networks approach to study mesoscopic neural networks *Slawomir J. Nasuto (Reading, United Kingdom)*
SS2.2	Auditory sensory and working memory in humans and non-human primates: Linking human and animal research through computational modelling *Reinhard König (Magdeburg, Germany)*
SS2.3	Power modulation of SSVEP *Maciej Łabęcki (Warszawa, Poland)*
SS2.4	Brain-computer interfaces and disorders of consciousness *Piotr Durka (Warszawa, Poland)*
18:00–18:30	**Special Events 2 and 3**
SE2	Komisja etyczna: progi i bariery *Anna Cabaj (Warszawa, Poland)*
SE3	Od mózgu po neurony – badania układu nerwowego z użyciem mikroskopii konfokalnej *Jarosław Korczyński* *(Kawa.ska company presentation)*
18:30–19:25	**Plenary Lecture**
PL6	Chromosomal Conformations in Human and Mouse Brain Affecting Cognition and Behavior *Schahram Akbarian (New York, USA)*
20:30	**Social Gathering** Endorfina Foksal Restaurant (Foksal 2)

AUGUST 31 (Thursday)

09:00–09:55	**Plenary Lecture**
PL7	Imaging the role of microglial activation in neurodegenerative disorders *David Brooks (Newcastle, Great Britain)*
09:55–10:20	**Coffee break**
10:20–12:20	**Symposia (morning session)**
S13	**Current research on serotonin 5-HT7 receptor 20 years after its discovery**
Chairperson:	*Grzegorz Hess (Kraków, Poland)*
S13.1	Low-basicity agonists of serotonin 5-HT7 receptors *Andrzej J. Bojarski (Kraków, Poland)*

S13.2 Activation of 5-HT7 receptors for serotonin modulates hippocampal synaptic plasticity in physiological conditions and in a mouse model of Fragile X Syndrome: involvement of cyclic AMP, intracellular kinases and protein synthesis
Lucia Ciranna (Catania, Italy)

S13.3 Synaptic remodeling depends on signaling between serotonin receptors and the extracellular matrix
Jakub Włodarczyk (Warszawa, Poland)

S13.4 5-HT7 receptor-dependent modulation of GABAergic and glutamatergic transmission in the dorsal raphe nucleus of the rat
Krzysztof Tokarski (Kraków, Poland)

S14 Understanding microglial functions in the central nervous system

Chairperson: *Bożena Kamińska (Warszawa, Poland)*

S14.1 Distinct microglial phenotypes in brain diseases
Helmut Kettenmann (Berlin, Germany)

S14.2 Mild neuroinflammatory profile without gliosis in mice – modelling age-related Parkinson's disease
Marina Pizzi (Brescia, Italy)

S14.3 Functional heterogeneity of microglia and macrophages in the ischemic brain
Bożena Kamińska (Warszawa, Poland)

S14.4 Minocycline affects neuropathic pain by regulation of kynurenic pathway – role of microglial cells
Ewelina Rojewska (Kraków, Poland)

S15 Molecular motors in neuronal function

Chairperson: *Maria J. Rędowicz (Warszawa, Poland)*

S15.1 Motor proteins in microcephaly and autophagy
Richard Vallee (New York, USA)

S15.2 Unconventional myosins as regulators of synaptic function and development
Wolfgang Wagner (Hamburg, Germany)

S15.3 Role of posttranslational modifications of tubulin in neuronal function
Andrzej Kasprzak (Warszawa, Poland)

S15.4 Role of myosin VI-DOCK7 interaction in neuronal cells
Maria J. Rędowicz (Warszawa, Poland)

12:25–13:10 Special Lectures 3 and 4: Breaking News

SL3 Acetyl-CoA and intracellular organelle cross-talk: from neurodevelopment to neurodegeneration
Luigi Puglielli (Madison, USA)

SL4	Rescuing sleepless flies – pharmacochaperoning of folding-deficient dopamine transporters, which give rise to infantile dystonia/Parkinsonism *Michael Freissmuth (Vienna, Austria)*
13:10–14:00	**Lunch** Centre of New Technologies (CeNT) (Banacha 2C)
14:10–14:55	**Plenary Lecture**
PL8	The Function of Synuclein *Robert Edwards (San Francisco, USA)*
14:55–15:15	**Closing Ceremony**
15:15–16:00	**PTBUN Governing Council Meeting**

PLENARY AND SPECIAL LECTURES

PL1. ORGANIZATION AND FUNCTION OF DESCENDING MOTOR CONTROL CIRCUITS

Silvia Arber[1,2]

[1] Biozentrum, University of Basel, Basel, Switzerland, [2] Friedrich Miescher Institute, Basel, Switzerland

Movement is the behavioral output of the nervous system. Animals carry out an enormous repertoire of distinct actions, spanning from seemingly simple repetitive tasks like walking to more complex movements such as forelimb manipulation tasks. This lecture will focus on recent work elucidating the organization and function of neuronal circuits at the core of regulating distinct motor behaviors. It will show that dedicated circuit modules within different brainstem nuclei and their interactions in the motor system play key roles in action diversification.

PL2. FEAR LEARNING AND THE BARREL CORTEX

Małgorzata Kossut

Nencki Institute of Experimental Biology, Warsaw, Poland

The sensory system linking mystacial vibrissae to barrels in somatosensory cortex is an object of many studies concerning brain plasticity. Sensory denervation, sensory deprivation and learning paradigms have been used to explore mechanisms of use-dependent plastic changes of cortical physiology, anatomy, neurochemistry and microstructure. The lecture will describe experiments examining changes in functioning of the barrel cortex evoked by classical conditioning in which stimulation of vibrissae was used as CS and tail shock as UCS. Plastic modification of the barrel cortex activation pattern following fear learning was revealed by 2-deoxyglucose autoradiography, showing expanded cortical representation of vibrissae to which CS was applied. This effect was NMDA receptor-dependent, and modified inhibitory and excitatory neurotransmission within the barrels. Changes in electrophysiological properties of neurons were observed, together with rapid inhibitory synaptogenesis. Activity dependent alterations were found in the ultrastructure of both inhibitory and excitatory synapses. The role of GABAergic transmission in learning-dependent plasticity of the barrel cortex will be discussed.

PL3. THE FUNCTIONAL LOGIC OF CORTICAL CIRCUITS

Mriganka Sur

Department of Brain and Cognitive Sciences, Picower Institute for Learning and Memory, Massachusetts Institute of Technology, Cambridge, USA

The computational power of the human brain derives from its neuronal wiring. Neuronal circuits are the engine of the brain, for they transform simple inputs into complex outputs underlying behavior and cognition. Novel technologies are transforming the analysis of brain circuits. Research in our laboratory has combined two-photon measurements of neuronal activity and dynamics in the intact mouse brain with manipulations of activity to discover specific and unique functions of inhibitory neuron classes and their circuits in cortical response tuning and gain control. Probing mechanisms of internal states, we have demonstrated a crucial role for cholinergic inputs to inhibitory-disinhibitory circuits in shaping the temporal dynamics of cortical activity, a mechanism that underlies neuronal desynchronization and decorrelation during arousal and attention. These discoveries demonstrate that cortical circuits contribute particular functions, and even 'diffuse' neurotransmitter systems act via cell-specific circuits to modulate cortical processing and brain states. Local and long-range circuits together mediate behavior: our experiments in awake behaving mice, combining large scale imaging across multiple areas and optogenetic manipulation, have revealed principles of information flow from sensory through parietal to motor and prefrontal cortex in mice during goal-directed behavior. The logic of these circuits reveals fundamental principles of information processing underlying sensorimotor transformations, and lays the groundwork for rich experimental and computational analyses of normal and abnormal brain function.

FINANCIAL SUPPORT: Supported by grants from NIH, NSF, and the Simons Foundation Autism Research Initiative.

PL4. CHANGING THE FATE. RESULTS COMING FROM EPILEPTOGENESIS IN TUBEROUS SCLEROSIS

Sergiusz Jóźwiak

Department of Pediatric Neurology, Warsaw Medical University, Warsaw, Poland

Tuberous sclerosis (TS) is a multisystem, genetic, neurocutaneous disorder associated with the development of benign tumours in several organs. Epilepsy affects 70–90% of patients. In the majority of patients epilepsy manifests in the first months of life and half of patients develop cognitive impairment, autism spectrum disorders or other neurodevelopmental disturbances. Our previous studies demonstrated that antiepileptic treatment before the onset of seizures but after electroencephalographic (EEG) deterioration results in significant decrease of clinical seizures, the risk of drug-resistant epilepsy, and relevant improvement in neurodevelopmental outcome. As recently shown the development of epilepsy is a long process called epileptogenesis. Seizures are usually preceded by changes in genes expression, neuronal death, activation

of inflammation, finally by changes in the EEG recordings. Intervention at this „latent" stage of epileptogenesis may change the fate of the TS children. The EPISTOP project (Full title: Long-term, prospective study evaluating clinical and molecular biomarkers of epileptogenesis in a genetic model of epilepsy – Tuberous sclerosis complex; www.Epistop.eu) is a multi-center prospective European study tracking epileptogenesis and epilepsy in infants with TS. These studies will allow for the further identification of clinical, molecular and genetic biomarkers that may be used to identify at-risk patients. In the presentation the concept of preventative intervention in epileptogenesis will be discussed.

FINANCIAL SUPPORT: We are grateful to all the partners of the EPISTOP consortium who participate in this multi-center European project. Part of the research leading to these results was funded by the European Community's Seventh Framework Programme (FP7/2007–2013) under grant agreement n°602391 – EPISTOP.

PL5. GENE SILENCING THERAPY FOR HUMAN NEURODEGENERATIVE DISEASE

Don W. Cleveland

Ludwig Institute, University of California at San Diego, La Jolla, USA

The genes whose mutation causes human neurodegenerative disease are widely expressed within neurons and non-neurons of the nervous system, producing damage not only within the most vulnerable neurons but also within their partner neurons, glia, and endothelia. Sustained gene silencing or altered pre-mRNA splicing broadly within neurons and non-neurons throughout the nervous system has been achieved using injection into the nervous system of clinically feasible "designer DNA drugs" known as antisense oligonucleotides (ASOs). Beginning with the founding example for inherited ALS caused by mutation in superoxide dismutase, single doses of this "designer DNA-based" drug approach have been shown to produce sustained, catalytic (RNase H-dependent) RNA degradation of a target gene, thereby producing slowing of disease progression of ALS in rodents or sustained partial disease reversal for Huntington's-like disease from single dose injection. Therapy with ASO injection is now in trial for ALS, Huntington's disease, and myotonic dystrophy. An additional trial using an ASO that corrects the splicing of the SMN2 gene has demonstrated efficacy in spinal muscular atrophy (SMA). A trial is anticipated to initiate in 2017 for the most frequent cause of both ALS and frontal temporal dementia. An extension of this approach is development of synthetic CRISPR RNAs to induce transient activation of Cas9 nuclease to cleave and permanently inactivate a selected target gene.

PL6. CHROMOSOMAL CONFORMATIONS IN HUMAN AND MOUSE BRAIN AFFECTING COGNITION AND BEHAVIOR

Schahram Akbarian

Department of Psychiatry and Department of Neuroscience, Icahn School of Medicine at Mount Sinai, New York, USA

Large-scale consortia provide increasingly detailed insights into the genetic and epigenetic risk architectures of common psychiatric disorders and offer vast amounts of molecular information, but with largely unexplored functional implications and therapeutic potential. Here I provide specific examples how cell-type specific epigenome mapping in human brain, in conjunction with studies in genetically engineered mice, could illuminate the role of chromatin structure and function for cognition and behavior. These include locus-specific disintegration of megabase scale chromosomal conformations and large topologically associated domains (superTADs) after genetic ablation of chromatin regulatory proteins in differentiated brain cells. These findings offer the exciting perspective that *in vivo* editing of enhancer and other regulatory non-coding DNA by RNA-guided nucleases including CRISPR-Cas, or designer transcription factors, could provide a pipeline for novel therapeutic approaches aimed at improving cognitive dysfunction in neurological and psychiatric disease.

FINANCIAL SUPPORT: Supported by Grants from the National Institutes of Health (USA).

PL7. IMAGING THE ROLE OF MICROGLIAL ACTIVATION IN NEURODEGENERATIVE DISORDERS

David J. Brooks[1,2]

[1] *Newcastle University, Newcastle upon Tyne, UK,* [2] *Aarhus University, Aarhus, Denmark*

The microglia comprise 20% of white cells and when activated by disease provide the natural immune defence of the brain. All neurodegenerative disorders are associated with microglial activation but the role of these cells in driving disease or protecting against the presence of pathology is still uncertain. When activated, microglia express the translocator protein (TSPO) which transports nutrients and modulates the membrane potential of mitochondria. PET ligands are now available that bind to TSPO allowing the distribution and time course of microglial activation to be imaged *in vivo*. In this lecture the spatial and temporal patterns of microglial activation in dementias and Parkinsonian syndromes will be illustrated and their relationship to clinical status will be discussed. It will be suggested that microglial activation may occur in tow phases – an initial protective phase which fails followed by a second tidal phase that acts to drive disease. Effective and non-toxic treatments which stimulate the first phase but suppress the second phase need to be further developed.

FINANCIAL SUPPORT: Medical research Council UK, Alzheimer Research UK, Danish Council for Independent Research, Lundbeck Foundation.

PL8. THE FUNCTION OF SYNUCLEIN

Jacob Bendor[1], Todd Logan[1], Robert Edwards[1,2,3]

[1] Departments of Neurology and Physiology, UCSF School of Medicine, San Francisco, USA, [2] Kavli Institute for Fundamental Neuroscience, San Francisco, USA, [3] Weill Institute for Neuroscience, San Francisco, USA

The presynaptic protein α-synuclein has a central role in multiple neurodegenerative disorders including Parkinson's disease (PD). Like many other proteins that accumulate in these disorders, however, the function of synuclein remains poorly understood. The presynaptic location of synuclein suggests a role in neurotransmitter release and over-expression inhibits synaptic vesicle exocytosis. However, knockout mice have shown little difference from wild type. Recent work has suggested that synuclein may act to bend membranes. However, we have observed no clear effect on endocytosis in triple knockout mice lacking all three synuclein isoforms. We have therefore focused on the process of exocytosis. By imaging the individual exocytic events of large dense core vesicles (LDCVs) in adrenal chromaffin cells and in neurons, we have found that both endogenous and over-expressed human synuclein promote dilation of the exocytic fusion pore. As with synaptic vesicles, over-expression inhibits LDCV fusion, but the synuclein does not increase the extent of exocytosis. Synuclein thus has two roles in exocytosis: inhibition (by over-expressed protein) and pore dilation (by the endogenous and over-expressed protein). To assess the significance of these findings for degeneration, we examined the effect of mutations associated with PD. Examining two of the best established mutations, we find that both inhibit LDCV exocytosis when over-expressed, just like wild type human α-synuclein. However, the mutations completely blocked the role of synuclein in pore dilation. Mutations that cause PD thus appear to act through a selective loss of normal function, without impairing the ability of synuclein to inhibit exocytosis or presumably to aggregate.

FINANCIAL SUPPORT: The Giannini Foundation, NIH, Weill Institute for Neuroscience.

SL1.1. HARMONIC DISCHARGE PATTERN REVEALS FUNDAMENTAL FREQUENCY (CA. 40 HZ) SYNCHRONISING ELEMENTS OF THE RAT SUBCORTICAL VISUAL SYSTEM

Łukasz Chrobok, Katarzyna Palus, Jagoda S. Jęczmień-Łazur, Tomasz Błasiak, Marian H. Lewandowski

Jagiellonian University, Institute of Zoology, Department of Neurophysiology and Chronobiology, Krakow, Poland

The lateral geniculate nucleus (LGN) is a retinorecipient thalamic structure serving both vision and non-vision forming functions. Subpopulation of LGN neurons is characterised by isoperiodic, infra slow oscillation (ISO; <0.01 Hz) in the rate of action potential firing. This ISO is common in the subcortical visual system and is synchronised among nuclei innervated by the same eye. Recently a new feature of light responsive neurons in the suprachiasmatic nuclei (SCN) has been described – harmonic distribution pattern (HDP) of interspike intervals (ISI), revealing fundamental frequency of ca. 30 Hz.

The aim of this study was to determine the existence of HDP in the firing of the rat LGN.

Single- or 32-channel extracellular recordings of neuronal firing were performed on *in vivo* (urethane anaesthesia) and *in vitro* preparations of Wistar rat brain. *In vivo* recordings were performed in experimentally controlled lighting conditions and during alternating brain states determined by EEG monitoring.

We have discovered harmonic distribution pattern (HDP) of interspike intervals (fundamental ISI ~25–30 ms) in half of the *in vivo* recorded LGN and OPN neurons that were characterised by ISOs, but not *in vitro*. HPD was resistant to sustained light changes, although altered by transient (5 s) light pulses. Inactivation of contralateral retina (TTX injection) abolished HDP observed in LGN. HDP was correlated within each LGN and OPN and also between ipsilateral nuclei.

For the first time we show that in LGN and OPN there is a subpopulation of neurons that fires in synchrony, governed by common HDP. Our results, combined with observation in SCN, allow as to propose the retina as a common source of HDP in light-responsive neurons and bring us closer to understanding the function of widespread retinal innervation of the mammalian brain.

FINANCIAL SUPPORT: Supported by MSHE grant: 0001/DIA/2014/43.

SL1.2. PRENATAL STRESS INDUCES CHANGES IN EXCITATORY AND INHIBITORY SYNAPTIC TRANSMISSION IN THE DORSAL RAPHE NUCLEUS OF ADOLESCENT RATS

Joanna G. Sowa[1], Grzegorz Hess[1,2]

[1] Institute of Pharmacology Polish Academy of Sciences, Department of Physiology, Krakow, Poland, [2] Jagiellonian University, Institute of Zoology and Biomedical Research, Krakow, Poland

Prenatal maternal stress (PS) adversely influences the development of the central nervous system. Its effects become evident later in life and may lead to mental and neurological disorders. The dorsal raphe nucleus (DRN) is a major source of serotonin in the mammalian brain. DRN plays a key role in regulation of the stress response and is

involved in the development of stress-related psychiatric disorders. Little is known of the effect of prenatal stress on the DRN. In particular, it is not known how PS influences excitatory and inhibitory synaptic transmission and the properties of neurons in the DRN.

The aim of this study was to determine the effects of prenatal stress on glutamatergic and GABAergic inputs to DRN serotonergic neurons of the rat.

Pregnant Sprague-Dawley rats were subjected daily to three restraint stress sessions, from 14th day of pregnancy until the delivery. The effects of this treatment were studied in slices of the DRN prepared from adolescent male offspring of control and stressed mothers. Whole-cell recordings were carried out from putative serotonergic neurons in DRN slices. Spontaneous excitatory (sEPSCs) and inhibitory (sIPSCs) postsynaptic currents were recorded to assess glutamatergic and GABA-ergic transmission, respectively.

Prenatal stress caused an increase in the frequency of sEPSCs and a decrease in the frequency of sIPSCs. Basic electrophysiological properties of serotonergic neurons in rat dorsal raphe nucleus, such as resting membrane potential, input resistance and excitability were not changed after prenatal stress.

These results suggest that prenatal maternal stress causes an enhancement of glutamatergic transmission and an attenuation of GABAergic transmission in the DRN of adolescent offspring rats. These effects are likely to affect the function of the serotonergic system.

FINANCIAL SUPPORT: This study was supported by grant 2015/17/N/NZ4/02455, National Science Centre Poland. Joanna Sowa is a holder of scholarship from the KNOW sponsored by Ministry of Science and Higher Education, Republic of Poland.

SL3. ACETYL-COA AND INTRACELLULAR ORGANELLE CROSS-TALK: FROM NEURODEVELOPMENT TO NEURODEGENERATION

Luigi Puglielli

Department of Medicine and Neuroscience, Waisman Center, University of Wisconsin-Madison, Madison, USA

The import of acetyl-CoA into the lumen of the endoplasmic reticulum (ER) by AT-1/SLC33A1 regulates Nε-lysine acetylation of ER-cargo proteins and is essential for the efficiency of the secretory pathway. Specifically, it regulates both quality control and autophagy-mediated disposal of protein aggregates (J Cell Sci 2010, 123, 3378; J Biol Chem 2014, 289, 32044; J Biol Chem 2012; 287: 29921; Brain 2016, 139, 937). Mice with reduced ER influx of acetyl-CoA display excessive induction of autophagy while mice with increased influx display increased efficiency of the secretory pathway. In both cases, lack of homeostatic balance leads to drastic phenotypes (J Neurosci 2014, 34, 6772; J Exp Med 2016, 213,1267). Importantly, a dysfunctional ER-acetylation machinery has been genetically linked to human diseases. To expand upon our findings, we generated three new mouse models: AT-1 sTg, ATase1-/-, and ATase2-/-. AT-1 sTg overexpress AT-1 systemically. They display defective proteostasis within the secretory pathway and a progeria-like phenotype. ATase1-/- and ATase2-/- display increased induction of autophagy with mild inflammatory infiltration of peripheral organs. Here, we will describe the phenotype of the above mice and report novel findings on the molecular mechanisms that regulate protein homeostasis down-stream of the ER acetylation machinery.

FINANCIAL SUPPORT: NIH.

SL4. RESCUEING SLEEPLESS FLIES – PHARMACOCHAPERONING OF FOLDING-DEFICIENT DOPAMINE TRANSPORTERS, WHICH GIVE RISE TO INFANTILE DYSTONIA/PARKINSONISM

Michael Freissmuth, Ali El-Kasaby, Ameya Kasture, Hafiz M. Mazhar Asjad, Sonja Sucic

Institute of Pharmacology, Centre of Physiology and Pharmacology, Medical University of Vienna, Vienna, Austria

Folding-defective mutants of the human dopamine transporter (DAT) cause a syndrome of infantile dystonia/Parkinsonism. We provide a proof-of-principle that the folding-deficit is amenable to correction *in vivo* by two means, the cognate DAT ligand noribogaine and the HSP70 inhibitor, pifithrin-μ: a mutation in the gene encoding dDAT was identified in the Zuker collection of Drosophila melanogaster, which leads to a sleepless phenotype in flies harboring the mutation dDAT-G108Q. We examined the structure of dDAT-G108Q by molecular dynamics simulations using the published crystal structure of dDAT as a starting point. These simulation provided evidence for structural instability of dDAT-G108Q consistent with a folding defect. We verified this conjecture by visulaizing heterologously expressed dDAT-G108Q and the human equivalent hDAT-G140Q in the endoplasmic reticulum and by showing that it was found in a complex with endogenous folding sensors (calnexin and HSP70-1A). Incubation of the cells with noribogaine (a DAT ligand selective for the inward facing state) and/or pifithrin-μ (an HSP70-inhibtor) restored folding of, and hence dopamine transport by, dDAT-G108Q and of hDAT-G140Q. The mutated versions of DAT were confined to the cell bodies of the dopaminergic neurons in the fly brain and failed to reach the axonal compartments. Axonal delivery was restored and sleep time increased to normal length (from 300 to 1000 min/d), if dDAT-G108Q expressing flies were treated with noribogaine and/or pifithrin-μ. Rescuing misfolded versions of DAT by pharmacochaperoning is of therapeutic interest: it may provide opportunities to remedy disorders arising

from folding-defective mutants of human DAT and of other related SLC6 transporters, e.g. of the human creatine transporter-1, which gives rise to mental retardation when mutated at the equivalent glycine residue.

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SPECIAL SYMPOSIA

SS1.1. ROLE OF PRENATAL HYPOXIA IN DEVELOPMENT OF NEURODEGENERATION-PRONE PHENOTYPE

Natalia N. Nalivaeva[1,2], Igor A. Zhuravin[1,3]

[1] I.M. Sechenov Institute of Evolutionary Physiology and Biochemistry of Russian Academy of Science, St. Petersburg, Russia, [2] School of Biomedical Sciences, University of Leeds, Leeds, UK, [3] Saint-Petersburg State Pediatric Medical University, St. Petersburg, Russia

Accumulating evidence suggests that prenatal hypoxia (PH) in critical periods of brain formation results in numerous changes in brain functioning at various stages of postnatal life. They involve morphological changes in brain structures involved in learning and memory as well as a decrease in brain adaptive potential and plasticity caused by disturbances in the process of formation of new contacts between cellular populations. In particular, PH decreases the number of labile dendritic spines in the cortex and hippocampus which underlie brain plasticity. This is underlined by epigenetic effects of PH on expression and processing of a variety of genes involved in normal brain development and functioning. Among proteins affected by PH is the major enzyme of the cholinergic system – acetylcholinesterase, and amyloid precursor protein, which have important roles in various brain functions. Disruption of their expression and metabolism caused by PH can result in both early cognitive dysfunctions and development of neurodegeneration in later life. Another group of enzymes involved in catabolism of neuropeptides, including amyloid-β peptide (Aβ) are also affected by PH. The decrease in the activity of neprilysin and insulin-degrading enzyme as well as of transport protein transthyretin after PH over the years could result in Aβ clearance deficit and accumulation of its toxic species causing neuronal cell death and development of neurodegeneration. PH also results in activation of brain caspases which affect numerous cellular events. Applying various approaches to restore expression of neuronal genes disrupted by PH during postnatal development opens an avenue for therapeutic compensation of cognitive dysfunctions and prevention of Aβ accumulation in ageing brain and the model of PH in rodents can be used as a reliable tool for assessment of their efficacy.

FINANCIAL SUPPORT: Supported by ARUK, RFBR 16-04-00694, Russia state budget (01201351571).

SS1.2. LIPIDIDIET – NUTRITIONAL INTERVENTION IN PRE-DEMENTIA ALZHEIMER'S DISEASE, FROM MOLECULAR MECHANISMS TO CLINICAL TRIAL RESULTS

Tobias Hartmann[1], Alina Solomon[2], Pieter Visser[3], Suzanne Hendrix[4], Marcus Grimm[1], Miia Kivipelto[2], Hilkka Soininen[5]

[1] Deutsches Institut fuer Demenzpraevention, Experimental Neurology, Saarland University, Saarbrücken, Germany, [2] Department of Clinical Geriatrics, NVS, Karolinska Institutet, Huddinge, Sweden, [3] Department of Neurology, Alzheimer Center, VU University Medical Center, The Netherlands, [4] Pentara Corporation, Salt Lake City UT, USA, [5] Neurocenter, Department of Neurology, Kuopio University Hospital, Kuopio, Finland

The Amyloid Precursor Protein (APP) is infamous for its role in Alzheimer's disease (AD) due to overproduction of a small proteolytic APP breakdown product the amyloid β (Aβ). In contrast to this pathological role, Aβ (and other APP cleavage products) have significant functions in lipid homeostasis that, as recent clinical data suggest, might hold a clue for treatment of Alzheimer's disease. Amyloid deposition is the first notable AD-linked pathology. With progression of the disease further synaptic and other cerebral pathologies accumulate. Studies performed *in vitro* and in AD-model animals suggest that multi-nutrient treatment would allow to synergistically target several of those pathologies as well as to enhance efficacy compared with individual nutrients. The aim was to provide neuroprotection by targeting disease processes in early AD, i.e., by supplying rate-limiting compounds for brain phospholipid synthesis and addressing multiple AD-related pathological processes *in vivo*. Studies in animal models showed that this multi-nutrient combination improved neuronal membrane composition, increased the formation of synapses, cholinergic neurotransmission, and cerebral blood flow and perfusion, preserved neuronal integrity, restored hippocampal neurogenesis, reduced β-amyloid pathology, and improved cognition. For clinical use a specific multi-nutrient combination (Fortasyn Connect/FC) was used. The LipiDiDiet study is a 6-year (2 year with a blinded extension up to 4 years), double-blind, parallel-group, multi-country RCT in subjects with prodromal AD, receiving FC or an iso-caloric control product once daily. A total of 311 subjects with prodromal AD were randomized. Study product compliance was high and there were no reasons for safety concerns. Main results include favorable effects of the nutrient combination on the Clinical Dementia Rating (sum of boxes) and reduced brain atrophy.

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SS1.3. IS GLUTAMATE DEHYDROGENASE ONE OF THE KEYS TO CONTROL BRAIN GLUTAMATE HOMEOSTASIS?

Helle S. Waagepetersen

Faculty of Health and Medical Sciences, University of Copenhagen, Denmark

Brain glutamate concentration needs to be balanced to avoid excitoxicity. Following glutamatergic neurotransmission astrocytes are responsible for clearance of the synaptic cleft via glutamate transporters. In the astrocyte the conversion of glutamate to glutamine is an essential part of the glutamate-glutamine cycle. But, a substantial amount of glutamate is oxidatively metabolized in the mitochondria, which to a large extent may be dependent on glutamate dehydrogenase (GDH). Thus, astrocytes are likely the main regulator of the brain glutamate concentration, but how do they do it? We have investigated the role of GDH in astrocytes with focus on energy and glutamate neurotransmitter homeostasis. We have used cultured astrocytes originating from CNS-specific GDH1 knock-out mice and cultures of astrocytes treated with siRNA against GDH. We find that an impaired GDH activity force glutamate to be only partially oxidized via the truncated TCA cycle and formation of another excitatory amino acid, aspartate. Astrocytes totally lacking GDH exhibit an increased glycolysis and impaired glucose oxidation, supporting that astrocytes are in a need for glutamate oxidation to sustain energy metabolism.

FINANCIAL SUPPORT: Lundbeck Foundation

SS1.4. DYSREGULATION OF CALCIUM HOMEOSTASIS AND OXIDATIVE STRESS: INTERRELATED MEDIATORS OF TETRABROMOBISPHENOL A TOXICITY IN PRIMARY CULTURES OF RAT CEREBELLAR GRANULE CELLS

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

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

There are alarming reports on cytotoxicity of the brominated flame retardant tetrabromobisphenol A (TBBPA) in the *in vitro* cellular models, that seem to be mediated by increases in the intracellular calcium concentration ([Ca2+]i) and oxidative stress. Still, the mechanisms of both these phenomena, their mutual cause-and-effect relationships and implication in the TBBPA-induced neuronal death are not clear. In our experiments the primary cultures of rat cerebellar granule cells (CGC) were acutely challenged with TBBPA. Such induced rises of [Ca2+]i appeared to result independently from the intracellular Ca2+ release via ryanodine receptors transformed into dysfunctional leak channels, and from Ca2+ influx mediated by NMDA receptors. These receptors seem to be activated indirectly, due to depolarization of neurons by TBBPA, which is mediated by the voltage-gated sodium channels and ionotropic glutamate receptors. TBBPA induced oxidative stress as evidenced by ROS production and decrease in GSH content and catalase activity. The pharmacological preventing of the TBBPA-induced rises in [Ca2+]i also entirely prevented oxidative stress induced by 10 μM TBBPA, while the effects of 25 μM TBBPA were only partially reduced. Application of free radical scavengers significantly reduced TBBPA-induced oxidative stress, but did not interfere with rises in [Ca2+]i. This indicates that TBBPA-induced increase in [Ca2+]i is a primary and major event triggering oxidative stress, however at higher μM concentrations a Ca2+-independent portion of oxidative stress emerges, and this effect seems to be directly induced by TBBPA. Furthermore, the separate application of inhibitors of TBBPA-induced Ca2+ transients and free radical scavengers, both provided a strong but incomplete cytoprotection, whereas combination of these substances completely prevented the death of neurons, showing that Ca2+ imbalance and oxidative stress are the triggers of acute TBBPA toxicity in CGC.

FINANCIAL SUPPORT: This study was supported by the Polish National Science Centre grant no. 2012/05/B/NZ7/03225.

SS1.5. MEMBRANE PROTEOLYSIS IN PATHOGENESIS OF ALZHEIMER'S DISEASE: FRIEND OR FOE?

Anthony J. Turner

School of Biomedical Sciences, University of Leeds, Leeds, UK

Membrane proteolytic events are fundamental to the pathogenesis of Alzheimer's disease (AD) and yet targeting these events has, to date, failed to produce any successful therapeutics. A number of factors underly these failures. These include the multiple physiological roles of the protease targets, late stage of clinical trials and toxicity of drugs developed. The amyloid cascade hypothesis formulated over 25 years ago has underpinned much of the subsequent research and AD drug development but has come under increasing pressures from lack of clinical success. Identification of new AD-related genes and biochemical pathways, such as intracellular trafficking, immunity and inflammatory cascades has also re-oriented the focus of current research. Yet still the amyloid hypothesis, initi-

ated by two-stage membrane proteolysis of the amyloid precursor protein (APP) by β- and γ-secretases, remains centre stage. The existence of multiple disease-promoting mutations in the APP gene, and of corresponding protective mutations, still highlights its relevance. The mechanistics of the primary proteolytic events in producing the neurotoxic amyloid β-peptides will be featured and current developments summarized. The significance of the alternative APP transcripts (APP695,751 and 770) will also be highlighted. Another major focus of development of anti-amyloid therapeutics has been peptide clearance both by proteolysis (e.g. by neprilysin (NEP), insulin-degrading enzyme etc.) and by transport mechanisms (e.g. ApoE, transthyretin (TTR)). Both NEP and TTR are subject to similar epigenetic regulation and manipulation of epigenetic pathways may also provide novel therapeutic targets e.g. selective inhibition of histone deacetylases (HDACs), or other protease activation strategies. In summary, membrane proteases have both neurodegeneration-promoting and neuroprotective roles and can act as friend or foe in the fight against AD. The future still remains optimistic for successful AD therapeutics despite a decade of setbacks.

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SS1.6. INTERACTIONS OF EARLY AND LATE NEUROTOXIC SIGNALS IN CHOLINERGIC NEURODEGENERATION

Andrzej Szutowicz

Medical University of Gdansk, Department of Laboratory Medicine, Gdansk, Poland

The loss of neurons and suppression of energy metabolism, in pathology-affected areas of the brain, are characteristic features of several neurodegenerative conditions including Alzheimer's disease, and vascular, dialysis, alcohol, liver, or thiamine deficiency encephalopathies. Multiple acute neurotoxic insults such as transient hypoxia, hypoglycemia, or xenobiotics generating excess of free radicals, glutamate-Zn excitotoxic stimulation, trace metal dis-homeostasis, inhibition of energy metabolism, may pave the path for subsequent stages of neurodegeneration. This presentation basing on cellular, animal models of neurotoxicity and clinical-laboratory medicine data, describes putative mechanisms linking early pathological alterations in energy-acetyl-CoA metabolism with late stages of different cholinergic encephalopathies. Preferential impairment of basal forebrain cholinergic neurons is blamed for appearance of cognitive deficits leading to dementia in final stages of these pathologies. This phenomenon may result from the fact that cholinergic neurons, unlike other ones utilize a direct key energy precursor metabolite – acetyl-CoA, derived from glucose, not only for ATP and N-acetylaspartate synthesis but also for acetylcholine production. Cholinergic neurons also possess greater than noncholinergic ones and glial cells zinc accumulation capacity. Such properties promote amyloidogenesis and processing of amyloid-β precursor protein, yielding accumulation of neurotoxic amyloid-β[1-42] oligomers. They may aggravate primary neurotoxic signals through interactions with extracellular and intracellular membranes and linked signal transduction [pathways. Amyloid-β exerted no direct inhibitory effects on pyruvate dehydrogenase and other enzymes of energy and ACh metabolism. These data indicate that several cytotoxic insults may focus on acetyl-Co-A metabolism as an ultimate target linked with consecutive stages of cholinergic neurodegeneration.

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SS2.1. COMPLEX NETWORKS APPROACH TO STUDY MESOSCOPIC NEURAL NETWORKS

Slawomir J. Nasuto

University of Reading, School of Biological Sciences, Biomedical Engineering Section, Whiteknights, UK

This work is a part of a wider research programme investigating the use of animats, hybrid systems consisting of robotic bodies controlled in a closed loop by neuronal cultures. The mesoscopic cortical cultured networks bridge the gap between low level neuronal properties and system-level behaviour. They are dependent on properties of single neurons, synapse types and connectivity patterns. At the same time the activity patterns emerging due to mesoscopic level interactions shape the entire brain dynamics. We studied the spontaneous development of mesoscopic cultures of rat cortical neurons using complex networks approaches. Cultures of cortical neurons were grown on Multi-Electrode Arrays and spontaneous activity was recorded during development (DIV's 7-35). Functional connectivity was obtained from the culture-wide bursts and typical complex network statistics were estimated. Cultures start with a random pattern of interactions which nevertheless develop small world characteristic as cultures mature, analogously to findings from *in vivo* studies. Connectivity evolution reveals that the burst networks are not completely random, although they do not show temporal dependencies. The reported results form a benchmark against which the effects of a closed loop on development of network interactions can be assessed. Animats offer an attractive platform linking network activity to behaviour. It allows to investigate the effects of closed loop interactions on neurobiological, dynamical and complex networks properties as well as to elucidate the functional role of the repertoire

of complex systems characteristics. Recent work though offers caveats regarding functional connectivity analysis (more broadly, any attempts to decode neural function from popular electrophysiological data analysis methods), prompting a need for more robust complex systems tools capable of unravelling variables playing causal roles in network function.

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SS2.2. AUDITORY SENSORY AND WORKING MEMORY IN HUMANS AND NON-HUMAN PRIMATES: LINKING HUMAN AND ANIMAL RESEARCH THROUGH COMPUTATIONAL MODELLING

Reinhard König[1], Patrick May[1], Ying Huang[2], Artur Matysiak[1], Aida Hajizadeh[1], Peter Heil[3,4], Michael Brosch[2,4]

[1] Leibniz Institute for Neurobiology, Special Lab Non-Invasive Brain Imaging, Magdeburg, Germany, [2] Leibniz Institute for Neurobiology, Special Lab Primate Neurobiology, Magdeburg, Germany, [3] Leibniz Institute for Neurobiology, Department Systems Physiology of Learning, Magdeburg, Germany, [4] Center for Behavioral Brain Sciences, Otto-von-Guericke University, Magdeburg, Germany

The aim of the study was to investigate whether the auditory cortex supports working memory, the short-term storage of information for goal-directed behavior. In order to achieve this, we carried out a series of experiments with non-human primates (Macaca fascicularis) and humans, who performed various tasks on two-sound sequences. We measured spiking activity of individual neurons and local field potentials in the auditory cortex of the monkeys, and the activity of neural populations in the auditory cortex of humans by means of magnetoencephalography (MEG). The experiments were designed in such a way that they enabled us to identify memory-related activity and disentangle it from activity related to other confounding factors, for example from activity associated with motor preparation. We found persistent auditory cortical activity in the silent period between the two sounds that was clearly related to the short-term storage of task-relevant information. Collectively, we found direct support in both species for the idea that temporary storage of information recruits the sensory areas which initially process the information. In our effort to understand the processes underlying auditory sensory and working memory, we are using the computational model of signal processing in the auditory cortex developed by May et al. In this model, the serial and parallel structure of auditory cortex is combined with short-term plasticity, and the dynamical units represent the mean spiking rates of local, excitatory and inhibitory neural populations. We show that the model is able to account for the observed phenomena related to sensory and working memory. In so doing, it bridges the gap between single- and multiunit measurements in monkeys and MEG experiments in humans.

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SS2.3. POWER MODULATION OF SSVEP

Maciej Łabęcki[1], Maria Nowicka[2], Piotr Suffczyński[1]

[1] University of Warsaw, Faculty of Physics, Department of Biomedical Physics, Warsaw, Poland, [2] Nencki Institute of Experimental Biology Polish Academy of Sciences, Warsaw, Poland

Steady State Visual Evoked Potentials are the steady-state responses elicited by flicker stimulation. Frequency of oscillation of these responses corresponds to the stimulus frequency and its harmonics. Since the very first reports on SSVEP in 1966, they have been commonly assumed to be stationary (i.e. steady-state) signals which power and other properties are stable over time. In our study we submitted human subjects to long term (i.e. 60 seconds) visual periodic stimulation. In most cases, the instantaneous power of SSVEP significantly evolved over time. Furthermore the temporal behavior of the response was strongly dependent on the stimulus frequency. The possible explanation and potential impact of these results is discussed.

SS2.4. BRAIN-COMPUTER INTERFACES AND DISORDERS OF CONSCIOUSNESS

Piotr J. Durka, Anna Chabuda, Marian Dovgialo, Anna Duszyk, Marcin Pietrzak, Piotr Różański, Magdalena Zieleniewska

Faculty of Physics, University of Warsaw, Warsaw, Poland

We will start with a brief introduction of the state of the art in EEG-based brain-computer interfaces (BCIs). Similar technologies, that is experimental paradigms and signal processing methods derived from the field of BCI, are believed to be promising candidates to solve one of the major problems of contemporary neuroscience, which is the lack of a stable method for assessment of patients with disorders of consciousness (DoC), commonly (and not quite correctly) addressed as "coma". This situation motivates the research project started recently in cooperation with a model hospital for children with severe brain damage (Warsaw's "Alarm Clock Clinic"). In the second part of this talk we will briefly present some of the preliminary results. P300 event-related potential is the classical electroenceph-

alographic indicator of conscious information processing. It occurs as a component in the EEG trials synchronized to those stimuli, that the subject was paying attention to, e.g. counting. In the standard P300-BCI paradigm, concentration on one of the subsequently flashing stimuli can be used as a conscious choice of one of the options, allowing for non-muscular transfer of information directly from the brain. If reliably detected in a DoC patient in response to the stimulus that the patient was asked to count, it proves the patient's ability to understand and follow commands. Also, it offers a possibility of establishing a non-muscular communication channel. A similar reasoning proves the usefulness of detection of the movement imagery reflections in EEG. Finally, brain's recovery can be also reflected in regaining the sleep pattern known from healthy subjects, also observable in EEG recordings. Detailed presentations of preliminary results from these approaches will be available in the poster session.

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SYMPOSIA

S1.1. ACTIVITY-DEPENDENT PLASTICITY IN MOTONEURONS DURING DEVELOPEMENT

Constanze Lenschow, Mélissa Sourrioux, Camille Quilgars, Jean-Rene Cazalets, Sandrine S. Bertrand

Institut de Neurosciences Integratives et Cognitives d'Aquitaine, CNRS UMR5287, University of Bordeaux, Bordeaux, France

In neuronal networks, synaptic strength is not constant but depends on the past activity of the synapse. This change in synaptic efficacy called activity-dependent synaptic plasticity (ADSP) is paramount to synaptic processing and maturation. However, identifying the ADSP capabilities of the numerous synapses converging onto spinal motoneurons (MNs) remain elusive. Using spinal cord slices from mice at two developmental stages, 1–4 and 8–12 postnatal days (P1–P4; P8–P12), we found that high-frequency stimulation of presumed reticulospinal neuron axons in the ventrolateral funiculus (VLF) induced either an NMDA receptor-dependent-long-term depression (LTD), a short-term depression (STD) or no synaptic modulation in limb MNs. Our study shows that P1–P4 cervical MNs expressed the same plasticity profiles as P8–P12 lumbar MNs rather than P1–P4 lumbar MNs indicating that ADSP expression at VLF-MN synapses undergoes a rostrocaudal maturation in the developing spinal cord. Interestingly, we observed that the form of ADSP expressed was related to the functional flexor or extensor MN subtype. In the spinal cord, metabotropic glutamate receptors (mGluRs) modulate synaptic transmission and undergo subtype-specific regulation of their expression and localization during development. In the second part of this study, we then investigated the impact of mGluR activation on ADSP expression in P1–P3 and P8–P12 MNs. We found that mGluR agonists differentially and selectively modulated ADSP at VLF-MN synapses and that this modulation is developmentally regulated. We then used mGluR activation as a tool to indirectly access the functional role of high-frequency-induced-synaptic plasticity at VLF-MN synapses in locomotor pattern generation.

S1.2. THE ROLE OF PERIPHERAL AFFERENT INPUTS AND THEIR INTERACTIONS WITH SEROTONIN IN THE CONTROL OF MOTONEURON ACTIVITY

Urszula Sławińska

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

It is known that neural circuitry in the spinal cord below a total transection is almost totally devoid of serotonin. As a consequence of spinal injury rats do not walk spontaneously. However, chronic spinal rats can be induced to perform proper plantar stepping by tail stimulation in the upright posture. Such plantar stepping is altered by removal of afferent feedback from the paws showing that sensory feedback from the foot facilitates the spinal central pattern generator (CPG) for locomotion when serotonergic innervation is missing. Although spinal rats can be induced to walk in the upright posture, they do not display recovery of quadrupedal locomotion in the horizontal posture typical for progression in rodents. Our data show that activation of 5-HT2A and 5-HT7/1A receptors using their agonists facilitates plantar stepping in the horizontal posture but interferes with upright stepping in paraplegic rats. In our next investigations we found that in intact adult freely moving rats intrathecal application of the selective 5-HT7 antagonist SB269970 induces hindlimb paralysis. This occurs without a direct effect on motoneurons as revealed by an investigation of reflex activity. The antagonist disrupted intra- and inter-limb coordination during locomotion in intact rats but not during fictive locomotion induced by stimulation of the mesencephalic locomotor region (MLR) in adult rat decerebrate preparations. During the recovery period, after transient blocking of MLR evoked fictive locomotion, the amplitude and frequency of rhythmic activity was reduced. The lack of effects on coordination by SB269970 application in paralyzed decerebrate rats with no afferent feedback indicates a critical role of 5-HT7 receptor mediated control of sensory pathways during locomotor activity. Our data show that for optimal coordinated locomotor movements in adult rats, in addition to activation of the serotonergic system, a potent afferent feedback from the foot seems to be necessary.

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S1.3. REDUCTION IN CHOLINERGIC AND GLUTAMATERGIC INNERVATION OF ANKLE EXTENSOR BUT NOT FLEXOR MOTONEURONS AFTER SPINALIZATION CALLS FOR SELECTIVE THERAPIES

Olga Gajewska-Woźniak

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

Five weeks after spinal cord transection (SCT) at low thoracic segments the number of cholinergic C-terminals, expressing VAChT and glutamatergic terminals of proprioceptive input expressing VGluT1 decreased profoundly on perikarya of soleus α-motoneurons (Sol Mns). However, their number apposing tibialis anterior (TA) Mns was not affected by SCT. Long-term locomotor training only partly counteracted deficit in the number of both cholinergic and VGluT1 glutamatergic terminals on Sol Mns. These observations point to high vulnerability of ankle extensor Mns to SCT and sensitivity of both excitatory inputs to the training. These prompted us to apply a selective method of enhancing proprioceptive signaling to ankle extensor Mns. In intact rats 7-days of electrical stimulation of low-threshold proprioceptive afferents in tibial nerve, verified by means of H-reflex, increased both the number and aggregate volume of cholinergic terminals on Mns of lateral gastrocnemius (LG), a synergist of Sol. It similarly affected proprioceptive glutamatergic innervation of LG Mns. However, this paradigm of activation of LG Mns applied shortly after spinal cord transection did not bring enrichment of their innervation, opening a discussion on optimal parameters of stimulation.

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S1.4. MODULATION OF MAMMALIAN MOTOR CONTROL NETWORKS BY GLIAL-DERIVED PURINES

Gareth B. Miles, David Acton, Emily C. Witts, Filipe Nascimento, Matthew J. Broadhead

School of Psychology & Neuroscience, University of St Andrews, St Andrews, UK

Modulation endows neural networks, such as central pattern generators (CPGs) controlling locomotion, with the flexibility required to adjust their output and the behaviours they control to suit varying environmental and organismal demands. Although such modulation is typically thought to originate from neuronal sources, we have recently revealed glial-derived modulation of spinal CPGs by utilising isolated mouse spinal cord preparations in which locomotor-related output can be recorded *in vitro*. In these preparations, both glial cell activation (via agonists for glial-specific protease-activated receptor-1) and glial cell ablation (using glial toxins), showed that glial cells release ATP in an activity–dependent manner, and that glial-derived ATP is subsequently degraded to adenosine, which in turn modulates the frequency of locomotor-related output via activation of neuronal A1 receptors. Interestingly, this glial-derived modulation is dependent on the co-activation of D1-type dopamine receptors. Whole-cell patch-clamp recordings showed that A1 receptor activation hyperpolarises interneurons and inhibits their synaptic input via presynaptic mechanisms, while A1 receptor activation depolarises motoneurons and has no direct effect on their synaptic inputs. This may allow for adaptation of the locomotor pattern generated by interneuronal networks whilst helping to ensure the maintenance of motor output. Overall, our data indicate that activity-dependent release of purines from glial cells provides negative feedback control of spinal motor networks to regulate, and perhaps stabilise, network output. Given that perturbations in both glial cell function and A1 receptor expression are implicated in neurodegenerative diseases such as Amyotrophic Lateral Sclerosis, our data may contribute to the understanding and eventual treatment of such conditions.

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S2.1. THE ROLE OF THE BASAL FOREBRAIN IN ASSOCIATIVE LEARNING

Balazs Hangya[1], Tamas Laszlovszky[1], Panna Hegedus[1], Sachin P. Ranade[2], Maja Lorenc[2], Adam Kepecs[2]

[1] Lendület Laboratory of Systems Neuroscience, Department of Cellular and Network Neurobiology, Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest, Hungary, [2] Cold Spring Harbor Laboratory, Cold Spring Harbor, USA

The basal forebrain (BF) gives rise to the central cholinergic neuromodulatory system that innervates the entire neocortex and is thought to regulate sensory processing, attention and learning. However, it is not known when cholinergic neurons are recruited during behavior and how their activity might support different aspects of cognition. Intermingled with the cholinergic system, the BF also provides a parallel, equally widespread GABAergic projection of largely unknown function. Here we recorded different cell types of the mouse BF in associative learn-

ing paradigms. Central cholinergic neurons were characterized as bursting and non-bursting cells. We found that both subtypes responded phasically to primary reward and punishment with remarkable speed and precision (18±2 ms), unexpected for a neuromodulatory system. Responses to reward were scaled by reinforcement surprise, raising the possibility that the cholinergic system also conveys cognitive information. Tonic firing properties changed during sleep-wake states but remained similar for bursting and non-bursting neurons, contradicting the current view of bursting cells transmitting phasic information and tonic, non-bursting neurons setting ambient acetylcholine levels. PV-expressing GABAergic neurons showed tonic, sustained responses starting already after the predictive cues and outlasting cholinergic activation. These results suggest that cholinergic neurons form a rapid, reliable and temporally precise signaling route for reinforcement feedback, which may be specifically enhanced or enabled by disinhibition mediated by BF GABAergic projections.

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S2.2. CELL-TYPE SPECIFIC THALAMO-AMYGDALAR INTERACTION UNDERLYING ASSOCIATIVE LEARNING

Ferenc Mátyás[1], Boglarka Barsy[1], Kinga Kocsis[1,2], Aletta Magyar[1], Ákos Babiczky[1], Vivien Kanti[1,3], Katalin Varga[1], Daniel Hillier[4], Ofer Yizhar[5]

[1] Research Centre for Natural Sciences, Institute of Cognitive Neuroscience and Psychology, Budapest, Hungary, [2] Roska Tamás Doctoral School of Sciences and Technology, Faculty of Information Technology and Bionics, Pázmány Péter Catholic University, Budapest, Hungary, [3] János Szentágothai School of Neurosciences, Semmelweis University, Budapest, Hungary, [4] Neural Circuit Laboratories, Friedrich Miescher Institute for Biomedical Research, Basel, Switzerland, [5] Department of Neurobiology, Weizmann Institute of Science, Rehovot, Israel

Associative learning is indispensable to elaborate adaptive behaviour. Lateral thalamic nuclei connected to the amygdala are shown to be involved in fear learning. However, the neural mechanisms and elements that control the acquisition and recall of conditioned fear memory are remained unclear. Here we apply circuit-based optogenetic, *in vivo* electrophysiological and neuroanatomical tracing methods to dissect this network and investigate its contribution to associative fear behaviour. The majority of the thalamic cells retrogradely labelled from the lateral aymgdala and the neigbouring amygdalostriatal transition area (AStr) were located in the supragenicular (SG) and the posterior intralaminar thalamic nuclei (PIL). Both thalamic cell groups co-express the calcium-binding protein calretinin (CR). Viral injection of SG/PIL in CR-Cre mice reveals strong axonal labelling in LA and AStr as well as in associative cortices, while the encompassed CR-negative auditory thalamic relay cells are strongly connected to the primary auditory cortex and only weakly to the amygdala. This pattern suggests that these two thalamic populations process distinct types of information regarding environmental (auditory/visual) cues. Applying bi-directional optogenetic control on axon terminals of CR thalamic cells in the amygdala, we find that the information carried by these cells mediates the formation, recall and extinction of fear memory. Furthermore, this route also encodes the contextual experience of the aversive situation. Finally, by performing *in vivo* extracellular recordings in freely behaving mice, we show that the activity of CR-positive thalamic cells correlates well with each stage of associative learning. Our findings demonstrate that the CR-expressing lateral thalamic cells connected to the amygdala are good candidates to mediate experience-dependent memory processes and thus, shape behavioural responses to the environment signals.

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S2.3. CENTRAL AMYGDALA MEDIATES SOCIAL MODULATION OF ON-GOING BEHAVIOR

Ewelina Knapska

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

Human empathy emerges over phylogeny from various behavioral precursors. One of the simplest is emotional contagion, i.e. sharing emotional states between individuals. Tuning one's emotional state to that of another increases the probability of similar behavior, which thereby allows for a rapid adaptation to environmental challenges. Emotional contagion, commonly observed in animals, is well described at the behavioral level, but the neural circuits necessary for sharing emotions are not well understood. To study neural circuits underlying emotional contagion we have developed behavioral rat models of adult, same-sex social interactions that induce positive emotions, active fear and passive fear. The neural circuits in the central nucleus of the amygdala (CeA) are crucial for both appetitively and aversively motivated non-social behaviors. In the latter case the CeA mediates both active and passive defensive responses. To test the hypothesis that the neural circuits of the CeA are necessary for socially transferred emotions of different

valence we used c-fos-driven targeting of channelrhodopsin and halorhodopsin to activate or inhibit neurons involved in social interactions. We show that activation of the CeA neurons involved in social interactions of different emotional valence in a novel environment resulted in distinct behavioral patterns. Activation of the CeA "positive" neurons increased exploration of the environment, activation of the "passive fear" neurons motivated rats to hide and activation of the "active fear" neurons enhanced risk assessment behavior. Inhibiting the CeA neurons led to opposite effects. Taken together, our results show that the neural circuits within the CeA control socially transmitted emotions and their impact on on-going behavior. Social emotions of different valence involve subpopulations of CeA neurons that are, at least partially, distinct.

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S2.4. THE ROLE OF GLUTAMATE RECEPTOR-DEPENDENT SIGNALING IN THE DOPAMINE SYSTEM IN REINFORCEMENT LEARNING AND ADAPTIVE DECISION-MAKING

Przemysław E. Cieślak

Institute of Pharmacology of the Polish Academy of Sciences, Krakow, Poland

Midbrain dopamine (DA) neurons, together with the major target of their projections – dopaminoceptive neurons in the frontal cortex and basal ganglia, provide a neural substrate for reinforcement learning and are involved in decision-making and action selection. Activity and plasticity in the DA system is largely dependent on excitatory glutamatergic transmission. Here, we sought to determine the role of glutamate receptors in the DA system by using genetically modified mice with cell-type specific ablation of NMDA or mGluR5 receptors in DA neurons and neurons expressing dopamine D1 receptors. Animals were tested in an adaptive decision-making task, that resembles a 'two-armed bandit problem', in which mouse is required to estimate by trial-and-error expected value of two alternatives associated with different reward probabilities (80% *vs.* 20%). During each session reward probabilities were reversed after 60 trials. In order to maximize the long-term sum of rewards, a mouse had to select alternative with higher success probability and adapt their choices to changes in reward contingencies. We observed that disruption of NMDA receptor-dependent signaling in DA neurons caused an initial impairment in error-driven learning and reduced the likelihood of returning to previously rewarded alternative. Moreover, loss of mGluR5 but not NMDA receptors in D1 receptor-expressing neurons decreased reward sensitivity, and as a consequence frequency of choosing alternative with higher reward probability. Finally, loss of NMDA receptors in DA neurons and mGluR5 receptors in D1 neurons caused a delay in decision time and increased latency to collect reward. In conclusion, our results suggest that glutamate receptor-dependent signaling in the DA system is necessary for quick and optimal decision-making.

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S3.1. HEPARAN SULFATE SULFOTRANSFERASES AND PATHOLOGICAL PHOSPHORYLATION OF TAU IN ALZHEIMER'S DISEASE

Dulce Papy-Garcia, Sandrine Chantepie, Auriane Maiza, Cecilia Vera, Minh Bao Huynh, Ouidir Mohand Ouidja

Cell Growth and Tissue Repair Laboratory (CRRET) CNRS 9215, Faculty Sciences and Technology, Université Paris Est Créteil, Paris, France

Heparan sulfates (HS) are the long and linear glycanic moieties of HS proteoglycans (HSPG). Although HSPG are classically located at the cell membrane and in the extracellular matrix, they are found to accumulate at the intracellular level in Alzheimer's disease (AD) degenerating neurons, in where they co-localize with tau in neurofibrillary tangles (NFT). The intracellular accumulation and co-localization of HS with tau in AD neurons suggest a possible role of these complex polysaccharides in the mechanisms leading to the abnormal phosphorylation and aggregation of tau in the neural cells. To explore this possibility, we investigated whether internalization of membrane-associated HS can occur in cell models of tauopathy, and studied the consequences of their internalization in the hyper phosphorylation of tau *in vitro* and *in vivo*. We also investigated HS structural insights associated to these events by analyzing the expression of HS biosynthetic enzymes in the AD brain. Our results show that the intracellular interaction of HS with tau induces the tau hyper phosphorylation through a mechanism involving conformational changes in the protein upon contact with HS. Analysis of the HS biosynthetic enzymes in the AD brain suggested the implication of HS 3-O-sulfation in the tau hyper phosphorylation event. Accordingly, inhibition of HS 3-O-sulfotransferase-2 (HS3ST2) in an animal model of tauopathy resulted in a strong reduction of abnormally phosphorylated tau epitopes and animal recovery. These results suggest that HS and their sulfotransferases play critical roles in the development of AD-related tau pathology.

S3.2. CSF BIOMARKERS OF ALZHEIMER'S DISEASE

Barbara Mroczko

Head of Department of Neurodegeneration Diagnostics, Medical University of Białystok, Poland

Alzheimer's disease (AD) is the most common cause of dementia. The well-established biomarkers of AD in cerebrospinal fluid (CSF) are: amyloid β1-42 (Aβ1-42), total-tau protein (T-tau) and phosphorylated tau (p-tau). These best validated CSF biomarkers are useful in the diagnosis and prediction of AD dementia, however they are not entirely specific for AD. Novel biomarkers that will allow for early recognition of an ongoing pathological process are critically needed in the diagnosis of AD patients. Some clinical investigations have proved that matrix metalloproteinases (MMPs) and their tissue inhibitors (TIMPs) play a role in the pathology of central nervous system, including AD. The presence of MMPs and their tissue inhibitors was found around amyloid plaques and neurofibrillary tangles. MMPs play an important role in the extracellular amyloid beta catabolism and may contribute to AD pathogenesis via a disruption of the blood brain barrier. Limited proteolysis of tau protein by MMP-9 increases tau oligomer formation. An assessment of functional relationship between the presence of the beta-amyloid peptide and the synthesis of inflammatory mediators within the amyloid plaques may be helpful to better understanding of the pathological mechanism of AD. Other novel biomarkers of AD are visinin-like protein 1 (VILIP-1), chitinase-3-like protein 1 (YKL-40) and selected Aβ isoforms. VILIP-1 is an indicator of neurodegeneration, while YKL-40 is a biomarker of neuroinflammation. It has been shown that ratios of Aβ isoforms (Aβ42/Aβ40 and Aβ42/Aβ38) may be useful in the discrimination between AD and non-AD MCI. The combined use of novel biomarkers with well-known CSF biomarkers could improve AD diagnostics and result in a higher level of sensitivity and diagnostic accuracy for discrimination between AD and patients with other neurological dementia disorders.

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S3.3. FROM BLOOD-BASED REDOX PROFILE TO THE VALIDATION OF A LEAD BIOMARKER FOR THE TIMELY DIAGNOSIS OF ALZHEIMER'S DISEASE

Giulia Abate[1], Marika Vezzoli[1], Antonello Novelli[2], Carmen Martinez[3], Antonio Guaita[4], Letizia Polito[4], Maurizio Memo[1], Daniela Uberti[1,5]

[1] Department of Molecular and Translational Medicine, University of Brescia, Brescia, Italy, [2] University of Oviedo, Oviedo, Spain, [3] Cabueñes General Hospital, Gijon, Spain, [4] Golgi Cenci Foundation, Abbiategrasso, Italy, [5] DIADEM LTD, Spin Off of Brescia University, Brescia, Italy

Alzheimer's disease is a devastating neurodegenerative disease, affecting nearly 44 millions of elderly worldwide. Early diagnosis is a crucial starting step in the management of the disease, allowing early interventions in the high-risk individuals before cognitive symptoms are over-claimed, and brain irreversible damaged. In fact, it is now well established that clinical manifestation of AD is preceded by a long prodromal stage, during which pathophysiological processes occur in the brain and peripheral tissues. Our research focused on the characterization of redox alterations as a signature of AD pathophysiology. In particular, the activity of antioxidant enzymes and the expression of post-translational redox-modified products were evaluated in blood samples of two independent studies. Initially, 10 variables related to redox steady state in a total of 88 blood samples from individuals with presymptomatic to large state Alzheimer's disease and healthy subjects (HS) were analyzed. Using visualizing data and machine learning methods we identified a peculiar blood-based redox profile characteristic of the disease. Through the clustering and random forest analysis, nitrated p53 isoform was identified as the variable that impacts more on AD classification, and cross-validated on an independent cohort derived from a retrospective longitudinal based population study "InveCe.Ab". Among approximately 1000 individuals of 75–79 years old, resident in Abbiategrasso, Italy, we processed, at different time points (baseline, 2 and 4 years later), plasma samples from 46 HS, and 27 MCI patients, some of which, included HS, converted in AD. The lead biomarker was validated using regression tree method that assessed the classification of unknown samples, carrying out a prediction of AD (sensitivity 86% and specificity 95%). Our data indicate that a highly specific biomarker related to blood based redox profile can characterize AD years before a clinical diagnosis can be made.

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S3.4. CIRCULATING MICRORNAS IN BLOOD AS BIOMARKERS OF EARLY ALZHEIMER'S DISEASE

Urszula Wojda

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

Among the major challenges related to Alzheimer's disease (AD) is identifying biomarkers of the early AD stage

in easily accessible blood samples, as an alternative to existing sophisticated (brain imaging) and invasive (cerebrospinal fluid, CSF) procedures for AD diagnosis. One of the promising approaches concentrates on circulating microRNAs (miRNAs). Using qRT-PCR we compared the miRNA profiles in the blood plasma of 15 mild cognitive impairment patients with early AD (MCI-AD), whose diagnoses were confirmed by CSF biomarkers, with 20 later AD patients and 15 non-demented, age-matched individuals (CTR). In the first screening, we assessed 179 plasma miRNAs. We confirmed 23 miRNAs reported earlier as AD biomarker candidates and found 26 novel differential miRNAs. For 15 statistically significant differential miRNAs, the TargetScan, MirTarBase and KEGG database analysis indicated putative targets among key proteins involved in AD pathology such as MAPT (tau), APP and enzymes of amyloidogenic proteolysis. These 15 miRNAs were verified in separate, subsequent AD, MCI-AD and CTR groups. Finally, 6 miRNAs were selected as the most promising biomarker candidates differentiating early AD from controls with the highest fold changes (from 1.32 to 14.72), consistent significance, specificities from 0.78 to 1 and sensitivities from 0.75 to 1), (patent pending, PCT/IB2016/052440). The identified miRNA panel in the blood could not only serve as an early non-invasive AD diagnostic, but could also indicate individualized therapy.

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S4.1. ROLE OF THE MTOR PATHWAY IN ANIMAL MODELS OF GENETIC AND ACQUIRED EPILEPSIES

Michael Wong

Department of Neurology, Washington University School of Medicine, St. Louis, USA

Current medical treatments are ineffective in at least one-third of all epilepsy patients and do not have proven disease-modifying or antiepileptogenic properties for preventing the development or progression of epilepsy. While available antiseizure drugs act primarily by suppressing neuronal activity via modulation of ion channels and neurotransmitter systems, more effective, disease-modifying therapies need to target novel mechanisms of action involved in the underlying process of epileptogenesis. The mechanistic target of rapamycin (mTOR) pathway represents a promising candidate for targeting anti-epileptogenic therapy. In the genetic disorder, tuberous sclerosis complex (TSC), epilepsy is very common and is often drug-resistant. The mTOR pathway has been strongly implicated in causing epileptogenesis in mouse models of TSC and mTOR inhibitors have both anti-seizure effects in decreasing existing seizures and anti-epileptogenic effects in preventing epilepsy in these models. Furthermore, there is evidence that the mTOR pathway may be involved in epileptogenesis in animal models of acquired epilepsy, such as following status epilepticus or traumatic brain injury. A number of downstream mechanisms have been implicated in mediating the effects of mTOR in epileptogenesis, including cell growth, synaptic plasticity, inflammation, neurogenesis, and translational regulation. This work in animal models has started to be translated to patients, as clinical trials of mTOR inhibitors for epilepsy in TSC patients are in progress.

S4.2. BRAIN LESIONS IN TUBEROUS SCLEROSIS COMPLEX: MOLECULAR PATHOGENESIS AND EPILEPTOGENESIS

Eleonora Aronica[1,2], EPISTOP Consortium

[1] Department of Neuropathology, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands, [2] Stichting Epilepsie Instellingen Nederland (SEIN), The Netherlands

Tuberous Sclerosis Complex (TSC) is a rare genetic disorder that results from a mutation in the TSC1 or TSC2 genes leading to constitutive activation of the mechanistic target of rapamycin complex 1 (mTORC1). TSC is associated with autism, intellectual disability and severe epilepsy. Cortical tubers are believed to represent the neuropathological substrates of these disabling manifestations in TSC. The expansion of neurosurgical epilepsy programs has offered the possibility of disposing clinically well-characterized human tissue, so that molecular mechanisms underlying the structural and functional reorganization within the epileptic focus can be investigated on a large scale. In our project a large cohort of TSC cases with representative cortical tubers and perituberal cortex has been collected and selected cases were used to generate a lesion classification system based on semi-automated histological quantification. Moreover, we used high-throughput RNA sequencing in combination with systems/computational analytic approaches to investigate the complexity of the TSC molecular network.. Through the analysis of the neuropathological and molecular and functional features specific of TSC brain lesions, a more defined picture of the relationship between the neuropathology and epileptogenesis can be achieved. Recent evidence support the hypothesis of developmental immaturity in TSC brain as unifying mechanism to explain the complex neurological and neuropsychiatric phenotypes and treatment challenges in TSC patients. Moreover, neuron-glia interactions involving inflammato-

ry molecules may also play a critical role in the generation of seizures and cognitive dysfunction. Both neuropathological and molecular features of TSC brain lesions will be discussed, highlighting the involvement of different, but often converging epileptogenic mechanisms.

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S4.3. REGULATION AND CELLULAR FUNCTIONS OF MTORC1 IN ANIMAL MODELS OF EPILEPSY

Jacek Jaworski[1], Matylda Macias[1], Magdalena Błażejczyk[1], Małgorzata Urbańska[1,2]

[1] International Institute of Molecular and Cell Biology, Warsaw, Poland, [2] Children's Memorial Health Institute, Department of Neurology and Epileptology, Warsaw, Poland

Introduction: Mammalian target of rapamycin (mTOR) is a protein kinase that regulates cellular metabolism. Adequate mTOR activity is needed for development as well as proper physiology of mature neurons. Changes in mTOR activity are often observed in neuropathology. Several groups reported that seizures increase mTOR activity, and mTOR contributes to spontaneous seizures. However, the current knowledge about 1) the spatiotemporal and 2) the subcellular pattern of mTOR activation as well as 3) mTOR downstream effectors in epilepsy is limited. Effects of mTOR insufficiency in seizures also remain under investigated. Aim: The aim of my team is to understand regulation and contribution of mTOR to epilepsy and pinpointing cellular mechanisms downstream mTOR. Methods: To study a role of mTOR in epilepsy we used models of pharmacological treatment with kainic acid (KA). We performed analysis of status epilepticus (SE) severity and progression. We analyzed with quantitative Western-blot and microarrays changes in signaling pathways and gene expression. Subcellular distribution of mTOR and its activity was analyzed by live microscopy. Results: We showed that SE induces mTOR first in neurons and next in astrocytes. At early times post seizures mTOR translocates to the nucleus, where its activity increases gradually. We showed that mTOR is involved in KA-dependent gene expression and genes regulated by mTOR regulate cytoskeleton. One of them, Elmo-1 regulates axonal growth and dendritic spine changes. Our research shows also that insufficient mTOR activity lead to increased sensitivity to KA. Conclusions: mTOR is an important player in epilepsy. One of the processes likely controlled by mTOR in epilepsy is transcription of genes responsible for cytoskeleton rearrangement. On the other hand, insufficient mTOR activity decreases threshold for epileptic-like neuronal activity.

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S4.4. MTOR INHIBITORS IN EPILEPSY MANAGEMENT: FROM BENCH TO BEDSIDE

Katarzyna Kotulska

The Children's Memorial Health Institute, Warsaw, Poland

Substantial and growing body of evidence now supports the role of mTOR pathway activation in epilepsy both in preclinical and clinical settings. In humans, mTOR activation has been showed to play an important role in seizures and epileptogenesis in numerous acquired and genetic epilepsies. Moreover, several studies indicate that mTOR signaling pathway is implicated in multidrug resistance resulting from P-gp expression in chronic epilepsy. Thus, the use of mTOR inhibitors is widely considered as an emerging potential therapy in many epilepsies. Tuberous sclerosis complex (TSC) is a genetic disorder characterized the development of hamartomas in various organs and tissues. The molecular hallmark of the disease is the overactivation of mTOR pathway resulting from inactivating mutations in either TSC1 or TSC2 genes. Epilepsy is present in 80–90% of TSC patients and usually appears in the first year of life. In the majority of affected patients, epilepsy is associated with developmental delay. Preclinical studies showed that mTOR inhibition with sirolimus might alleviate or prevent seizures in animal models of TSC. In recent years, many case reports showed beneficial effect of mTOR inhibitors in TSC patients with drug-resistant epilepsy. EXIST-3 trial was the first clinical study to show efficacy of everolimus in drug-resistant epilepsy associated with TSC. There are few other trials of mTOR inhibitors in epilepsy associated with TSC, focal cortical dysplasias, and Sturge-Weber ongoing. Given the data from basic research and the results of first clinical studies, mTOR inhibitors should be considered as promising therapeutic option in drug-resistant epilepsies.

S5.1. ROLE OF LOCAL PALMITOYLATION MACHINERY IN THE POSTSYNAPTIC NANODOMAIN ORGANIZATION

Masaki Fukata[1,2], Norihiko Yokoi[1,2], Yuko Fukata[1,2]

[1] National Institutes of Natural Sciences, National Institute for Physiological Sciences, Department of Molecular and Cellular Physiology, Division of Membrane Physiology, Okazaki, Japan, [2] SOKENDAI, The Graduate University for Advanced Studies, School of Life Science, Department of Physiological Sciences, Okazaki, Japan

Protein palmitoylation, the most common lipid modification, dynamically regulates neuronal protein localiza-

tion and function. Its unique reversibility is conferred by DHHC-type palmitoyl acyl transferases (palmitoylating enzymes) and palmitoyl-protein thioesterases (depalmitoylating enzymes). PSD-95 represents a major palmitoylated postsynaptic scaffolding protein that assembles various synaptic components such as AMPA- and NMDA-type glutamate receptors at the postsynaptic specialized membrane domain (postsynaptic density, PSD). Recently, we found that PSD-95 is partitioned into subsynaptic nanodomains and that PSD-95 in nanodomains undergoes continuous de/repalmitoylation cycles, thereby defining the geometry of PSDs. We found that a subset of metabolic serine hydrolases, ABHD17A, 17B and 17C, specifically depalmitoylate PSD-95 in heterologous cells. Expression of the plasma membrane-localized ABHD17 in hippocampal neurons directly binds to PSD-95 through its catalytic region and dramatically disperses PSD-95 clusters. Furthermore, taking advantage of the acyl-PEGyl exchange gel shift (APEGS) method, we quantitatively monitored the palmitoylation stoichiometry and the depalmitoylation kinetics of representative synaptic proteins, PSD-95, GluA1, GluN2A, mGluR5, Gαq, and HRas. Uniquely, most of the PSD-95 population undergoes rapid palmitoylation cycles. We also found that inhibition of ABHD17 expression dramatically delays the kinetics of PSD-95 depalmitoylation. We propose that local palmitoylation machinery composed of synaptic DHHC palmitoylating enzymes and ABHD17 finely controls the amount of synaptic PSD-95 and thereby organizes the postsynaptic nanodomains.

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S5.2. THE ROLE OF S-PALMITOYLATION AND S-NITROSYLATION INTERPLAY IN THE CHRONIC STRESS DISORDERS

Monika Zaręba-Kozioł, Anna Bartkowiak-Kaczmarek, Adam Krzystyniak, Monika Bijata, Izabela Figiel, Jakub Włodarczyk

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

Chronic stress exposure is a key environmental factor for development of neuropsychiatric disorders such as major depression and anxiety disorders. Chronic stress-related emotional and cognitive impairment is associated with alternations in synaptic organization. One of the best described mechanism of synaptic proteins regulation are posttranslational modifications. S-palmitoylation is the covalent lipid reversible modification of cysteine with palmitate which regulates diverse aspects of neuronal protein trafficking and function. The reversible nature of palmitoylation allows proteins to associate with membranes, what regulate their sorting, localization and functions. Intracellular protein S-palmitoylation is controlled by family of protein acyl-transferases and palmitoyl-thioesterases. Recent study shows alternative mechanism of S-palmitoylation regulation by S-nitrosylation. S-nitrosylation is the covalent modification of cysteine by a nitric oxide (NO). The main aim is to understand the functional consequences of alerted protein S-palmitoylation and S-nitrosylation interplay induced by chronic restraint stress. Using mass spectrometry based approaches we profiled endogenous S-palmitoylation and S-nitrosylation. We identified massive changes at the level of proteins and exact sites of modifications in the mouse model of chronic stress. In the physiological conditions we observed excellent competitive effect, over 50% of cysteines were identified only in the one form S-nitrosylated or S-palmitoylated. After chronic stress, we demonstrated that almost all identified proteins were simultaneously modified by palmitate and NO. Summarizing, our results suggest that altered mechanism of interplay between S-palmitoylation and S-nitrosylation of synaptic proteins might be one of the main events associated with chronic stress disorder, leading to destabilization in synaptic networks.

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S5.3. PERISYNAPTIC ASTROCYTE MORPHOLOGY SHAPES HIPPOCAMPAL GLUTAMATE SIGNALLING

Christian Henneberger

Institute of Cellular Neurosciences, University of Bonn Medical School, Bonn, Germany

Uptake of glutamate by perisynaptic astrocyte processes limits glutamate escape into extrasynaptic space and thus determines the spatial fidelity of synaptic transmission. Importantly, the coverage of synapses by perisynaptic astrocyte processes can vary strongly between nearby synapses. However, the factors that determine local astrocytic coverage of synapses and the functional significance of variable coverage remain largely unknown. Interestingly, expansion microscopy of dendritic spines of pyramidal cells in the hippocampal CA1 region and adjacent astrocyte processes revealed a negative correlation between the spine size and the abundance of perisynaptic glutamate transporters. Functional tests using two-photon excitation imaging of extracellular glutamate and intracellular Ca^{2+} provided evidence for more efficient glutamate uptake at smaller spines. This indicates that the spine size may determine the efficiency of local glutamate uptake and thus the

spatial precision of synaptic transmission. It also implies that astrocytic coverage of spines should decrease after induction of spine growth. The latter is a common observation after induction of synaptic long-term potentiation (LTP). We provide evidence that LTP induction indeed withdraws astrocyte processes from synapses. On the functional level, this withdrawal led to increased escape of glutamate into extrasynaptic space (detected by optical glutamate sensors). In addition, increased glutamate spill-over onto high-affinity N-methyl-D-aspartate receptors at inactive synapses was observed after LTP induction. Our observations indicate that spine size and synaptic plasticity dynamically determine the spatial configuration of synapses and perisynaptic astrocyte processes. On the functional level and as a consequence, glutamate uptake is less efficient at larger postsynaptic spines and after LTP induction, which increases the probability of glutamate to escape active synapses and to invade neighbouring synapses.

FINANCIAL SUPPORT: NRW-Rückkehrerprogramm, HFSP, DFG.

S5.4. SYNAPTIC PLASTICITY AT BASAL AND APICAL DENDRITES OF HIPPOCAMPAL NEURONS ENGAGE UNIQUE INTRACELLULAR CASCADES AND MATRIX METALLOPROTEASES SUBTYPES

Tomasz Wójtowicz[1], Patrycja Brzdak[1,2], Olga Wójcicka[1], Jerzy W. Mozrzymas[1,2]

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

Cognitive processes such as learning and memory require functional modifications within neural circuits which involve reorganization of existing synaptic connections and modulation of its strength. In addition, neurons can significantly enhance information storage capacity by scaling dendritic and somatic excitability (e.g. EPSP-to-spike potentiation). Proteolysis of extracellular matrix constituents and membrane proteins by matrix metalloproteases (MMP) has recently emerged as a key element in these processes. We identified NMDARs as a target for MMP-3 but not MMP-2/9 immediately following LTP induction. We next applied confocal imaging for nuclear cFos protein in brain slices fixed immediately following electrophysiology studies and Ca2+ imaging for somatodendritic NMDAR-mediated Ca2+ waves. We concluded that long-term hippocampal E-S potentiation limited to stratum radiatum inputs required MMP-3 activity in a narrow time window following enhanced neuronal activity that promotes NMDAR-mediated postsynaptic Ca2+ entry and activation of downstream signaling cascades leading to immediate early genes transcription. Most recently we discovered that in striking contrast to apical dendrites, synaptic plasticity induced at basal dendrites was insensitive to a wide range of broad and subtype specific MMP inhibitors. Thus, stratum radiatum synapses required MMP-3, alpha 5-integrins or protease-activated receptor 1 (PAR-1) and PKC kinase activity for modulation of NMDARs function, unlike stratum oriens synapses.

FINANCIAL SUPPORT: National Science Center grant no. SONATA/2014/13/D/NZ4/03045.

S6.1. TRANSDUCING NEURONAL ACTIVITY AT MULTIPLE SCALES: EMERGING OPPORTUNITIES FROM NEUROELECTRONICS AND NANOSTRUCTURES

Luca Berdondini

Department of Neuroscience and Brain Technologies, Fondazione Istituto Italiano di Tecnologia, Genova, Italy

Brain functions and pathological dysfunctions emerge over time by involving a complex dynamics of intracellular and intercellular signalling interactions. This signalling involves multiple and interrelated spatial and temporal scales and it occurs within large cellular networks formed by heterogeneous types of connected and variable spiking neurons. The challenge of developing neurotechnologies enabling to precisely monitor and selectively perturb these biological signals at cellular and possibly sub-cellular resolutions within large networks has occupied neuroscientists and engineers for decades, but current methods are still limited. In particular, while techniques for perturbing neuronal signals by acting at molecular or cellular scales have remarkably progressed over recent years, resolving and monitoring these signals at multiple scales simultaneously within cellular networks and brain circuits remains a fundamental challenge. We aim at developing neurotechnologies and experimental methodologies for monitoring neuronal signals within neuronal systems *in vitro* and brain circuits *in vivo*, and able to resolve the signal contribution of a large number of single neurons. Our methodology consists in realizing planar and micro-structured microelectronic devices providing dense arrays of microelectrodes and on-chip circuits for signal conditioning and multiplexing. We are also exploring the potential of on-chip plasmonic 3D nanostructures for chemical spectroscopy, cell poration and nanofluidic intracellular interfacing. In this seminar, I will present results achieved so far by applying CMOS electrode array devices on increasingly complex *in vitro* and *ex vivo* neuronal systems as well as very recent results obtained with the ongoing develop-

ment of implantable CMOS-probes. This will illustrate emerging experimental opportunities offered by these emerging devices for neuroscience research and applications.

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S6.2. PRESENT AND FUTURE OF RETINAL IMPLANTS

Paweł Hottowy

AGH University of Science and Technology, Faculty of Physics and Applied Computer Science, Krakow, Poland

The retina is a key element of the visual system. The image captured by the eye is processed by the photoreceptors, specialized interneurons and finally by the ganglion cells that encode the visual information in sequences of the action potential and send this information to the brain. Some retinal diseases lead to loss of sight due to degeneration of the photoreceptors. However, the interneurons and the ganglion cells remain alive even in advanced stages of the disease, and keep the ability to process the visual information and send it out to the brain. Here comes the idea for visual prosthesis: replace the photoreceptors by a camera, deliver the visual information to the alive cells by stimulating them electrically, and it will propagate to the brain providing an artificial sight. The first generaion of the retinal prosthetic devices are already available for the patients. Unfortunately, one important limitation of current implants is low spatial resolution of the electrical stimulation. Large electrodes activate simultaneously large groups of neurons, what results in low resolution of the visual information. Furthermore, uncontrolled stimulation of different cell types makes it difficult for the brain to interpret the visual information, and unwanted stimulation of axons can further reduce the visual acuity. I will discuss design, performance and limitations of the current state-of-the-art prosthetic devices, as well as perspectives for development of next generation devices. I will also try to answer the following question: is it possible to build a retinal implant that could transfer to the brain visual information identical to that initiated in the healthy retina processing a complex visual scene?

FINANCIAL SUPPORT: This work was supported by Polish National Science Centre grant DEC-2013/10/M/NZ4/00268.

S6.3. NETWORK AND BEHAVIORAL DYNAMICS OF SENSORY INTEGRATION IN THE RODENT HIPPOCAMPAL SYSTEM

Anton Sirota

Ludwig-Maximilians-Universität München, München, Germany

What are the systems-level mechanisms allowing for formation of memories? The conceptual picture that emerges is that the representation of a novel object or event is incorporated into representation of the spatio-temporal context in the hippocampus, a structure critical for memory. The accepted broad mechanistic framework for this process is that perceived information about the world is transferred from multimodal neocortical areas to the hippocampal region where it is actively encoded. Both transfer of information to and encoding in the hippocampus relies on active sampling of the external sensory inputs and internal network dynamics. However the quantitative link between diverse exploratory behaviour that rodents use to actively sample external sensory inputs during learning, oscillatory network dynamics that controls information flow and hippocampal population code for space and memory is not established. We use marker-based high-resolution tracking of rat biological motion to quantitatively and objectively segment and classify its exploratory behaviour. We combine behaviour analysis with multichannel extracellular recording of populations of neurons and oscillatory dynamics in entorhino-hippocampal circuits. In the talk I will present our recent advances in these project along several directions. First, I will show how high-resolution tracking gives rise to quantification of known behaviours and discovery of new behavioural motifs. Second, how population activity of hippocampal neurons is changing dynamically with changes of exploratory states. Third, I give comprehensive overview of the oscillatory synchronization dynamics across entorhinal-hippocampal circuits. Taken together the constraints imposed by spontaneous exploratory behaviour and network dynamics on activity of hippocampal neurons give rise to a novel temporal framework for the analysis of the mechanisms of memory encoding.

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S6.4. CONCEPTUAL AND COMPUTATIONAL CHALLENGES IN MASSIVE MULTIELECTRODE DATA ANALYSIS

Daniel K. Wójcik

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

Extracellular potential recorded in the brain typically reflects activity of multiple cells and processes happening in multiple spatial and temporal scales, depending on the type of electrode and geometric relation between the setups and the tissue. While easy to record, it is notoriously difficult to interpret due to the long range of the electric field. When multiple recordings are available it is possible to estimate the local distribution of current sources (CSD analysis). Our recent method, kernel Current Source Density, allows to estimate CSD from arbitrary distribution of contacts, however, when the number of contacts is large conecptual and computational problems make the CSD analysis difficult. Discuss the challenges appearing in CSD analysis of multielectrode recordings in complex setups. Reproducible kernel Hilbert spaces, singular value decomposition, Python. A wavelet-style multiscale approach to the CSD analysis leads to optimal use of high density probes. For data coming from single cells, morphological information allows one to obtain estimates of CSD distribution along the cell morphology. It is possible to combine recordings of different type, such as ECoG and SEEG, to improve localization of specific phenomena. Kernel CSD analysis and its variants may significantly improve understanding and interpretation of extracellularly recorded brain activity.

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S7.1. THE PATHOLOGICAL CONSEQUENCES OF DESMIN MUTATIONS: LESSONS FROM MAN AND MICE

Rolf Schröder

Institute of Neuropathology, University Hospital Erlangen, Erlangen, Germany

The intermediate filament protein desmin is a central component of the three-dimensional extra-sarcomeric cytoskeleton in muscle cells, which interlinks neighboring myofibrils and connects the myofibrillar apparatus with the subsarcolemmal cytoskeleton, myonuclei, mitochondria, intercalated discs as well as myotendinous and neuromuscular junctions. The pivotal role of desmin for the structural and functional integrity of striated muscle tissue is highlighted by the observation that mutations of the human desmin gene on chromosome 2q35 cause autosomal-dominant, autosomal-recessive, and sporadic myopathies and cardiomyopathies with marked phenotypic variability. To date, no specific treatment is available for this severely disabling and often lethal disease. To understand the key pathological effects of mutant desmin on the structure and function of striated muscle tissue in early, pre-symptomatic disease stages. Morphological, biochemical, proteomic, genetic and biomechanical analyses of cells and striated muscle tissues expressing mutant desmin (R349P desmin knock-in mice; human muscle biopsies). Mutant desmin inflicts a complex, multilevel pathology with deleterious effects on the formation and maintenance of the extra-sarcomeric intermediate filament network, mitochondrial functions and biomechanical stress resistance. Our analyses demonstrate that mutant desmin already leads to a disruption of the extra-sarcomeric intermediate filament network and severe mitochondrial pathology in the early disease stages of desminopathies.

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S7.2. DUCHENNE MUSCULAR DYSTROPHY – THE SLOW DEATH OF A DOGMA

Dariusz C. Górecki

University of Portsmouth, School of Pharmacy and Biomedical Sciences, Portsmouth, UK

Duchenne muscular dystrophy (DMD) is the most common inherited muscle disease leading to severe disability and death of young men. The central hypothesis states that the DMD pathology results from the myofibre sarcolemma fragility due to loss of dystrophin and the main focus of pre-clinical and clinical research is on its restoration there. Unfortunately, so far no treatment improved the long-term outcome. Findings from our and other laboratories obtained using molecular and functional approaches *in vitro* and *in vivo* contradict the central hypothesis by demonstrating that loss of dystrophin in adult muscle does not necessarily triggers myofibre damage. Moreover, central to the argument presented here, we have evidence that already in myogenic cells, believed not to be affected at this stage of differentiation, DMD mutations produce significant abnormalities. These include cell proliferation, differentiation, energy metabolism, Ca^{2+} homeostasis and death, leading to impaired muscle regeneration. We have also shown that DMD mutations alter extracellular ATP (eATP) signalling via P2RX7 purinoceptor upregulation, leading to death of dystrophic myoblasts. Blockade of P2RX7 in the mdx mouse model of DMD produced significant improvements. As targeting signalling pathways using pharmacological agents is more achievable than restoration of structural proteins, P2RX7 is an attractive translational therapeutic target. Clearly, understanding how DMD mutations alter such a range of functions in myogenic cells is vital for developing effective therapies. We hope that starting a discussion may

bring to light further results that will help re-evaluating the established belief. We have to consider whether the prevailing dogma is not steering us into a wrong direction and whether we could deliver a treatment more quickly and cheaply if alternative strategies are investigated.

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S7.3. MYOTONIC DYSTROPHY: MOLECULAR PATHOMECHANISM AND THERAPEUTIC STRATEGIES

Krzysztof Sobczak

Department of Gene Expression, Institute of Molecular Biology and Biotechnology, Adam Mickiewicz University, Poznan, Poland

Myotonic dystrophies (DMs) are autosomal dominant disorders caused by an expansion of either CTG or CCTG nucleotide repeats in two different genes. The mutated transcripts containing toxic, expanded CUG repeats (CUGexp) or CCUG repeats (CCUGexp) accumulate in nuclei forming RNA foci and sequester some nuclear proteins regulating RNA metabolism, mostly muscleblind-like proteins (MBNLs). MBNLs function as factors regulating RNA metabolism at multiple developmental stages. Using microscopic techniques we showed that MBNL-CUGexp complexes are highly dynamic structures composed of tightly packed, although mobile, MBNL proteins that modulate RNA foci morphology. We also showed that sequestration of MBNL proteins in DM1 and DM2 results in aberrant alternative splicing of hundreds of pre-mRNAs. Some of them showed graded changes that correlated with strength of muscle weakness in patients. They may serve as biomarkers of disease severity and therapeutic response in DM. We also found that among alternative exons significantly misregulated in DM are exons forming alternative 3' untranslated regions (3'UTRs). Depletion of MBNL proteins in DM leads to misregulation of thousands of alternative polyadenylation events. These findings reveal an additional developmental function for MBNL proteins and demonstrate that DM is characterized by misregulation of pre-mRNA processing at multiple levels. We also tested several strategies to eliminate or reduce the toxic effect of CUGexp in different DM1 models. They include the siRNA-induced degradation of CUGexp and inhibition of nuclear protein sequestration by antisense oligomers (AONs) which specifically bind to CUGexp. Therapeutic AONs and siRNAs induce 1) reduction of the number and size of CUGexp foci, 2) reduction of MBNL sequestration and correction of MBNL-dependent alternative splicing and 3) significant reduction of myotonia. Our data showed that short AONs and siRNA are potential therapeutic agent in DM1 treatment.

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S7.4. GENETICS OF MITOCHONDRIAL DISEASES – FROM MITOCHONDRIAL DNA TO WHOLE EXOME STUDIES

Katarzyna Tońska[1], Magdalena Kaliszewska[1], Joanna Rusecka[1], Biruta Kierdaszuk[2], Małgorzata Rydzanicz[3], Piotr Stawiński[3], Rafał Płoski[3], Anna Kamińska[2]

[1] Institute of Genetics and Biotechnology, Faculty of Biology, University of Warsaw, Warsaw, Poland, [2] Department of Neurology, Medical University of Warsaw, Warsaw, Poland, [3] Department of Medical Genetics, Medical University of Warsaw, Warsaw, Poland

Mitochondrial diseases, caused by dysfunction of the respiratory chain are characterised by very high clinical as well as genetic heterogeneity. In most of the cases multiple organs and systems are involved with special place taken by muscular and nervous systems due to their high respiratory requirements. From the genetic point of view mitochondrial diseases are exceptionally difficult to study. As the respiratory chain function is secured by the cooperation of up to 1500 proteins, the number of genes in which mutations may lead to OXPHOS dysfunction may be close to that number. Another difficulty is that the respiratory chain subunits are encoded by two different genomes. 13 of them are localised in mitochondrial DNA (mtDNA) – small, multicopy maternally inherited molecule. The remaining 70 are nuclear encoded. This means that the mutations responsible for mitochondrial diseases may be inherited both in a mendelian and a maternal way. A group of the diseases caused by mutations in nuclear genes encoding proteins responsible for mtDNA maintenance is worth mentioning. mtDNA depletion or multiple deletions are observed as a result of such mutations. POLG and C10orf2 mutations are the most frequent in Polish patients. Next generation sequencing (NGS) enabled detailed analysis of both genomes. The application of NGS to mtDNA analysis in our hands has proven to be an effective tool to capture known as well as novel pathogenic variants. Due to the high number of reads and high coverage it also allows the detection of low levels of heteroplasmy. WES was applied to analyse genetic background of the disease in adult patients with progressive external ophthalmoplegia, multiple mtDNA deletions and negative screening for POLG and C10orf2 mutations. The preliminary results indicate that the success ratio is much lower than in paediatric patients.

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S8.1. CORTICAL INHIBITION: THE GATE-KEEPER OF ASSOCIATIVE MEMORIES

Helen Barron[1,2,3]

[1] Merton College, University of Oxford, Oxford, UK, [2] Oxford Centre for Functional MRI of the Brain, University of Oxford, Oxford, UK, [3] MRC Brain Network Dynamics Unity, University of Oxford, Oxford, UK

Individual memories are thought to be stored by ensembles of neurons that are reactivated during recall. While these memory ensembles can now be labelled and manipulated in the rodent brain, they are difficult to measure in humans using non-invasive methods. Here, by adopting a multifaceted approach, I will show how indirect measures of memory ensembles can be obtained from the human brain. Data from ultra-high field MR spectroscopy, fMRI and transcranial direct current stimulation (tDCS) will be presented to demonstrate how these techniques can be combined to investigate storage of associative memories. By measuring and manipulating neocortical GABA in the human brain I will then show evidence to suggest that associative memories are stored in balanced excitatory-inhibitory ensembles. The inhibitory component of memory ensemble ensures that memories lie dormant unless neocortical excitability is modulated but also appears to play a critical role in protecting overlapping memories from interference.

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S8.2. EXPERIENCE-DEPENDENT ALTERATIONS IN INHIBITION USING HIGH-THROUGHPUT AND INPUT-SPECIFIC FLUORESCENCE SYNAPSE IMAGING

Dika A. Kuljis[1,4,5], Megumi T. Matsushita[1], Eunsol Park[1], Cheryl A. Telmer[2,3], Marcel P. Bruchez[1,2,3], Alison L. Barth[1,5]

[1] Biological Sciences Department, Carnegie Mellon University, Pittsburgh, USA, [2] Department of Chemistry, Carnegie Mellon University, Pittsburgh, USA, [3] Biosensor and Imaging Center, Carnegie Mellon University, Pittsburgh, USA, [4] Neurobiology Department[4], University of Pittsburgh, Pittsburgh, USA, [5] Center for the Neural Basis of Cognition, University of Pittsburgh, Pittsburgh, USA

Fluorescence-based synapse detection enables dense, high-throughput and multichannel analysis of synapse properties and connections in brain tissue. Using fluorogen activating proteins (FAPs) or YFP coupled to a neuroligin tether, we have developed genetically-encoded reagents for fluorescence-labeling of post-synaptic sites. Sparse viral expression of YFP-post or FAP-post in mouse somatosensory (barrel) cortex enables compartment-specific quantitation of synapses across the surface of an individual neuron, as well as cell-type specific presynaptic input assignment. High-resolution, 3D confocal stacks were used for semi-automated, high-throughput assignment of YFP-post and FAP-post synaptic puncta across specific neuron cell-types. Using transgenic mice where specific subtypes of presynaptic inhibitory neurons were fluorescently-labeled with YFP, far red-fluorescence of FAP-post synaptic puncta and dTomato-filled post-synaptic pyramidal cells could be aligned to specific presynaptic partners using tricolor colocalization. Fluorescent post-synaptic puncta properties were evaluated to generate metrics reflecting synapse location, size, shape, and fluorescence intensity that could be used to differentiate synapses from different inhibitory sources. We used these quantitative metrics to evaluate changes in inhibitory inputs after sensory association training for pyramidal neurons in barrel cortex. Genetically-encoded fluorescence-based synaptic labeling reagents provide a powerful approach to enable high-throughput and automated analysis of synapse organization in brain tissue across development, learning, and disease states.

S8.3. MODULATION OF EXCITATORY SYNAPTIC INPUT TO SOMATOSTATIN-EXPRESSING NEURONS

Joanna Urban-Ciećko[1,2], Jean-Sebastien Jouhanneau[3,4], James F.A. Poulet[3,4], Alison L. Barth[2]

[1] Nencki Institute of Experimental Biology Polish Academy of Sciences, Department of Molecular and Cellular Neurobiology, Warsaw, Poland, [2] Carnegie Mellon University, Department of Biological Sciences and Center for the Neural Basis of Cognition, Pittsburgh, USA, [3] Max Delbrück Center for Molecular Medicine (MDC), Department of Neuroscience, Berlin-Buch, Berlin, Germany, [4] Charité-Universitätsmedizin Berlin, Cluster of Excellence NeuroCure, Neuroscience Research Center, Berlin, Germany

Disynaptic inhibition through somatostatin (SST) interneurons is a powerful potential mechanism for gain control in cortical circuits. However, excitatory drive to SST neurons is remarkably weak. Here we investigate the modulation of local pyramidal (Pyr) inputs onto SST interneurons of layer 2/3 in mouse barrel cortex, using *in vitro* and *in vivo* whole-cell recordings from Pyr-SST pairs with a combination of pharmacological screening and optogenetic activation of specific modulatory pathways. We show that presynaptic nicotinic acetylcholine receptor activation rapidly enhances local excitatory inputs onto SST neurons through PKA-dependent pathway. Precisely-timed, brief optogenetic activation of cholinergic fibers was sufficient to account for the enhancement of synaptic efficacy induced by pharmacological activation of Ach receptors. Importantly, these effects were synapse-specific and did not occur at local excitatory connections between pyramidal neurons, indicating that cholinergic fibers selectively modulate synaptic transmission toward enhanced SST neurons-mediated inhibition. Our results show that brain state can selectively

alter network function through the input-specific modulation of specific synaptic motifs.

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S8.4. RECENT ADVANCES IN PHARMACOLOGY OF PLASMA MEMBRANE GABA TRANSPORTERS

Kinga Sałat

Jagiellonian University Medical College, Department of Pharmacodynamics, Krakow, Poland

Plasma membrane transporters for γ-aminobutyric acid (GABA) belong to the solute carrier 6 (SLC6) family of proteins able to perform transport of amino acids and their derivatives into cells. These molecules use cotransport of extracellular sodium ions as a driving force for substrate transport against chemical gradient. The four cloned plasma membrane GABA transporters (GAT1-4) have distinct localization in the central nervous system (CNS) and in peripheral tissues being an essential part of the GABAergic system able to control neuronal excitability by counterbalancing the effects of excitatory neurotransmitters. The use of a selective GAT1 inhibitor – tiagabine is a therapeutic strategy to abolish seizures but recent studies utilizing GAT1-knockout animals or GAT1 inhibitors (tiagabine, NO-711, DDPM-2571) have shown that the inhibition of GAT1 could provide therapeutic benefits related to improvement of mood disorders (anxiety, depression), cognitive decline, acute or neuropathic pain, sleep disorders and others. Strong efforts of medicinal chemistry focused on the discovery of non-GAT1-selective tools to study the biological role of GAT2-4 and to establish potential therapeutic use of their inhibitors. However in this area little progress has been achieved as these compounds were not able to discriminate between GAT1-4 subtype. Hence, the functional role of GAT2-4 and their therapeutic potential remains not fully understood and so far only few compounds, such as EF1502 – a GAT1/GAT2 inhibitor and (S)-SNAP-5114, a semiselective GAT4 inhibitor proved their anticonvulsant activity in animal models. The results of these studies confirmed that GAT2 and GAT4 might be also a promising antiepileptic drug target for compounds administered either alone, or in combination with tiagabine. Taken together, although most of the current research is focused on GAT1 inhibition and seizure control, also non-GAT1 transporters are interesting drug targets in various CNS disorders.

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S9.1. AGING OF A BRAIN NEURAL STEM CELL NICHE

Joanne C. Conover

Department of Physiology and Neurobiology, University of Connecticut, Connecticut, USA

In the anterior forebrain, along the lateral wall of the lateral ventricles, a neurogenic stem cell niche is found in a region referred to as the ventricular-subventricular zone (V-SVZ). In rodents, robust V-SVZ neurogenesis provides new neurons to the olfactory bulb throughout adulthood; however, with increasing age stem cell numbers are reduced and neurogenic capacity is significantly diminished, but new olfactory bulb neurons continue to be produced even in old age. Humans, in contrast, show little to no new neurogenesis after two years of age and whether V-SVZ neural stem cells persist in the adult human brain remains unclear. Investigations into the cytoarchitectural organization and molecular controls that regulate the V-SVZ stem cell niche can thus inspire and influence strategies for future regenerative and replacement therapies in cases of brain aging, injury or disease. I will present functional and organizational differences in the V-SVZ stem cell niche of mice and humans, and examine how aging affects the V-SVZ niche and its associated functions.

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S9.2. THE COMPLEX ROLE OF REGULAR EXERCISE ON BRAIN FUNCTION

Zsolt Radak

Research Center of Sport Science, University of Physical Education, Budapest, Hungary

Regular exercise has systemic beneficial effects, including the promotion of brain function. The adaptive response to regular exercise involves the up-regulation of the enzymatic antioxidant system and modulation of oxidative damage. Reactive oxygen species (ROS) are important regulators of cell signaling. Exercise, via intensity- dependent modulation of metabolism and/or directly activated ROS generating enzymes, modulates the cellular redox state of the brain. ROS mediated alteration of lipids, protein, and DNA could directly affect brain function, while exercise modulates the accumulation of oxidative damage. Oxidative alteration of macromolecules can activate signaling processes, membrane remodeling, and gene transcription. ROS are also involved in the self-renewal and differentiation of neuronal stem cells and the exercise-mediated neurogenesis could be partly associated with ROS production. Exercise directly activates brain derived neurotrophic factor, neuron growth factor and

vascular endothelial growth factor which play an important role in synaptic plasticity, memory and neurogenesis. Moreover, regular exercise can change the bacterial content and activity of microbiome, which might have an effect of brain function and the prevention of neurodegenerative diseases. Overall, regular exercise physical exercise has neuroprotective role with wide range of signaling molecular pathways.

S9.3. EXERCISE, BLOOD-BRAIN BARRIER INTEGRITY, AND HIPPOCAMPAL NEUROGENESIS

Michał Toborek[1,2]

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

With a growing number of associations between daily activities and disease development, recent research has focused on understanding the contribution of lifestyle choices such as exercise. Several groups have demonstrated that exercise can improve activity, strength, aerobic capacity and emotional health. Our studies focused on the role of exercise in neurogenesis of neural progenitor cells (NPC) in the hippocampus in the context of the blood-brain barrier (BBB) integrity. The rationale is related to the fact that a substantial number of NPC are in direct proximity to the endothelium of brain microvessels, which form the BBB. The hippocampal dentate gyrus is an important site of adult neurogenesis, including the formation, survival, and integration of newly born neurons into the mature granule cell synaptic circuitry. Evidence indicates that adult hippocampal neurogenesis is important for learning and memory and is affected by disease conditions associated with cognitive impairment, depression, and anxiety. In a model of chronic methamphetamine administration, we observed decreased expression of tight junction proteins and increased BBB permeability in the hippocampus. These changes were associated with the development of significant aberrations of neural differentiation, such as a reduction in proliferating NPC and their conversion to neurons. Exercise protected against these effects by enhancing the expression of tight junction proteins, stabilizing the BBB integrity, and enhancing the neural differentiation. In addition, exercise protected against methamphetamine-induced systemic increase in inflammatory cytokine levels. These results suggest that exercise can attenuate aberrant neurotoxicity by protecting against the BBB disruption and related microenvironmental changes in the hippocampus.

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S9.4. INFLAMMATORY RESPONSE IN THE CNS: FRIEND OR FOE?

Jerzy Leszek

Wroclaw Medical University, Department of Psychiatry, Wrocław, Poland

Chronic inflammatory reactions are consistently present in neurodegeneration of Alzheimer's type and are considered important factors that accelerate progression of the disease. Inflammatory reactions could be both beneficial and detrimental to the brain, depending on strengths of their activation in various stages of neurodegeneration. Mild activation of microglia and astrocytes usually reveals neuroprotective effects and ameliorates early symptoms of neurodegeneration; for instance, released cytokines help maintain synaptic plasticity and modulate neuronal excitability, and stimulated toll-like receptors(TLRs) promote neurogenesis and neurite outgrowth. However, strong activation of glial cells gives rise to cytokine overexpression/dysregulation, which accelerates neurodegeneration. TLRs and receptors for advanced glycation end product(RAGE), play a central role in perpetuation of inflammation. RAGE activation should be perceived as a primary mechanism which determines self-perpetuated chronic inflammation, and RAGE cooperation with TLRs amplifies inflammatory signaling. Altered mutual regulation of p53 protein, a major tumor suppressor, and NF-kB, the major regulation of inflammation, seems to be crucial for the shift from beneficial to detrimental effects of neuroinflammatory reactions in neurodegeneration. Therapeutic intervention in the p53-NF-kB axis and modulation of RAGE-TLR crosstalk activity are future challenges to cope with neurodegeneration.

S10.1. HUMAN NEURAL STEM CELLS SOURCES FOR CELL THERAPIES IN THE CNS AND A SYNOPSIS OF THE EXPERIENCE FROM PHASE I CLINICAL TRIALS

Angelo L. Vescovi[2,3,4,5,6], Letizia Mazzini[1], Maurizio Gelati[2,3], Daniela Ferrari[4], Daniela C. Profico[2,3], Gianmarco Muzi[2], Jessica Rosati[3], Maurizio Leone[3], Francesco Petruzzelli[3]

[1] Department of Neurology, Eastern Piedmont University, Maggiore della Carità Hospital, Novara, Italy, [2] Laboratorio Cellule Staminali, Cell Factory e Biobanca, Terni Hospital, Terni, Italy, [3] IRCCS Casa Sollievo della Sofferenza, viale dei Cappuccini, Foggia, Italy, [4] Biotechnology and Biosciences Department Bicocca University, Milan, Italy, [5] Fondazione Cellule Staminali di Terni, Terni Hospital, Terni, Italy, [6] Associazione Revert Onlus, Milan, Italy

Cell-based approaches remain one of the most promising areas of investigation for the development of effective experimental therapies for CNS disorders at large. A wide variety of cell donor sources, spanning from cells from the surrenal gland to primary fetal

brain cells have been now tested in clinical settings. Thus, a lot of emphasis goes on the source of donor cells to be used under different therapeutic settings, for many of the cell systems used so fare pose serious procurement, technical or ethical limitations or concerns. Appropriate answers to this situation may be found with stem cells. The inherent functional plasticity of stem cells allows them to carry out a plethora of potential therapeutic actions, spanning the replacement of dead cells, immunomodulation, anti-inflammatory, trophic, homeostatic, scavenging and toxicity-blunting effects. Here we will describe our cGMP protocol and experience with using human fetal brain stem cells (hNSCSs) for the establishment of continuous, stable, plentiful and standardized cell lines that are amenable for certification under clinical good manufacturing practice standards (European Medicine Agencies). We report how such cells successfully obtained cGMP certification (aM 143/2016) and were used in a Phase I clinical trial (EudraCT 2009-014484-39) in which 18 ALS patients received multiple grafts of these cells. The trial was completed successfully, follow up exceeding two years landmark. Safety and efficacy data will be discussed briefly, followed by considerations on the design of an upstarting phase I clinical trial with intracerebroventricular injection of the same cells in secondary progressive multiple sclerosis patients (EudraCt 2015-004855-37). We will also illustrate the establishment of hNSCs from induced pluripotent human cells (hiNSCs) and their comparison to native hNSCs and their perspective cGMP certification for potential use in autologous transplantation upcoming clinical trials.

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S10.2. DEFINING RECOVERY NEUROBIOLOGY OF INJURED SPINAL CORD BY STEM CELL-BASED MULTIMODAL APPROACHES

Yang D. Teng[1,2,3]

[1] Department of Physcial Medicine & Rehabilitation, Harvard Medical School/ Spaulding Rehabilitation Hospital Network, Cambridge, USA, [2] Department of Neurosurgery, Harvard Medical School/Brigham & Women's Hospital, Cambridge, USA, [3] Spinal Cord Injury Research Program, VA Boston Healthcare System, Boston, UK

Emerging evidence increasingly suggests that stem cells may help repair the central nervous system through multiple mechanistic strategies that are often concurrent (i.e., Functional Multipotency of Stem Cells). They may serve not only as tissue engineering mediators of cellular reconstitution, but also as vectors for the delivery of molecules and genes. We have now developed a platform technology to determine therapeutic mechanisms of human mesenchymal stromal stem cells (hMSCs) in a dorsal root ganglion coculture system and an intraspinal cord implantation model. The unique poly(lactic-co-glycolic) acid scaffolding augments hMSC stemness, engraftment, and function without neural transdifferentiation or mesenchymal lineage development, resulting in robust motosensory improvement, pain and tissue damage mitigation, and myelin preservation in adult rat spinal cord after injury. The scaffolded hMSC-derived neurotrophism, neurogenesis, angiogenesis, antiautoimmunity, and antiinflammation support the propriospinal network, neuromuscular junctions, and serotonergic reticulospinal reinnervation to activate the central pattern generator for restoring hindlimb locomotion. Our findings illuminate "Recovery Neurobiology" — i.e., the injured spinal cord may deploy polysynaptic neural circuits different from normal adulthood pathways for postinjury improvement. I will discuss that how tailored polymer implants containing hMSCs or human neural progenitor cells (hNPCs) may hold significant promise for providing a broad range of insight regarding essential neurological mechanisms required for repairing the adult mammalian spinal cord after injury. Our findings may provide a stem cell-based multimodal approach to investigating and formulating therapeutic strategies to achieve clinically meaningful improvement for SCI and neurodegenerative diseases.

FINANCIAL SUPPORT: VA (1-I01-RX000308-01), DoD, CASIS-NASA (GA-2015-222), and a Cele H. and William B. Rubin Family Fund, Inc. Grant for the Gordon Program.

S10.3. STEM CELLS MIGRATE FROM THE BRAIN TO THE PERIPHERY USING LYMPHATIC VESSELS TO SEQUESTER STROKE-INDUCED INFLAMMATION

Xu Kaya, Jea Young Lee, PoJung Lin, Stefano Pianta, Fernando Vale, Harry van Loveren, Yuji Kaneko, Cesar V. Borlongan

University of South Florida Morsani College of Medicine, Tampa, USA

Investigations of stem cell therapy for neurological disorders have primarily focused on the grafted cells' effects within the local brain tissue. Despite mounting evidence of a massive peripheral inflammatory response accompanying stroke, the ability of intracerebrally transplanted cells to migrate to the periphery and sequester systemic inflammation remains unexplored. We previously reported that intravenously transplanted human bone marrow stem cells (hBMSCs) preferentially migrate to spleen, subsequently abrogating chronic inflammation in stroke. Here, we tested the hypothesis that intracerebrally transplanted stem cells in the brain of adult rats subjected

to experimental stroke can migrate to the spleen, a vital organ that confers peripheral inflammation after stroke. Immunofluorescence microscopy revealed stem cells engrafted in the brain, but interestingly a specialized band of stem of cells homed to the spleen via lymphatic vessels, seemingly propelled by inflammatory signals. Mechanism-based *in vitro* studies using hBMSCs co-cultured with lymphatic endothelial cells or microglia, and treated with TNF-alpha further implicated the key role of the lymphatic system in directing stem cell migration and in dampening inflammation. Altogether, the results suggest a robust therapeutic outcome in stroke can be achieved by targeting the systemic inflammatory response. This study is the first to demonstrate brain-to-periphery migration of stem cells, advancing the novel concept of harnessing the lymphatic system in mobilizing stem cells to sequester peripheral inflammation as a brain repair strategy.

FINANCIAL SUPPORT: CVB is supported by the National Institutes of Health.

S10.4. PRECLINICAL CHARACTERISTICS AND REGENERATIVE POTENTIAL OF ADIPOSE – DERIVED MSC

Anna Sarnowska[1], Anna Figiel-Dąbrowska[1], Elżbieta Szczepanik[2], Magdalena Kuźma[4], Hanna Mierzewska[2], Dorota Antczak-Marach[2], Małgorzata Kruk[2], Iwona Terczyńska[2], Natalia Krześniak[3], Ewa Sawicka[2], Adam Nowak[4], Bartłomiej Noszczyk[3], Anna Kamińska[4], Andrzej Marchel[4], Krystyna Domanska-Janik[1]

[1] Mossakowski Medical Research Centre Polish Academy of Sciences, Warsaw, Poland, [2] The Institute of Mother and Child, Warsaw, Poland, [3] Centre of Postgraduate Medical Education, Warsaw, Poland, [4] Medical University of Warsaw, Warsaw, Poland

While the rapid development of stem cell-based therapies are nowadays running, the reliable protocols leading to safe and effective way for cell isolation, expansion and commitment *in vitro* according to distinguished therapeutic purposes still need more consensus and standardization. In order to obtain particular disease-committed therapeutic cells we have screened various culture conditions, especially new systems involving lowered oxygen tension and small molecule treatments which may "rejuvenates" MSC. We also looked on the changes in grow dynamics of long-term cultures in various oxygen concentrations to dissect changes that predict genetic instability of cultivated cells. The standardization of such type of culture (by additional criteria beyond the framework developed by ISCT) should further enhance life-span, expansion and differentiation potential of MSCs needed for more effective regeneration of diseased brain. In recent years we have also entered into the clinic with individual medical experiments (in accordance with the guidelines described by A. Korczyn' Sieratzki – Chair of Neurology Tel Aviv University 2010) based on mesenchymal regenerative cell therapy to elaborate save therapeutic procedures for autoimmune epilepsy, ALS and vast nerve injury. First results are promising and indicate regenerative and immunomodulatory properties of transplanted MSC.

FINANCIAL SUPPORT: The work was supported by National Centre for Research and Development grant No STRATEGMED 1/234261/2/NCBR/2014.

S11.1. MOLECULAR MECHANISM OF RETINAL GANGLION CELLS DEGENERATION AND NEUROPROTECTION

Dorota Skowronska-Krawczyk

Shiley Eye Institute, Department of Ophthalmology, University of California, San Diego, USA

Glaucoma is a blinding neurodegenerative disease, whose risk factors include elevated intraocular pressure (IOP), age, and genetics. Glaucoma is characterized by accelerated and progressive retinal ganglion cell (RGC) death. Despite decades of research, the mechanism of RGC death in glaucoma is still unknown. We found that the genetic effect of the SIX6 risk variant is enhanced by another major POAG risk gene, p16INK4a (cyclin-dependent kinase inhibitor 2A, isoform INK4a). We further showed that __p16Ink4a elevated expression is linked to retinal ganglion cells (RGCs) aging and death. The p16Ink4a gene is repressed in most normal adult tissues, and becomes activated only at times of tissue damage, cellular stress or aging. In young healthy organisms expression of p16INK4a is low or undetectable, but it increases dramatically in most tissues during natural aging. Consistent with this, the activation of p16INK4a is observed in most senescent cells. Interestingly, markers of cellular senescence dramatically increase in glaucomatous human eye and in mouse models, including elevated secretion of deleterious senescence associated secretory phenotype (SASP) molecules. During our talk we will present the evidence that removal of the senescent cells using transgenic mouse model protects the RGCs from cell death providing preliminary data for future investigations aiming at finding senolytic drugs that can be used to treat glaucoma patients.

S11.2. CTBP1: A NEW PRESYNAPSE-TO-NUCLEUS MESSENGER LINKING THE CELLULAR METABOLIC STATE WITH THE ACTIVITY-DEPENDENT GENE EXPRESSION

Anna Fejtova[1,2], Daniela Ivanova[2], Santosh Pothula[2], Anika Dirks[3]

[1] Molecular Psychiatry, Dep. of Psychiatry, University Hospital Erlangen, Erlangen, Germany, [2] RG Presynaptic Plasticity, Leibniz Institute for Neurobiology, Magdeburg, Germany, [3] Departament of Neurochemistry and Molecular Biology, Leibniz Institute for Neurobiology, Magdeburg, Germany

Persistent experience-induced changes in brain performance are connected with reconfiguration of neuronal gene expression patterns, which lead to structural and functional alterations of brain circuits. How signals about neuronal activity levels (especially in the axons and presynapses) are converted into changes of gene expression in neuronal nuclei is still poorly understood. In this talk I will present our data pointing to a novel neuronal function of C-terminal binding protein 1 (CtBP1) in the activity-induced presynapse to nucleus signaling, which controls expression of neuronal activity-regulated genes. CtBP1 is a nuclear co-repressor with distinct localization to presynaptic compartment and to nuclei in neurons. We found that synaptic retention and nuclear abundance of CtBP1 are tightly coupled and regulated by neuronal activity. The nuclear shuttling of CtBP1 requires its active retrograde transport mediated by axonal importin beta and karyopherin-mediated nuclear import and export and critically influences expression of activity-regulated genes. Presynaptic anchoring of CtBP1 is mediated by its direct interaction with active zone proteins Bassoon and Piccolo. This association is regulated by neuronal activity via modulation of the cellular NAD/NADH balance. The synaptic retention of CtBP1 restrains the availability of CtBP1 for nuclear import and thereby contributes to the regulation of activity-dependent gene expression in neurons. This novel CtBP1-signalling pathway links the synaptic function, cellular metabolic state and expressional control of neuronal activity-regulated genes, provides new mechanism for experience-driven shaping of neuronal circuits and is likely involved in neuropathology induced by epilepsy-, stroke- or neurodegeneration-linked neuronal damage.

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S11.3. COMPLEX REGULATION OF THE MATRIX METALLOPROTEINASE-9 (MMP-9) EXPRESSION IN THE NORMAL AND EPILEPTIC HIPPOCAMPUS

Marcin Rylski

Department of Clinical Cytology, the Centre of Postgraduate Medical Education, Warsaw, Poland

MMP-9 is involved in different aspects of the brain activity-driven physiology and pathology. It will be presented multifactorial and interweaved molecular mechanisms related to epigenetic regulators (like DNA methylation, DNA hydroxymethylation, histone modifications, miRNA), transcription factors and mRNA stabilizing proteins, which control MMP-9 expression in the normally as well as excessively acitved hippocampus.

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S11.4. TRANSCRIPTION FACTOR SRF CONTROLS NEURONAL PLASTICITY

Katarzyna Kalita

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

Gene expression induced by neuronal activity is essential for the establishment of long-lasting changes in synaptic connectivity. The cell nucleus, where an active gene transcription occurs, plays the key role as a signal integrator. Transcription factors controlling process of physiological and pathological plasticity remain underinvestigated. This presentation will focus on our recent studies showing that Serum Response Factor, SRF plays a prominent role in regulating expression of genes during development and adult neuronal plasticity. Using genome-wide approach, we identified transcription program regulated by SRF and identified genes that may function as "molecular brakes" giving negative feedback in response to strong neuronal stimulation. Thus, the lack of SRF could contribute to the development of hyperexcitability associated disorder like epilepsy. Moreover, the role of SRF in the regulation of structural and physiological plasticity during development will be discussed.

FINANCIAL SUPPORT: Supported by Polish National Science Center grant (SONATA BIS 2) DEC-2012/07/E/NZ3/01814.

S12.1. BRAIN MECHANISMS FOR THE INTEGRAL CONTROL OF METABOLISM, PUBERTY AND FERTILITY

Manuel Tena-Sempere[1,2,3,4]

[1] *Maimonides Institute of Biomedical Research of Cordoba (IMIBIC), Cordoba, Spain,* [2] *Department of Cell Biology, Physiology and Immunology, University of Cordoba, Cordoba, Spain,* [3] *CIBEROBN, Instituto de Salud Carlos III, Madrid, Spain,* [4] *FiDiPro Program, University of Turku, Turku, Finland*

Puberty and reproductive function are essential for the perpetuation of the species and, hence, are under the control of sophisticated regulatory networks, which integrate central and peripheral signals, as well as external cues. Among numerous regulators, puberty and fertility are highly sensitive to metabolic signals, and different metabolic stressors, ranging from subnutrition to morbid obesity, are known to have a discernible impact on the reproductive axis. In turn, gonadal factors substantially influence body weight and metabolism along the lifespan,

acting at central and peripheral levels. While the neuro-endocrine substrate for such a close bidirectional relationship remains ill defined, our knowledge of the mechanisms whereby whole body metabolism and reproductive function are reciprocally controlled has recently expanded significantly. This has been due, to a large extent, to the discovery of the reproductive effects of leptin and other metabolic hormones (e.g., ghrelin, PYY3-36 or GLP-1), as well as the identification of the key reproductive roles of the neuropeptide, kisspeptin, that seemingly plays an important function as relay for the metabolic control of puberty and fertility at central levels. In this presentation, we will briefly summary these recent developments and will focus our attention on recent findings illustrating the roles of brain circuits involving cellular energy sensors, such as AMPK, and neuropeptide partners of kisspeptin, such as melanocortins, in the integral regulation of puberty, reproduction and body weight homeostasis.

S12.2. SEASONAL PLASTICITY OF THE BRAIN: THE USE OF SHEEP MODEL TO STUDY LEPTIN RESISTANCE AND OBESITY

Dorota A. Zięba, Małgorzata Szczęsna, Katarzyna Kirsz, Edyta Molik

Agricultural University of Krakow, Department of Animal Biotechnology, Laboratory of Biotechnology and Genomics, Krakow, Poland

The core of the leptin resistance hypothesis promulgated several years ago to explain obesity as a result of environmental causes consists of two tenets: the extinction of leptin-induced intracellular signaling downstream of leptin binding to the long form of the neuronal receptor LTRb in the hypothalamus and the impedance to leptin entry imposed at the blood-brain barrier (BBB). A recent comprehensive investigation concluded that a central leptin insufficiency associated with obesity can be attributed to a decreased efficiency of BBB leptin transport and not to leptin insensitivity within the hypothalamus. Interestingly, anorectic leptin's effects are counteracted in some individuals by a natural resistance associated with hyperleptinemia, which is related to changes in hypothalamic sensitivity to leptin associated with, for example, seasonal reproduction, malnutrition or obesity. In sheep, it was observed that the hypothalamus is resistant to leptin in some periods, and this phenomenon is related to the adaptation of these animals to annual changes in energy supply and demand. However, a broad range of ambiguities exists regarding the implications that the intracellular signaling of signal transducer and activator of transcription-2/suppressor of cytokine signaling 3 (STAT2/SOCS3) imparts central leptin resistance. Furthermore, several plausible alternative possibilities have been proposed, such as compensatory functional and anatomical reorganizations in the appetite regulating network (ARN), rearrangements in the afferent hormonal feedback signaling involved in weight homeostasis and modifications in leptin transport to the hypothalamus across the BBB. Taken together, these observations suggest that the contention that impaired intracellular signaling downstream of leptin entry into the ARN expedites environmentally induced obesity remains unsubstantiated and requires further evidence.

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S12.3. MAPPING SYNAPTIC INPUTS TO KISSPEPTIN NEURONS USING A CONDITIONAL TRANSNEURONAL VIRAL TRACER

William H. Colledge, Shel Hwa-Yeo

Physiology, Development and Neuroscience, University of Cambridge, Cambridge, UK

Kisspeptin neuropeptides, encoded by the Kiss1 gene, are key regulators of the mammalian reproductive axis by potently stimulating GnRH release. Kiss1 neurons are located in two main areas of the hypothalamus: the AVPV region, which controls the preovulatory LH surge and the ARC region, which controls basal GnRH pulsatility. One of the fundamental steps in understanding how the reproductive axis is co-ordinated with other physiological processes is an accurate description of the neuronal circuitry communicating with Kiss1 neurons. We have generated a transgenic mouse line that expresses the CRE recombinase specifically in Kiss1 neurons. We have used this mouse line to undertake conditional viral tracing with a genetically modified pseudorabies virus (Ba2001) to define afferent neuronal inputs to Kiss1 neurons. Several of these neuronal populations have been implicated as physiologically relevant in controlling the reproductive axis. These include the suprachiasmatic nucleus, which communicates information about day length; the subfornical organ, which provides information about peripheral metabolic status; the amygdala, which responds to pheromone signals and POMC and NPY neurons in the ARC, which regulate feeding behaviour. We are currently studying these connections to define their functional relevance in regulating Kiss1 neuronal activity.

FINANCIAL SUPPORT: This work was funded by a BBSRC grant (BB/K003178/1).

S12.4. KNDY NEURONS AND REPRODUCTIVE DYSFUNCTIONS IN ANIMAL MODELS OF OBESITY AND DIABETES

Joanna H. Śliwowska

Laboratory of Neurobiology, Department of Veterinary Medicine and Animal Husbandry, Poznan University of Life Sciences, Poznan, Poland

The current obesity and type 2 diabetes epidemic is thought to be largely attributable to excessive consumption of foods and the lack of or very limited physical activity. As obesity and diabetes are widespread throughout the globe, the World Health Organization recognized both diseases among the biggest public health problems. Besides primary metabolic health problems occurring in people with obesity and diabetes, there are numerous secondary problems, including major disruptions of the reproductive system, manifested by disrupted menstrual cycles in women, decreased testosterone levels and spermatogenesis in men, hypogonadism, premature child birth, miscarriages and infertility. However, there is still a fundamental lack of synthetic knowledge considering integration of metabolic and reproductive systems in obese and diabetic patients. Basic research is essential if we are to uncover the mechanisms responsible for metabolic and reproductive failure in cases of obesity and diabetes. Reproduction is influenced by metabolic cues and is governed by the hypothalamic-pituitary-gonadal (HPG) axis and kisspeptin plays an important role in the integration of metabolic and reproductive systems. However, kisspeptin does not act alone to regulate reproduction. A subset of neurons was identified in the arcuate nucleus of the hypothalamus (ARC) that co-localize, in addition to kisspeptin, the neuropeptides, neurokinin B (NKB), and dynorphin (DYN), so called "KNDy neurons". In this talk recent data on possible mechanisms responsible for disruptions of reproduction in animal models of diet-induced obesity and diabetes will be presented.

FINANCIAL SUPPORT: NCN OPUS grant 2015/17/B/NZ4/02021.

S13.1. LOW-BASICITY AGONISTS OF SEROTONIN 5-HT7 RECEPTORS

Adam S. Hogendorf[1], Agata Hogendorf[1], Rafał Kurczab[1], Grzegorz Satała[1], Tomasz Lenda[1], Maria Walczak[2], Gniewomir Latacz[2], Jadwiga Handzlik[2], Katarzyna Kieć-Kononowicz[2], Joanna Wierońska[1], Monika Woźniak[1], Paulina Cieślik[1], Ryszard Bugno[1], Jakub Staroń[1], Andrzej J. Bojarski[1]

[1] Institute of Pharmacology Polish Academy of Sciences, Krakow, Poland, [2] Faculty of Pharmacy, Jagiellonian University Medical College, Krakow, Poland

The 5-HT7R is involved in many physiological processes, i.e. the regulation of body temperature, circadian rhythm, learning and memory, as well as pathophysiological processes such as mood disorders, anxiety, schizophrenia and pain. None of the known 5-HT7R agonists qualify as perfect radioligand candidates, mainly due to their poor selectivity, metabolic stability or non-optimal ADME properties. Development of new ligands of 5-HT7R of the unique structure and properties. The compounds were concisely synthesized using van Leusen multi-component protocol and receptor affinity (5-HT7, 1A, 2A, 6, and D2) was tested in radioligand binding assays. The intrinsic clearance was determined using human liver microsomes, whereas cytotoxicity was measured on HEK-293 and HepG2 cells. The pharmacokinetics of a lead compound was tested on CD-1 mice at 5 mg/kg dose (i.p.). The compound ability to reverse MK-801 induced impairment in novel object recognition test was conducted on Albino Swiss mice. Results: We have developed a series of 5-HT7R ligands, which are one of the very few examples of low-basicity aminergic receptor agonists. Lead compounds exhibited high affinity for the 5-HT7R as well as high efficacy as agonists, excellent selectivity over related CNS targets, high metabolic stability and low toxicity. Docking to 5-HT7R homology models indicated a plausible binding mode which explain the unusually high selectivity. A rapid absorption to the blood, high blood-brain barrier permeation and a very high peak concentration in the brain were found for the lead compound. It was also found active in the NOR test. Because the compounds fulfill all the requirements needed for a PET radioligand a synthetic method which enables the incorporation of 11C isotope was developed. The obtained group of selective, low-basicity 5-HT7R receptor agonists has a great potential to be developed as pharmacological tools, radioligands or drug candidates.

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S13.2. ACTIVATION OF 5-HT7 RECEPTORS FOR SEROTONIN MODULATES HIPPOCAMPAL SYNAPTIC PLASTICITY IN PHYSIOLOGICAL CONDITIONS AND IN A MOUSE MODEL OF FRAGILE X SYNDROME: INVOLVEMENT OF CYCLIC AMP, INTRACELLULAR KINASES AND PROTEIN SYNTHESIS

Lara Costa[1], Lara M. Sardone[2], Michela Spatuzza[3], Carmela M. Bonaccorso[4], Simona D'Antoni[3], Daniela Puzzo[2], Walter Gulisano[2], Marcello Leopoldo[5], Enza Lacivita[5], Maria V. Catania[3,4], Lucia Ciranna[2]

[1] Dept. of Clinical and Experimental Medicine, University of Messina, Messina, Italy, [2] Dept. of Biomedical and Biotechnological Sciences, University of Catania, Catania, Italy, [3] Institute of Neurological Sciences (ISN), Natlional Research Council (CNR), Catania, Italy, [4] Lab. of Neurobiology, IRCCS Oasi Maria Santissima, Troina (EN), Italy, [5] Dept. of Pharmacy, University of Bari, Bari, Italy

The neurotransmitter serotonin (5-HT) is widely diffused in the central nervous system and controls many brain functions, among which mood and cognition. Fragile X Syndrome is the most common form of inherited intel-

lectual disability, frequently associated with epilepsy and autism. Fmr1 KO mice, an animal model of Fragile X Syndrome, display excessive metabotropic glutamate receptor-mediated long-term depression (mGluR-LTD), altered dendritic spine morphology, learning deficit and autistic behavior. We have shown that activation of serotonin 5-HT7 receptors (5-HT7Rs) reverses mGluR-LTD in wild-type (wt) and Fmr1 KO mice. To identify the mechanisms underlying 5-HT7R-mediated effects, we used patch clamp on hippocampal slices from wt and Fmr1 KO mice to test the effects of 5-HT7R agonists on mGluR-LTD in the presence of specific blockers of intracellular messengers. Our results show that 5-HT7R activation reverses mGluR-LTD in wt and Fmr1 KO slices acting through a cAMP-dependent mechanism involving ERK, Cdk5 and GSK3 kinases and protein synthesis; we are currently investigating at which level these pathways interact. Consistent with our *in vitro* results on hippocampal slices, we found that *in vivo* systemic administration of a 5-HT7R agonist improved learning in wt mice and rescued dendritic spine morphology, learning and behavior in Fmr1 KO mice. Taken together, our data show that 5-HT7 receptors play a crucial role in learning and memory in physiological conditions and indicate that activation of 5-HT7 receptors might become a novel therapeutic strategy for Fragile X Syndrome.

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S13.3. SYNAPTIC REMODELING DEPENDS ON SIGNALING BETWEEN SEROTONIN RECEPTORS AND THE EXTRACELLULAR MATRIX

Jakub Włodarczyk[1], Monika Bijata[1], Josephine Labus[2], Daria Guseva[2], Piotr Michaluk[3], Dmitri A. Rusakov[3], Alexander Dityatev[4,5], Joanna Dzwonek[6], Evgeni Ponimaskin[2]

[1] Department of Molecular and Cellular Neurobiology, Nencki Institute of Experimental Biology Polish Academy of Science, Warsaw, Poland, [2] Cellular Neurophysiology, Center of Physiology, Hannover Medical School, Hannover, Germany, [3] UCL Institute of Neurology, University College of London, London, UK, [4] Molecular Neuroplasticity Group, German Center for Neurodegenerative Diseases (DZNE), Magdeburg, Germany, [5] Medical Faculty, Otto-von-Guericke University, Magdeburg, Germany, [6] Department of Neurophysiology, Nencki Institute of Experimental Biology of Polish Academy of Science, Warsaw, Poland

The rewiring of synaptic circuitry pertinent to memory formation in the brain has often been associated with morphological changes in dendritic spines and extracellular matrix (ECM) remodeling. Here, we linked these processes by uncovering the signaling pathway involving the serotonin 5-HT7 receptors (5 HT7R) the matrix metalloproteinase-9 (MMP-9), the hyaluronan receptor CD44, and the small GTPase Cdc42. We highlight a physical interaction between 5-HT7R and CD44 (identified as a novel MMP 9 substrate in neurons) on the nanoscale, and find that 5-HT7R stimulation increases local MMP 9 activity triggering dendritic spines remodeling, synaptic pruning and impairment of long-term potentiation (LTP). The underlying molecular machinery involves 5-HT7R-mediated activation of MMP-9, which leads to CD44 cleavage followed by Cdc42 activation. Pharmacological/genetic suppression of this pathway rescues the 5-HT7R-induced synaptic changes and the deficit in LTP. Our results thus reveal causal interactions in a previously unknown molecular mechanism regulating neuronal plasticity.

FINANCIAL SUPPORT: The work was supported by the National Science Centre (grant no. DEC-2012/06/M/NZ3/00163), TANGO1/269352/NCBR/2015, Deutsche Forschungsgemeinschaft (grant no. PO732, excellence cluster REBIRTH), and ERA-NET Neuron/BMBF funding for the TargetECM project to E.P and A.D.

S13.4. 5-HT7 RECEPTOR-DEPENDENT MODULATION OF GABAERGIC AND GLUTAMATERGIC TRANSMISSION IN THE DORSAL RAPHE NUCLEUS OF THE RAT

Krzysztof Tokarski[1], Magdalena Kusek[1], Joanna G. Sowa[1], Grzegorz Hess[1,2]

[1] Department of Physiology, Institute of Pharmacology Polish Academy of Sciences, Krakow, Poland, [2] Institute of Zoology and Biomedical Research, Jagiellonian University, Krakow, Poland

The 5-HT7 receptor is one of the several serotonin (5-HT) receptor subtypes that are expressed in the dorsal raphe nucleus (DRN). Some earlier findings suggested that 5-HT7 receptors in the DRN are localized on the GABAergic interneurons and glutamatergic terminals which modulate the activity of 5-HT DRN projection neurons. The present study was aimed at finding how the 5-HT7 receptor modulates the GABAergic and glutamatergic synaptic inputs to 5-HT DRN neurons, and whether blockade of the 5-HT7 receptor would affect the release of 5-HT in the target structure. Male Wistar rats with microdialysis probes implanted in the prefrontal cortex (PFC) received injections of the 5-HT7 receptor antagonist SB 269970, which induced an increase in the levels of 5-HT and its metabolite, 5 hydroxyindoleacetic acid (5-HIAA) in the PFC. In another set of experiments whole-cell recordings from presumed projection neurons were carried out from DRN slices. SB 269970 application resulted in depolarization and in an increase in the firing frequency of the cells. In order to activate 5-HT7 receptors, 5-carboxamidotryptamine (5-CT) was applied in the presence of a selective 5-HT1A receptor antagonist WAY100635. Hyperpolarization of cells and a decrease in the firing frequency were observed after activation of the 5-HT7 receptor. Application of 5-CT induced a concentration-dependent increase in the frequency of sIPSCs and

a decrease in sEPSCs frequency in recorded neurons. Blockade of 5-HT7 receptors caused opposite effects.

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S14.1. DISTINCT MICROGLIAL PHENOTYPES IN BRAIN DISEASES

Helmut Kettenmann

Max Delbrueck Center for Molecular Medicine in the Helmholtz Association, Berlin, Germany

Ten years ago we promoted the concept that microglial activation is not an all or none process but is highly diverse depending on the type of pathology and time point during the pathologic process. We have, therefore, studied aspects of microglial properties in mouse models of Alzheimer's Disease, schizophrenia, and glioma. In Alzheimer's Disease two functions of microglial cells are impaired, namely the phagocytic activity and the ability to respond to a local injury. Phagocytic activity is controlled by P2Y6 receptors and we recently found that also purinergic signaling is impaired. An impairment of phagocytic activity was also found in microglia isolated from a mouse model of schizophrenia. In glioma, microglial and invading monocytes accumulate and these glioma associated brain macrophages (GAMs) phagocytic activity is increased. The GAM phenotype is altered in a very characteristic manner not reflecting the classical M1 or M2 phenotype of activation. We found two mechanisms altered in GAMs which helped to promote glioma growth, namely the upregulation of metalloproteases MT1/MMP and MMP9. This supports the hypothesis that microglial cells can obtain diverse phenotypes depending on the pathologic state.

S14.2. MILD NEUROINFLAMMATORY PROFILE WITHOUT GLIOSIS IN MICE – MODELLING AGE-RELATED PARKINSON'S DISEASE

Marina Pizzi

University of Brescia, Brescia, Italy

Awaiting for submission.

S14.3. FUNCTIONAL HETEROGENEITY OF MICROGLIA AND MACROPHAGES IN THE ISCHEMIC BRAIN

Bożena Kamińska

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

Microglia are myeloid cells residing in the central nervous system (CNS) that rapidly respond to signals originating in the injured or infected brain. Microglia respond differently to challenges and acquire different functional phenotypes with extremes: an inflammatory, detrimental M1 or immunosuppressive, cytoprotective M2 phenotype. Post-ischemic inflammation plays a key role in secondary and delayed neuronal damage and death, and involves activation of microglia, perivascular and peripheral macrophages, that accumulate in the ischemic brain and contribute to disease. The molecular signature of myeloid cells in the inflammed brain, and specific roles of different populations are unclear. Lack of robust markers that differentiate microglia from macrophages makes it less feasible. We developed effective, flow cytometry-based methods for sorting myeloid subpopulations that accumulate in the hemispheres of sham-operated and ischemic rats undergoing transient 90 min MCA (middle cerebral artery) occlusion. We analyzed transcriptomes of specific populations by RNAseq and evaluated their functional phenotypes. This analysis revealed a prevalence of inflammatory M1 microglia 1 day after MCAo and accumulation of M2-immunosuppressive macrophages 3–7 days after MCAo. Ablation of peripheral macrophages reduced abundance of M2 immunosuppressive, Arg1 expressing macrophages. Using immunofluorescence and confocal microscopy we demonstrated that with time Arg1+ macrophages lose their M2-phenotype and become inflammatory iNos expressing cells. Moreover, we found that macrophages of perivascular space and meninges (defined by CD163+ staining) are a distinct myeloid subpopulation in the brain, and these cells proliferate and migrate to the ischemic parenchyma after MCAo. Altogether, we demonstrate distinct functional properties and transcriptional networks in brain macrophages, that suggests functional plasticity and distinctive functions in neuroinflammation and repair.

S14.4. MINOCYCLINE AFFECTS NEUROPATHIC PAIN BY REGULATION OF KYNURENIC PATHWAY – ROLE OF MICROGLIAL CELLS

Ewelina Rojewska, Anna Piotrowska, Agnieszka M. Jurga, Wioletta Makuch, Barbara Przewłocka, Joanna Mika

Institute of Pharmacology Polish Academy of Sciences, Department of Pain Pharmacology, Krakow, Poland

The participation of kynurenine system in the pathology of neurodegenerative and autoimmune diseases was studied, but the significance of this pathway in neuropathic pain have been poorly studied. The kynurenine pathway has already been shown to exist mainly in macrophages and microglia. Growing evidence suggests that spinal microglia are crucial in the neuropathic pain. Mino-

cycline, a microglial inhibitor, is a substances with diverse mechanisms of action that modulate the neuroimmune system have been shown to relieve neuropathic pain. The aim of our study was to examine the role of kynurenine 3-monooxygenase (Kmo) in a rat model of neuropathy. Chronic constriction injury (CCI) of the sciatic nerve was performed according to Bennett and Xie (1988). Behavioral studies consisted of the tactile and thermal hypersensitivity measurements, biochemical studies comprised the RT-PCR and/or Western blot analysis in the tissue (spinal cord, DRG) and primary glia cultures. The experiments were carried out according to IASP rules. Using microarray and qRT-PCR methods, we showed that intraperitoneal administration of minocycline decreased neuropathic pain in rats and in parallel the spinal 3-monooxygenase kynurenine expression (Kmo). Further, minocycline administration diminished the lipopolysaccharide (LPS)-induced upregulation of Kmo mRNA in primary microglial cell cultures. Moreover, we verified that not only indirect inhibition of Kmo using minocycline but also direct inhibition using Kmo inhibitors (JM6,Ro61-6048) decreased neuropathic pain intensity. Ro61-6048 administration reduced in the spinal cord and/or the DRG the protein levels of IBA-1, IL-6, IL-1beta and NOS2. Interestingly, Kmo inhibitors potentiated the analgesic properties of morphine. Summing up, our results suggest that the kynurenine pathway is an important mediator of neuropathic pain pathology.

FINANCIAL SUPPORT: Supported by National Science Centre grant-Sonata 2015/17/D/NZ4/02284 and statutory funds. AP is a scholarship holder from the KNOW sponsored by Ministry of Science and Higher Education, Republic of Poland.

S15.1. MOTOR PROTEINS IN MICROCEPHALY AND AUTOPHAGY

Richard Vallee, Noopur Khobrekar

Department of Pathology & Cell Biology, Columbia University, New York, USA

Microtubule motor proteins play important roles in a very diverse range of biological functions. Recent work from our lab has revealed contributions by cytoplasmic dynein and kinesins to neurogenesis and neuronal migration during embryonic rat brain development. We found cytoplasmic dynein and its regulators LIS1, Nde1, Ndel1 to be responsible for apical nuclear migration in Radial Glial Progenitor cells (RGPs), and the kinesin Kif1a to be responsible for basal nuclear migration. Inhibition of dynein regulatory genes blocked apical nuclear migration and mitotic entry in the RGP cells, leading to microcephaly. Inhibition of Kif1a also causes microcephaly. In this case knockdown or mutation of the motor protein decreased the ratio of asymmetric:symmetric RGP divisions, and interfered with BDNF-mediated neuronal migration, which, strikingly, could be rescued in brain slice preparations by BDNF application (2). In separate studies, we have identified a novel role in cultured neurons for a cytoplasmic dynein adaptor protein in autophagosome biogenesis and transport. The adaptor, furthermore, is activated by mTOR inhibition. Our results suggest that, in response to cellular stress, the adaptor catalyzes autophagosome biogenesis and then transports mature autophagosomes and autophagolysosomes to the cell body. The adapter appears to represent the first mTOR-regulated link between the autophagy and motor protein pathways.

S15.2. UNCONVENTIONAL MYOSINS AS REGULATORS OF SYNAPTIC FUNCTION AND DEVELOPMENT

Wolfgang Wagner[1], Kristina Lippmann[2], Christopher Alexander[3], John A. Hammer III[3], Sönke Hornig[1], Sandra Freitag[1], Martijn Schonewille[4], Franco Lombino[1], Mona Roesler[1], Jürgen R. Schwarz[1], Chris De Zeeuw[4], Jens Eilers[2], Matthias Kneussel[1]

[1] Department of Molecular Neurogenetics, Center for Molecular Neurobiology (ZMNH), UKE, Hamburg, Germany, [2] Carl Ludwig Institute for Physiology, Medical Faculty, University of Leipzig, Leipzig, Germany, [3] Cell Biology and Physiology Center, National Heart, Lung and Blood Institute, NIH, Bethesda, USA, [4] Department of Neuroscience, Erasmus MC, Rotterdam, The Netherlands

Myosins are a large family of functionally diverse cytoskeletal motors that use actin filaments as tracks and that produce movement and force. Actin filaments are highly enriched within dendritic spines, the tiny postsynaptic compartments that carry excitatory synapses. By investigating the roles of unconventional myosins, we aim to shed light on the actin-dependent development and function of excitatory neuronal synapses. We employ cerebellar Purkinje cells (PCs) as a neuronal model system. Importantly, postsynaptic plasticity in these central cerebellar signal integrators appears to be crucial for motor learning. Interestingly, we were able to provide direct evidence that the processive class V myosin, myosin Va, is a point-to-point organelle transporter that moves endoplasmic reticulum as cargo into the dendritic spines of PCs. The spine endoplasmic reticulum supports local calcium signaling that is thought to be required for synaptic long-term depression at parallel fiber to Purkinje cell synapses (PF-PC LTD). We also examined the role of myosin VI, the only myosin known to move towards the minus end of the actin filament. Our data reveal that myosin VI affects AMPAR trafficking and dynamics in PCs. Importantly, we also find that myosin VI is crucial for postsynaptic function and plasticity of PCs. Nevertheless, myosin VI expression in PCs does not appear to be essential for motor coordination or cerebellum-dependent motor learning since myosin VI knock-

out specifically in PCs does not lead to impairments in this respect. Taken together, using cerebellar PCs as a model system, we are able to provide novel insights into the cellular mechanism of action of unconventional myosins and elucidate their roles for postsynaptic development and function.

FINANCIAL SUPPORT: Marie Curie FP7 Integration Grant (PCIG11-GA-2012-321905) within the 7th European Union Framework Programme; DFG Research Unit FOR2419, project Wa3716/1-1; Landesforschungsförderung Hamburg FV27.

S15.3. ROLE OF POSTTRANSLATIONAL MODIFICATIONS OF TUBULIN IN NEURONAL FUNCTION

Andrzej A. Kasprzak[1], Beata Kliszcz[1], Michał Szkop[1], Dorota Włoga[2], Grzegorz M. Wilczyński[3]

[1] Institute of Experimental Biology, Department of Biochemistry, Warsaw, Poland, [2] Institute of Experimental Biology, Department of Cell Biology, Warsaw, Poland, [3] Institute of Experimental Biology, Department of Neurophysiology, Warsaw, Poland

The axon is a slender projection of a neuron that conducts electrical impulses to synapses in muscles or other neurons. A number of cellular components such as mitochondria and vesicles are transported along the axon by microtubule-based molecular motors (kinesins and dyneins). The most important factors controlling the axonal transport are the structure and function of the motors involved and the condition of the rails (the microtubule, MT). In the last decade, a compelling evidence has been provided that MTs contain marks (post-translational modifications, PTMs) that indicate what kind of activity should take place in a given MT segment. These cues are read and interpreted by molecular motors, MAPS and other proteins. Kinesin-1, the major motor that transports cargoes along MTs is a homodimer with a pair of MT-binding sites on each end of the molecule. In some conditions, kinesin-1, besides interacting with MT using its N-terminal motor domains, can also bind another MT by its tail site producing sliding of one MT relative to another. This type of pair sliding can be used to sort MTs in the same way it occurs in the mitotic spindle and also act as an efficient way to move large amounts of tubulin, in the form of short MTs. Both activities have been observed in Drosophila cultured neurons. The effects of PTMs on the interaction of the motor domain with MT are to some extent characterized, but the effects of PTMs in the cargo-MT on the MT pair sliding have never been examined. Currently, we are exploring the impact of two well-known PTMs (detyrosination and polyglutamylation) and one recently reported (polyamination) on MT-MT sliding. To that end, we have developed an assay in which the sliding is observed *in vitro* and quantified by kymographic analysis. Surprisingly, the velocity of the sliding was highly variable along the track indicating that its efficiency may be sensitive to many cellular and developmental mechanisms.

FINANCIAL SUPPORT: Supported in part by NCN Grant 2014/13/B/NZ1/03995.

S15.4. ROLE OF MYOSIN VI-DOCK7 INTERACTION IN NEURONAL CELLS

Maria J. Rędowicz

Nencki Institute of Experimental Biology, Warsaw, Poland

Myosin VI (MVI) is a unique unconventional myosin as unlike all other myosins it walks towards the minus (pointed) end of actin filaments. It is involved in many cellular functions related to intracellular transport and organization of the actin cytoskeleton. MVI involvement in a given function depends on its interactions with its tissue/cell specific partners. Several studies show that MVI plays a role in glutamate receptor internalization, synaptic transmission and synaptic vesicle recycling. Our search for MVI partners resulted in identification of DOCK7 as its potential partner in neuronal-lineage PC12 cells. Since DOCK7, a protein with GEF activity towards Rac1 and Cdc42, is crucial for axon formation, we aimed at characterization of physiological relevance of this novel interaction in neuronal cells, including neurosecretory PC12 cells and primary culture neurons. We confirmed that this interaction occurred also in neurons, and biochemically characterized MVI-DOCK7 binding sites. The presence of MVI was necessary for both DOCK7 localization and activity as well as for NGF-stimulated protrusion formation, as revealed for PC12 cells. Studies on primary culture neurons revealed that co-localization of both proteins was maintained during the culture time-course and was visible within cell body, neurites, dendritic spines and neurite growth cones. Also, lack or depletion of MVI affected the dendritic arbor formation and morphology of axonal growth cone. MVI-DOCK7 co-localization was also visible in the brain. Studies on brains of Snell's waltzer mice (SV) that do not synthesize MVI also revealed a decrease of DOCK7 activity measured by the levels its own and its downstream effectors phosphorylation. Of note, SV mice exhibit several neuronal dysfunctions such as deafness, circling and head tossing behavior. Taken together, our data indicate that MVI-DOCK7 interaction plays important role in the neuronal system.

FINANCIAL SUPPORT: The work was supported by a grant UMO-2012/05/B/NZ3/01996 from the National Science Centre and statutory funds for the Nencki Institute of Experimental Biology from Ministry of Science and Higher Education.

POSTERS

POSTER SESSION 1

P1.1. RATS REDOX STATUS CHANGES TO LOW ETHANOL (HORMETIC) DOSE EXPOSURE

Diana Museridze, Igor Svanidze, Lali Gegenava, Tamar Sanikidze, Nino Gvinadze, Ketevan Aptsiauri

I. Beritashvili Center of Experimental Biomedicine, Departement of Neurotoxicology, Tbilisi, Georgia

INTRODUCTION: In the aspect of the hormesis concept, ethanol is considered to be a classic example, as the consumption of low or moderate doses of ethanol is associated with positive phenomena. Alcohol consumption by women during pregnancy leads to the formation of Fetal Alcohol Syndrome (FAS), that causes the violation of the basic processes of neurogenesis. Under the conditions of hormesis the stimulation of cell endogenous protective mechanisms causes the neuroprotective effect of low doses of toxic substances and allows the therapeutic use of hormesis. Therefore, it is important research of alcohol hormetic effect on the nervous system.

AIM(S): The aim of this study was to determine the neuroprotective effect of hormetic dose of ethanol in the offspring of female white rats during pregnancy intraperitoneally treated with ethanol at low doses.

METHOD(S): We studied free radicals signal intensity in cerebral cortex and hippocampus of 60-day-old offspring of female white rats, treated hormetic dose (0.25 g/kg) of ethanol during pregnancy. The intensity of free radicals by electron paramagnetic resonance (EPR) method was determined with use of spin-trap α-phenyl-tertbutilnitron (PBN) for lipoperoxide radicals (LOO·) and the free nitric oxide (NO) for sodium diethil-ditio-carbomate (DEDTC).

RESULTS: The significant changes in EPR signals of spin-trapped LOO· in the cortex and hippocampus samples of experimental group rats were not detected. The intensity of NO EPR signal was statistically reliably increased in the cortex and was not changed in the hippocampus. A number of studies show the increase in iNOS and nNOS protein synthesis and the activity under the influence of alcohol. Probably the low (hormetic) dose of alcohol induces only slight activation of NO-synthase, resulted in 7% increase of free NO content in the cortex.

CONCLUSIONS: In the present series of experiments was not observed intensification of free radical oxidation in brain tissues.

FINANCIAL SUPPORT: The study was supported by the Sh. Rustaveli National Science Foundation of Georgia.

P1.2. CIRCULATING MICRORNA AS A BIOMARKER OF EPILEPTOGENESIS AND EPILEPSY IN THE RAT MODEL OF TEMPORAL LOBE EPILEPSY

Kinga Szydłowska, Karolina Nizińska, Anna Bot, Katarzyna Łukasiuk

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

INTRODUCTION: Epilepsy frequently develops as a result of brain insult, for example: brain injury, status epilepticus, or stroke, however currently there are no tools allowing us to predict which patients suffering from trauma will eventually develop epilepsy or how severe it is going to be. In recent years small non-coding RNAs are proposed as biomarkers for neurological diseases. Particularly microRNAs are interesting candidates, as several of them were described changing their levels in the brain of epileptic subjects. There is evidence suggesting that microRNAs levels are altered also in the plasma, making them attractive candidates for peripheral biomarkers of epilepsy.

AIM(S): This study was conducted to evaluate usefulness of plasma miRNAs as biomarkers of epileptogenesis and epilepsy.

METHOD(S): In our studies we used the rat model of temporal lobe epilepsy. The status epilepticus was evoked by the stimulation of left lateral nucleus of amygdala. Animals were continuously video and EEG monitored for 6 months. Blood was collected at 14, 30, 60, and 90 days after stimulation from tail vein. Blood plasma was separated and processed using Affymetrix miRNA 4.1 array strip microarrays.

RESULTS: We have compared miRNA levels between sham operated (n=12) and stimulated animals (n=15); $p<0.01$ was used as a cut off. We have detected 14 miRNA differentiating between sham operated and stimulated animals at 14 days, 6 at 30 d, 16 at 60d, and 11 at 90 days. We have also compared the miRNAs levels between animals with high (30–70 seizures/day) and low (1–5 seizures/day) number of seizures. We found differences in levels of 11 miRNA at 14 d, 7 at 30 d, 11 at 60 d and 8 at 90 d (at $p<0.01$).

CONCLUSIONS: Levels of miRNA in plasma are altered during epileptogenesis and differentiate between animals with frequent and rare seizures. miRNA may become a useful biomarker of epileptogenesis/epilepsy as well as severity of the disease.

FINANCIAL SUPPORT: This work was supported by the FP7-HEALTH project 602102 (EPITARGET) and Polish Ministry of Science and Education grant W19/7.PR/2014.

P1.3. HUR STABILIZES MRNA FOR MMP-9 (MATRIX METALLOPROTEINASE-9) CONTRIBUTING TO ITS EPILEPTOGENESIS--RELATED UPREGULATION IN THE HIPPOCAMPUS

Katarzyna Żybura-Broda, Małgorzata Wolder-Gontarek, Agnieszka Bogusz, Magdalena Ambrozek-Latecka, Artur Choroś, Joanna Wilemska, Marcin Rylski

Centre of Postgraduate Medical Education, Department of Clinical Cytology, Warsaw, Poland

INTRODUCTION: Epilepsy is one of the most common neurological disorders in humans. Precise pathogenesis of epilepsy is complex and unclear. The Matrix Metalloproteinase-9 (MMP-9) is a proepileptic protein involved in a formation of aberrant brain neuronal networks during epileptogenesis, what finally leads to the development of seizures. Despite of its essential role in etiology of epilepsy, regulation of the MMP-9 expression during epileptogenesis is almost unknown. Similarly, completely obscured is a dependence of the MMP-9 expression on the mRNA stabilization mechanisms in the epilepsy.

AIM(S): Our goal was to determine mechanisms responsible for the MMP-9 mRNA stability changes occurring in the rat hippocampus during epileptogenesis.

METHOD(S): We used two models to study the mRNA stabilization-dependent regulation of MMP-9 during epileptogenesis: the pentylenotetrazole (PTZ)-dependent kindling in rats (*in vivo* pharmacological model of epileptogenesis) and the generation of the spontaneous recurrent epileptform discharges (SREDs) in cultured rat hippocampal neurons (*in vitro* model of epilepsy).

RESULTS: Considering the MMP-9 mRNA expression profile and results obtained using the RNA degradation assay, we observed significant stabilization of the MMP-9 mRNA during epileptogenesis, and corresponding to this phenomenon, a gradual upregulation of its hippocampal mRNA expression during epileptogenesis. Interestingly, our data collected with REMSA supershift assays, RIPA, protein mass spectrometry as well as functional HuR overexpression and depletion studies have showed that HuR directly binds to the ARE1 and ARE4 sites in the 3'UTR of MMP-9 mRNA and therefore stabilize MMP-9 mRNA.

CONCLUSIONS: The epileptogenesis-evoked upregulation of MMP-9 expression in the rat hippocampus is clearly and strongly dependent on its mRNA stabilization mediated by HuR action related to its direct binding to the ARE1 and ARE4 sites in the 3'UTR of MMP-9 mRNA.

FINANCIAL SUPPORT: This work was supported by the Polish National Science Centre grant no. 2012/05/B/N23/01943.

P1.4. DOPAMINE, SEROTONIN AND NOREPINEPHRINE TRANSPORTERS BINDING IN MOTOR BRAIN STRUCTURES OF 6-OHDA-LESIONED RATS TREATED CHRONICALLY WITH AMITRIPTYLINE AND L-DOPA

Kinga Kamińska, Jadwiga Wardas, Jolanta Konieczny, Tomasz Lenda, Elżbieta Lorenc-Koci

Department of Neuropsycho-pharmacology, Institute of Pharmacology Polish Academy of Sciences, Krakow, Poland

INTRODUCTION: Depression frequently accompanied to Parkinson's disease (PD).

AIM(S): The aim of the study was to examine the effects of chronic treatment with amitriptyline(AMI) and L-DOPA on binding to dopamine (DAT), serotonin (SERT) and norepinephrine (NET) transporters in the substantia nigra (SN) and striatum (STR) of the unilaterally 6-OHDA-lesioned rats.

METHOD(S): Experiments were performed on Wistar Han rats receiving unilaterally 16 µg/4 µl of 6-OHDA into the medial forebrain bundle (MFB). Two weeks later, rats exhibiting at least 100 contralateral turns/1 h in the apomorphine test were treated with AMI (10 mg/kg) and L-DOPA (12 mg/kg), alone or in combination, once daily for 21 consecutive days. The rats were sacrificed 1h after the last injection, their brains were dissected and frozen. The binding of [3H] GBR 12,935 to DAT, [3H] citalopram to SERT and [3H] nisoxetine to NET was assayed on nigral and striatal tissue sections.

RESULTS: Injection of 6-OHDA into MFB caused a decline in [3H] GBR 12,935 binding to DAT in the ipsilateral SN and STR. On the contralateral side comes to up-regulation of DAT expression both in the STR and SN. AMI but not L-DOPA alone, lowered DAT expression in the contralateral STR. In the contralateral SN, DAT expression in drug treated groups was maintained at a control level. In the SN, the unilateral lesion of dopaminergic innervation caused a significant up-regulation of [3H] citalopram binding to SERT on both sides while in the STR only on the contralateral side. In both structures, L-DOPA did not change [3H] citalopram binding to SERT while AMI, alone or in combination, decreased it markedly on both sides. L-DOPA also decreased NET expression in the STR on both sides while AMI, alone or in combination maintained at the control level.

CONCLUSIONS: Our data indicates that AMI can modulate the release of L-DOPA derived dopamine from serotonergic terminals on ipsilateral side and serotonin on contralateral one. The obtained data is discussed within the context of motor functions in PD.

P1.5. ANTI-APOPTOTIC AND PRO-SURVIVAL EFFECT OF GROUP II METABOTROPIC GLUTAMATE RECEPTORS (MGLUR2/3) ACTIVATION IN AN ANIMAL MODEL OF BIRTH ASPHYXIA

Elżbieta Salińska, Apolonia Ziembowicz, Ewelina Bratek
Department of Neurochemistry, Mossakowski Medical Research Centre Polish Academy of Sciences, Warsaw, Poland

INTRODUCTION: Birth asphyxia results in serious damage of central nervous system or neonatal death. It was shown recently that group II metabotropic glutamate receptors (mGluR2/3) activation results in neuroprotection but the exact mechanism of this effect is not clear.

AIM(S): The aim of present study was to investigate whether neuroprotective effect of mGluR2/3 activation is connected with inhibition of apoptosis and activation of pro-survival neurotrophic factors.

METHOD(S): We used hypoxia-ischemia (HI) on 7-day old rat pups as animal model of birth asphyxia. Animals were anesthetized and the left common carotid artery was isolated, double-ligated and cut between the ligatures. After 60 min of recovery the pups were subjected to hypoxia (7.4% oxygen in nitrogen for 75 min.). Control pups were sham-operated. Animals were injected i.p. with specific mGluR2 (LY 379268) and mGluR3 (NAAG) agonists 1h or 6h after HI (5 mg/kg of b.w.). The weight deficit of the ischemic brain hemisphere was measured and the expression of Bax, Bcl-2, HTR/OMI was examined. The damage in the hippocampal CA1 region was examined by Cresyl violet staining. Differences in the expression of neurotrophic factors (BDNF, GDNF, TGF –beta) were measured using ELISA.

RESULTS: Our results show that application of mGluR2/3 agonists after HI reduce brain damage. Both applied agonists decreased weight loss in ischemic hemisphere independently on the time of application by 50% and reduced the damage of CA1 region of hippocampus. Both mGluR2/3 agonists inhibited HI induced increase in expression of Bax and HTR/OMI and restored, decreased after HI, expression of Bcl-2. LY379268 and NAAG applied 1h or 6h after HI increased TGF beta expression and expression of BDNF and GDNF in the ischemic brain hemisphere.

CONCLUSIONS: Neuroprotective effect of mGluR2/3 activation after HI insult is connected with reduction of apoptotic processes and activation of pro-survival neurotrophic factors.

FINANCIAL SUPPORT: This work was made under MMRC-KNOW 2013–2017 project.

P1.6. PROLONGED SOCS3 GENE RESPONSE FOLLOWING SOMAN INTOXICATION IN THE RAT BRAIN

Edyta Paruszewska, Magdalena Łapot, Marek Brytan, Bożena Antkowiak, Marek Kowalczyk, Magdalena Ciechanowska
The General Karol Kaczkowski Military Institute of Hygiene and Epidemiology, Warsaw, Poland

INTRODUCTION: In comparison with well documented data concerning the mechanisms of acute neurotoxic action of nerve agents such as soman (GD), the immunomodulatory properties of these compounds are still poorly understood, especially considering their long-term effects. One promising candidate for mediation of GD-induced immunomodulation seems to be a suppressor of cytokine signaling 3 (SOCS 3) – an intracellular protein which exhibits a wide variety of physiological effects on immune cell function. There also exists strong evidence to support SOCS3 as a crucial regulator of many disease processes in the central nervous system.

AIM(S): The aim of the present study was to determine whether perinatal exposure to GD exerted distant action on the expression of mRNA encoding SOCS3 in selected tissue of the rats brain.

METHOD(S): Studies were conducted on maternal generation (F0) and on first filial generation (F1) of Wistar rats. F0 animals were treated subcutaneously with a low (0.2×LD50) repeated dose of soman (o-pinacolyl methylphosphonofluoridate). GD was administrated first, in pregnancy, and subsequently during the lactation period. Six months after termination of GD exposure animals were anesthetised and immediately hippocampus, cerebellum and piriform cortex were obtained for subsequent analysis. Real-Time PCR with SYBR Green dye was used to evaluate the level of SOCS3 mRNA in selected structures of the brain.

RESULTS: Intoxication with GD decreased significantly SOCS3 mRNA levels in the cerebellum and in the piriform cortex in F0 females and in their offspring of both sexes. The analogous tendency, but without statistical significance, was observed in the hippocampus of all experimental animals.

CONCLUSIONS: The current data do not clarify distant signs and symptoms of soman exposure; however, a decrease in expression of SOCS3 following intoxication with GD may suggest a functional role of this protein in pathogenesis of GD-induced neurological disorders.

FINANCIAL SUPPORT: This work was supported by Polish Ministry of Science and Higher Education No O R00 0042 08, "Soldier as a precise weapon – packages and sets".

P1.7. LPS INJECTION ON THE 6TH AND 30TH POSTNATAL DAYS MODULATES DIFFERENTLY SEIZURE-INDUCED MICROGLIAL TRANSFORMATION IN ADULT RATS' BRAIN CORTEX. MORPHOMETRIC ANALYSIS

Emilia Kosonowska, Krzysztof Janeczko, Zuzanna Setkowicz
Jagiellonian University, Institute of Zoology, Department of Neuroanatomy, Krakow, Poland

INTRODUCTION: According to previous studies, neuroinflammation may lead to an increase in seizure susceptibility

and trigger epileptogenesis. However, emerging experimental evidence indicates that early age inflammation acting as a preconditioning factor may also have protective effects. Microglia are the immune competent cells of the CNS. After inflammation cells retract their branches and transform into macrophages. Parameters of this morphological changes can be used as parameters of tissue reactivity to inflammation and seizures.

AIM(S): The aim of this study was to examine the long term effects of systemic inflammation induced at different postnatal developmental stages on the range of morphological changes in microglial cells within brain cortex in response to status epilepticus experimentally evoked in adulthood.

METHOD(S): Wistar rats were injected intraperitoneally with LPS on postnatal days 6 (P06) or 30 (P30). Two-month-old animals were injected with pilocarpine to evoke status epilepticus and sacrificed three days later. Brain sections were then processed for Iba-1 immunohistochemistry. A set of photographs were taken from several locations in the brain cortex and the automated Sholl analysis and morphometric measurements were performed.

RESULTS: LPS injection alone on P06 and P30 causes significant decrease of critical radius and enclosing radius and significant increase of solidity. After seizures induced in adulthood statistically significant differences in all examined morphological parameters in both LPS-treated groups were observed. The morphology of microglia in rats' cortex in P06 and P30 group after seizures is significantly closer to morphology of microglial cells in naïve rats.

CONCLUSIONS: This imply that microglia in adult rat's cortex after LPS injection alone in P06 or P30 are more ramified closer to the cell body. Also the longest processes are shorter after LPS injection than in naïve rats. This might result from long-term changes in nervous-tissue reactivity – preconditioning.

FINANCIAL SUPPORT: Supported by NCS GRANT: UMO-2012/05/B/NZ4/02406.

P1.8. STRATEGY IN A PROBABILISTIC CHOICE TASK DEPENDS ON TYPE OF REWARD OFFERED

Jan Rodriguez Parkitna, Łukasz Szumiec

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

INTRODUCTION: Natural rewards and addictive substances both act on the brain's reward system, however it remains unclear whether they reinforce actions through the same mechanisms.

AIM(S): Our goal was to compare the strategy employed by mice when choosing between two actions with variable chance to access alcohol or sweetened solution.

METHOD(S): We have developed a novel method to assess behavioral strategy employed by mice when selecting between options associated with different probabilities of receiving a reward. A group of animals is implanted with radiofrequency chips and introduced to an IntelliCage, where their activity in cage corners is continuously recorded. After a period of adaptation, mice are offered access to saccharin or alcohol solution in two opposite corners, with free access to water in the remaining corners. Initially, upon entering a rewarded corner there is a 90% chance that access to saccharine or alcohol solution will be granted after 2 seconds. As the procedure progresses, the probability changes periodically between 90% and 30% and cycles through all possible combinations between the two rewarded corners.

RESULTS: We tested choice between solutions 0.1% (w/v) saccharin, 4% (v/v) ethanol or a combination of them. In case of the sweetened water reward, the main factor affecting choice was time elapsed from last decision. The effect of the outcome of the previous choice was also significant, but overall smaller. In case of the alcohol solution, the effects of time and previous outcome were weaker, animals were more likely to repeat previous choice. We also observed that irrespective of strategy choices follow a specific time pattern, with majority occurring at discrete intervals.

CONCLUSIONS: The type of reward strongly affected animals' behavioral strategy. Probabilistic access to sweetened water was associated with high probability of switching between choices, while access to alcohol solution led to frequent repeating of the same choice.

FINANCIAL SUPPORT: NCN Sonata Bis UMO-2012/07/E/NZ3/01785.

P1.9. GLUTAMATE, GLUTAMINE AND GABA LEVELS IN THE RAT HIPPOCAMPUS IN THE PHARMACOLOGICAL MODELS OF AUTISM: A MICRODIALYSIS STUDY

Dominik Diamandakis[1], Elżbieta Ziemińska[1], Wojciech Hilgier[1], Robert K. Filipkowski[1], Rafał Polowy[1], Beata Toczyłowska[2], Jerzy W. Łazarewicz[1]

[1] Mossakowski Medical Research Centre Polish Academy of Sciences, Warsaw, Poland, [2] Nalecz Institute of Biocybernetics and Biomedical Engineering Polish Academy of Sciences, Warsaw, Poland

INTRODUCTION: The disorders of the glutamatergic neurotransmission have been implicated in the pathogenesis of autism, but data on brain content of glutamate (Glu) in patients and animal models are inconsistent.

AIM(S): Aim of this study is to evaluate changes in the brain content of Glu, glutamine (Gln) and GABA in the rat models of pharmacologically-induced autism.

METHOD(S): The rat females at the 11th day of gestation were given orally 800 mg/kg b.w. of valproic acid (VPA) or 500 mg/kg b.w. of thalidomide (THAL). The pups at PND 9 were submitted to ultrasonic vocalization (USV) test, and at PND 30, under anesthesia, to *in vivo* unilateral microdi-

alysis of the hippocampus with a calcium-containing medium. The samples of dialysate representing the basal level followed by a 40 min pulse of 100 mM KCl were collected. The contralateral hippocampi were prepared and homogenized. After derivatization of the amino acids with o-phtalaldehyde, the samples were submitted to HPLC analysis with a fluorescence detection.

RESULTS: The results of USV tests showed that the pups prenatally treated with VPA, and to a greater extent with THAL, less frequently produced USV calls, which is regarded as impairment in social communication, a symptom characteristic of autism. In the male rats of the VPA and THAL groups, a total content of Glu increased to 143% and 158%, respectively, and also Gln and GABA contents were significantly elevated. All these values remained unchanged in the female rats. Basal levels of Glu, Gln and GABA in the dialysates of the hippocampi in the experimental groups did not differ from controls, however in VPA-treated male rats during application of 100 mM KCl a reduction by 59% of Gln concentration and tendency to increase GABA level were found.

CONCLUSIONS: The results demonstrate increased content of glutamate in the hippocampus of rats in two chemical models of autism, support a hypothesis on the role of the glutamatergic disturbances in the pathogenesis of autism.

FINANCIAL SUPPORT: This study was supported by the Polish National Science Centre, grant no. 2014/15/B/NZ4/04490.

P1.10. ANALGESIC AND ANTIALLODYNIC PROPERTIES OF NEW 3,3-DIPHENYL-PROPIONAMIDES WITH ANTICONVULSANT ACTIVITY

Anna Rapacz[1], Jolanta Obniska[2]

[1] Department of Pharmacodynamics, Faculty of Pharmacy, Jagiellonian University, Medical College, Kracow, Poland, [2] Department of Medicinal Chemistry, Faculty of Pharmacy, Jagiellonian University, Medical College, Kracow, Poland

INTRODUCTION: Apart from epilepsy treatment, anticonvulsant drugs are also extensively used as efficacious therapy of diverse non-epileptic conditions, including pain (neuropathic pain, migraine prophylaxis), neuromuscular disorders and psychiatric disorders (anxiety, bipolar affective disorder). Therefore, continued preclinical searching for new anticonvulsant drugs with collateral antinociceptive activity are expected since they lead to further advancements in the treatment of epilepsy, as well as neuropathic pain.

AIM(S): The aim of the study was to examine analgesic activity of three new 3,3-diphenyl-propionamides, which demonstrated in the previous research anticonvulsant activity in the MES (the maximal electroshock seizure) and scPTZ (subcutaneous pentylenetetrazole) tests in mice.

METHOD(S): The antinociceptive properties were estimated in four models of pain in mice – the hot plate test (acute pain), the formalin test (tonic pain), the oxaliplatin-induced neuropathy (neuropathic pain), and the streptozotocin-induced diabetic neuropathy.

RESULTS: In the hot plate test the greatest effect possessed compound JOA 122, which at a dose of 30 mg/kg significantly prolonged the latency time to pain reaction. In the formalin test a significant antinociceptive activity was observed for compounds JOA 122 and JOA 123 in the second (late) phase. In this phase the compound JOA 122 tested at the doses 1, 10, 30 mg/kg reduced duration of licking response. Compound JOA 123 attenuated the nocifensive response in this phase only at the dose 30 mg/kg. In the oxaliplatin-induced neuropathy, as well as streptozotocin-induced neuropathy tested compounds at the dose of 30 mg/kg attenuated tactile allodynia, since they significantly elevated the pain sensitivity threshold in the acute phase.

CONCLUSIONS: The results obtained in the current studies proved that in the group of novel 3,3-diphenyl-propionamides new anticonvulsants with collateral analgesic properties can be found.

FINANCIAL SUPPORT: Supported by the grant of the Polish National Scientific Centre, Poland (Grant No. DEC-2013/11/B/NZ7/02081).

P1.11. EFFECTS OF SOME ENVIRONMENTAL COMPONENTS OF AIR POLLUTION ON SELECTED PARAMETERS OF METABOLIC ACTIVITY OF NEURONAL AND MACROPHAGAL CELLS IN CONTEXT OF POTENTIAL ROLE IN A PATHOMECHANISM OF NEURODEGENERATIVE DISORDERS

Adam Roman, Monika Jankowska, Irena Nalepa

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

INTRODUCTION: A number of literature reports pointed out a connection between neurodegenerative disorders morbidity and air pollution with particulate matter (PM) containing transition metals. PM exerts their effect by activation of immune system and oxidative stress induction.

AIM(S): The aim of this study was to assess whether exposure to PM is associated with proinflammatory activation of macrophagal cells and negative neuronal cells condition including reactive oxygen species (ROS) generation.

METHOD(S): Particulate matter NIST1648A (standard material) and LAp120 (NIST1648A devoid of organic components) were investigated for their biological potency in mouse macrophagal cell line RAW 264.7 and in human

neuronal cell line SH-SY5Y. It was assessed using metabolic activity assay (resazurin reduction (RES) test), ROS generation assay (DCFH-DA test), nitric oxide (NO) synthesis and superoxide anion (O2-) release (NBT reduction test).

RESULTS: At highest concentration (100 μg/ml) and longest incubation time (48 h), NIST1648A slightly increased metabolic activity, NO synthesis and O2-release in macrophagal cells. In these conditions LAp120 decreased metabolic activity and O2-release without influence of NO synthesis. Both forms of PM shortly increased the ROS generation. In neuronal cells both forms of the PM resulted in increase of ROS generation and decreased metabolic activity.

CONCLUSIONS: The PM tested in this study possess a potential to direct activation of innate immunity and to direct damage of neuronal cells probably by oxidative stress induction.

FINANCIAL SUPPORT: The study was supported by APARIC project – NCN grant No. 2015/16/W/ST5/00005, and statutory funds of Institute of Pharmacology Polish Academy of Sciences.

P1.12. THE INFLUENCE OF SILVER NANOPARTICLES ON BLOOD-BRAIN BARRIER FUNCTION AND MORPHOLOGY IN ADULT RATS

Grzegorz Sulkowski[1], Beata Dąbrowska-Bouta[1], Jolanta Orzelska-Górka[2], Małgorzata Frontczak-Baniewicz[3], Lidia Strużyńska[1]

[1] Mossakowski Medical Research Centre Polish Academy of Sciences, Department of Neurochemistry, Laboratory of Pathoneurochemistry, Warsaw, Poland, [2] Medical University of Lublin, Department of Pharmacology and Pharmacodynamics, Lublin, Poland, [3] Mossakowski Medical Research Centre Polish Academy of Sciences, Electron Microscopy Platform, Warsaw, Poland

INTRODUCTION: Neurotoxicity of silver nanoparticles has been confirmed in a lot of *in vitro* and *in vivo* studies using different experimental models. However, the mechanisms of the toxic action have not been fully clarified. Since nanoparticles have the ability to enter the brain and significantly accumulate in this organ, it is important to investigate their neurotoxic mechanisms.

AIM(S): We examined the effect of prolonged exposure on blood-brain barrier (BBB) ultrastructure and expression of tight junctions protein components as opposed to the ionic silver.

METHOD(S): In the current study we exposed adult rats to a low dose (0.2 mg/kg b.w.) of small (10 nm) citrate-stabilized silver nanoparticles (AgNPs).

RESULTS: The BBB is a highly specialized structure composed of a basement membrane and microvascular endothelial cells which interact with pericytes, perivascular artrocytes and neurons forming neurovascular unit. Administration of AgNPs over a two-week period resulted in changes in BBB ultrastructure and integrity. TEM analysis revealed accumulation of AgNPs inside endothelial cells of microvessels, mainly in lysosomes. Ultrastructural features of enhanced permeability of cerebral microvessels were observed such as enhanced activity of pinocytotic vesicular system and swollen perivascular astrocytic end-feets. This suggests uptake of fluid and its transfer to parenchyma which further results in perivascular edema. Additionally, we observed changes in the level of mRNA of the main tight junction proteins such as claudine, ocludine, and ZO1 as well as PDGF and its receptor PDGFbR which constitute the signaling pathway between endothelial cells and pericytes. All these characteristic protein components are responsible for the integrity of BBB.

CONCLUSIONS: The results of the current study demonstrate that exposure of adult rats to AgNPs induces BBB dysfunction leading to the enhanced permeability of cerebral microvessels.

P1.13. GLUTAMATE, GLUTAMINE AND GABA LEVELS IN THE RAT HIPPOCAMPUS IN THE PHARMACOLOGICAL MODELS OF AUTISM: MRS AND NMR STUDY

Elżbieta Ziemińska[1], Robert K. Filipkowski[1], Rafał Polowy[1], Jarosław Orzeł[1], Beata Toczyłowska[2], Michał J. Górka[3], Jerzy W. Łazarewicz[1]

[1] Mossakowski Medical Research Centre Polish Academy of Sciences, Warsaw, Poland, [2] Nalecz Institute of Biocybernetics and Biomedical Engineering Polish Academy of Sciences, Warsaw, Poland, [3] Division of Biophysics, Institute of Experimental Physics, Faculty of Physics, University of Warsaw, Warsaw, Poland

INTRODUCTION: An imbalance in excitatory/inhibitory neurotransmission has been implicated in the pathogenesis of autism.

AIM(S): We tested this hypothesis by measuring with Magnetic Resonance Spectroscopy (MRS) and Nuclear Magnetic Resonance (NMR) the content of glutamate (Glu), glutamine (Gln) and GABA in the rat hippocampus in two pharmacological models of autism.

METHOD(S): The rat females at the 11th day of gestation were given orally 800 mg/kg of valproic acid (VPA) or 500 mg/kg of thalidomide (THAL). The pups at PND 9 were submitted to ultrasonic vocalization (USV) test, and at PND 30, to MRS studies using the 7 T Bruker BioSpec 70/30 Avance III system. Spectrum was acquired with the short echo time PRESS sequence (TR/TE=2500/20 ms, 512 averages, 2048 points, scan time=17 min) with VAPOR water suppression, the outer volume suppression, frequency drift correction (flip angle 5°) and eddy current correction. Metabolite concentrations were estimated using the LCModel software. The amino acids from homogenates of rat hippocampi were extracted for NMR studies using the HCl-Bligh and Dyer procedure. Three-trimethylsilyl propionic acid

(1 mM) was used as an internal reference signal. All NMR spectra were acquired at 25°C on a Avance III HD 500 MHz (Bruker) spectrometer.

RESULTS: The results of USV tests showed that the "autistic pups" produced less calls/animal (VPA-122, THAL-33) as compared to control animals (295). MRS studies demonstrated increase by 21% and 20% in Glu content in the hippocampus of male rats from both, VPA- and THAL-treated groups, whereas Gln and GABA were on the control levels. NMR studies showed gender-dependent differences in Glu content in VPA-group (by 36%) and THAL-group *vs.* control (by 16%); increased level of Gln in males from both groups (by 47% and 74%) and increased (by 86%) level of GABA in male VPA-treated rats.

CONCLUSIONS: These results are consistent with a hypothesis on the role of the imbalance in glutamatergic *vs.* GABAergic neurotransmission in the pathogenesis of autism.

FINANCIAL SUPPORT: This study was supported by the Polish National Science Centre, grant no. 2014/15/B/NZ4/04490.

P1.14. THE BRAIN ANATOMY IMAGING AFTER PROLONGED KETOGENIC DIET FEEDING IN RATS

Kinga Gzieło[1], Michał Kiełbiński[1], Krzysztof Jasiński[2], Zuzanna Setkowicz[1]

[1] Jagiellonian University, Institute of Zoology, Department of Neuroanatomy, Krakow, Poland, [2] Polish Academy of Science, Institute of Nuclear Physics, Department of Magnetic Resonance Imaging, Krakow, Poland

INTRODUCTION: Ketogenic diet (KD) results in mild to moderate ketosis, which in turn can significantly change the metabolic balance in the brain. The effects of KD are broadly studied in search of potential clinical uses, such as reducing seizure severity in epilepsy, or providing adjunctive therapy for cancer, Alzheimer's or Parkinson's disease. The exact mechanism of those putative neuroprotective effects of KD is, however, still poorly understood.

AIM(S): Here, we have checked if prolonged ketogenic diet changed beta hydroxy butyrate(BHB) and epididymal fat levels. The crucial thing was to determine how the ketogenic diet affects brain volume and anatomy.

METHOD(S): Male Wistar rats were assigned into two experimental groups: one was given KD for 4 months (n=10), the other (n=11) was fed normal laboratory chow (N). After 4 months, rats were sacrificed. Blood samples were collected and BHB levels measured with ELISA. T2-weighed *ex vivo* images of extracted brains, taken with a 9.4 T magnetic resonance scanner were obtained at the Institute of Nuclear Physics, at XY resolution of 0.025 mm and voxel depth of 0.25 mm. Using a computer-assisted Cavalieri method, the volumes of the entire brain, hippocampus and brainstem structures (midbrain, pons) were estimated. Volumes were compared between groups to show differentially affected regions. Student's t-tests was used for statystical analysis.

RESULTS: We have observed increased epididymal fat and elevated BHB levels in KD in comparison to the N group ($p<0{,}000001$). Additionally we have found a significant reduction in overall pontine volume in the KD group after the 4-month feeding period.

CONCLUSIONS: Our results indicate that the prolonged ketogenic feeding was successful in inducing metabolic changes in KD animals. Observed differences in pontine volume in rats fed a ketogenic diet may lead to modification of feeding behavior. These imapairments in food-intake process may be strictly involved with parabrachial structures which are engaged in regulating appetitive behavior.

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P1.15. MMP-9 ACTIVITY IN ANIMAL MODEL OF TRAUMATIC BRAIN INJURY

Barbara Pijet, Marzena Stefaniuk, Emilia Rejmak-Kozicka, Leszek Kaczmarek

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

INTRODUCTION: Epilepsy in 20% of cases, develops as an effect of traumatic brain injury (TBI). Recent evidences indicate important role of extracellular matrix metalloproteinase-9 (MMP-9) in neuronal circuitry remodeling and synaptic plasticity.

AIM(S): The aim of the present study was to evaluate the MMP-9 activity changes, dendritic spines density after brain injury and the influence of MMP-9 expression level on spontaneous seizures appearance after TBI.

METHOD(S): We used Controlled Cortical Impact (CCI) as a model of TBI in animals with altered MMP-9 levels (lacking: MMP-9 KO; overexpressing: MMP-9 OE) and their WT siblings. 12 weeks after CCI animals were subjected to continuous video-EEG monitoring. To verify MMP-9 changes after TBI: gel zymography and in situ hybridization were used. For dendritic spine alterations staining using liophilic dye DiI were performed.

RESULTS: TBI resulted in progressive cortex (Cx) degeneration during 30 days after TBI. This effect was MMP-9 dependent. In MMP-9 KO animals degeneration volume degree was significantly smaller compared to wildtype siblings and MMP-9 OE mice. Gel zymography analysis showed time-associated elevation of MMP-9 activity in ipsilateral Cx and Hp, also in the thalamus samples during 3 days after CCI. Moreover, in situ hy-

bridization showed increase of MMP-9 mRNA expression which reached the peak 6 hours post-CCI. Density of the dendritic spines measured 7 and 14 d after CCI was significantly decreased in ipsilateral Cx and CA1. EEG recordings with threshold test showed decreased seizure latency in MMP-9 OE mice while increased in MMP-9 KO. Interestingly total seizure number was the highest in MMP-9 OE animals.

CONCLUSIONS: We described the correlation between TBI and MMP-9 activity and action post trauma. We indicated that MMP-9 might be an important factor for major dendritic spine reshaping, observed after brain injury, which in consequence may lead to altered sensibility of neuronal circuits to trigger seizures.

FINANCIAL SUPPORT: This work was supported by PBS Program founded by The National Centre of Research and Development.

P1.16. HSP90 CO-CHAPERONE, SGT1, IN BRAIN OF PATIENTS WITH PARKINSON'S DISEASE

Anastasiia Bohush[1], Anna Filipek[1], Serge Weis[2]

[1] Department of Molecular and Cellular Neurobiology, Nencki Institute of Experimental Biology Polish Academy of Sciences, Warsaw, Poland, [2] Neuromed Campus, Kepler University Hospital, Johannes Kepler University, Neuropathology, Linz, Austria

INTRODUCTION: Parkinson's disease (PD) is characterized by the presence in the brain of inclusions formed from misfolded α-synuclein. It has been suggested that formation of these inclusions is inhibited by chaperones such as Hsp90 and its co-chaperones.

AIM(S): In this work we analyzed the localization and level of Hsp90 co-chaperone, SGT1, in the brain of PD patients.

METHOD(S): Studies were performed on the specimens derived from 5 control individuals and 5 patients with PD. Localization of SGT1 was determined by immunohistochemistry while the level of Sgt1 protein and mRNA by Western blot and qRT-PCR, respectively. Neuroblastoma NB2a cells and PLA were applied to estimate the interaction between α-synuclein and SGT1.

RESULTS: We have determined the localization of SGT1 in different brain regions. We also found that the level of SGT1 is upregulated in cortex and downregulated in putamen of patients with PD. We have demonstrated that SGT1 transiently interacts with α-synuclein in NB2a cells.

CONCLUSIONS: Our results suggest that a Hsp90 co-chaperone, SGT1, is involved in the pathogenesis of PD.

FINANCIAL SUPPORT: This work has been supported by the EU Horizon 2020, Marie Sklodowska-Curie grant No 665735 and by the Polish Ministry of Science and Higher Education grant No 3548/H2020/COFUND/2016/2.

P1.17. MMP-9 INHIBITORS AS A NEW DRUG THAT BLOCKS THE DEVELOPMENT OF EPILEPSY

Emilia Rejmak-Kozicka, Danylo Khomiak, Leszek Kaczmarek

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

INTRODUCTION: Matrix metalloproteinase-9 (MMP-9) is a member of matrix metalloproteinase family that remodels the extracellular matrix. Recently, cumulative evidence indicates that MMP-9 is upregulated in experimental epilepsy models. Increased MMP-9 is also implicated in clinical epilepsy studies.

AIM(S): Thus, we hypothesize that MMP-9 may be a novel therapeutic target for epilepsy and some agents, such as OAT1 or OAT2, may be potential antiepileptogenic drugs.

METHOD(S): First we estimated IC50 of selected inhibitors using recombinant MMP-9 and DQ gelatin, fluorescent substrate of MMP-9. Next, we estimated the level of cleavage of MMP-9 substrate – Nectin-3. To determine whether Nectin-3 cleavage might be blocked by MMP-9 inhibitors, we added to hippocampal neurons different concentrations of inhibitors upon 50 μM glutamate stimulation. Extracts from whole cell lysates were analyzed on Western blots. We also adapted gel zymography, method for estimating the level of MMP-9. Due to the MMP-9 low brain expression level and its secretion on the synapse upon neuronal stimulation we decided to inject mice with PTZ (50 mg/kg, 15 min). Next we added inhibitors of MMP-9 directly to developing buffer.

RESULTS: IC50 value for OAT1 is 0,5 nM and for OAT2 is 13 nM. Glutamate-dependent stimulation of Nectin-3 cleavage was abolished only in the presence of OAT1 in 5 μM concentration in culture. Further, the gel zymography analysis showed inhibition of MMP-9 activity in the gel with OAT1 in the buffer. The inhibitor's specificity towards MMP-9 was supported by the absence of MMP-2 activity in this probe, which is another abundant in the brain metalloproteinase showing gelatinase activity.

CONCLUSIONS: This results strongly confirm that OAT1 is specific towards MMP-9 and could be used in animal models of epileptogenesis.

FINANCIAL SUPPORT: PBS3/A8/36/2015 from The National Centre for Research and Development.

P1.18. EFFECTS OF MYRIOCIN AND FTY720 ON GENE EXPRESSION OF SPHINGOLIPID METABOLISM ENZYMES IN ANIMAL MODEL OF ALZHEIMER'S DISEASE

Henryk Jęśko[1], Przemysław L. Wencel[2], Robert P. Strosznajder[2]

[1] Department of Cellular Signalling, Mossakowski Medical Research Centre Polish Academy of Sciences, Warsaw, Poland, [2] Laboratory of Preclinical Research and Environmental Agents, Department of Neurosurgery, Mossakowski Medical Research Centre Polish Academy of Sciences, Warsaw, Poland

INTRODUCTION: Sphingolipid imbalance has been observed in Alzheimer's disease (AD) (accumulation of the pro-apoptotic ceramide, and loss of the protective sphingosine-1-phosphate – S1P) being correlated with the progress of neurodegeneration. Deregulated sphingolipid homeostasis may lead to neuronal death. Therefore enzymes regulating sphingolipid metabolism gain attention as highly promising targets in AD research/therapy.

AIM(S): We examined the influence of myriocin and FTY720 on gene expression of enzymes metabolizing ceramide/sphingosine-1-phosphate in a mouse transgenic AD model.

METHOD(S): mRNAs were measured with real-time PCR in the cerebral cortex of 6-months old FVB mice overexpressing human Aβ precursor protein (APP) treated with myriocin, a ceramide biosynthesis inhibitor, and FTY720 a sphingosine analog and sphingosine-1-phosphate receptor modulator.

RESULTS: Myriocin has increased the expression of ceramidases ACER2 and -3, ceramide kinase (CERK), sphingosine kinase 2 (SPHK2) and S1P receptors (S1PR1 and -5) in APP mice. These results suggest a metabolic shift from ceramide towards the survival-promoting ceramide-1-phosphate and S1P. However, both Bcl-2 and Bax were increased, leaving the question open. The mock-transfected animals seemed to respond to treatment with a shift towards ceramide accumulation and dephosphorylation of S1P into sphingosine. FTY720 treatment of APP animals increased mRNA levels of ceramide synthases (CERS2 and 6), SPHK1 and 2, and proteins from Bcl-2 family.

CONCLUSIONS: Our results suggest that myriocin and FTY720 treatment may lead to widespread modification of gene expression in the sphingolipid rheostat and signaling pathways, which requires further research to fully understand their mechanisms of action.

FINANCIAL SUPPORT: Supported by the National Science Center grant no NCN/15/B/NZ3/01049.

P1.19. NOVEL PEPTIDOMIMETIC MMP-9 INHIBITORS AS POTENTIAL ANTI-EPILEPTOGENIC THERAPEUTICS

Danylo Khomiak[1], Alexandra Bertran[2], Emilia Rejmak[1], Leszek Kaczmarek[1]

[1] Department of Neurophysiology, Nencki Institute of Experimental Biology, Warsaw, Poland, [2] Iproteos, Barcelona Science Park, Barcelona, Spain

INTRODUCTION: Epilepsy is the most widespread neurological disorder (prevalence – 50 million). The recent discoveries suggest that remodelling of the brain extracellular matrix, executed by extracellularly operating proteases, may play a fundamental role in the pathogenesis of epilepsy. One of them, MMP-9 has been particularly linked to epileptogenesis and the functional involvement of MMP-9 in kainic acid and pentylenetetrazole-kindling models of temporal lobe epilepsy demonstrated.

AIM(S): Considering the inhibition MMP-9 as a promising therapeutic strategy, MMP-9 Inhibitors of peptidomimetic nature IPR-X, IPR-Y and IPR-Z were developed at Iproteos and initial conjoint testing of these compounds has been performed.

METHOD(S): Compounds ability to penetrate blood-brain barrier was analyzed by PAMPA (Parallel artificial membrane permeability assay. Stability to degradation in liver and cytotoxicity were tested by exposing to rat liver microsomes and by MTT assay, respectively. Selectivity and potency of IPR-X-Z were pre-estimated by fluorescent assay using DQ-gelatin and recombinant MMP-9. Finally, the inhibitors' ability to inhibit cleavage of of MMP-9 substrate – Nectin-3 was tested in primary hippocampal cell culture upon 50 μM glutamate stimulation.

RESULTS: PAMPA assay studies yielded in 7.7–13.3% penetration percentage for artificial blood-brain barrier. The values of intrinsic clearance for IPR-X-Z determined by stability in liver microsomes assay ranged from 33 to 46 μL/min/mg protein, indicating moderate degradation. Compounds proved to be non-toxic in MTT cytotoxicity assay, except of IPR-Z at the highest concentration tested (200 μM). IC50 values from DQ-gelatinase assay were shown to be 4–10 μM. In hippocampal cell cultures compounds inhibited MMP-9 dependent nectin-3 cleavage with 60% of total band intensity remaining on western blot.

CONCLUSIONS: The obtained results confirm that IPR-X, IPR-Y and IPR-Z are specific and potent towards MMP-9 and could be further tested in animal models of epileptogenesis.

FINANCIAL SUPPORT: This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No 642881.

P1.20. MORPHOLOGICAL FEATURES OF THE INTERNAL ORGANS OF THE MATURE AND IMMATURE RATS IN THE ACUTE PERIOD OF DIFFUSIVE CRANIOCEREBRAL TRAUMA

Ruslan Prus, Olena Kholodkova, Danylo Ternovyi, Yulia Sadovska, Iryna Horiuk

Odessa National Medical University, Department of human anatomy, Odessa, Ukraine

INTRODUCTION: Craniocerebral trauma (CCT) is important problem of modern medicine and plays a dominant role in morbidity and mortality of the population. It is known the severity of CCT can be burdened by comorbid conditions, the degree of expression of which directly depends on age.

AIM(S): The aim: to study the morphological features of the liver, kidneys and myocardium of mature and imma-

ture rats after diffusive CCT in the period of acute reaction to trauma (1 hour).

METHOD(S): The study was conducted on 20 mature and 20 immature rats. For both groups, intact comparison groups of the corresponding age were isolated. The rats of both groups were simulated with mechanical CCT of mild severity, by free falling of the load in the parieto-occipital region of the skull. The organ blocks were prepared for microscopic examination by standard methods.

RESULTS: 1 hour after the CCT in the liver of mature rats, were observed liver enlargement, vasodilatation, diapedesis hemorrhage phenomena were noted. In immature rats, the liver was of normal size, but there was focal voiding of the vessels, as well as the phenomenon of diapedesis hemorrhage. In the kidneys of mature rats, lymphohystiocytic infiltration of the glomeruli was noted, as well as diapedesis steeping of the stroma. In immature rats, the capsule of the glomerulus and their wrinkling, the foci of necrosis of the glomeruli expressed diapedesis hemorrhages at the border of the cortical and cerebral substance are observed. In the heart of mature rats, single miosymplasts are traced. Vessels of small and medium caliber are dilated and plethora. Immature rats have small foci of necrosis, located mainly under the endocardium.

CONCLUSIONS: Thus, the results of the study show that in all the organs studied after CCT in the acute phase, changes in the type of hemocirculation disturbances are noted in mature rats: stasis, infiltration, dilated vessels. In immature rats, changes in the organs are more pronounced than in mature rats. Changes are mainly ischemic and necrotic.

FINANCIAL SUPPORT: PhD student non-member. One-day participation (28.08.2017). Total cost: 250 PLN.

P1.21. PHOTOTHROMBOTIC STROKE INDUCES LONG-LASTING CHANGES IN MRNA EXPRESSION OF ENZYMES INVOLVED IN POLYSACCHARIDE METABOLISM IN THE BRAIN AREAS REMOTE TO INFARCT

Anna K. Greda, Dorota Nowicka

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

INTRODUCTION: Perineuronal nets (PNNs) are brain extracellular matrix (ECM) formations enveloping selected neurons. They stabilize synaptic connections, thus limiting plasticity. Decrease in PNNs densities was observed after stroke and may be considered an attempt to create favorable conditions for neuroplasticity. PNNs are composed mainly of sugar moieties, therefore we hypothesize the role of polysaccharide modifying enzymes in observed phenomenon.

AIM(S): We investigated the changes in gene expression and protein localization of enzymes involved in hyaluronic acid and chondroitin sulfate metabolism.

METHOD(S): The expression of genes was analyzed in the remote area at 7 days (d), 1 and 3 months (m) after unilateral photothrombosis. To investigate spatiotemporal mRNA expression qPCR method was employed. Immunohistochemical staining was used to analyze cellular localization of investigated enzymes.

RESULTS: We observed increased expression of hyaluronan synthase 2 (HAS2) 7d post-stroke within both hemispheres. Also, changes in mRNA level of chondroitin sulfate synthesis-mediating enzymes occurred, namely, elevation of beta-1,3-glucuronyltransferase 2 (B3GAT2) and simultaneous decrease in B3GAT1 and B3GAT3 expression. Progressive reduction of chondroitin sulfate N-acetylgalactosaminyltransferase 1 (ChGn-1) expression was observed from 7d to 1 month in the contralateral homotopic area. Moreover, 7d after stroke decrease in chondroitin-4-sulfotranseferase 1 (C4ST1) accompanied by increase in arylsulfatase B (ARSB) mRNA level was observed. Importantly, protein of investigated enzymes, which mRNA level alteration occurred at shorter time point, were still observed 1m after stroke.

CONCLUSIONS: Obtained data indicate prolonged time window for modification of polysaccharide components of the brain ECM that may affect rearrangement of synaptic connections after stroke.

FINANCIAL SUPPORT: This work was supported by National Science Centre (Poland) grants: 2012/05/B/NZ3/00851 and 2015/17/N/NZ3/02244.

P1.22. BENEFICIAL EFFECT OF HIGH FREQUENCY STIMULATION OF SUBTHALAMIC NUCLEUS ON VERMICELLI HANDLING TEST BEHAVIOR AFTER PARTIAL NIGRAL DEPLETION IN RATS

Beata Grembecka, Ewa Dzbeńska, Natalia Ziółkowska, Irena Majkutewicz, Karolina Plucińska, Kacper Ptaszek, Danuta Wrona

Department of Animal and Human Physiology, Faculty of Biology, University of Gdansk, Gdansk, Poland

INTRODUCTION: Loss of dopaminergic neurons in substantia nigra pars compacta (SNpc) lead to motor deficits observed in patients with Parkinson's disease (PD). The neurosurgical therapy of choice is high frequency stimulation of subthalamic nucleus (HFS-STN) improved motor control. The motor impairment depends on the progression of nigral degeneration and in rats model of PD may be measured by Vermicelli handling test (VHT).

AIM(S): The purpose of this study was to evaluate the influence of HFS-STN on VHT behavior in rats with early PD model, induced by 6-OHDA infusion into SNpc.

METHOD(S): Male Wistar rats (n=12) were implanted unilaterally for HFS-STN and received a intranigral infusion of 6-OHDA. 5 days before infusion rats were trained on handle 7 cm lengths of vermicelli pasta and acclimated to video recording. Then, rats were subjected to HFS-STN for 7 days (1 h daily) at intensity just below triggering forelimb dyskinesia or SHAM stimulation. The VHT was providing in both groups each day. The number of adjustments made with each forepaw per each pasta piece, which allow definite Vermicelli asymmetry ratio (VAR) and time to eat were analyzed. PD model have been verified by the detection of tyrosine hydroxylase positive neurons in substantia nigra pars compacta. For a statistical analysis of the results, SPSS software was used.

RESULTS: U-Man Whitney tests showed that HFS-STN stimulated rats consumed the pasta significantly faster than the SHAM ($p \leq 0.001$) across days 1st, 2nd, 5th, 6th, and 7th after 6-OHDA infusion. Interestingly, the VAR was higher in HFS-STN rats in 1st and 4th ($p \leq 0.001$ and $p \leq 0.01$) days in comparison to SHAM animals. The atypical behaviors were not observed.

CONCLUSIONS: The HFS-STN applied in partial dopamine depleted rats influence on time of pasta eating and enhanced asymmetries in forepaw adjustments. The obtained results suggest that faster eating after HFS-STN may be related with amelioration of orofacial movements or increased motivation for food, but not with forepaw manipulation improvement.

FINANCIAL SUPPORT: Supported by the Department of Animal and Human Physiology found 530-L124-D248-16.

P1.23. THE ROLE OF CYCLIN-DEPENDENT KINASE 5 IN REGULATING EARLY INFLAMMATORY SIGNALING IN THE MOUSE BRAIN DURING AMYLOID BETA TOXICITY

Grzegorz A. Czapski, Anna Wilkaniec,
Magdalena Gąssowska-Dobrowolska, Agata Adamczyk
Department of Cellular Signalling, Mossakowski Medical Research Centre Polish Academy of Sciences, Warsaw, Poland

INTRODUCTION: Cyclin-dependent kinase 5 (Cdk5) belongs to the family of serine/threonine kinases and plays a fundamental role in brain development and functioning, but its deregulated activity has also been implicated in various neurodegenerative disorders, including Alzheimer's disease (AD). Moreover, our recent study demonstrated the involvement of Cdk5 in regulating inflammatory processes in the brain during peripheral activation of immune system. However, the relationship between AD, Cdk5 and neuroinflammation is poorly understood.

AIM(S): The aim of this study was to investigate the involvement of Cdk5 in regulating neuroinflammation in mouse model of amyloid beta (Aβ) toxicity.

METHOD(S): Here, we used the experimental model, based on single intracerebroventricular injection of Aβ1-42 oligomers, enabling the production of Alzheimer-like behavioral abnormalities and resembling some molecular events occurring during early stage of AD. The brain tissue was analyzed up to 35 days post-injection. The role of Cdk5 in inflammatory process activation was evaluated using the pharmacological Cdk5 inhibitor roscovitine.

RESULTS: Our results demonstrated that injection of Aβ1-42 oligomers induces long-lasting activation of microglia and astrocytes in hippocampus. Analysis of mRNA level for inflammation-related genes (e.g. Tnf-α, IL-1β, IL-6) showed rapid rise as early as 3 h after injection of Aβ1-42. Notably, injection of scrambled Aβ1-42 had no effect on expression of inflammatory mediators. Furthermore, Aβ1-42 promotes p25 generation, indicative of increased Cdk5 activity. Importantly, inhibition of Cdk5 with roscovitine significantly reduced gene expression and/or protein level of Tnf-α, IL-1β, IL-6, IL-10 and Nos2.

CONCLUSIONS: Our data indicated that Cdk5 plays an important role in Aβ toxicity via controlling brain inflammatory processes. These findings provide important new insights into the molecular mechanisms linking neuroinflammation with the pathogenesis of Alzheimer's disease.

FINANCIAL SUPPORT: This study was supported by The NCN Grant 2011/03/B/NZ3/04549.

P1.24. ROLE OF UNCONVENTIONAL MYOSIN VI IN LOCALIZATION AND ACTIVITY OF DOCK7 IN THE BRAIN

Vira Chumak[1,2], Maria J. Rędowicz[1]
[1] Nencki Institute of Experimental Biology Polish Academy of Sciences, Warsaw, Poland, [2] Institute of Cell Biology National Academy of Science Ukraine, Lviv, Ukraine

INTRODUCTION: Myosin VI (MVI) is a unique unconventional motor that moves toward the minus end of actin filaments. It is involved in endocytosis, cellular trafficking, cell migration and adhesion. The spontaneous mutation of a mouse Myo6 gene resulted in a characteristic circling phenotype (termed as Snell's waltzer, SV) with sensorineural deafness and neurological symptoms accompanied by abnormalities in other organs. DOCK7 (dedicator of cytokinesis 7), a guanidine nucleotide exchange factor (GEF) for Rac1 and Cdc42 GTP that plays a role in axon formation and neuronal polarization, is a binding partner of MVI. We also characterized the MVI-DOCK7 interaction sites and showed that in PC12 cells the interaction was important for DOCK7 activity and NGF-stimulated protrusion formation.

AIM(S): The main aim of this work is elucidation of the role of MVI in brain development and function.

METHOD(S): Western-blot, confocal microscopy.

RESULTS: In hippocampus of WT brains, DOCK7 colocalized with MVI mainly in the perinuclear region. In the SV brains, DOCK7 distribution was more diffusive, not resembling the puncti-like defined structures visible in the WT samples. Also, the absence of MVI affected DOCK7 activity as revealed by estimation of the levels of phosphorylated (active) forms of DOCK7 and its downstream effector SAPK/JNK kinase. Moreover, a significant increase of the levels of GFAP (glial fibrillary acidic protein) and caspase-3 were observed in the in hippocampus and cerebral cortex of SV brains.

CONCLUSIONS: MVI is important both for DOCK7 distribution and activity, and that this interaction could play important role(s) in neuronal functions.

P1.25. MORPHOLOGICAL CHANGES OF CA1 AND CA3 PYRAMIDAL NEURONS IN RAT HIPPOCAMPAL SLICE CULTURES FOLLOWING TTYH1 OVEREXPRESSION

Małgorzata Górniak-Walas[1], Aleksandra Kaliszewska[1], Szymon Łęski[2], Katarzyna Łukasiuk[1]

[1] Department of Molecular and Cellular Neurobiology, Nencki Institute of Experimental Biology Polish Academy of Sciences, Warsaw, Poland, [2] Department of Neurophysiology, Nencki Institute of Experimental Biology Polish Academy of Sciences, Warsaw, Poland

INTRODUCTION: Tweety homolog1 (Ttyh1) is a presumed volume-regulated Cl-channel. It has been proposed to participate in the regulation of neuronal morphology.

AIM(S): We aimed to examine dendritic arborization and spine morphology of pyramidal neurons following TTYH1 overexpression in organotypic hippocampal cultures.

METHOD(S): Rat organotypic cultures were co-transfected with TTYH1-GFP-Synapsin and RFP-β-actin constructs, using biolistic transfection (Gene-Gun, BioRad). Neuronal reconstructions of CA1 and CA3 pyramidal cells were obtained with confocal microscopy and Neuromantic software. Morphometric assessments of individual neurons were performed with Sholl method. L-measure software was used to extract more complex quantitative measurements from neuronal reconstructions. Changes in spine morphology and density on CA1 and CA3 neurons were studied with SpineMagick software.

RESULTS: Sholl method did not reveal signifficant differences in dendritic arborization of neurons overexpressing TTYH1 compared to control neurons. L-measure revealed that CA3 neurons overexpressing TTYH1 showed increased average branch length in the seventh branch order of apical dendrites ($P<0.05$) and increased number of branches in the third branch order of basal dendrites ($P<0.01$). CA1 pyramidal neurons overexpressing TTYH1 showed reduced average branch length in the third ($P<0.05$) and fourth ($P<0.001$) branch orders of basal dendrites. TTYH1 overexpression led to increased number of stubby spines on CA1 neurons (apical proximal and distal dendrites: $P<0.01$; basal dendrites: $P<0.05$) and CA3 neurons (apical proximal dendrites: $P<0.01$). Decrease in the number of long spines on CA1 neurons (apical proximal and distal dendrites: $P<0.01$) and CA3 neurons was confirmed (apical proximal dendrites: $P<0.05$).

CONCLUSIONS: The influence of TTYH1 overexpression on dendritic complexity and spines morphology suggests that TTYH1 protein may be involved in neuronal plasticity.

FINANCIAL SUPPORT: This research was supported by Polish National Science Centre Grant 2011/03/B/NZ4/00302.

P1.26. ROLE OF MMP-9 IN SCHIZOPHRENIA-LIKE BEHAVIORS IN RODENTS

Behnam Vafadari, Leszek Kaczmarek

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

INTRODUCTION: Schizophrenia is recognized by 3 symptoms, classified as positive, negative and cognitive. Positive symptoms may be modelled in experimental animal models by hyperlocomotion, whereas in negative symptoms lack of interest in rewards and problems in social behavior can be demonstrated. Finally, poor working memory may correlate with cognitive symptoms of schizophrenia.

AIM(S): We employed mouse models of schizophrenia for positive, cognitive and negative symptoms to investigate the role of diminished MMP-9 and stress in pathogenesis of schizophrenia in these animals.

METHOD(S): Mice with genetically lowered MMP-9 levels in heterozygotes (+/–, MMP-9 HET) were employed, along their wild type (WT, +/+) littermates. Since early-life stress is regarded as a factor promoting schizophrenia, we subjected the mice, in some experiments, to daily (for 21 days) encounter with an aggressive conspecific and then behavioral phenotyping were done.

RESULTS: Alterations in the level of active MMP-9 in the brain result in increased sensitivity to locomotor hyperactivity induced by MK-801. On the other hand chronic stress, potentiates negative symptoms of schizophrenia in MMP-9 Het mice such as depressive behaviors and social behaviors impairment. Cognitive symptoms such as poor working memory can be seen in MMP-9 HET control mice.

CONCLUSIONS: These results support the notion that MMP-9 alterations in brain may play a role in schizophrenia.

FINANCIAL SUPPORT: Extrabrain EU FP7.

P1.27. ALTERATION OF MTOR SIGNALLING PATHWAY IN RATS PRENATALLY EXPOSED TO VALPROIC ACID

Agata Adamczyk[1], Magdalena Cieślik[1], Magdalena Gąssowska-Dobrowolska[1], Henryk Jęśko[1], Robert K. Filipkowski[2], Paweł M. Boguszewski[3]

[1] Department of Cellular Signalling, Mossakowski Medical Research Centre Polish Academy of Sciences, Warsaw, Poland, [2] Behavior and Metabolism Research Laboratory, Mossakowski Medical Research Centre Polish Academy of Sciences, Warsaw, Poland, [3] Laboratory of Animal Models, Neurobiology Center, Nencki Institute of Experimental Biology Polish Academy of Sciences, Warsaw, Poland

INTRODUCTION: Autism is a neurodevelopmental disorder characterised by impaired social interaction, deficits in communication and stereotyped behaviours with synaptic dysfunction suggested as the major causative factor. However, the molecular mechanisms of synapses impairment remain unclear. The most recent studies point to mTOR, a regulator of protein synthesis at spines, as a potential molecular basis of autism.

AIM(S): Here, we investigated whether the Akt/mTOR pathway is damaged in rats prenatally exposed to valproic acid (VPA), an animal model exhibiting autistic-like behaviour.

METHOD(S): Pregnant Wistar rats were injected i.p. with a single dose of 400 mg/kg VPA on embryonic day 12.5. Autism-like behaviours were verified by measuring ultrasonic vocalizations and elevated plus maze test. Gene expression and protein levels were analysed using real-time PCR and western blot methods, respectively.

RESULTS: Our behavioural investigations have shown impaired communication and increased anxiety in VPA group. Along with the behavioural changes we observed alteration of mTOR signalling in the cerebral cortex, hippocampus and cerebellum of autistic model rats. Enhancement of phospho-mTOR protein level was the most pronounced in the hippocampus, where the phosphorylation of mTOR targets was observed: increased p-4E-BP1, and reduced phospho-p70S6K. These changes were accompanied by an increase in p-Akt protein level. Activation of mTOR in the cerebellum caused an increase of p-4E-BP1, but not of p70S6K. The mild but significant rise in phosphorylated mTOR in the cerebral cortex did not lead to any changes in p70 or 4E-BP1 phosphorylation. Synaptosomes isolated from VPA subjects revealed significant abnormalities in their ultrastructure including unidentified electron-dense matrix material as well as fragile and malformed the post-synaptic densities.

CONCLUSIONS: These results suggested that altered signalling via Akt/mTOR/p70S6K/p-4E-BP1 may result in disturbed spine protein synthesis and thereby lead to synaptic dysfunction.

FINANCIAL SUPPORT: Supported from MMRC statutory theme 8.

P1.28. CLASSIFICATION OF TIME-FREQUENCY EEG RESPONSES OF PATIENTS WITH DISORDERS OF CONSCIOUSNESS

Anna Chabuda[1], Piotr Różański[2], Marian Dovgialo[1], Anna Duszyk[1], Marcin Pietrzak[1], Magdalena Zieleniewska[1], Piotr J. Durka[1]

[1] Faculty of Physics, University of Warsaw, Warsaw, Poland, [2] College of Inter-Faculty Individual Studies in Mathematics and Natural Sciences (MISMaP), University of Warsaw, Warsaw, Poland

INTRODUCTION: Event-related (de-)synchronization (ER(D)S) is a short-lasting modulation of specific frequency bands (e.g. alpha band) of EEG, which occurs in response for external stimuli (visual, haptic) or with motor imagery and execution of movements. Since it's both time- and frequency-specific, it is typically analysed in time-frequency space, using Fourier or wavelet methods.

AIM(S): As part of the research project of University of Warsaw's Faculty of Physics and Warsaw's "Alarm Clock Clinic" (Klinika Budzik), we try to assess whether the presence of a ER(D)S in EEG can be used as an indicator of consciousness in DOC (disorders of consciousness) patients.

METHOD(S): Results from two paradigms are presented: 1) motor imagery experiments consisting of a series of auditory commands ("move your hand/leg") and, 2) haptic stimulation sessions of vibrations applied to patient's shoulder/hand, while the patient was instructed to focus on stimuli delivered to given location. EEG (23 electrodes from extended 10-20 system) and EMG signals were recorded. EEG data were analysed in time-frequency space to identify whether any statistically significant ER(D)S had occurred.

RESULTS: Assessments of possible conscious responses reflected in EEG were correlated with the patients' CRS-R (Coma Recovery Scale-Revised) diagnosis and, in case of ER(D)S, with corresponding EMG signal. We present results of several possible indicators, based both on the statistical significance of time-frequency features, as well as on the cross-validated classification accuracy.

CONCLUSIONS: While the major problem we encountered was caused by severe contamination of EEG with involuntary movement artifacts, we noted that EEG patterns of DOC patients are far from uniform, which is related not only to the patients' neurological state, but also to physical skull defects and reconstructions. Instead of the classical approach of comparing the patterns of patients to the control group, we propose to look for any statistically stable traces of conscious responses.

FINANCIAL SUPPORT: This research was supported by the Polish National Science Centre grant 2015/17/B/ST7/03784.

P1.29. SPHINGOSINE KINASE-1, THE NEW TARGET IN THE NEUROPROTECTIVE EFFECT OF FINGOLIMOD AND PRAMIPEXOLE IN PARKINSON'S DISEASE ANIMAL MODEL

Joanna Motyl[1], Przemysław L. Wencel[2], Łukasz Przykaza[3], Piotr Kosson[4], Paweł M. Boguszewski[5], Joanna B. Strosznajder[1]

[1] Department of Cellular Signalling, Mossakowski Medical Research Centre Polish Academy of Sciences, Warsaw, Poland, [2] Laboratory of Preclinical Research and Environmental Agents, Department of Neurosurgery, Mossakowski Medical Research Centre Polish Academy of Sciences, Warsaw, Poland, [3] Laboratory of Experimental Neurosurgery, Department of Neurosurgery, Mossakowski Medical Research Centre Polish Academy of Sciences, Warsaw, Poland, [4] Laboratory for Genetically Modified Animals, Mossakowski Medical Research Centre Polish Academy of Sciences, Warsaw, Poland, [5] Laboratory of Limbic System, Department of Neurophysiology, Nencki Institute of Experimental Biology Polish Academy of Sciences, Warsaw, Poland

INTRODUCTION: Sphingosine kinase (Sphk1) synthetizing sphingosine-1-phoshate (S1P) is a key enzyme responsible for the regulation of cell fate. Sphk1/S1P could be the attractive target in Parkinson's disease (PD) neuroprotective therapy. Our previous data showed inhibition of Sphk1 expression/activity in PD *in vitro* model and indicated neuroprotective effect of S1P analog phospho-fingolimod (FTY720-P).

AIM(S): The aim of current research was to investigate the effect of FTY720 and dopamine D2/D3 receptors agonist – pramipexole (PPX) on Sphk1 dependent molecular pathway(s) in selected parts of the brain and on locomotor activity in PD animal model.

METHOD(S): Neurotoxin 1-methyl-4-phenyl-1,2,3,6--tetrahydropyridine (MPTP, 40 mg/kg) was administrated i.p. to adult C57BL/6 mice. FTY720 (1 mg/kg) or PPX (1 mg/kg) were injected i.p. during 10 days. Behavioral tests (open field, rota-rod) were performed. Midbrain and striatum were separated. The immunochemical, spectrofluorometrical, and QPCR methods were applied.

RESULTS: Our data indicated that PD mice exhibited significant loss of dopaminergic nerve terminals within striatum, evaluated by reduced tyrosine hydroxylase immunoreactivity level (TH-IR). Moreover we found the lower level of mRNA/ immunoreactivity and activity of Sphk1 in the midbrain of PD mice. Both FTY720 and PPX significantly increased TH-IR in MPTP mice striatum. FTY720 and PPX protected against MPTP-evoked Sphk1 alterations and significantly elevated pro-survival Akt kinase phosphorylation, which indicated its activation. Subsequently, FTY720 increased BAD protein phosphorylation in MPTP mice midbrain, which may protect cells against BAD-mediated death. Then it was observed that FTY-720 and PPX improved locomotor impairment in PD mice.

CONCLUSIONS: Our data indicated the new neuroprotective mechanism of PPX and FTY720 action connected with sphingolipid signaling and demonstrated beneficial properties of these compounds on movement alterations in PD animal model.

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P1.30. ROLE OF RAB11 IN THE MTOR-MEDIATED AUTOPHAGY IN THE DENDRITIC SPINES

Aleksandra Janusz-Kamińska, Katarzyna Świtoń, Jacek Jaworski

International Institute of Molecular and Cell Biology, Laboratory of Molecular and Cell Neurobiology, Warsaw, Poland

INTRODUCTION: Rab11a is a protein that belongs to the small GTPase superfamily. It is known to be involved in the endocytic recycling. In neurons, it was demonstrated to participate in TrkB receptor translocation to the postsynaptic density in the chemical long-term potentiation (c-LTP) upon BDNF stimulation and in AMPA receptor recycling. Recycling endosomes were also shown to contribute to early steps in autophagy, and Rab11 depletion inhibited autophagosome formation in human HEK293A cells. Lack of mTOR-driven autophagy was implied to cause defective dendritic spine pruning and faulty synaptic plasticity in autistic spectrum disorders.

AIM(S): We sought to investigate how Rab11 strikes a balance between endocytic recycling and autophagy in synaptic plasticity via mTOR dependent pathway.

METHOD(S): So far, we have performed live imaging of primary hippocampal neurons on the confocal spinning disc microscope to estimate the mobility of Rab11-mCherry in the dendritic spines. By super-resolution immunofluorescence imaging we have investigated colocalization of Rab11a with early autophagy marker Atg9a, recycling endosome marker syntaxin13 and Hook 1 protein in the dendritic spines. We have also co-immunoprecipitated EGFP-Rab11 with myc-mTOR.

RESULTS: We have shown that Rab11 vesicles decrease their mobility upon mTOR inhibition in the dendritic spines of primary hippocampal neurons. We have also confirmed that EGFP-Rab11 is pulled down with myc-mTOR in the immunoprecipitation experiment. Preliminary analysis indicated increased Rab11 colocalization with autophagy markers upon mTOR inhibition.

CONCLUSIONS: Altogether our results point to the potential role of Rab11 in the mTOR-dependent autophagy in the synaptic plasticity.

FINANCIAL SUPPORT: This research was funded by Polish National Science Centre Sonata Bis (2012/07/E/NZ3/00503).

P1.31. INFLUENCE OF SYSTEMIC INFLAMMATION AND FLUCTUATIONS OF BLOOD GLUCOSE LEVEL ON AMYLOIDOPATHY PROGRESSION IN MOUSE MODEL OF SPORADIC ALZHEIMER'S DISEASE

Angelika Więckowska, Małgorzata Wydrych, Anna Mietelska-Porowska, Urszula Wojda

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

INTRODUCTION: Prominent neuropathologic features of Alzheimer's Disease (AD) are the appearance of senile plaques composed of amyloid peptides and neurofibrillary tangles derived from Tau protein. Induction of main risk factors before the appearance of typical neuropathological AD hallmarks can help to track the sequence of different and complicated early molecular mechanisms of the sporadic form of human AD (SAD).

AIM(S): Our aim is to establish a mouse model that would mimic molecular mechanisms leading to SAD by induction of systemic neuroinflammation and insulin resistance in transgenic mice with mutated human gene encoding amyloid precursor protein (Tg APP). Additionally, we would like to check whether the same experimental conditions may induce AD hallmarks in wild type mice, that may be a proof of lifestyle factors influence on AD development.

METHOD(S): In order to induce neuroinflammation and evaluate the influence of insulin dysregulation in the brain, Tg APP and C57BL mice were injected with LPS, and diabetes was induced by high-fat diet feeding, or streptozocin injection. Every two weeks blood glucose level and body weight were checked. To characterize the metabolic phenotype and immunostaining pattern of neuroinflammatory markers and amyloid β, mice blood and brain tissue were used.

RESULTS: We show effects of systemic administration of infectious agent in neuroinflammation in the brain and body weight and blood biochemical pattern related to high-fat diet and their relation with amyloidopathy progression in the brain.

CONCLUSIONS: The data verify if lifestyle conditions including ongoing systemic inflammation and metabolic changes related to unhealthy diet may accelerate amyloidopathy progression. Studied factors may cause changes not only in Tg APP mice but also lead to the development of AD hallmarks in brain of mice without mutations in APP gene. Results might provide the evidence that the proposed animal model may be an effective tool to study the molecular mechanisms of early stages of SAD progression.

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P1.32. MOLECULAR BIOMARKERS OF ADDICTION IN A MODEL OF LONG-TERM MORPHINE SELF-ADMINISTRATION IN MICE

Urszula Skupio, Magdalena Sikora, Michał Korostyński, Agnieszka Wawrzczak-Bargieła, Marcin Piechota, Ryszard Przewłocki

Department of Molecular Neuropharmacology, Institute of Pharmacology of the Polish Academy of Sciences, Krakow, Poland

INTRODUCTION: Chronic exposure to opioids induces various adaptations in brain physiology that lead to formation of dependence and addiction. Commonly used approaches for modeling morphine dependence, such as conditioned place preference and morphine self-administration typically last less than two weeks, which is presumably too short to observe long-lasting alterations in the brain that accompany drug addiction.

AIM(S): In the present study, we aimed to establish a novel model of long-term morphine self-administration in C57BL/6J mice. Our second goal was to identify molecular biomarkers, specific transcriptional patterns and signs of genetic predispositions to opiate addiction.

METHOD(S): We used automated IntelliCage system to observe the animals in groups. The animals in two separate cages were allowed access to morphine or saccharin solutions for 3 months. We tested animals for symptoms of addiction using paradigms like progressive ratio schedule and intermittent access. Gene expression profiles were evaluated in the striatum using whole-genome microarrays and qPCR.

RESULTS: The animals drinking morphine showed addiction-related behavioral pattern when compared with control animals. The analysis of molecular changes revealed long-lasting alterations in gene expression profiles between the analyzed groups of animals. Interestingly, correlation analyses between individual gene expression levels and motivation allowed to identify genes (Epha5, Ncam) that possibly indicate predisposition to addiction-like behaviors.

CONCLUSIONS: Our model represents a novel approach for investigating both behavioral and molecular mechanisms of addiction. Mice drinking morphine exhibit many of the addiction-like symptoms compared to control animals. Prolonged morphine intake resulted in adaptive processes in the brain that manifested as altered transcriptional sensitivity to opioids.

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P1.33. ABNORMAL AMPA RECEPTOR TRAFFICKING IN A MOUSE MODEL OF FRAGILE X SYNDROME

Magdalena Chojnacka[1,2], Anna Beroun[3], Bozena Kuzniewska[2], Leszek Kaczmarek[3], Magdalena Dziembowska[2]

[1] Laboratory of RNA Biology and Functional Genomics, Institute of Biochemistry and Biophysics, Warsaw, Poland, [2] Laboratory of Molecular Basis of Synaptic Plasticity, Centre of New Technologies, University of Warsaw, Warsaw, Poland, [3] Laboratory of Neurobiology, Nencki Institute of Experimental Biology Polish Academy of Sciences, Warsaw, Poland

INTRODUCTION: Fragile X syndrome (FXS) a common inherited form of mental retardation and autism is caused by lack of expression of fragile X mental retardation protein (FMRP). FMRP is RNA-binding protein that regulates local translation of many synaptic proteins, including AMPA-type glutamate receptors subunits. Accumulated evidence indicate that proper rates of exocytosis and endocytosis of glutamate receptors play a key role in synaptic plasticity. However, current state of knowledge of AMPA receptor trafficking in FXS models is incomplete.

AIM(S): The aim of this study was to analyze AMPA receptor trafficking in a mouse model of fragile X syndrome.

METHOD(S): We used synaptoneurosomes (SN) isolated from Fmr1 KO and wild-type (WT) mice and stimulated them *in vitro* with NMDA/glutamate. To determine levels of surface and intracellular GluR1, GluR2 and GluR3 we used crosslinking of SN with BS3 reagent followed by western blot analysis. To confirm our biochemical results we investigated the synaptic calcium-permeable AMPA receptors using whole-cell patch-clamp recordings.

RESULTS: We found that SN stimulation produced an increase in the surface glutamate receptor subunits only in WT mice. We also found that surface GluR2 protein level was significantly higher in Fmr1 KO SN in basal conditions, when compared to WT. The electrophysiological experiments confirmed higher abundance of GluR2-containing AMPA receptors in the hippocampus of Fmr1 KO mice.

CONCLUSIONS: Our results indicate that Fmr1 KO mice exhibit abnormal AMPA receptor trafficking and it is demonstrated by elevated amount GluR2.

P1.34. OVERNIGHT EEG PROFILES IN MONITORING THE PROCESS OF REGAINING CONSCIOUSNESS

Magdalena Zieleniewska[1], Tadeusz Piotrowski[2], Anna Duszyk[1], Krzysztof Piwoński[1], Anna Chabuda[1], Marian Dovgialo[1], Marcin Pietrzak[1], Piotr Różański[1], Piotr J. Durka[1]

[1] *University of Warsaw, Faculty of Physics, Warsaw, Poland,* [2] *Department of Psychiatry, Medical University of Warsaw, Warsaw, Poland*

INTRODUCTION: Previous research on disorders of consciousness (DOC) phenomena indicated significant changes in circadian activity and sleep architecture that correlated with patient's diagnosis. Although polysomnography seems to provide a valuable tool in assessing consciousness level, the main obstacle is the absence of specific staging criteria in scoring sleep patterns of patients with DOC and the inaccuracy of neuropsychological diagnosis.

AIM(S): The aim of the study was to identify potential quantitative EEG indices in polysomnographic sleep patterns in patients regaining consciousness with main focus on slow wave activity regulation (SWA) and sleep spindles. Besides visual scoring of PSG recordings, we also performed an automatic SWA and sleep spindles detection and parametrization based on the matching pursuit (MP) algorithm.

METHOD(S): Preliminary results of one MCS patient are presented. Overnight multichannel EEG recordings and neuropsychological examination with Coma Recovery Scale-Revised (CRS-R) were performed every 2 months during patient's one-year stay in a model hospital for children with severe brain damages. Each recording was visually scored by an expert with modified AASM sleep scoring criteria, adjusted to specific characteristics of pediatric DOC sleep patterns. We also performed automatic analysis of EEG sleep profiles based on the MP algorithm.

RESULTS: Overall, the overnight EEG profiles of SWA and sleep spindles correlated with visual scores and neuropsychological assessment with CRS-R. Apart from that, for one patient (whose data are hereby presented), some of the SWA and sleep spindles characteristics preceded improvement in the CRS-R diagnosis. These effects were not clearly detectable in the visual assessment of the polysomnograms.

CONCLUSIONS: Preliminary results indicate that automatic parametrization of sleep structures, obtained from the MP algorithm, might provide a valuable tool in monitoring patient's consciousness level during the rehabilitation process.

FINANCIAL SUPPORT: This research was supported by the Polish National Science Centre grant 2015/17/N/ST7/03769.

P1.35. PERFORMANCE OF A MATCHING PURSUIT BASED CLASSIFIER ON VEP: A POTENTIAL PARAMETER FOR ASSESSMENT OF THE STATE OF PATIENTS WITH DISORDERS OF CONSCIOUSNESS

Marian Dovgialo, Anna Chabuda, Anna Duszyk, Marcin Pietrzak, Piotr Różański, Magdalena Zieleniewska, Piotr J. Durka

University of Warsaw, Faculty of Physics, Warsaw, Poland

INTRODUCTION: P300 event-related potential reflects the brain response to external stimuli. Attention paid to one of many repeatedly presented stimuli can be detected from the relative strengths of the responses; if the subject actively counts the occurrences of one of the stimuli (target), specific waveform response, namely the P300 potential, may be observed, and it will be absent during the unattended (non-target) stimuli. This difference can be used as an indicator of conscious information processing in unresponsive patients suffering from disorders of consciousness (DoC). However, P300 waveforms recorded from those patients may significantly differ from the classical shape known from healthy subjects.

AIM(S): We test the possibility of replacing the classical indicators used for assessing the difference between responses to target and non-target stimuli by cross-valida-

tion of a classifier detecting responses, based upon multivariate matching pursuit (MMP) parameterization.

METHOD(S): Visual P300 potentials were recorded in a standard paradigm from a group of healthy subjects and patients in different states of disorder of consciousness. MMP algorithm was used as parametrization, and based upon a subset of recorded data a classifier was trained to distinguish responses to target and non-target stimuli.

RESULTS: Cross-validation performance of the classifier measured as the area under corresponding ROC curves discriminates the healthy group from DoC patients, and in some cases correlates with the severity of DoC.

CONCLUSIONS: Replacing estimation of the statistical significance of the average P300 amplitudes by the performance of MMP-based classification assessed by cross-validation allows for nonparametric detection of conscious responses in DoC patients, whose ERPs do not always exhibit the classical components.

FINANCIAL SUPPORT: This research was supported by the Polish National Science Centre grant 2015/17/B/ST7/03784.

P1.36. ANTICONVULSANT EFFECTS OF SELECTED ANTIEPILEPTIC DRUGS IN MICE EXPOSED TO CAFFEINE DURING PREGNANCY AND BREASTFEEDING IN THE MAXIMAL ELECTROSHOCK-INDUCED SEIZURE MODEL

Barbara Miziak, Iwona Radzik, Stanisław J. Czuczwar
Department of Pathophysiology, Medical University of Lublin, Poland

INTRODUCTION: Caffeine presence in coffee, tea and some drugs makes it the most commonly consumed stimulant drug. Available data show that consumption of caffeine during pregnancy and in the early postpartum period while breastfeeding can affect the offspring's behaviour at the later stages of life.

AIM(S): The aim was to test the impact of caffeine fed to pregnant and breastfeeding mice on the anticonvulsant activity of selected antiepileptic drugs (AEDs): sodium valproate (VPA), topiramate (TPM) and carbamazepine (CBZ), against the maximal electroshock-induced seizures (MES).

METHOD(S): From the 3 day after fertilization, pregnant mice received caffeine dissolved in drinking water throughout the pregnancy, and then throughout breastfeeding. The experiments were carried out on adult offspring (mice in their 8th week of age). The control groups comprised mice, born by females that received only pure water throughout the pregnancy and then throughout breastfeeding while experimental groups – mice, born by females that drank caffeine (0.3 g/l). Seizures were induced by alternating current (25 mA, 50 Hz, stimulus duration 0.2 s) delivered by a generator via ear electrodes. Undesirable effects of the combined treatments were examined in the chimney test, passive avoidance task, and grip strength test.

RESULTS: In the groups exposed to caffeine, there was a significant impairment of the anticonvulsant action of both, VPA and CBZ. The respective ED50s were increased from 155.5 (146.2–165.4) to 175.5 (164.1–187.7) and from 8.4 (6.9–10.3) to 11.6 (10.5–12.9) mg/kg, respectively. As regards TPM, no significant impact of exposure to caffeine on the effectiveness of this AED has been observed. The neurotoxic effects of AEDs were not affected by caffeine exposure.

CONCLUSIONS: The experiments show that exposure to caffeine has a statistically relevant influence on the anticonvulsant action of VPA and CBZ against MES test carried out in the exposed offspring.

FINANCIAL SUPPORT: DS 475.

P1.37. THE ROLE OF ASTROCYTIC GLUCOCORTICOID RECEPTOR IN BEHAVIORAL EFFECTS OF OPIOIDS

Łucja Kudła, Urszula Skupio, Łucja Marut, Magdalena Tertil, Agnieszka Wawrzczak-Bargieła, Ryszard Przewłocki
Department of Molecular Neuropharmacology, Institute of Pharmacology Polish Academy of Sciences, Krakow, Poland

INTRODUCTION: Glucocorticoid receptor (GR)-dependent mechanisms are considered to affect behavioral effects of multiple drugs of abuse, including opioids. Recent evidence points to the important role of astrocytes in mediating GR-dependent effects in the brain. However, the exact mechanisms of astrocytic GR contribution to behavioral response to opioids remain unknown.

AIM(S): Here, we aimed to evaluate effects of opioid receptors ligands in astrocytic GR knockout mice. We assessed the animals in nociception and addiction assays.

METHOD(S): We used transgenic mice in which GR is selectively ablated in astrocytes expressing connexin 30 (C×30×GR flox/flox) and non-transgenic littermates. To investigate nociceptive sensitivity and morphine-induced analgesia, animals were assessed in tail flick test. To evaluate addiction-like behavior, morphine tolerance and naloxone-precipitated morphine withdrawal symptoms were measured. Moreover, sensitivity to opioid reward was tested in conditioned place preference (CPP) paradigm and response to aversive properties of naloxone was measured using conditioned place aversion (CPA) test.

RESULTS: Mutant and control mice presented similar nociceptive sensitivity, did not differ in morphine analgesia, developed similar opioid tolerance and morphine-induced CPP. However, when subjected to naloxone-precipitated morphine withdrawal, mutants showed decreased number of jumps, indicating attenuated physical signs of opioid withdrawal. What is more, astrocytic GR knockout mice did not acquire nalox-

one-induced CPA, suggesting alternations in behavioral response to naloxone-evoked aversion.

CONCLUSIONS: Our data indicate that astrocytic GR may be involved in regulation of naloxone-induced aversion and morphine withdrawal. However, knockout of GR in astrocytes does not influence pain sensitivity, morphine analgesia, tolerance and reward-associated memory. In conclusion, our results shed a light on the causal role of GR-dependent signaling in astrocytes in mediating behavioral effects of opioids.

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P1.38. GLUCOCORTICOID RECEPTOR STIMULATION MODULATES MORPHINE ADDICTION-LIKE SYMPTOMS

Łucja Marut, Urszula Skupio, Ryszard Przewłocki

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

INTRODUCTION: Morphine is widely used painkiller, however misuse of morphine may lead to the addiction. Stress system and glucocorticoids are thought to be involved in various aspects of addiction-like behavior. In animal models stressors and glucocorticoids facilitate acquisition of drug self-administration, increase their rewarding potential and promote relapse.

AIM(S): The involvement of stress system in addiction is widely studied however the specific role of glucocorticoid receptor (GR) is still not fully understood. The aim of this study was to evaluate effects of GR stimulation on addiction-like symptoms induced by morphine.

METHOD(S): We used dexamethasone (DEX), selective glucocorticoid receptor agonist, in self-administration (SA) and conditioned place preference (CPP) paradigms in mice. Mice were allowed to self-administer increasing doses of morphine. To test the influence of GR stimulation on morphine intake, we administered DEX (4 mg/kg). Also effects of DEX on morphine place preference was evaluated. 1 hour before morphine conditioning mice were pretreated with either DEX (4 mg/kg) or saline (SAL).

RESULTS: DEX treatment resulted in significant increase in morphine intake. This effect seems to be specific for the drug, as at the same time, DEX treatment caused decrease in water intake. Interestingly, in CPP paradigm DEX pretreatment resulted in significant decrease of time spent in morphine-paired compartment of the apparatus. In control conditions SAL pretreatment did not affect morphine place preference.

CONCLUSIONS: In CPP paradigm DEX attenuate morphine place preference that is a measure of drug rewarding properties. GR stimulation led to enhanced morphine SA. This result may indicate that after DEX mice need more drug to achieve the same reward. Taking together our result may suggest that GR stimulation decrease rewarding potential of morphine.

FINANCIAL SUPPORT: Funding for this study was provided by Polish National Science Centre Grants: 2013/08/A/NZ3/00848.

P1.39. IMPACT OF FEAR MEMORY CONSOLIDATION AND RETRIEVAL ON THE MRNA EXPRESSION OF G PROTEINS IN MICE BRAIN

Agnieszka Zelek-Molik[1], Marco Costanzi[2], Katarzyna Rafa-Zabłocka[1], Grzegorz Kreiner[1], Adam Roman[1], Vincenzo Cestari[3], Irena Nalepa[1]

[1] Institute of Pharmacology Polish Academy of Sciences, Department of Brain Biochemistry, Krakow, Poland, [2] Free University Maria Ss. Assunta (LUMSA), Department of Human Sciences, Rome, Italy, [3] Sapienza University Rome, Department of Psychology, Rome, Italy

INTRODUCTION: Fear evoked signaling disturbances among hippocampus (HP), nuclei of amygdala (Amy) and prefrontal cortex (PFC) underlie anxiety related disorders, but their molecular mechanism remains elusive. Heterotrimeric G proteins (GP) based on intracellular activity of alpha subunit (Gα) are divided into four families: G(s) stimulating cAMP generation; G(i/o) inhibiting cAMP pathway; G(Q/11) increasing Ca++ level; G(12/13) activating monomeric GP-Rho.

AIM(S): In the present study, the effects of fear memory consolidation and retrieval on the mRNA expression of Gα from all GP families were assessed in HP, Amy and PFC.

METHOD(S): C57BL/6J mice were subject to 1-day fear conditioning (FC) procedure followed by contextual (CTX) or cued (Cs) retrieval test of freezing behavior. Morphine (1mg/kg/ip) injected immediately after FC was used to prevent fear consolidation process. RealTime PCR technique was adopted to measure mRNA expression of Gα subunits: 1 h after FC, 24 h later, 1 h after CTX or Cs retrieval test.

RESULTS: In HP, the increased levels of Gα(s), (12) and (11) were observed 1 h after FC. The Gα(s) mRNA decreased (vs. control) when consolidation was stabilized as well after Cs retrieval. Elevated Gα(12) mRNA, as observed 1h after FC, returned to control level at fear memory stabilization and raised again with CTX retrieval. The increase in Gα(11) persisted 24 h after FC and after CTX (but not Cs) retrieval. In PFC, the CTX retrieval was accompanied by a decrease in Gα(i2) and (i3) mRNA levels. In Amy, no specific change to fear memory process was observed.

CONCLUSIONS: FC evoked changes in Gα mRNA expression are observed mainly in HP and mostly connected to CTX learning. Results suggest that activated signaling pathways from Gα(s) and Gα(12) are necessary to begin

fear memory consolidation process in HP while signal transduction via Gα(11) is implicated in maintenance of fear consolidation.

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P1.40. STUDIES OF CACYBP/SIP PROTEIN IN β-CATENIN DYSREGULATION USING YAC128 HD MICE AND THE CACYBP ZEBRAFISH KNOCKOUT

Magdalena Czeredys[1], Anna Romaszko[1,2], Jacek Kuźnicki[1]

[1] International Institute of Molecular and Cell Biology in Warsaw, Laboratory of Neurodegeneration, Warsaw, Poland, [2] Warsaw University of Life Sciences, Biotechnology, Warsaw, Poland

INTRODUCTION: Mutated huntingtin has been shown to affect gene expression in brains of Huntington's disease (HD) mice models. One of these genes is CacyBP/SIP encoding calcyclin-binding protein (CacyBP/SIP), which is 2-fold overexpressed in the striatum of YAC128 mice, a model of HD. A higher increase in the CacyBP/SIP dimer than in the monomer was found in the striatum of 3-month-old HD mice. Moreover, we detected a decrease in total protein ubiquitination, while the level of β-catenin was higher in the striatum of HD transgenic mice as compared to wild-type mice.

AIM(S): To determine the effect of increased dimerization of CacyBP/SIP protein in YAC128 model and the effect of decreased level of Cacybp in zebrafish on β-catenin signaling.

METHOD(S): Medium Spiny Neurons (MSNs) and glial cultures isolated from the striatum of YAC128 and wild-type mice were used to analyze the level of β-catenin and protein ubiquitination by western blotting. Proximity Ligation Assay (PLA) was used to study CacyBP/SIP dimerization in these cultures. Zebrafish lines with knockout of cacybp were generated using CRISPR/Cas9 technology.

RESULTS: We did not detect any changes in β-catenin or total protein ubiquitination in MSN or glial cultures. We observed the presence of CacyBP/SIP dimers using PLA. Currently, we are studying if the increased level of CacyBP/SIP dimers affects β-catenin and its ubiquitination in YAC128 MSNs. Preliminary data from the cacybp zebrafish knockout shows disturbances in β-catenin protein level in total protein extracts.

CONCLUSIONS: In MSNs and glial cells from YAC128 mice we did not find any changes in β-catenin and protein ubiquitination, which were observed in the striatum of adult HD mice. Increased dimerization of CacyBP might disturb degradation of β-catenin during aging in HD. The cacybp zebrafish knockouts will allow us to find out if Cacybp is involved in β-catenin signaling and what are its other potential functions.

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P1.41. MATERNAL IMMUNE ACTIVATION ALTERS SYNAPTIC PROTEIN EXPRESSION AND CAUSES SOCIAL INTERACTION IMPAIRMENT IN RAT OFFSPRING

Magdalena Cieślik[1], Magdalena Gąssowska-Dobrowolska[1], Henryk Jęśko[1], Paweł M. Boguszewski[2], Robert K. Filipkowski[3], Agata Adamczyk[1]

[1] Department of Cellular Signalling, Mossakowski Medical Research Centre Polish Academy of Sciences, Warsaw, Poland, [2] Laboratory of Animal Models, Nencki Institute of Experimental Biology Polish Academy of Sciences, Warsaw, Poland, [3] Behavior and Metabolism Research Laboratory, Mossakowski Medical Research Centre Polish Academy of Sciences, Warsaw, Poland

INTRODUCTION: Emerging epidemiology data indicate that maternal immune activation (MIA) resulting from inflammatory stimuli such as bacterial infections during pregnancy may constitute a risk factor for multiple neurodevelopmental diseases including autism spectrum disorders (ASD). Genetic and environmental variation, inflammation during early development, and their interaction can influence synaptic dysfunction in ASD. However, the molecular links between infection-induced fetal development alterations and the risk of ASD are still unclear.

AIM(S): The aim of this study was to investigate the effect of MIA on the expression and protein level of key synaptic proteins along with the autism-associated behavior in male rat offspring.

METHOD(S): Pregnant Wistar rat dams were injected intraperitoneally (i.p.) at gestational day 9.5 with 0.1 mg/kg lipopolysaccharide (LPS), which induces immune response similar to that against gram-negative bacteria.

RESULTS: Our data shown impaired social interaction, tested by the play behaviors (Tickling test on post-natal day PND 45–50). However, we did not observe any changes in ultrasonic vocalization (9–11 PND) and bedding preference (PND 15) in MIA offspring. Along with the social interaction changes, MIA has induced presynaptic protein alterations in adolescent rat offspring. These alterations included decreased level of synaptobrevin and syntaxin-1, the key components of SNARE complex, as well as higher level of synapsin. Together with changes in presynaptic proteins, MIA induced reduction in PSD-95 and down-regulation of SHANK family proteins. Moreover, alteration in the protein level of phospho-Akt, and 4E-BP1 was found in MIA subjects.

CONCLUSIONS: It is possible that variations of Akt/mTOR pathway are responsible for aberrant synthesis of key synaptic proteins. The altered synthesis of these pro-

teins would generate changes in synaptic structure and function, contributing to ASD-like behaviors.

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P1.42. P2X7 RECEPTOR MEDIATES EXTRACELLULAR ALPHA-SYNUCLEIN-INDUCED MITOCHONDRIAL DYSFUNCTION IN NEUROBLASTOMA SH-SY5Y CELLS

Anna Wilkaniec[1], Anna M. Lenkiewicz[1], Grzegorz A. Czapski[1], Grzegorz Sulkowski[2], Agata Adamczyk[1]

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

INTRODUCTION: α-Synuclein (ASN) accumulation and mitochondrial dysfunction are central to the pathogenesis of most forms of Parkinson's disease (PD) and appear to intersect, but how the two are related to each other has remained elusive. Recent research emphasised the important role of purinergic signalling dysfunction in PD. While the significant role of purinergic P2 family receptors in mitochondrial dysfunction is well known, the interaction of extracellular soluble ASN with purinergic receptors as well as the involvement of this interaction on mitochondria are not yet studied.

AIM(S): The aim of this study was to investigate the effect of ASN on P2 purinergic signalling and the involvement of purinergic receptors in mitochondrial dysfunction.

METHOD(S): As a research model we used neuroblastoma SH-SY5Y cell line as well as rat synaptoneurosomes treated with exogenous soluble ASN. The experiments were performed using spectrofluorometric, radiochemical and immunochemical methods.

RESULTS: We found that exogenous ASN directly interacts with purinergic P2X7 receptor leading to its activation and intracellular free calcium mobilization in neuronal cells and nerve endings. Activation of P2X7 receptors leads to pannexin 1 recruitment and increased ATP release. Furthermore, ASN treatment induced mitochondrial dysfunction: changes in mitochondrial redox state, decrease in mitochondria membrane potential and elevation of mitochondrial superoxide production. This resulted in decreased synthesis of ATP and ultimately cell death. Importantly, treatment with non-selective (PPADS) or selective (AZ 11645373) P2X7 antagonist reversed the ASN-induced mitochondrial damage and prevented SH-SY5Y cells death.

CONCLUSIONS: Our data indicated that P2X7 receptor activation is responsible for ASN-induced mitochondrial dysfunction. Thus, interference with P2X7 signalling seems to be a promising strategy for the prevention or therapy of PD and other neurodegenerative disorders.

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P1.43. THE INFLUENCE OF 12 WEEKS HIGH FAT DIET AND LOW DOSE STREPTOZOTOCIN-INDUCED DIABETES ON CONTRACTILE PROPERTIES OF MOTOR UNITS IN MEDIAL GASTROCNEMIUS MUSCLE OF RAT

Katarzyna Kryściak, Hanna Drzymała-Celichowska, Piotr Krutki, Dawid Łochyński, Jan Celichowski

Poznan University of Physical Education, Department of Neurobiology, Poznan, Poland

INTRODUCTION: Obesity and diabetes, with associated conditions termed as metabolic syndrome are growing problem worldwide. Consequences of impaired glucose metabolism affect the whole organism including neuromuscular system. Diabetic neuropathy influences predominantly sensory system and to smaller degree motor system. Additionally, skeletal muscles as involved in glucose metabolism are exposed to processes related to insulin resistance.

AIM(S): The study was aimed to investigate effects of high fat diet and streptozotocin-induced diabetes on motor unit's (MUs) contractile properties in rat medial gastrocnemius (MG) muscle.

METHOD(S): Male rats weighting about 180 g were randomly assigned to 3 groups: C, untreated, control, on standard laboratory diet (n=10); HFD, on high fat diet for 12 weeks (n=10); and STZ, on high fat diet for 8 weeks, then injected with a single dose of STZ – 35 mg/kg (n=13). Contractile properties of MUs were investigated in electrophysiological experiments.

RESULTS: Both interventions increased the glucose level in the blood but evoked no changes in MG mass. Proportions of the 3 MUs types (FF; fast fatigable, FR; fast resistant and S; slow) were not changed neither in HFD nor STZ compared to C but contractile properties differed significantly in HFD and/or STZ in relation to C. For both fast MU types the twitch time parameters in HFD and STZ were longer, and the twitch-to-tetanus ratio was higher in STZ. The force frequency curves were shifted to lower frequencies in HFD and STZ compared do C, and their slope increased in HFD compared to C group. Furthermore, for FR MUs the force potentiation was lower in STZ compared to C. For S MUs higher tetanus force in HFD compared to C was noted.

CONCLUSIONS: Although high fat diet and low dose of streptozotocin have not changed the MG mass and MU proportions, the impaired glucose metabolism modified force-regulation mechanisms of fast MUs in studied muscle.

P1.44. NITRIC OXIDE SYNTHASE INHIBITORS PROTECT AGAINST THE DEVELOPMENT OF SENSITIZATION TO MEPHEDRONE IN MICE

Sylwia Talarek, Joanna Listos, Jolanta Orzelska-Górka, Gabriela Bielecka, Jolanta Kotlińska

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

INTRODUCTION: Mephedrone is a cathinone derivative that possesses powerful psychostimulant and hallucinogenic effects. It is known that mephedrone may act by increasing release and reuptake inhibition of serotonine and dopamine. Mephedrone has a high abuse and health risk liability, with increased tolerance, sensitization, impaired control and a compulsion to use, the predominant reported dependence symptoms. However, the precise mechanisms underlying its psychoactive effects remain unclear. NO is produced from L-arginine by a reaction catalyzed by NO synthase in response to activation of excitatory amino acid receptors. It acts as an endogenous activator of guanylate cyclase and thereby increases the level of an intracellular second messenger, cGMP. NO, a novel neuronal messenger, is involved in a number of physiological and pathophysiological processes. Recent studies indicate that NO may play a role in tolerance, dependence and sensitization to the addictive drugs such as opioids, ethanol, psychostimulants and nicotine.

AIM(S): The present studies were undertaken to determine the influence of NO synthase inhibitors: NG-nitro-L-arginine methyl ester (L-NAME), non-selective NO synthase inhibitor and 7-nitroindazole, selective inhibitor of neuronal NO synthase, in the development of sensitization to locomotor activity following repeated mephedrone administration in mice.

METHOD(S): Sensitization to locomotor activity was induced by chronic administration of mephedrone (2.5 mg/kg/day ip, 5 days) in male albino Swiss mice. L-NAME (25, 50 mg/kg) and 7-nitroindazole (10, 20 mg/kg) were injected ip for 5 days, 20 min before mephedrone administration. After a 7-day interval, acute dose of mehedrone (2.5 mg/kg) was injected and locomotor activity was assessed for 30 min.

RESULTS: The present experiments demonstrated that coadministration of NO synthase inhibitors: L-NAME and 7-nitroindazole with mephedrone for 5 days protect against the development of mephedrone-induced sensitization to locomotor activity in mice.

CONCLUSIONS: The results of the present study suggest that NO may play a role in the development of sensitization to mephedrone in mice.

FINANCIAL SUPPORT: The reported study was supported by Founds for Statutory Activity of Medical University of Lublin, Poland.

P1.45. INFLUENCE OF INHIBITORS OF DIPEPTIDYL PEPTIDASE-4 (DPP-4) ON NALOXONE-INDUCED MORPHINE WITHDRAWAL SIGNS IN RATS

Joanna Listos, Małgorzata Łupina, Sylwia Talarek, Antonina Mazur, Jolanta Kotlińska

Department of Pharmacology and Pharmacodynamics, Faculty of Pharmacy with Medical Analytics Division, Medical University Of Lublin, Lublin, Poland

INTRODUCTION: Inhibitors of dipeptidyl peptidase-4 (DPP-4), named as gliptins, are a class of oral antihyperglycemic drugs. They block enzyme – DPP-4, elevating glucagon-like peptide-1 (GLP-1) level. Commonly DPP-4 inhibitors are used to treat diabetes mellitus. An increasing number of data demonstrate that GLP-1 acts as neuropeptide in brain. The exact mechanism of GLP-1 in brain is unclear. It has been demonstrated, that GLP-1 is bound to presynaptic receptors on glutamatergic terminals facilitating glutamate release, without triggering direct postsynaptic effects. GLP-1 is involved in food intake and GLP-1 receptors are located in mesolimbic structures so it is possible that GLP-1 modulators may be involved in addiction.

AIM(S): In the present experiment the influence of DPP-4 inhibitor, linagliptin, on morphine withdrawal signs was studied in rats.

METHOD(S): Morphine dependence in rats was obtained by administration of increasing doses of morphine, for 8 days. On the 9th day, the subsequent dose of morphine was injected. 1 hour later, naloxone was administered for induction of morphine withdrawal signs in rats. Then, animals were placed into cylinders and number of jumpings was recorded. In order to evaluate the influence of GLP-1 on the expression of morphine withdrawal signs, linagliptin (10 and 20 mg/kg) was administered in rats on the 9th day of the experiment, before the morphine dose. In order to assess the effect of GLP-1 on the acquisition of morphine withdrawal signs, linagliptin (10 and 20 mg/kg) was injected once a day for 8 consecutive days, before morphine injection.

RESULTS: In the present study we demonstrated that linagliptin significantly and dose-dependently reduced the expression of morphine withdrawal signs in rats, and only higher dose of linagliptin markedly reduced the acquisition of morphine withdrawal signs in animals.

CONCLUSIONS: The present study provides that DPP-4 inhibitor, linagliptin, may be considered as a valuable tool in searching for new strategies in therapy of morphine dependence.

FINANCIAL SUPPORT: The study was financed by funds of Medical University of Lublin.

P1.46. FRAGILE X MENTAL RETARDATION PROTEIN REGULATES SYNAPTIC TRANSLATION OF NEUROLIGINS

Joanna J. Podsiadlowska[1,2], Bozena Kuzniewska[1], Jacek Milek[1], Katarzyna Urbanska[1], Magdalena Dziembowska[1]

[1] Laboratory of Molecular Basis of Synaptic Plasticity, Centre of New Technologies, University of Warsaw, Warsaw, Poland, [2] Postgraduate School of Molecular Medicine, Medical University of Warsaw, Warsaw, Poland

INTRODUCTION: Neuroligins (NLGNs) are postsynaptic cell adhesion proteins which bind to their presynaptic partners neurexins across the synaptic cleft. Thus, NLGNs are crucial for the formation, maturation and maintenance of synapses. In rodents, neuroligins are encoded by four genes: Nlgn1, Nlgn2, Nlgn3 and Nlgn4. The mutations in Nlgn3 and Nlgn4 genes are associated with autistic phenotype. Another cause of autistic behaviors, fragile X syndrome, results from the lack of fragile X mental retardation protein (FMRP). FMRP binds to neuronal mRNAs and regulate local translation of transcripts that play an important role in synaptic signaling and plasticity.

AIM(S): We aimed to determine if synaptic translation of Nlgn1, Nlgn2 and Nlgn3 mRNAs is regulated by FMRP.

METHOD(S): We used Fmr1 knock-out mice (Fmr1 KO) and their wild type (WT) littermates to isolate synaptoneurosomes, which were stimulated *in vitro* to induce local protein synthesis. We performed FMRP IP on synaptoneurosomes and FISH combined with FMRP immunostaining on cultured neurons to investigate Nlgns mRNAs interaction with FMRP. The polyribosome fractionation was used to elucidate if FMRP regulates Nlgns mRNAs local translation. To study the surface versus intracellular NLGNs distribution at WT and Fmr1 KO synapses we have chosen chemical crosslinking and biotinylation assays, followed by Western blotting.

RESULTS: We show that mRNAs for three studied neuroligins interact directly with FMRP in synaptoneurosomes and Nlgn1, Nlgn2, Nlgn3 mRNAs colocalize with FMRP in dendritic granules of cultured hippocampal neurons. The Nlgn1, Nlgn2 and Nlgn3 mRNAs associate with translating polyribosomes in response to synaptic stimulation and Fmr1 KO mice exhibit upregulated local translation due to the lack of FMRP. Finally, the excessive local synthesis of NLGN proteins at Fmr1 KO synapses leads to their elevated level on the postsynaptic membrane.

CONCLUSIONS: Nlgn1, Nlgn2 and Nlgn3 mRNAs are locally translated at the synapse and FMRP regulates this process.

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P1.47. MAGNETIC RESONANCE ASSESSMENT OF THE HIPPOCAMPAL VOLUME IN CANINE IDIOPATHIC EPILEPSY AS A POSSIBLE MODEL FOR HUMAN MESIAL TEMPORAL LOBE EPILEPSY

Adriana Czerwik[1], Marta Płonek[1], Józef Nicpoń[2], Marcin Wrzosek[1]

[1] University of Environmental and Life Sciences, The Faculty of Veterinary Medicine, Department of Internal Medicine and Clinic for Horses, Dogs and Cats, Wrocław, Poland, [2] University of Environmental and Life Sciences, The Faculty of Veterinary Medicine, Center of Experimental Diagnostics and Innovative Biomedical Technologie, Wrocław, Poland

INTRODUCTION: Idiopathic epilepsy (IE) is one of the most common neurological diseases in humans and dogs. In humans the most frequent form is mesial temporal lobe epilepsy (mesial TLE) and is characterised by hippocampal sclerosis (HS). Hippocampal atrophy and/or necrosis in IE dogs may be equivalent to HS, but TLE is yet not diagnosed in dogs.

AIM(S): The aim of the study was the magnetic resonance comparison of the volume of the hippocampus dogs with IE with healthy group.

METHOD(S): The study was carried on 18 dogs, divided into groups: A, dogs with confirmed IE (n=9, 6 males, 3 females, mean age 65 months [range 8–108]) and B; dogs with no history of seizures, normal blood and brain MRI (n=9, 7 males, 2 females, mean age 88 months [range 36–120]). All dogs underwent brain MRI (1.5T, Philips, Ingenia). The results were calculated using MR slice volumetric (cm^3) analysis of the right and left hippocampus (OsiriX 8, Switzerland), using a semi-automatic method (T2W transverse images, TR/TE 6032/100 ms, FOV 140 mm, layer thickness 3.00 mm, GAP 1.0 mm, voxel size 0.45/0.58/2.00 mm). Statistical analysis was carried out using Statistica software (version 12.5). Data distribution was assessed using the Shapiro-Wilk test. Normally distributed data was assessed using the Student's t-test. Non-normally distributed data was assessed using Mann-Whitney U test. Statistical significance was set at $p<0.05$.

RESULTS: There was a statistically significant difference in the size of the left and right hippocampus between group A and B (left $p=0.001$, right $p=0.007$). No difference was found in the inter hemispheric volumes of the hippocampus in both groups ($p>0.05$).

CONCLUSIONS: We found significant differences in the hippocampal volume in IE dogs visible in MRI, suggesting hippocampal atrophy. This finding may resemble human mesial TLE, which makes tdogs a possible naturally occurring animal model for human mesial TLE.

P1.48. FKRP GENE MUTATIONS AND CANDIDATE DISEASE-MODIFYING GENES IN POLISH PATIENTS WITH LIMB-GIRDLE MUSCULAR DYSTROPHY – RESULTS OF WHOLE-EXOME SEQUENCING STUDY

Anna Macias[1], Jakub Fichna[2], Michał Korostyński[3], Marcin Piechota[3], Anna Potulska-Chromik[1], Maria J. Rędowicz[4], Cezary Zekanowski[2], Anna M. Kaminska[1], Anna Kostera-Pruszczyk[1]

[1] Department of Neurology, Medical University of Warsaw, Warsaw, Poland, [2] Department of Neurodegenerative Disorders, Mossakowski Medical Research Center Polish Academy of Sciences, Warsaw, Poland, [3] Department of Molecular Neuropharmacology, Institute of Pharmacology Polish Academy of Sciences, Krakow, Poland, [4] Laboratory of Molecular Basis of Cell Motility, Department of Biochemistry, Nencki Institute of Experimental Biology Polish Academy of Sciences, Warsaw, Poland

INTRODUCTION: Limb-girdle muscular dystrophies (LGMD) are hereditary progressive disorders of skeletal muscles. Currently 33 LGMD types are recognized. For up to 50% of LGMD patients the causal genetic defect remains unknown. There is considerable phenotypic variability, even among patients with identical causal mutation. Mutations in fukutin-related protein (FKRP) gene are responsible for an autosomal recessive type 2 I of LGMD, which is a relatively frequent type of LGMD in Europe.

AIM(S): The aim of this work was to assess frequency of LGMD2I in Polish LGMD patients, characterize the pathogenic mutations, clinical phenotype and possible disease modifying genes.

METHOD(S): The study involved 85 patients with LGMD diagnosis based on clinical assessment and muscle biopsy. Whole exome sequencing of peripheral blood DNA was performed. Filtering of the identified variants was based on allele frequency, association with Human Phenotype Ontology terms and predicted pathogenicity. Selected variants were confirmed using a direct fluorescence-based sequencing.

RESULTS: Homozygous or compound heterozygous mutations in FKRP gene were found in 7/85 patients. L276I mutation was the most common one – found in 6/7 LGMD2I patients, 3 of them were homozygous. We could observe considerable phenotypic variability. Candidate disease-modifying genes were COL6A3, COL12A1, PLEC, SYNE1. In 2 patients with particularly severe course of the disease, heterozygous mutation in genes involved In glycosylation process was found (LARGE, ISPD, ITGA7). Two patients were found to be heterozygous for mutations in DYSF gene.

CONCLUSIONS: LGMD2I is a common type of LGMD in Polish population. The most common mutation in FKRP gene is L276I. Heterozygocity for mutations in other LGMD genes is high in this group of patients. New generation sequencing methods are a valuable tool for identifying causal mutations, but also for finding candidate disease-modifying genes, which can help to elucidate mechanisms of LGMD.

P1.49. POST-ANOXIC LEVEL OF HIF-1α AND BDNF IN NEONATAL RAT BRAIN DEPENDS ON BODY TEMPERATURE

Justyna Rogalska[1], Hanna Kletkiewicz[1], Malwina Hyjek[2,3], Krzysztof Jaworski[4], Agnieszka Siejka[1], Anna Nowakowska[1]

[1] Department of Animal Physiology, Faculty of Biology and Environmental Protection, Nicolaus Copernicus University, Toruń, Poland, [2] Department of Cell Biology, Faculty of Biology and Environmental Protection, Nicolaus Copernicus University, Toruń, Poland, [3] Centre For Modern Interdisciplinary Technologies, Nicolaus Copernicus University, Toruń, Poland, [4] Chair of Plant Physiology and Biotechnology, Nicolaus Copernicus University, Toruń, Poland

INTRODUCTION: Complications after neonatal asphyxia are the most common cause of subsequent neurological disorders. Transcriptional hypoxia-inducible factor-1α (HIF-1α) plays the fundamental role in adaptive processes in response to hypoxia. Moreover, the most crucial role in neuronal plasticity is attributed to brain-derived neurotrophic factor (BDNF). Newborn mammals showing reduced physiological body temperature are protected against perinatal asphyxia-induced neurotoxicity. The processes underlying neuroprotective effects of decreased body temperature might include the increased levels of HIF-1α and BDNF.

AIM(S): Therefore, we aimed at experimental verification of the hypothesis, that the body temperature during perinatal anoxia affects the level of HIF-1α and BDNF.

METHOD(S): Two-day-old newborn rats were exposed to anoxia in 100% nitrogen atmosphere for 10 min in different thermal conditions, which allow them to regulate the rectal temperature at the level of i. 33°C (physiological to rat neonates), ii. 37°C (level typical of healthy adult rats), or iii. 39°C (febrile adult rats). Hippocampal and cortex levels of HIF-1α and BDNF were determined 1) immediately after anoxia, 2) 3 days after anoxia, and 3) 7 days after anoxia.

RESULTS: There were no postanoxic changes in the level of BDNF in newborn rats kept at body temperature of 33°C. In contrast, at hyperthermic thermal conditions the level of the neurotrophin was decreased. Thermal conditions during neonatal anoxia affected the cerebral level of HIF-1α. The highest level of anoxia-induced HIF-1α production was recorded in animals having physiological body temperature in comparison with that in hyperthermic animals.

CONCLUSIONS: Since HIF-1 and BDNF have been recently regarded as promising therapeutical targets against brain lesions due to hypoxia/ischemia, presented data imply that to achieve a full effect of neuroprotection, the thermal conditions during and after the insult should be taken into consideration.

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P1.50. CYCLIC NAPHTHYRIDINE DIMERS AS THERAPEUTIC AGENTS FOR FRAGILE X-ASSOCIATED TREMOR/ATAXIA SYNDROME

Patryk Konieczny[1], Sanjukta Mukherjee[2], Chikara Dohno[2], Kazuhiko Nakatani[2], Krzysztof Sobczak[1]

[1] Department of Gene Expression, Institute of Molecular Biology and Biotechnology, Adam Mickiewicz University, Poznan, Poland, [2] Department of Regulatory Bioorganic Chemistry, The Institute of Scientific and Industrial Research, Osaka University, Osaka, Japan

INTRODUCTION: Fragile X-associated tremor/ataxia syndrome (FXTAS) is a late-onset neurodegenerative disorder caused by expansion of CGG trinucleotides in the 5'UTR of the FMR1 gene. The patients, with the number of the repeats ranging from 55 to 200, show specific manifestation of clinical symptoms that include intention tremor, cerebellar ataxia, neuropathic pain, parkinsonian features and cognitive deficits, with the underlying brain atrophy and white matter disease of particular regions. The mutation occurs with high frequency in women (1:150–300) and men (1:400–850), with the current estimate of 16–20% female and 40–75% male carriers to develop FXTAS after reaching 50 years of age. As of now, accumulation of the toxic, RAN translation polyglycine (polyGLY) product, expressed from the expanded CGG repeats, is considered to be the main triggering factor of neurodegenerative processes in FXTAS patients.

AIM(S): We aimed at estimating if cyclic naphthyridine dimers could be used to block expression of toxic polyGLY in cells expressing transcripts carrying expanded CGG repeats. We also targeted at evaluation whether this potentially therapeutic intervention could be achieved without affecting generation of FMRP, a protein translated independently from polyGLY from the mutated FMR1 transcript.

METHOD(S): We employed forced expression of plasmids carrying expanded CGG triplets and sequences coding for eGFP or luciferase cloned either in or out of frame to the repeats, to evaluate the relative expression of polyGLY and FMRP following administration of cyclic naphthyridine dimers.

RESULTS: Our cell culture experiments revealed that cyclic naphthyridine dimers efficiently block expression of polyGLY without affecting the overall RNA content of transcripts carrying expanded CGG repeats.

CONCLUSIONS: Cyclic naphthyridine dimers efficiently block expression of the toxic RAN translation product generated from forced expressed plasmids carrying expanded CGG repeats in cell culture experiments.

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P1.51. LONG-TERM EFFECTS OF SUBDURAL INJECTIONS OF AN ANTLEROGENIC CELL HOMOGENATE (ACH) IN A PORCINE MODEL OF SPINAL CORD INJURY (SCI)

Marcin Wrzosek[1], Marta Płonek[1], Józef Nicpoń[2], Anna Chełmońska-Soyta[3], Przemysław Podgórski[4], Marek Cegielski[5], Tomasz Gębarowski[6], Anna Mietelska-Porowska[7], Urszula Wojda[7], Olga Gajewska-Woźniak[8], Małgorzata Skup[8]

[1] Department of Internal Medicine and Clinic of Horses, Dogs and Cats, The Faculty of Veterinary Medicine Wrocław University of Environmental and Life Sciences, Wrocław, Poland, [2] Center of Experimental Diagnostics and Innovative Biomedical Technologies, The Faculty of Veterinary Medicine Wrocław University of Environmental and Life Sciences, Wrocław, Poland, [3] Department of Immunology, Pathophysiology and Veterinary Preventive Medicine, The Faculty of Veterinary Medicine Wrocław University of Environmental and Life Sciences, Wrocław, Poland, [4] Department of General Radiology and Interventional Radiology and Neuroradiology, Wrocław Medical University, Wrocław, Poland, [5] Department of Histology and Embryology, Wrocław Medical University, Wrocław, Poland, [6] Department of Basic Medical Sciences, Wrocław Medical University, Wrocław, Poland, [7] Laboratory of Preclinical Studies of Higher Standard, Neurobiology Center, Nencki Institute of Experimental Biology Polish Academy of Sciences, Warsaw, Poland, [8] Department of Neurophysiology, Nencki Institute of Experimental Biology Polish Academy of Sciences, Warsaw, Poland

INTRODUCTION: The potential use of stem cells in spinal cord regeneration is widely discussed. Xenogenic implantation of antlerogenic stem cell homogenate (ACH) was reported to improve cartilage and cornea regeneration.

AIM(S): A multilevel spinal cord reaction assessment to an ACH implantation in a spinal cord injury (SCI) porcine model was undertaken.

METHOD(S): ACH (cell line MIC-1; 10×106 cells/ml) was obtained using sonification. Five groups were studied: A-sham, B-negative control, C–E with subdural ACH injection, applied immediately after SCI (C), and 1h (D) and 24 h (E) after SCI. Before (P0), directly after (P1), 2 weeks (P2) and 8 weeks (P3) after contusion, CBC and standard blood biochemistry, TP and CSF pleocytosis, UCHL-1, TNF-alfa, MBP, IL-8, IL-6, IL-1β in the serum and CSF were compared. The degree of SCI on MRI (1.5T, Philips, Ingenia) and MR-DTI parameters (FA, ADC) were also evaluated. Post-mortem histopathology and IHC labeling for an astroglial (GFAP) and microglial (IBA) reaction were performed. All of the above analyses were double-blind and randomized.

RESULTS: The majority of the CSF changes were found only in the late postlesion period (P3). The lack of serum IL-1β changes during the entire experiment in all animals, together with the HP and IHC findings, point to a lack of pro-inflammatory reaction to the subdural ACH implanta-

tion. Decreased levels of cell degeneration markers (MBP, TNF alfa, IL-8) in the CSF of the animals where ACH was used suggest that it has potential neuroprotective activity.

CONCLUSIONS: MR and MR-DTI results and a small astrocyte and microglial response in group C (subdural ACH implantation directly after the SCI), suggest a potential beneficial influence of ACH on the neuronal tissue at the injury site. However, due to the data inhomogeneity, a longer observation on a larger group of animals should be conducted.

FINANCIAL SUPPORT: This study was conducted in a National Center for Research and Development project (UOD-DEM-1-352/001).

P1.52. THE EFFECT OF α-SYNUCLEIN ON INITIATION OF INFLAMMATORY REACTION IN THE MURINE BRAIN

Ilona Joniec-Maciejak[1], Adriana Wawer[1], Anna Sznejder-Pachołek[1], Ewa Wojnar[1], Dagmara Mirowska-Guzel[1,2]

[1] Department of Experimental and Clinical Pharmacology, Medical University of Warsaw, Warsaw, Poland, [2] Institute of Psychiatry and Neurology, Warsaw, Poland

INTRODUCTION: Parkinson's disease (PD), one of the most common neurological disorder, is characterized by the loss of dopaminergic neurons in substantia nigra and striatum (ST). The typical reaction of central nervous system (CNS) on neurodegenerative processes is microglia activation and the inflammatory reaction. The data suggests that increased level of α-synuclein (ASN), a small protein which is the major component of Lewy bodies, can induce microglia activation. Activated microglial cells release proinflammatory and potentially cytotoxic substances like cytokines. Till now, little is known about *in vivo* effects of exogenous ASN monomers on initiation of neuroinflammation and neurodegeneration.

AIM(S): The aim of the present study was to examine the effect of increased ASN monomers concentration on microglia response and expression of pro- and anti-inflammatory cytokines (interleukin 1α (IL-1α), IL-10, IL-12) in the ST.

METHOD(S): Male and female C57Bl/10 Tar mice 9 month-old were used in this study. Human recombinant ASN was bilaterally administered into ST (single treatment – 4 μg / structure, 8 μg per brain) and mice were decapitated after 4 or 12 weeks post injection. The changes in the level of inflammatory factors in ST were evaluated using Real-Time PCR and enzyme-linked immunosorbent assay (ELISA).

RESULTS: We observed increased level of a microglia marker – ionized calcium-binding adapter molecule 1 (IBA1) protein after ASN injection into ST. We noticed also some differences in the level of one of the most important pro inflammatory cytokines – IL-1α.

CONCLUSIONS: Our study showed that monomers of ASN are strongly involved in the inflammatory reaction in the murine CNS. Further studies are required to reveal the detailed mechanism of the influence of ASN on neuroinflammation in course of Parkinson's disease.

FINANCIAL SUPPORT: This study was supported by Grant No 1M9/PM 2/16 (Medical University of Warsaw). Research subject was implemented with CePT infrastructure financed by the European Union – The Europan Regional Development Fund within the operational programme "Innovative economy for 2007–2013.

P1.53. THE INFLUENCE OF α-SYNUCLEIN ON NEURODEGENERATIVE PROCESSES IN THE MURINE BRAIN

Ewa Wojnar[1], Adriana Wawer[1], Ilona Joniec-Maciejak[1], Anna Sznejder-Pachołek[1], Dagmara Mirowska-Guzel[1,2]

[1] Department of Experimental and Clinical Pharmacology, Medical University of Warsaw, Warsaw, Poland, [2] Institute of Psychiatry and Neurology, Warsaw, Poland

INTRODUCTION: Parkinson's disease (PD) is one of the most common neurodegenerative disorders. It is characterized by a progressive loss of dopaminergic neurons accompanied by a decreased concentration of dopamine (DA) and its metabolites in the striatum (ST). Experimental and clinical data indicate that α-synuclein (ASN) plays an important role in many processes observed in the brains of patients with PD, such as disorder homeostasis of dopamine (DA) or initiate of oxidative stress. Changes in ASN levels due to its aggregation, overexpression or decreased expression may disrupt DA homeostasis and contribute to the neurodegeneration process observed in PD.

AIM(S): The aim of the present study was to investigate the influence of cerebral injection of ASN on neurotransmitters level in ST. We also examine the expression of tyrosine hydroxylase gene (TH, the rate-limiting enzyme of catecholamine biosynthesis) and tissue transglutaminase 2 gene (TG2; an enzyme involved in aggregation of ASN).

METHOD(S): Male and female C57Bl/10 Tar mice 9 month-old were used in this study. Human recombinant ASN was bilaterally administered into ST (4 μg/structure, 8 μg per brain) and mice were decapitated after 4 or 12 weeks post injection. Concentration of striatal neurotransmitters were measured by high performance liquid chromatography (HPLC). The gene expressions were examined by Real Time PCR.

RESULTS: Intracerebral administration of ASN monomers led to changes in concentrations of striatal neurotransmitters but do not affect the expression of TH gene.

The ASN administered intracerebrally into ST increases striatal expression of TG2 gene, which can lead to enhanced ASN aggregation.

CONCLUSIONS: The biochemical changes observed after ASN administration may initiate further neurodegenerative processes and probably represent a very early stage in development of PD. Further research must be conducted to better understand the crucial role of ASN in the neurodegenerative process in PD.

FINANCIAL SUPPORT: This study was supported by Grant No. 1M9/PM 2/16 (Medical University of Warsaw). Research subject was implemented with CePT infrastructure financed by the European Union – The Europan Regional Development Fund within the operational programme "Innovative economy for 2007–2013.

P1.54. DTI U-FIBRE TRACK DENSITY QUANTIFICATION LOCALISES EPILEPTOGENIC ZONE IN CRYPTOGENIC FOCAL EPILEPSY

Joanna Goc, Elisabeth Hartl, Soheyl Noachtar, Christian Vollmar
Deptament of Neurology, Epilepsy Centre, University of Munich Hospital, Munich, Germany

INTRODUCTION: Pharmacoresistant focal epilepsy might be treated surgically by removing the seizure onset zone. However, in cryptogenic epilepsy no structural lesion is visible in clinical MRI, challenging the exact diagnosis and planning of resective surgery. Here, we present a new DTI analysis approach based on quantification of track density images of short-association fibers, called U-fibers. This approach identifies previously undetected structural abnormalities.

AIM(S): The aim of this study was to develop a new approach that localizes the epileptogenic zone in patients with cryptogenic focal epilepsy.

METHOD(S): Diffusion Tensor Imaging (DTI) data was acquired for 23 patients with cryptogenic focal epilepsy and 31 healthy participants on a GE Signa HDx 3T Scanner with 64 diffusion weighted directions, a b-value of 1000 s/mm² and 2.4 mm slice thickness. Whole brain streamline tracking was performed and the population of U-fibre tracks was selected from which fibre density maps were created. These density maps were spatially normalized to a common space. Statistical comparison to a population of healthy controls was carried out to identify clusters of reduced fibre density in individual patients.

RESULTS: The quantification of U-fiber track density images resulted in clusters of reduced fiber density, which might reflect the microstructural changes in the vicinity of the epileptogenic zone (EZ). This approach correctly identified the EZ in 86% of patients with specificity of 83%.

CONCLUSIONS: Quantification of U-fiber track density images localizes the EZ in cFE, with higher sensitivity and specificity than previously reported studies. This approach can improve the surgical planning of the electrode implantation, necessary for in cFE patients.

FINANCIAL SUPPORT: This study was supported by the Friedrich-Baur-Stiftung and the Herta-Riehr Stiftung.

P1.55. ANTICONVULSANT EFFECT OF RESVERATROL IN THE CONDITIONS OF ACUTE PENTILENTETRAZOL-INDUCED SEIZURES

Yurii Boiko[1], Oksana Boiko[1], Alexey Shandra[1], Irina Kravchenko[2,3]
[1] Department of Physiology, Odessa National Medical University, Odessa, Ukraine, [2] Department of Pharmaceutical Chemistry, Odessa National University Mechnikova I.I., Odessa, Ukraine, [3] Department of Organic and Pharmaceutical Technology, Odessa National Politechnical University, Odessa, Ukraine

INTRODUCTION: The incidence of seizure syndromes including those epilepsy associated actualizes the search of new anticonvulsants. Resveratrol has antioxidant, cardioprotective, anti-cancer and neuroprotective properties. It was also shown that resveratrol can be used as the drug reducing the risk of chronic epilepsy.

AIM(S): The objective of this study was to evaluate the effect of resveratrol on acute seizure activity induced by intravenous pentylenetetrazol administration.

METHOD(S): To study the anticonvulsant effect, young male mice, weighing 18–20 g, CBA line kept in standard vivarium conditions, were used. The introduction of trans-resveratrol was carried out orally in a dose of 50, 100 and 200 mg/kg. Acute seizure activity was induced by administration of 0.1% aqueous pentylenetetrazol solution in the tail vein 30, 90 and 120 minutes after the administration of resveratrol. The effect of the anticonvulsant action of resveratrol was assessed by the changes in minimum pentylenetetrazol dose causing clonic-tonic convulsions (DCTC) and tonic extension (DCE) in the experimental animals. Efficient pentylenetetrazol doses for experimental groups of animals are expressed in % of similar pentylenetetrazol doses causing clonic-tonic convulsions and tonic extensions in the control group.

RESULTS: Resveratrol administration with about 30 minutes exposure increased in the same way DCTC and DCE parameters for all doses studied. Thus, DCTC was by 36–45% bigger and DCE by 41–56% bigger than the corresponding parameters in the control group of animals. When resveratrol is administered at 90 and 120 minutes, the most pronounced anticonvulsant effect was fixed for the dose of 200 mg/kg (DCTC – 188%, DCE – 195%). For the doses of 50 and 100 mg/kg at 90 and 120

minute exposure, the anticonvulsant effect was 12–25% lower than for the dose of 200 mg/kg.

CONCLUSIONS: The findings in present study by suggest an antiepileptic potential of resveratrol in chronic condition or in other seizure model.

P1.56. CHRONIC PROGRESSIVE EXTERNAL OPHTHALMOPLEGIA IN POLISH PATIENTS – CLINICAL AND GENETIC CHARACTERISTICS

Biruta Kierdaszuk[1], Magdalena Kaliszewska[2], Katarzyna Tońska[2], Ewa Bartnik[2], Anna M. Kaminska[1], Anna Kostera-Pruszczyk[1]

[1] Department of Neurology, Medical University of Warsaw, Warsaw, Poland, [2] Institute of Genetics and Biotechnology, Faculty of Biology, University of Warsaw, Warsaw, Poland

INTRODUCTION: Mitochondrial encephalomyopathies comprise a group of heterogeneous disorders which may result from mutations in mitochondrial (mtDNA) and nuclear genome (nDNA). From a variety of symptoms progressive external ophthalmoplegia (PEO) seems to be the most common.

AIM(S): The aim of this study was the clinical and genetic characteristics of Polish patients with progressive external ophthalmoplegia.

METHOD(S): Clinical, electrophysiological, neuroradiological and morphological data of 45 patients aged 11 to 76 years were analyzed. Genetic studies of mtDNA were performed in all patients. Among nDNA genes POLG was studied in 15 and C10orf2 in 6 patients.

RESULTS: 16 patients with ptosis and PEO were included to chronic progressive external ophthalmoplegia (CPEO) group and 13 with ptosis, PEO and limb or trunk muscles' weakness to CPEO+ group. There were 11 patients with PEO and the central nervous system impairment classified as mitochondrial encephalomyopathy (ME), 4 patients with Kearns-Sayre syndrome (KSS) and one patient with sensory ataxic neuropathy, dysarthria, ophthalmoparesis (SANDO) syndrome. Genetic studies of mtDNA revealed already known single or multiple mtDNA deletions in all patients and in most cases they were detected in the muscle tissue. Genetic analysis of nDNA genes confirmed mutations in POLG gene in 6 patients. There were 3 CPEO patients with p.[Arg309Leu];[Gln968Glu], p.[Ala518Thr];[=] and p.[Trp748Ser];[Ser998Pro] mutations, and 2 CPEO+ patients with p.[Thr251Ile;Pro587Leu];[Thr251Ile;Pro587Leu] and p.[Thr251Ile;Pro587Leu];[Lys1191Asn] mutations. In patient with SANDO syndrome the mutation p.[Arg290Cys];[Arg309Cys] in POLG gene was confirmed. Additionally the analysis of the C10orf2 gene proved the mutation p.[Arg374Gln];[=] in one CPEO patient.

CONCLUSIONS: Genetic studies of both mtDNA and nDNA are necessary for diagnosis of chronic progressive external ophthalmoplegia and its genetic counseling.

P1.57. MYOFIBRILLAR MYOPATHIES IN POLAND: CLINICAL, HISTOPATHOLOGICAL AND GENETIC STUDIES

Anna Potulska-Chromik[1], Jakub Fichna[2], Justyna Karolczak[3], Mariusz Berdyński[2], Przemysław Miszta[4], Agata Sikorska[3], Maria J. Rędowicz[3], Anna Kostera-Pruszczyk[1], Anna Kaminska[1]

[1] Department of Neurology, Medical University of Warsaw, Warsaw, Poland, [2] Department of Neurodegenerative Disorders, Mossakowski Medical Research Center Polish Academy of Sciences, Warsaw, Poland, [3] Department of Biochemistry, Nencki Institute of Experimental Biology Polish Academy of Sciences, Warsaw, Poland, [4] Faculty of Chemistry & Biological and Chemical Research Centre, University of Warsaw, Poland

INTRODUCTION: Myofibrillar myopathies (MFMs) are hereditary muscle diseases characterized by distinctive histopathology of myofibrillar disintegration and abnormal protein aggregation. Seven genes: DES, CRYAB, MYOT, FLNC, LDB3, BAG3, PLEC encoding proteins associated with Z disc are considered responsible for MFMs. However in about half of patients, the gene defect is still unknown.

AIM(S): The aim of this study was to describe the clinical and histopathological features of genetically confirmed MFM.

METHOD(S): 13 patients from 4 families with MFM were systematically identified and clinically studied. The families were not known to be related. In all suspected MFM patients (one proband from each family) disintegration of myofibrils and accumulation of degradation products into inclusions containing desmin were detected in muscle biopsy. However differentiation between MFM subtypes on the basis of clinical/pathological phenotype alone was impossible. Therefore, subtype identification was performed using molecular studies.

RESULTS: All patients presented with progressive muscle weakness with distal-proximal distribution in the lower limbs. CK was normal or slightly elevated. Finally three mutations were identified: two in DES: (Q348P) and (A357_E359del) and one in CRYAB (D109A). In two families with desminopathy caused by A357_E359del mutation cardiac arrhythmias was observed (paternal uncle with similar symptoms died due to cardiac arrhythmia). Dilated cardiomyopathy was confirmed by echocardiography in family with CRYAB D109A. In this family respiratory insufficiency as well as early cataract were diagnosed.

CONCLUSIONS: Molecular identification of MFM is crucial for final diagnosis. The awareness of MFM type could be life-saving by means of appropriate treatment

such as 1) inserting of a pacemaker in case of significant heart conduction defects and arrhythmia or 2) initiation of noninvasive ventilation in case of chronic respiratory failure.

P1.58. NGS AS A DIAGNOSTIC TOOL FOR UNEXPLAINED LIMB-GIRDLE MUSCLES WEAKNESS: RESULTS OF EUROPEAN MYO-SEQ PROJECT IN 75 POLISH PATIENTS

Anna Łusakowska[1], Katherine Johnson[2], Ana Töpf[2], Marta Bertoli[2], Lauren Phillips[2], Volker Straub[2], Monkol Lek[3,4], Liwen Xu[3,4], Daniel G. MacArthur[3,4], Anna Macias[1], Anna Potulska-Chromik[1], Biruta Kierdaszuk[1], Katarzyna Janiszewska[1], Ewelina Arendt[5], Przemysław Grochowski[5], Anna Kamińska[1], Anna Kostera-Pruszczyk[1]

[1] Department of Neurology, Medical University of Warsaw, Warsaw, Poland, [2] John Walton Muscular Dystrophy Research Centre, Institute of Genetic Medicine, Newcastle University, International Centre for Life, Central Parkway, Newcastle upon Tyne, UK, [3] Analytic and Translational Genetics Unit, Massachusetts General Hospital, Boston, USA, [4] Program in Medical and Population Genetics, Broad Institute of Harvard and MIT, Cambridge, USA, [5] Students' Scientific Group by the Department of Neurology, Medical University of Warsaw, Warsaw, Poland

INTRODUCTION: Hereditary muscle disorders are a genetically heterogeneous group of rare diseases with overlapping phenotypes causing difficulties in establishing a diagnosis. Genetic testing is the only reliable tool to confirm a prompt diagnosis. Genetically confirmed diagnosis is required for the future targeted therapies and the genetic counselling. Department of Neurology, Warsaw Medical University, participated in a European multicenter project MYO-SEQ led by Institute of Genetic Medicine, Newcastle University.

AIM(S): The main aim of the project was to establish accurate diagnoses in patients with unexplained limb-girdle muscle weakness by applying New Generation Sequencing(NGS). MATERIAL Patients included in the study were at least 10 years old, presented with unexplained limb-girdle or respiratory muscle weakness and/or elevated serum CK activity. Based on these criteria we identified 75 patients treated at our Department of Neurology.

METHOD(S): With the patients' consent, their encoded DNA samples and anonymous clinical data were sent to the MYO-SEQ coordinating center for a whole exome sequencing using NGS. A detailed analysis of potential mutations was restricted to 169 gene associated with neuromuscular disorders. When the molecular result were obtained, a detail clinical-genetic analysis was done.

RESULTS: In total of 75 tested samples, 50 (66,7%) showed specific mutations responsible for the patients' symptoms, including 45 (60,0%) with mutation in a single gene. In 5 samples (6,7%), mutations in more than one gene were found. In two patients the treatable diseases were identified: Pompe disease and congenital myasthenic syndrome. In 25 (33,3%) samples, no strong candidate gene was identified.

CONCLUSIONS: NGS offers an accurate and reliable methodology to establish a diagnosis in rare inherited muscle diseases.When the new molecular therapies become available, NGS test should be included in a standard diagnostic procedure of myopathies.

P1.59. THE RESPONSE OF CARDIOVASCULAR SYSTEM TO COLD PRESSOR TEST IN ATHLETES AND NON-ATHLETES

Katarzyna Leznicka[1], Maciej Pawlak[2]

[1] Szczecin University, Department of Physical Culture and Health Promotion, Szczecin, Poland, [2] Poznan University of Physical Education, Department of Physiology, Biochemistry and Hygiene, Poznan, Poland

INTRODUCTION: Numerous studies have indicated that intense physical effort is one of the key factors determining individual sensitivity to pain.

AIM(S): Assessment of pain threshold and pain tolerance in boxers and non-athletes evaluated with Cold Pressor Test (CPT) taking into account cardiovascular indices.

METHOD(S): The study involved 261 men aged 18–28. The first group consisted of 80 athletes, after at least five years of practicing boxing. The control group consisted of 181 students of the Faculty of Physical Culture, University of Szczecin. Pain tolerance and threshold were assessed using CPT. The Cold Pressor Test is a widely used experimental pain procedure to determine a person's pain threshold and pain tolerance, defined as the elapsed time until voluntary withdrawal of the hand. Systolic and diastolic blood pressure and pulse were measured three times: 1) prior to the test, 2) when the pain threshold was reported, 3) at the end of the test.

RESULTS: CPT indicated showed that boxers were much more tolerant to pain compare to non-athletes. All three measurements (s. Methods) showed higher heart rates in the control group. Reporting pain threshold, boxers had significantly lower heart rates compared to the control group. In both groups systematic increase of the systolic and diastolic blood pressure during CPT caused by pain stimulus (cold water) was observed, Systolic blood pressure was significantly lower in the control group after the test in comparison with boxers.

CONCLUSIONS: Our observations differ to some extent from the findings presented up to now and confirm the essential role of physical activity in the constant adrenergic stimulation.

FINANCIAL SUPPORT: I confirm that my study was given neither external financial support nor funding by any organization.

P1.60. PLAYING WITH COLORS: WHITE/YELLOW SPECTRAL SENSITIVITY OF THE RAT OLIVARY PRETECTAL NUCLEUS NEURONS

Patrycja Orłowska-Feuer[1,2], Magdalena K. Smyk[1,2], Marian H. Lewandowski[2]

[1] Malopolska Centre of Biotechnology, Jagiellonian University Krakow, Krakow, Poland, [2] Department of Neurophysiology and Chronobiology, Chair of Animal Physiology, Institute of Zoology and Biomedical Research, Jagiellonian University Krakow, Krakow, Poland

INTRODUCTION: Twice a day, at dawn and dusk, animals experience considerable changes in the amount and spectral composition of light. Interestingly, both of them, so irradiance as well as colour, contribute to photoentrainment and are used by rodents to encode the time of the day. The olivary pretectal nucleus (OPN) is a retinorecipient midbrain structure responsible for pupillary light reflex and is suggested to play a role in photoentrainment.

AIM(S): The aim of the study was to investigate whether cutting off the short wavelengths of light resulting in color changes influences light-induced neuronal activity in the rat OPN.

METHOD(S): To address this issue multielectrode *in vivo* recordings from the OPN of urethane anesthetized Long Evans rats were performed. Recordings were combined with light stimulations of different irradiance and spectral composition: full light spectrum provided by xenon lamp ("white light") *vs.* blockade of blue light by yellow filter ("yellow light"; cut off at 490 nm).

RESULTS: Both light stimulations induced a robust increase in multiunit activity across the pretectum area, with three different types of neuronal responses clearly seen: sustained, transient ON and OFF. Interestingly, sustained cells were able to encode light intensities independently of yellow filter usage. However, their mean activity during light pulses decreased in yellow light across all irradiances.

CONCLUSIONS: To our knowledge this is the first study showing that spectral composition of light matters not only for the suprachiasmatic nucleus where the main biological clock is localised, but also for other structures of the non-image forming visual system.

FINANCIAL SUPPORT: Supported by the grant 2013/08/W/NZ3/00700 obtained from the National Science Centre in Poland.

P1.61. EXPOSURE TO ELECTROMAGNETIC FIELD IMPROVES REGENERATION OF NERVOUS SYSTEM IN INSECT – ELECTROPHYSIOLOGICAL EVIDENCES

Maria Stankiewicz[1], Milena Jankowska[1], Ana P. Zanatta[2], Justyna Rogalska[1], Joanna Wyszkowska[1]

[1] Faculty of Biology and Environment Protection, Nicolaus Copernicus University, Toruń, Poland, [2] Laboratory of Neurobiology and Toxinology, Federal University of Pampa, São Gabriel, Brazil

INTRODUCTION: Cerci mechanoreceptors act as input into the insect escape system. They permit insects to recognize the direction of mechanical stimuli action. Amputation of one cercus elicits a disturbance of space-orientation of the insect and alters its locomotor activity.

AIM(S): The aim of the study was: 1) to examine whether untouched one cercus can functionally replace the second one removed, and 2) to determine the influence of electromagnetic field (EMF, 7 mT, 50 Hz, exposition 1 h/day during 3 weeks) on activity of the remaining cercus.

METHOD(S): Recordings of the global bioelectrical activity of cercal nerve were performed using tungsten extracellular electrodes.

RESULTS: Effect of wind puff stimulation was dependent on a direction of stimulus application. Under control conditions, stimulation of the left cercus from the right side caused ca. 50% smaller response (recorded from cercal nerve) than that of induced by stimulation from the left side. Similar side-dependent effect was observed for the other (right) cercus – stimulation from the left side induced much lower effect than stimulation from the right side. In the following experiments, the right cercus was removed and observations of cercal nerve bioelectrical activity were performed 24 hours after amputation, and then 1, 2 and 3 weeks later. The ratio of the magnitude of responses following stimulation from the right side to the stimulation from the left side was assessed. Twenty four hours after the amputation, and then 1 and 3 weeks later the ratio in unexposed insects equals 0.37, 0.45 and 0.53, respectively. During exposure to EMF the ratio was 0.54 after 1 week and 0.69 after 3 weeks from amputation.

CONCLUSIONS: Results suggest that the function of amputated cercus can be replaced by the other one and exposure to EMF facilitates this process.

FINANCIAL SUPPORT: Work was supported by grant of Nicolaus Copernicus University.

P1.62. RELATIONSHIP BETWEEN CORTICAL ACTIVATION AND THE DORSAL LATERAL GENICULATE NUCLEUS ACTIVITY UNDER URETHANE ANAESTHESIA IN THE RAT

Jagoda S. Jęczmień-Łazur[1,2], Patrycja Orłowska-Feuer[1,2], Marian H. Lewandowski[2]

[1] The Malopolska Centre of Biotechnology (MCB), Jagiellonian University in Krakow, Krakow, Poland, [2] Department of Neurophysiology and Chronobiology, Institute of Zoology, Jagiellonian University in Krakow, Krakow, Poland

INTRODUCTION: Retinal signals pass through the dorsal lateral geniculate nucleus (dLGN) of the thalamus to target the primary visual cortex. Besides the driver input from the eye, dLGN also receives substantial modulatory projections from layer 6 of cortex and the brainstem, which exert strong

influence on the dLGN cells. Thalamic neurons have two switching modes of firing activity: tonic and burst. Bursts comprise a number of closely spaced action potentials, followed by a long refractory period between bursts.

AIM(S): The aim of the present study was to determine whether activity of the rat dLGN depends on alternating brain states in terms of its spontaneous activity, firing mode (tonic and bursting) and light-induced responses.

METHOD(S): Extracellular single-unit *in vivo* and EEG recordings combined with white light stimulations were performed in 19 adult Long Evans rats under urethane anaesthesia. Light-induced responses, bursting parameters and correlation between spontaneous neuronal activity and EEG were analysed.

RESULTS: In total, 22 light-responsive neurons were recorded and all of them were characterized by burst firing mode detected in both EEG phases. Spontaneous activity of 68% of cells was modulated by EEG changes with significant decrease during the deactivation. Moreover, during that phase the percentage of bursts was higher, while neuronal responses to light were significantly reduced.

CONCLUSIONS: The dorsal lateral geniculate nucleus of the thalamus comprises of two subpopulations of light-sensitive cells, which are distinguished by their sensitivity to cyclic brain alternations under urethane anaesthesia. Interestingly, bursting cells within dLGN are involved in visual signal transmission in a state dependent manner.

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P1.63. REPEATED CORTICOSTERONE TREATMENT INCREASES THIN SPINE DENSITY IN PYRAMIDAL NEURONS OF DEEP LAYERS OF THE RAT PRIMARY MOTOR CORTEX

Anna Guguła[1], Anna Błasiak[1], Joanna Kula[1], Alan Kania[1], Grzegorz Tylko[1], Grzegorz Hess[1,2]

[1] Department of Neurophysiology and Chronobiology, Institute of Zoology and Biomedical Research, Jagiellonian University in Krakow, Krakow, Poland, [2] Department of Physiology, Institute of Pharmacology Polish Academy of Sciences, Krakow, Poland

INTRODUCTION: Stress-related elevated glucocorticoid level disrupts performance of motor tests in rats but underlying neuronal mechanisms remain unknown. Repeated corticosterone injections serve as an animal model of prolonged stress. Recently we have shown that treatment with corticosterone influences both the electrophysiology and morphology of pyramidal neurons of rat primary motor cortex (M1). It enhances spontaneous glutamate release and has no effect on dendritic spine density in superficial layers of M1, whereas in deep layers it increases spine density but does not affect electrophysiology.

AIM(S): The current study aimed at identification of spine types in different layers of M1 and consequent judgment on their ability to form functional synapses. Investigating the density of various spine types would clarify the apparent discrepancy between our previous electrophysiological and morphological data.

METHOD(S): Rats were injected with corticosterone for 7 days, twice-daily (control group received the vehicle) and then decapitated. Brains were removed and stained using the Golgi-Cox method. Images of layer II/III and V M1 pyramidal neurons were obtained and deconvolved. Mushroom, stubby and thin spines and filopodia were counted manually on representative dendrites from the apical and basal part of the neurons.

RESULTS: In layer II/III of M1 the density of each morphological spine type remained unaltered by corticosterone treatment, however, it significantly elevated the density of thin spines in layer V neurons. Other spine types were not affected.

CONCLUSIONS: These data suggest that previously observed increase in M1 layer V spine density was caused exclusively by thin spine number upsurge. Thin spines are considered immature and do not form functional synaptic connections, what further validates our previous electrophysiological data. Lack of morphological changes in layer II/III is congruent with the proposed corticosterone-induced pre-synaptic mechanism of enhanced glutamate release.

P1.64. SUBPOPULATION OF C-FIBER-ACTIVATED LAMINA I PROJECTION NEURONS WITH UNIQUE INPUT-OUTPUT CHARACTERISTICS FOR THE NMDAR-DEPENDENT NOCICEPTIVE CODING

Kirill Agashkov[1], Volodymyr Krotov[1], Margarita Krasniakova[1], Diana Shevchuk[1], Anna Liashenko[1], Boris Safronov[3], Nana Voitenko[1], Pavel Belan[2]

[1] Bogomoletz Institute of Physiology, Laboratory of Sensory Signaling, Kiev, Ukraine, [2] Bogomoletz Institute of Physiology, Laboratory of Molecular Biophysics, Kiev, Ukraine, [3] Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, Portugal

INTRODUCTION: Spinal lamina I projection neurons (PNs) are key elements of the pain processing system, which relay peripheral input to supraspinal structures generating sensation of pain. The population of PNs is small (~5% of lamina I neurons) but very heterogeneous according to their intrinsic and synaptic properties.

AIM(S): Investigate mechanisms of acute nociception encoding and evaluate input-output characteristics of lamina I PNs.

METHOD(S): Whole-cell recordings from lamina I PNs retrogradely-labeled via the lateral PB area in an intact spinal cord preparation with attached dorsal roots.

RESULTS: We identified a specific group of PNs (16%) with unique properties. In these PNs (SB-PNs), a stimulation of nociceptive afferents evoked gradual strength-dependent amplification of afferent input expressed as an increase in the number of generated APs. Upon a root stimulation at C-fiber-intensity, the SB-PN group generated more than 80% of spikes of the entire population of PNs, thus, being the major group of PNs codifying acute pain sensation. We have also identified several mechanisms of this nociceptive input amplification. First, SB-PNs are intensively innervated by the high-threshold A delta- and C-afferents providing robust and reliable spike generation. Second, the nociceptive input was amplified by intrinsic bursting capabilities of SB PNs. Third, the afferent input was prolonged (to 0.-1.5 s) and potentiated (to -45 mV to -20 mV) by the NMDAR-dependent synaptic component forming intrinsic plateau potentials generated by SB-PNs. The afferent stimulation increased, for several seconds, spontaneous excitatory drive to SB-PNs that became suprathreshold and evoked series of spikes.

CONCLUSIONS: We have described a new type of lamina I PNs efficiently transmitting the main part of primary nociceptive input to supraspinal structures playing an important role in acute pain generation. A complex interplay between synaptic, intrinsic and network activities underlies unique nociceptive encoding features of this group of PNs.

FINANCIAL SUPPORT: Supported by IBRO.

P1.65. SPINAL BDNF OVEREXPRESSION PARTIALLY PROTECTS SYNAPTIC MACHINERY OF NEUROMUSCULAR JUNCTION FROM DISINTEGRATION AFTER COMPLETE SPINAL CORD TRANSECTION IN ADULT RATS: NEUROCHEMICAL AND MORPHOLOGICAL CHANGES EVALUATED BY HISTOCHEMICAL TECHNIQUES

Anna Głowacka[1], Olga Gajewska-Woźniak[1], Krzysztof M. Bernadzki[2], Julita Czarkowska-Bauch[1], Małgorzata Skup[1]

[1] Department of Neurophysiology, Nencki Institute of Experimental Biology Polish Academy of Sciences, Warsaw, Poland, [2] Department of Cell Biology, Nencki Institute of Experimental Biology Polish Academy of Sciences, Warsaw, Poland

INTRODUCTION: Complete spinal cord transection (SCT) leads to loss of motor control due to disruption of supraspinal tracts and altered functioning of both central and peripheral synapses. We showed that SCT at low thoracic segments causes deficiency in cholinergic input to ankle extensor (soleus) motoneurons, whereas brain-derived neurotrophic factor (BDNF) overexpression below the lesion site increases markers of spinal neurotransmission and improves locomotor performance. These findings raise the question if SCT impairs also integrity of peripheral synapses in soleus muscle and if BDNF can counteract lesion effects.

AIM(S): To disclose the impact of SCT and BDNF overexpression on pre- (VAChT and S-100) and postsynaptic (nAChR) components of neuromuscular junction (NMJ) in soleus muscle.

METHOD(S): VAChT and S-100 were detected immunohistochemically and acetylcholine receptors were visualized with fluorescently labeled bungarotoxin on free-floating muscle fibers 2 weeks after SCT and intraspinal injection of PBS (n=6) or BDNF (n=7). Images acquired on Zeiss confocal microscope were deconvoluted with Huygens Professional and analyzed with 3D Imaris Software to evaluate NMJ morphology.

RESULTS: SCT reduced the number of contacts of normal morphology to 39% which was accompanied by decrease in NMJs size. BDNF overexpression resulted in preservation of 73% of normal contacts, but did not prevent NMJ shrinkage. VAChT-labeled synaptic vesicles marking motoneuron terminals were visibly more dispersed after SCT than in controls. BDNF did not affect this dispersion.

CONCLUSIONS: Spinal BDNF overexpression partially prevents NMJs from denervation, albeit does not counteract the reduced size of NMJ. It needs further investigation whether motor improvement is the effect of direct neuroprotective role of BDNF on NMJs or the result of altered signaling at central synapses.

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P1.66. HIGH RESOLUTION MRI ANATOMY OF THE CAT VISUAL SYSTEM AT 7 TESLA

Anna Kozak[1], Bartosz Kossowski[3], Jarosław Orzeł[2], Marlena Wełniak-Kamińska[2], Kalina Burnat[1], Michał Fiedorowicz[2]

[1] Department of Molecular and Cellular Neurobiology, Nencki Institute of Experimental Biology Polish Academy of Sciences, Warsaw, Poland, [2] Mossakowski Medical Research Centre Polish Academy of Sciences, Warsaw, Poland, [3] Laboratory of Brain Imaging, Nencki Institute of Experimental Biology Polish Academy of Sciences, Warsaw, Poland

INTRODUCTION: Majority of our knowledge about human visual system comes from cat or monkey studies. Feline models of visual diseases, such Macular Degeneration and congenital cataract accurately recreate many aspects of human impariments allowing for comparative study of neuropathology and the testing of the novel therapeutics. Advances in human visual system research frequently remain to depend upon animal modeling. However, filling the gap between body of knowledge about human and animal anatomy requires developing of imaging methods, providing more accurate comparisons.

AIM(S): Here we describe *in vivo* visualization of the feline visual system that were previously only visible *post mortem*.

METHOD(S): T2-weighted (TR=3500 ms, TE=30 ms) turbo spin echo (TurboRARE-T2) images were acquired using 7 Tesla Bruker BioSpec 70/30 USR (Ettlingen, Germany). Anatomic structures were identified based on feline histology. We applied in situ hybridization to measure the expression of the activity reporter gene zif268 as a function of the visual activation in the visual system of the cat. As a control histology staining was performed.

RESULTS: T2-weighted, high resolution MR images of feline visual system are provided in sagittal and dorsal planes. Comparison with traditional high resolution imaging methods (in situ hybridization and Nissl staining) is shown.

CONCLUSIONS: Presented data establish normal appearance of detailed anatomical structures of the feline brain. As feline models reproduces anatomy of human visual system most faithfully, this data provide reference when evaluating neurologic disease or testing efficacy of novel therapeutics in animal models.

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P1.67. EFFECT OF TEMPORARY INACTIVATION OF RAT VISUAL CORTEX ON VISUAL RESPONSES IN THE SUPERIOR COLLICULUS

Katarzyna Kordecka[1], Andrzej Foik[2], Agnieszka Wierzbicka[1,3], Wioletta J. Waleszczyk[1]

[1] Department of Neurophysiology, Nencki Institute of Experimental Biology Polish Academy of Sciences, Warsaw, Poland, [2] University of California, Irvine, USA, [3] University of Warsaw, Faculty of Physics, Warsaw, Poland

INTRODUCTION: It is generally accepted that neuronal plasticity can be induced at the cortical level. In our previous study we observed that relatively strong visual stimulation enhanced responses both at the cortical and subcortical level. The backward projection from the visual cortex to superior colliculus (SC) may facilitate the reinforcement of response in this midbrain structure.

AIM(S): In the current study we examined how inactivation of the visual cortex affects responses in the SC after visual training.

METHOD(S): Visual evoked potentials (VEPs) were recorded in anesthetized rats (n=5) from the primary visual cortex (VCx) and the SC, contralateral to stimulated eye, in response to flashing white-light-emitting diodes (LEDs) placed 10 cm in front of the rat. Monocular visual stimulation consisted of series of 300 repetitions of light flashes with 2 s intervals, presented every 15 minutes through 3 hours. In order to temporary block the activity of the cortex after 3-hour visual stimulation, a well above the contralateral VCx was fulfilled with xylocaine solution (2.5%). During cortical inactivation a single series of visual stimulation (300 stimulus repetitions) was presented and the SC VEP amplitudes were analysed.

RESULTS: Chemical inactivation resulted in strong attenuation of cortical VEP amplitudes. In the case of the SC, cortical deactivation did not cause any significant difference in VEP amplitudes as compared to responses after 3 h of visual training. Collicular VEPs were still at the high level and significantly differed from control recording at the beginning of training, which indicates a minor impact of the VCx on response enhancement in the SC.

CONCLUSIONS: Temporary deactivation of the visual cortex didn't result in decline of VEP amplitudes in the SC, which indicates that increase of responses in SC after visual training is most likely due to enhancement of the retinal input to the SC.

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P1.68. THE DREADD AGONIST, CNO, DOES NOT AFFECT LEARNING-DEPENDENT CORTICAL PLASTICITY

Grzegorz Dobrzański, Renata Zakrzewska, Małgorzata Kossut

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

INTRODUCTION: Associative fear learning, in which stimulation of vibrissae is paired with tail shock results in increased functional cortical representation of the row of whiskers activated during the conditioning. Expansion of the functional cortical representation was revealed with 2-deoxyglucose autoradiography. The chemogenetic DREADD technique allows for precise manipulation of the brain circuits and is based on exclusive activation of designer receptors by designer drug – CNO (Clozapine N-oxide). CNO, which is believed to be pharmacologically inert in mice and rats but not in humans, recently was found to produce some behavioural effects in one of the rat's strain.

AIM(S): Taking into account the possible unspecific results in our chemogenetic experiments in mice, we aimed to determine if CNO administered alone can influence the learning-dependent plasticity.

METHOD(S): A group of wild type, C57BL/6J mice underwent behavioural training consisting of 3 sessions of conditioning in three consecutive days. 30 minutes before each session mice were injected intraperitoneally with CNO (1 mg/kg). 24 hours after the third session 2-deoxyglucose procedure was performed. Autoradiograms of tangential brain sections containing the barrel field were analyzed and functional representation of the conditioned row

of whiskers and contralateral row on the other side of the snout were mapped.

RESULTS: Analysis showed the increased representation of the trained row in the fourth layer of barrel cortex in conditioned hemisphere in comparison to control one. Cortical activity was also observed in other structures like secondary somatosensory cortex and auditory cortex, which replicate the pattern of activation observed in previous experiments.

CONCLUSIONS: The results suggest that CNO administered alone does not influence the learning--dependent cortical plasticity and can be applied in chemogenetic experiments within this experimental model of learning in mice.

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P1.69. PROPERTIES OF RHYTHMIC FIRING OF MOTONEURONS ARE ALTERED IN RESPONSE TO WEIGHT-LIFTING TRAINING IN RATS

Piotr Krutki[1], Włodzimierz Mrówczyński[1], Marcin Bączyk[1], Dawid Łochyński[2], Jan Celichowski[1]

[1] Department of Neurobiology, Poznan University of Physical Education, Poznan, Poland, [2] Department of Musculoskeletal Rehabilitation, Poznan University of Physical Education, Poznan, Poland

INTRODUCTION: Repeated short-term and high--intensity exercises with a progressive external load are defined as strength or resistance training, which is responsible for an increase in muscle mass and force.

AIM(S): The aim of this study was to determine whether strength training induces adaptive changes in firing properties of motoneurons (MNs) innervating the trained muscles.

METHOD(S): The study was performed on adult male Wistar rats. Animals from the training group were subjected to a five-week voluntary progressive weight-lifting program, while control rats were restricted to standard cage activity. Intracellular recordings from lumbar spinal MNs innervating gastrocnemius and soleus muscles were made under pentobarbital anesthesia.

RESULTS: The strength training evoked adaptive changes in both slow and fast-type MNs, indicating their increased excitability: a higher input resistance, a lower rheobase, a decrease in the minimum currents required to evoke rhythmic firing. The maximum frequencies of the early-state firing (ESF) and of the steady-state firing (SSF) were increased. Moreover, higher ESF and SSF slopes of the frequency-current relationship were observed in MNs of the trained group. Higher maximum firing rates of MNs as well as higher discharge frequencies evoked at the same level of intracellular depolarization current imply higher levels of tetanic forces developed by motor units over the operating range of force production after the strength training.

CONCLUSIONS: This study provides evidence that the changes in spinal excitability following strength training observed in humans may be due to changes in the intrinsic properties of the MNs. The findings largely explain why some adaptations in the twitch and tetanus force development of motor units could be observed in response to the dynamic resistance training without qualitative changes in the muscle myosin heavy-chain expression.

FINANCIAL SUPPORT: The study was supported by the National Science Center grant 2013/11/B/NZ7/01518.

P1.70. SEX DIFFERENCES IN THE NUMBER AND MORPHOMETRIC PROPERTIES OF MUSCLE SPINDLES IN THE RAT MEDIAL GASTROCNEMIUS MUSCLE

Magdalena Gartych[1], Dorota Bukowska[1], Barbara Mierzejewska-Krzyżowska[2], Jan Celichowski[1]

[1] Poznan University of Physical Education, Department of Neurobiology, Poznan, Poland, [2] Poznan University of Physical Education, Faculty of Physical Culture in Gorzów Wielkopolski, Department of Biomedical Sciences and Health Sciences, Division of Morphological Sciences, Biology and Health Sciences, Gorzów Wielkopolski, Poland

INTRODUCTION: There are considerable differences in a number and density of muscle spindles in various skeletal muscles. Considerable sex differences in muscle mass and diameter of extrafusal muscle fibres suggest that muscle spindle density in muscles and morphometric properties of spindles: the diameter, the number and diameters of intrafusal muscle fibres are also different in males and females. Similar number of γ-motoneurons in male and female rats suggest similar number of muscle spindles but their lower density in males.

AIM(S): The aim of the study was to check sex differences in the number, the density and morphometric properties of muscle spindles in rat medial gastrocnemius muscle.

METHOD(S): Medial gastrocnemius muscles were excised from two male and two female three-month old Wistar rats. Muscles were stored in 4% formalin solution and then cut into 10 and 20 μm slices stained with methylene blue and magenta. The light microscopy (Nikon microscope with camera and NIS Elements program) was used to calculate a number of muscle spindles in the muscle. The morphometric properties of spindles were measured on the equatorial regions.

RESULTS: The number of muscle spindles is similar in male (13), in female (13–14) muscles. However, the density of spindles was different: 86–92 in males and 51–57 mg of the muscle mass per one spindle in females. There were

also slight differences in a number of intrafusal muscle fibres in one spindle (in males: 4.3; range 3–8 and in females: 4.5; range 2–7). The diameters of intrafusal fibres on the equatorial regions amounted to: 6.7±2.1 μm in males and 6.6±2.6 μm in females. However, it was noticed that diameters of the male muscle spindles are larger than in the female ones: 21.4±7.3 μm and 20.2±5.7 μm, respectively.

CONCLUSIONS: The main sex difference concerns density of muscle spindles which is lower in males than in females.

P1.71. ORIENTATION TUNING IN MOUSE PRIMARY VISUAL CORTEX – INTRINSIC SIGNAL OPTICAL IMAGING STUDY

Ida Raciborska, Katarzyna Kordecka, Anna Posłuszny, Wioletta J. Waleszczyk

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

INTRODUCTION: Accumulating body of research has shown a cardinal bias for preference of spatially oriented targets in different species including humans, indicating greater neuronal responses in the primary visual cortex for horizontal or vertical contours in opposite to oblique ones.

AIM(S): We used intrinsic signal optical imaging, a popular tool to map cortical function in rodents to verify the hypothesis whether a cardinal bias is present also in mouse primary visual cortex.

METHOD(S): The experiments were performed on 7 week old wild mice under isoflurane anaesthesia. Intrinsic signals were recorded using CCD camera set above the visual cortex. Visual stimuli, square-wave black-and-white gratings (spatial frequency 0.05 cycle/degree, and temporal frequency 2 Hz, four orientations: 0, 45, 90, 135 degree) drifting in two directions, back and forth, were presented in random order with uniform grey images in 16 trials. Imaging was performed under the control of Imager 3001 system. Data were collected with 10 Hz resolution from 1 s before stimulus onset, during 7 s of visual stimulation and to 1 s after stimulus offset with 7 s interval between recordings.

RESULTS: Using the described protocol of visual stimulation and data collection we could successfully map cortical responses to visual stimuli of different orientations. Collected images showed the strongest responses for horizontally and vertically oriented gratings.

CONCLUSIONS: Our results support the hypothesis of the bias toward cardinal orientation preference in mouse visual cortex.

FINANCIAL SUPPORT: Supported by the Polish National Science Center grant Symfonia 1 (2013/08/W/NZ4/00691).

P1.72. A COMPARISON OF METHODS FOR ANALYSIS OF TEMPORAL FREQUENCY TUNING IN VISUAL RESPONSES

Agnieszka Wierzbicka[1,2], Katarzyna Kordecka[1], Jarosław Żygierewicz[2], Wioletta J. Waleszczyk[1]

[1] Nencki Institute of Experimental Biology Polish Academy of Sciences, Warsaw, Poland, [2] Faculty of Physics, University of Warsaw, Warsaw, Poland

INTRODUCTION: There are numerous methods to study neuronal processing of information about temporal frequency content of visual stimuli. The two most fundamental methods are 1) direct measurement of response amplitude, e.g. an amplitude of averaged visual evoked potential, and 2) assessment of response magnitude after transformation of electrophysiological signal from time to frequency domain.

AIM(S): The aim of this study was to find an appropriate analysis method to characterize cortical responses to visual stimuli of various temporal frequencies.

METHOD(S): Visual responses were recorded from both primary visual cortices, contra- and ipsilateral to the stimulated eye, using multichannel linear electrode arrays during electrophysiology experiments performed on anesthetized rats. As a visual stimulus we used 2-ms-long LED flashes delivered at two frequencies: 1 and 7 Hz.

RESULTS: We found that for frequency of 1 Hz it is difficult to draw conclusions based on power spectrum alone. For frequency of 7 Hz the assessment of evoked potential in time domain was highly inaccurate.

CONCLUSIONS: For 1 Hz the estimation of the visual evoked potential amplitude by direct measurement should be also performed. For 7 Hz the analysis should be performed after transformation of the signal from the time to frequency domain. Our results also indicate the advantages of the Welch method in comparison to the periodogram to analyze signals in the frequency domain.

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P1.73. SINUSOIDAL MECHANICAL RESPONSES FROM SINGLE MOTOR UNITS OF RAT MEDIAL GASTROCNEMIUS MUSCLE

Hanna Drzymała-Celichowska[1,2], Katarzyna Kryściak[1], Piotr Kaczmarek[3], Claudio Orizio[4], Piotr Krutki[1], Jan Celichowski[1]

[1] Department of Neurobiology, University School of Physical Education in Poznan, Poland, [2] Department of Biochemistry, University School of Physical Education in Poznan, Poland, [3] Institute of Control and Information Engineering, Faculty of Electrical Engineering, Poznan University of Technology, Poznan, Poland, [4] Department of Clinical and Experimental Sciences, University of Brescia, Brescia, Italy

INTRODUCTION: The relationship between the output force and motor command depends on the intrinsic

dynamic responses of motor units (MUs), which can be characterized by evoking sinusoidal force responses at variable rate.

AIM(S): The main goal of this study was to determine whether sinusoidal modulation of the stimulation frequency for different types of MUs results in reliable sinusoidal force changes.

METHOD(S): Experiments were performed on adult Wistar rats under general anesthesia. MUs were functionally isolated by electrical stimulation of single axons from the ventral roots of spinal nerves and then were stimulated by changing the stimulation frequency sinusoidally at several rates of frequency modulation (0.1–0.2–0.3–0.5–0.7–1.0–1.5–2.0 Hz for slow and 0.4–0.8–1.2–2.0–2.8–4.0–6.0–8.0 Hz for fast MUs). The stimulation frequency range was individually matched for each MU to evoke unfused tetanic contractions.

RESULTS: The amplitudes of force fluctuations related to modulations of stimulation frequency were measured and presented as a function of a rate of frequency changes. When the rate increased, the amplitude exponentially decreased and the decrease exceeded 20% at a rate 2.8–4.0 Hz for fast resistant (FR) MUs and 0.3–0.5 Hz for slow (S) ones.

CONCLUSIONS: In conclusions, these results indicate that high rate changes in rate coding are efficient for fast but not for slow MUs. These findings provide a novel tool that may expand our knowledge of the specificity of MU types through the dynamic responses of different MUs, with potential implications for study of changes that occur with training, aging or neuromuscular diseases.

P1.74. FACTORS CONTRIBUTING TO SAG IN UNFUSED TETANIC CONTRACTIONS OF FAST MOTOR UNITS IN RAT

Jan Celichowski[1], Katarzyna Kryściak[1], Rositsa Raikova[3], Piotr Krutki[1], Hanna Drzymała-Celichowska[1,2]

[1] Department of Neurobiology, Poznan University of Physical Education, Poznan, Poland, [2] Department of Biochemistry, Poznan University of Physical Education, Poznan, Poland, [3] Institute of Biophysics and Biomedical Engineering, Bulgarian Academy of Sciences, Sofia, Bulgaria

INTRODUCTION: Sag phenomenon is observed in fast motor units (MUs) as a transitional decline in force during unfused tetanic contractions, however its mechanisms are not known.

AIM(S): The goal of the study was to identify factors contributing to sag in two types of fast MUs, FF and FR ones, with emphasis on differences between them.

METHOD(S): Experiments were performed on adult Wistar anesthetized rats. MUs were activated by electrical stimulation of single axons isolated from ventral roots of spinal nerves. The unfused tetanic contractions of fast MUs were recorded.

RESULTS: The mathematical decomposition of unfused tetanic contractions of FF and FR MUs into twitch-shape responses to consecutive stimuli was conducted. The decomposition indicated substantial changes predominantly in force and additionally in time parameters of successive twitch-like components, responsible for a sag profile in tetanic curve. Namely, initially the force increased and the highest force was observed in a response to the 2nd–3rd stimulus for FF units, while after the 3rd–7th stimulus for FR MUs and later decreased leading to the sag. In the second series of experiments, a repeatability of the sag in tetanic contractions of the same MU in a muscle with preserved blood circulation and under ischemic conditions was tested. Sag restitution was present in muscles with the circulation preserved but it was prevented by occlusion of blood vessels, indicating that sag depends on an availability of an energy source which can be restituted under aerobic conditions.

CONCLUSIONS: The study indicated that sag profile of unfused tetanic contractions is predominantly an effect of early increase in amplitudes of several initial responses followed by a decrease in their amplitudes and that these changes are stronger and longer in time scale in FR than in FF MUs. The results concerning repeatability of the phenomenon suggest that most probable source of energy for initial force increase is phosphocreatine.

P1.75. EFFECTS OF SHORT- AND LONG-TERM CARNOSINE TREATMENT ON CONTRACTILE PROPERTIES OF MOTOR UNITS IN AGED RATS

Maciej Pawlak[1], Dominik Kaczmarek[1,2], Dawid Łochyński[3], Magdalena Gartych[2], Tomasz Podgórski[1], Anna-Maria Borucka[1], Inge Everaert[4], Wim Derave[4], Jan Celichowski[2]

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

INTRODUCTION: Carnosine (beta-alanyl-L-histidine) is predominantly present in skeletal muscle but also in other excitable tissues. It was suggested that muscle carnosine concentration can decrease with ageing. There is growing evidence that supplementation of carnosine can be effective for the treatment of age related disorders as well as neurological disorders e.g. Alzheimer's disease or Parkinson's disease.

AIM(S): Here we investigate the effects of orally supplemented carnosine in aged rats on motor units (MUs) contractile properties.

METHOD(S): 42 male Wistar rats aged 15 months were randomly assigned to three groups: control (CON; n=15), treated with carnosine for 8 months (CAR8M; n=15) or treated with

carnosine for the last 10 weeks of the study (CAR10W; n=12). After 8 months all survived animals (n=10, 9 and 8 in CON, CAR8M and CAR10W group, respectively) were used 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), twitch force (TwF) and force profile during the two separate fatigue tests were analysed.

RESULTS: Our study revealed that in FF units, the force was maintained at a higher level during the first fatigue test in CAR8M group and during the first minute of this test in CAR10W group. In addition, in control rats the force of FR units declined more during the second fatigue test. The TwF and TetF did not differ between groups in fast MUs but TwF was significantly higher in slow MUs of rats treated with carnosine for 8 months. However, in these animals the force of slow units declined more during the initial 20 seconds of the first fatigue test.

CONCLUSIONS: In conclusion, carnosine treatment in aged animals seems to induce beneficial changes in MU contractile properties, causing improvement in the force maintenance in fast units and enhancement of the twitch force in slow units.

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P1.76. ROLE OF THE TCF7L2 TRANSCRIPTION FACTOR IN REGULATING THE DEVELOPMENT OF THALAMUS AND GROWTH OF THALAMIC AXONS

Marcin Lipiec[1,2], Kamil Koziński[1], Tomasz Zajkowski[1], Andrzej Nagalski[1], Marta B. Wiśniewska[1]

[1] Centre of New Technologies, Laboratory of Molecular Neurobiology, University of Warsaw, Warsaw, Poland, [2] Faculty of Biology, University of Warsaw, Warsaw, Poland

INTRODUCTION: The thalamus integrates sensory information and is involved in the selection of behavioural responses. These functions require a proper development of specific thalamic nuclei and establishment of neural connections between the thalamus and the cortex. The molecular mechanisms of thalamic development are poorly characterised. Our studies suggested, that this process may strongly depend on the TCF7L2 transcription factor. However, the actual function of TCF7L2 in directing thalamic development was not established.

AIM(S): The aim of our research was to determine the role of TCF7L2 in the development of thalamic cytoarchitecture, molecular anatomy and thalamocortical connections.

METHOD(S): In the study, we used Tcf7l2-/- mouse embryos (E18.5). The embryos were sacrificed, their brains isolated and fixed. In one set of experiments the brains were frozen and cut with the use of cryotome. Then, slices were used either for Nissl staining to visualise anatomical structure, or in situ hybridisation for gene expression analysis. In another set of experiments, fixed brains were dissected into two hemispheres, after which a small DiI crystal was inserted into the thalamus. After incubation, the hemispheres were cut with a vibratome and DiI-stained axons were visualised under fluorescent microscope.

RESULTS: We show here, that TCF7L2-deficent mice displayed major changes in the anatomy of the thalamus and habenulae, as well as partial malformations of the striatum. Furthermore, Tcf7l2-/- mice most often completely failed to produce thalamocortical axons; if some were visible, they did not reach their cortical targets.

CONCLUSIONS: The study demonstrated a critical involvement of TCF7L2 in thalamic nucleogenesis and establishment of thalamocortical axons.

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P1.77. DIETARY HYPERHOMOCYSTEINEMIA REDUCES BRAIN AQP4 EXPRESSION AND COGNITIVE PERFORMANCE IN OLD MICE

Joanna Suszyńska-Zajczyk[1], Joanna Śliwowska[2], Hieronim Jakubowski[1,3]

[1] Department of Biochemistry and Biotechnology, Poznan University of Life Sciences, Poznan, Poland, [2] Laboratory of Neurobiology, Department of Veterinary Medicine and Animal Sciences, Poznan University of Life Sciences, Poznan, Poland, [3] Department of Microbiology, Biochemistry and Molecular Genetics, Rutgers-New Jersey Medical School, International Center for Public Health, Newark, USA

INTRODUCTION: Elevated plasma total homocysteine (tHcy), i.e. hyperhomocysteinemia (HHcy), is associated with brain pathologies such as vascular dementia, cognitive decline and Alzheimer's disease and glial functions. Learning and memory may also be indirectly regulated by water homeostasis in the brain.

AIM(S): Determine how the mouse brain and behaviour are affected by chronic long-term dietary HHcy.

METHOD(S): HHcy was induced in 8-month-old C57BL/6 male mice by providing 1% methionine in drinking water for 12 months. Control mice did not receive methionine. At 20 months of age, weight, urinary tHcy, and behavioural phenotypes were recorded. Cognitive impairment was assessed by the Novel Object Recognition (NOR) test. Mice were sacrificed, perfused, and brains were collected for aquaporin-4 (AQP4) immunohistochemistry.

RESULTS: HHcy mice had lower body weight than controls (31.4±1.1 *vs.* 35.1±1.3 g, p=0.04). HHcy mice had 32-fold higher urinary tHcy than controls (493±57 *vs.* 15.3±4.9 μM, p<0.0001). HHcy mice showed neurodegener-

ation phenotypes with impaired limb-clasping reflex and coordination in the ledge test (index 3.5 *vs.* 1.6, p=0.02). Short-term memory in HHcy mice was impaired in the NOR test. In familiarization session (FS) HHcy mice presented uneven contact duration with both objects (fold change 1.5±0.4), while in test session (TS) time spent with familiar and novel object did not differ (10.45±1.27 *vs.* 9.55±1.27 s, p>0.05). In control mice, no preference for one of the two objects was observed in FS, while in TS, mice were able to detect novel object (12.44±1.67 *vs.* 7.56±1.67 s, p<0.01). Mean recognition index for novel object was lower in HHcy mice relative to controls (0.52±0.04 *vs.* 0.62±0.07, p<0.05). HHcy brains had lower number of AQP4-positive vessels in the cerebral cortex, compared to controls (27.5±3.8 *vs.* 41.6±4.7, p<0.0001).

CONCLUSIONS: These findings show that long-term exposure to HHcy impairs mouse brain functions and that these effects may be mediated by changes in water homeostasis.

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P1.78. VOLTAGE-GATED POTASSIUM CHANNEL KCNB1 PLAYS A ROLE IN DEVELOPMENT OF HEARING AND VESTIBULAR FUNCTION IN ZEBRAFISH

Justyna Jędrychowska, Jacek Kuźnicki, Vladimir Korzh

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

INTRODUCTION: Voltage-gated potassium channels selectively regulate transport of potassium ions along electrochemical gradient in plasma membrane. They are involved in many crucial biological processes in both excitable and non-excitable cells, including action potential, apoptosis, cell proliferation and differentiation, neurotransmitter or hormone release and cardiac activity. Hence, their dysfunctions lead to severe human disorders, e.g. deafness or epilepsy. In our study we focused on the zebrafish Kcnb1 (Kv2.1), a member of the Kv2 subfamily of voltage-gated potassium channels. Recently, a novel function of Kcnb1 in formation of the brain ventricular system in zebrafish has been found. This study demonstrated that during development Kcnb1 is expressed also in the ear and eye. Based on its expression pattern we suggest that Kcnb1 is important for development of the ear, where it may be required for hearing and spatial orientation.

AIM(S): The aim of this study was to use the zebrafish mutant of Kcnb1 to investigate a role of Kcnb1 during development of the ear.

METHOD(S): We used morphometric and behavioral analyses to study development of the ear and check hearing and spatial orientation in Kcnb1 mutant embryos and larvae.

RESULTS: The otic vesicle and otoliths of Kcnb1 mutant develop relatively normal. We observed significant length reduction of the otic vesicle in mutant embryos. Mutants demonstrate significant hearing defects and uncoordinated balance movements.

CONCLUSIONS: Unlike clear morphological abnormality of the brain ventricular system, development of the otic vesicle in Kcnb1 zebrafish mutant is relatively uneventful. Kcnb1 requirement in the ear manifests itself in mutants as abnormal hearing and vestibular function.

FINANCIAL SUPPORT: This work was supported by funds from OPUS grant to Vladimir Korzh from National Science Centre (OPUS UMO-2016/21/B/N23/00354).

P1.79. ADVANCED PATERNAL AGE AFFECTS ULTRASOUND VOCALIZATION IN ADULT MOUSE OFFSPRING

Marta Ziętek[1], Artur H. Świergiel[1], Silvestre Sampino[2]

[1] *University of Gdansk, Faculty of Biology, Department of Animal and Human Physiology, Laboratory of Physiology of Behavior and Stress, Gdansk, Poland,*
[2] *Institute of Genetics and Animal Breeding Polish Academy of Sciences, Department of Experimental Embryology, Jastrzębiec, Poland*

INTRODUCTION: Advanced paternal age (APA) is a risk factor for conceiving children with autism spectrum disorders. Social deficits, altered communication and repetitive behaviors are key diagnostic symptoms of autism.

AIM(S): In this study we used a mouse model to investigate the effects of APA on offspring communicative behaviors - ultrasound vocalization (USV).

METHOD(S): 4–5 month old Swiss males conceived by fathers of 3 different ages – 12 month old (Advanced Paternal Age, APA n=32), 2 month old (Young Paternal Age, YPA-1 n=17), 5 month old (YPA-2 n=16) - and mothers aged 3 months, were subjected to the resident-intruder test to evoke USVs emission. The tested mouse, which had been previously isolated for 7 days, was habituated for 7 minutes to a soundproof chamber. An intruder (C57/CBA 2–4 month old male) was then introduced to the home-cage of the tested mouse. Ultrasound vocalizations were recorded for 180 s using an ultrasound-sensitive microphone placed 10 cm above the cage and were analysed using Avisoft SASLab software.

RESULTS: There were no statistically significant differences among groups in the latency to start USV emission. The percentage of vocalizing mice in APA was 87,5%, YPA-1 – 53% and YPA-2 – 87,5%. APA mice displayed increased number of USVs (p=0.003), increased duration and decreased sound amplitude of USVs, compared to YPA mice (p<0.001 and p<0.0001, respectively). No significant changes were observed in minimum and maximum USV frequen-

cies. Furthermore, USVs were classified based on their waveform pattern. Mice conceived by differently aged fathers exhibited different repertoires of vocalizations.

CONCLUSIONS: Overall, paternal age affects USV patterns in adult offspring. Heritable de novo mutations and/or epigenetic alterations transmitted by the sperm may underlie the phenotypic changes observed in offspring.

FINANCIAL SUPPORT: This work was supported by the Polish National Science Center (2014/15/D/NZ4/04274) and in part by Statutory Research Fund of the Department of Animal and Human Physiology of University of Gdansk.

P1.80. PROFILE EXPRESSION OF STIM2.1, A NEWLY DISCOVERED STIM2 SPLICE VARIANT IN IMMUNE CELLS, IN MOUSE BRAIN STRUCTURES AT DIFFERENT TIMES OF DEVELOPMENT

Łukasz Majewski[1], Iga Wasilewska[1], Barbara A. Niemeyer[2], Jacek Kuźnicki[1]

[1] Laboratory of Neurodegeneration, International Institute of Molecular and Cell Biology in Warsaw, Warsaw, Poland, [2] Center for Integrative Physiology and Molecular Medicine, Saarland University, Homburg, Germany

INTRODUCTION: STIM1 and STIM2 mediate the process of store-operated calcium entry (SOCE) by interacting with the ion channels in the cell membrane – ORAIs. SOCE is a process by which the depletion of Ca^{2+} from the ER causes an influx of Ca^{2+} from the extracellular space to replenish the intracellular stores. In T cells there are two splice variants of STIM2 – STIM2.1 and STIM2.2, which play opposite roles in regulation of SOCE. According to a paper by Niemeyer group, STIM2.1, in contrast to STIM2.2, has an inhibitory effect on Store-Operated Calcium Entry (SOCE).

AIM(S): One of our objectives was to check the distribution of STIM2.1 and STIM2.2 in mouse brain structures at different times of development. We also investigated the influence of neuronal-specific STIM1 and ORAI1 overexpression on STIM2 mRNA level in the brain.

METHOD(S): Using quantitative RealTime-PCR we compared the expression of STIM2 isoforms in wildtype and our novel transgenic mouse lines overexpressing STIM1 or ORAI1 specifically in brain neurons.

RESULTS: We show that STIM2.1 splice variant is expressed in mouse brain at a low level with the highest STIM2.1/STIM2.2 ratio in the olfactory bulbs. These are the only structures in which expression of both STIM2 splice variants increases with aging. We also observed that overexpression of STIM1 in neurons tends to modify the expression of STIM2 isoforms in a region specific manner, eg. it decreases its level in the hippocampus, while increases in substantia nigra. Neither expression of STIM2.2 nor STIM2.1 isoform was affected by ORAI1 overexpression in brain neurons.

CONCLUSIONS: Our data show for the first time the expression of STIM2 isoforms in mouse brain structures at different time points. The observation that STIM1 shapes the expression of STIM2 isoforms in a region specific manner could contribute to a better understanding of the interplay between these two key elements of SOCE in neurons.

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P1.81. EXPRESSION OF GENES INVOLVED IN SOCE IN DANIO RERIO

Iga Wasilewska, Jacek Kuźnicki

Laboratory of Neurodegeneration, International Institute of Molecular and Cell Biology, Warsaw, Poland

INTRODUCTION: Store-Operated Calcium Entry (SOCE) is a major calcium influx mechanism in non-excitable cells, however, there is increasing evidence indicating its significance in neurons. STIM proteins, that are calcium sensors localized in the endoplasmic reticulum membrane, are crucial for this process. Dysregulated calcium homeostasis is a feature of many neurodegenerative disorders. Recently, it was shown that the level of STIM2 is reduced in the hippocampus in mice model of Alzheimer's disease (AD) and in cortical samples from sporadic AD patients. Moreover, expression of STIM2 in mice brain decreases with aging.

AIM(S): The aim of this study was to determine the expression pattern of stims and orais in zebrafish. This will allow us to investigate Stim2 functions in neurons and to prepare models of some pathologies. Genetically modified zebrafish lines are often used as models of human diseases.

METHOD(S): Using quantitative RT-PCR we estimated the mRNA level of stims and orais in larvae and in various tissues obtained from adult zebrafish. Using CRISPR/Cas9 technology stim2b-/- zebrafish line was generated and then crossed with Tg(HuC:GCaMP5G) line. Changes in Ca^{2+} level in wild-type and k/o larvae were measured *in vivo* using Light-Sheet microscopy with GCaMP5G calcium probe expressed in neurons.

RESULTS: Similarly to what was observed in rodents, orai2 dominates in the brain, nevertheless, there are some differences between mammalian and zebrafish expression pattern regarding stims. It was shown that Stim2 is mainly expressed in the mouse brain, while in zebrafish both stim2 isoforms dominate in muscles, however their level in

the brain is still relatively high. Interestingly, stim1b and stim2b as well as orai1a and orai1b expression significantly increases with aging.

CONCLUSIONS: The analysis of calcium homeostasis and gene expression in stim2b-/- fish indicates that this calcium ER sensor plays an important role in zebrafish neurons.

P1.82. GABAERGIC SYSTEM IN AGING. FOCUS ON SOMATOSTATIN CONTAINING INTERNEURONS

Aleksandra Różycka, Renata Zakrzewska, Monika Liguz-Lęcznar
Department of Molecular and Cellular Neurobiology, Nencki Institute of Experimental Biology Polish Academy of Sciences, Warsaw, Poland

INTRODUCTION: Molecular aging, defined as an age-related transcriptome changes, and biochemical protein-related alterations within synapses weaken the plastic potential of neurons. Previously, we have shown an age-related impairment of learning-related functional plasticity in mouse somatosensory cortex (SI), induced by associative fear learning and visualized with brain mapping using 2-deoxyglucose technique.

AIM(S): The aim of the study was to investigate age-related changes in somatostatin-containing GABAergic interneurons, which are involved in learning – related plasticity in mouse SI.

METHOD(S): Learning-related plasticity was induced with classical conditioning, where tactile stimulus to large sensory whiskers was coupled to the tail shock. Two groups of mice were used in the experiments: young (3 months old) and aged (1 year old). We have investigated mRNA and protein level of GAD67 (enzyme synthesizing GABA) and SOM (somatostatin) in mouse SI using q-RT-PCR and ELISA, respectively. Using immunofluorescence we compared the number of both types of neurons in SI.

RESULTS: Analysis of q-RT-PCR results revealed no change in investigated mRNAs levels between young and aged mice. We also observed an upregulation of GAD67 and GABA levels after training in young but not in aged animals. Immunohistochemistry results showed an increase in the number of GAD67+ cells, however, we did not observe an elevation in the number of SOM+/GAD67+ cells.

CONCLUSIONS: Increase in GAD67+ neurons density after sensory training in aged animals without parallel upregulation in GAD67 and GABA levels suggests lower GABA synthesis resulting in reduced effectiveness of aged GABAergic neurons. Lack of increase in SOM+ neurons density after sensory training in aged mice, suggest that upregulation of SOM+ cells is necessary for training induced plasticity.

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P1.83. DIVERSE ROLES OF LEF1 IN THE DEVELOPMENT OF THALAMUS, PRETECTUM AND OPTIC TECTUM

Nikola Brozko[1,2], Marta Krolak[1,3], Andrzej Nagalski[1], Ryan MacDonald[4], Marta B. Wiśniewska[1]
[1] Laboratory of Molecular Neurobiology, Centre of New Technologies, University of Warsaw, Poland, [2] The Postgraduate School of Molecular Medicine, Medical University of Warsaw, Poland, [3] Faculty of Biology, Department of Animal Physiology, University of Warsaw, Poland, [4] Department of Infection, Immunity & Cardiovascular Disease, University of Sheffield, UK

INTRODUCTION: Lef1 is an effector of the canonical Wnt pathway that has been implicated in brain development at many stages. Lef1 is expressed specifically in the dorsal diencephalon and mesencephalon from the early stages onwards in many vertebrates. However, its role in the development of these brain parts has not been investigated so far.

AIM(S): I used zebrafish as a model organism to examine the role of the widely expressed Lef1 in regulating the specification of neurons in distinct domains in the diencephalon and mesencephalon.

METHOD(S): Firstly, I analyzed the spatiotemporal expression patterns of Lef1 proteins in zebrafish brain cryosections. Then I performed knockdowns of lef1 using Morpholinos, and analyzed the expression of markers that are specific for diverse progenitors (at stage 30hpf) and neurons (at stage 3dpf) in the brain. To this end I used fluorescent in situ hybridization (ISH) and visualized the larvae under confocal microscopy.

RESULTS: Immunostaining revealed a strong expression of the Lef1 protein in the brain at 2dpf. ISH at the stage of progenitor domains (shh, dbx1a) showed that lef1 is not involved in their formation in diencephalon (thalamus – Th, pretectum – Pt) and mesencephalon (optic tectum – TeO). However, I observed serious impairments in expression of ascl1a and neurog1, genes characteristic for different classes of prospective neurons in the primordium of the Th, Pt and TeO. Because ascl1 is expressed in GABAergic progenitors, I hypothesized that Lef1 is involved in the specification of GABAergic neurons. I verified it at the stage of 3dpf and observed an expansion of the tcf7l2 expression that is a marker of the caudal Th, into the GABAergic rostral Th (nkx2.2a). Moreover I noted a depletion of GABAergic neurons in Pt and TeO.

CONCLUSIONS: Concluding, my results implicate Lef1 in establishing the boundaries of the caudal part of Th and in the generation of GABAergic neurons in Pt and TeO. The mechanisms by which Lef1 participates in these events are yet to be understood.

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P1.84. DEVELOPMENTAL CHANGES IN RESTING-STATE EEG ACTIVITY IN ADHD: A CROSS-SECTIONAL STUDY

Katarzyna Giertuga[1], Marta Z. Zakrzewska[2], Maksymilian Bielecki[3], Ewa Racicka[4], Małgorzata Kossut[1,3], Anita Cybulska-Klosowicz[1]

[1] Laboratory of Neuroplasticity, Department of Molecular and Cellular Neurobiology, Nencki Institute of Experimental Biology Polish Academy of Sciences, Warsaw, Poland, [2] Gösta Ekman Laboratory, Department of Psychology, Stockholm University, Stockholm, Sweden, [3] Faculty of Psychology, SWPS University of Social Sciences and Humanities, Warsaw, Poland, [4] Department of Child Psychiatry, Medical University of Warsaw, Warsaw, Poland

INTRODUCTION: Numerous studies indicate that Attention Deficit/Hyperactivity Disorder (ADHD) is related to some developmental trends, as its symptoms change widely over time. There is a disagreement whether ADHD is related to deviations in brain development or to a delay in brain maturation. The model of deviated brain development suggests that the ADHD brain matures in a fundamentally different way, and does not reach normal maturity at any developmental stage. In contrast, the delayed brain maturation model assumes that the ADHD brain indeed matures in a different, delayed way in comparison to healthy age-matched controls, yet eventually reaches proper maturation.

AIM(S): We investigated developmental changes in resting-state EEG activity to find evidence supporting one of the alternative models.

METHOD(S): A total number of 141 participants took part in the study: 67 ADHD and 74 healthy controls. We recorded 5 minutes of resting-state EEG. Each participant's power estimates were averaged across clusters of electrodes and across frequency bands: delta, theta, alpha, and beta. The absolute power of each frequency was analyzed. To test the combined effect of age and ADHD diagnosis on EEG power spectrum, we performed a regression analysis.

RESULTS: The results revealed a typical developmental effect of decreasing absolute EEG power with increasing age. Absolute EEG power was found to decrease linearly especially for delta and theta frequencies in both groups. We also observed differences between groups. The ADHD group had significantly lower absolute power in all frequency bands, with the most pronounced difference in lower theta absolute power.

CONCLUSIONS: This study revealed that the resting-EEG developmental pattern was similar in ADHD and healthy controls. Even so, the ADHD group had consistently lower absolute EEG power, mostly in the theta frequency band. Our results are in line with deviant brain maturation hypothesis, as ADHD brain activity would not be considered the same as in healthy controls at any age.

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P1.85. CEREBELLUM DEVELOPMENT IN THE GREY SHORT-TAILED OPOSSUM

Beata Tepper[1], Katarzyna Bartkowska[1], Krzysztof Turlejski[2], Ruzanna Djavadian[1]

[1] Nencki Institute of Experimental Biology Polish Academy of Sciences, Warsaw, Poland, [2] Cardinal Stefan Wyszynski University in Warsaw, Warsaw, Poland

INTRODUCTION: The grey short-tailed opossum, Monodelphis domestica is a laboratory animal useful in developmental research due to its slow and mostly postnatal growth. Little is known about marsupials brain development.

AIM(S): The aim of the study was to investigate cerebellum development in the Monodelphis opossum.

METHOD(S): We performed Nissl staining on the brain sections from opossums in different age to see the overall pattern of cerebellum development. To find out when progenitor cells are present in this structure, we performed bromodeoxyuridine (BrdU) intraperitoneal injections in different time points. Phenotype of proliferating cells was identified by double immunofluorescence staining for a neuronal marker, NeuN or an astrocyte marker, GFAP and BrdU.

RESULTS: We found that in the newborn Monodelphis opossum cerebellum is not yet morphologically formed. Immunostainig of BrdU-positive cells showed that Purkinje cells were generated between postnatal day (P) 1–5, whereas the highest rate of granule cells generation occurred between P11–P40. Double immunostaining revealed that the majority of BrdU-positive cells in the opossum cerebellum generated from P11 to P50 was neurons, as they showed colocalization with NeuN immunoreactivity.

CONCLUSIONS: Our study shows that cerebellum development in the opossum is longer and more extended in time than in rodents.

FINANCIAL SUPPORT: The work was supported by the National Science Center grant 2015/17/B/NZ4/02410.

P1.86. GENES EXPRESSED IN THE OPOSSUM NEOCORTEX DURING DEVELOPMENT OF INTERHEMISPHERIC CONNECTIONS

Katarzyna Bartkowska[1], Roman Wunderlich[2], Victor Tarabykin[2], Krzysztof Turlejski[3], Ruzanna Djavadian[1]

[1] Department of Molecular and Cellular Neurobiology, Nencki Institute of Experimental Biology Polish Academy of Sciences, Warsaw, Poland, [2] Charité – Medical University of Berlin, Institute of Cell Biology and Neurobiology, Berlin, Germany, [3] Faculty of Biology and Environmental Sciences, Cardinal Stefan Wyszynski University in Warsaw, Warsaw, Poland

INTRODUCTION: All mammals have the six-layered brain neocortex and neurons of upper layers (III/II) forming interhemispheric connections link the two cerebral hemispheres. In marsupials the interhemispheric connections of the neocortex pass via the anterior commissure,

while in eutherians they form additionally new, much shorter fiber pathway, the corpus callosum.

AIM(S): The aim of this study was to define differences in molecular signals guiding elongation of interhemispheric neocortical axons during formation of corticocortical connections in the mouse, representative of eutherians and the opossum, representative of marsupials.

METHOD(S): Using immunofluorescence labeling we detected the special AT-rich DNA-binding protein 2 (Satb2) expression in the opossum brain between postnatal days (P) 14 and P19. This pattern of expression was similar to that in the mouse between embryonic days (E) 16 and E18, which corresponds to the same developmental stage of opossums. Some genes that are essential for guiding neocortical commissural axons in mice were studied by in situ hybridization method.

RESULTS: We detected expression of many genes such as DCC, Unc5c, EphA5, EphA7, EphB1, Sema6D, Slit1, PlxA4 that exhibit the same patterns in the mouse and the opossum brains. However, the expression of EphA4 and Sema3c in both mouse and opossum cortical neurons was different. The expression of EphA4 and Sema3c was restricted to upper layers neurons of the opossum, while EphA4 was strongly expressed in the ventricular and subventrical neurons of the mouse and Sema3c was expressed in the intermediate zone of mouse neocortex.

CONCLUSIONS: Thus, we suggest that EphA4 and Sema3c are candidate genes for controlling and establishing interhemispheric connection in the opossum brain. But the question remains open: can these genes regulate formation of the cortical commissure before midline interaction.

FINANCIAL SUPPORT: This work was supported by the National Science Center grant 2016/22/M/NZ4/00670.

P1.87. ALTERED ACTIVITY OF NEURAL PROGENITORS IN THE SUBVENTRICULAR ZONE OF THE CYCLIN D2-DEFICIENT MICE

Rafał Płatek, Piotr Rogujski, Jarosław Mazuryk, Leszek Kaczmarek, Artur Czupryn

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

INTRODUCTION: Adult neurogenesis has been widely studied in mammals due to potential implications for therapeutical use. There are two main neurogenic regions in the adult mammalian forebrain: 1) the subventricular zone (SVZ), which is the thin cellular lining of the lateral ventricles, and 2) the subgranular zone (SGZ) of the dentate gyrus of the hippocampus. Experimental models with defective adult neurogenesis enable to decipher molecular components critical for neuronal replacement. On the other hand, there is a limited number of animal models for studying impaired neurogenesis. Mice lacking cyclin D2 were reported to show dramatically reduced number of actively proliferating cells in SGZ, however, molecular and cellular mechanisms of proliferation deficiency in the SVZ still need to be characterized.

AIM(S): In our study, we investigated the role of cyclin D2 in SVZ neural progenitors proliferation activity, migration of neuroblasts and their differentiation to olfactory bulb interneurons.

METHOD(S): In order to assess in details proliferation activity, wildtype (WT) and cyclin D2-knockout (cD2-KO) mice were injected with EdU, a thymidine analogue, and analyzed together with endogenous proliferation marker Ki67, enabling quantification of cells at different cell cycle stages.

RESULTS: We observed a significant reduction in the number of EdU(+) and/or Ki67(+) progenitors along anterior-posterior and dorsal-ventral axis of the SVZ. We also revealed differences in expression of transcription factors between cD2-WT and cD2-KO mice, bearing in mind SVZ mosaic organization and that certain SVZ domains produce certain subpopulations of interneurons.

CONCLUSIONS: Understanding of the mechanisms governing adult neurogenesis at the cellular and molecular level may lead towards cell-based therapies in neurodegenerative diseases or after brain injuries.

FINANCIAL SUPPORT: Supported by the National Science Center (NCN) grant no UMO-2012/07/B/ NZ4/01733 and partially by the National Center for Research and Development (NCBiR) grant Strategmed Regennova (235077/9/ NCBR/2014).

P1.88. INFLUENCE OF OBESITY AND TYPE 2 DIABETES ON THE NUMBER OF KISSPEPTIN-, NEUROKININ B- AND DYNORPHIN A-IMMUNOREACTIVE NEURONS IN THE ARCUATE NUCLEUS OF FEMALE RATS

Kamil Ziarniak[1], Paweł Kołodziejski[2], Ewa Pruszyńska-Oszmałek[2], Julia Matuszewska[1], Joanna H. Śliwowska[1]

[1] Laboratory of Neurobiology, Faculty of Veterinary Medicine and Animal Science, Poznan University of Life Sciences, Poznan, Poland, [2] Department of Animal Physiology and Biochemistry, Faculty of Veterinary Medicine and Animal Science, Poznan University of Life Sciences, Poznan, Poland

INTRODUCTION: There is a strong evidence that neurons co-expressing kisspeptin (KP), neurokinin B (NKB) and dynorphin (Dyn), so called KNDy neurons, are important factors governing the hypothalamic-pituitary-gonadal axis. These neurons are present in the arcuate nucleus of the hypothalamus (ARC), which is also a region involved in energy homeostasis. It was shown that expression of KP, NKB and Dyn is dependent on hormonal and metabolic status. We have previous found that type 2 diabetes but not diet-induced obesity increases number of KP-, NKB- and

Dyn-immunoreactive (-ir) neurons in the ARC of male rats. Hence, it seemed likely that metabolic and hormonal alterations (obesity, diabetes) would also alter the number of KNDy neurons in females.

AIM(S): We hypothesized that obesity and/or diabetes will alter the number of KP-, NKB- and Dyn-ir neurons in the ARC of female rats.

METHOD(S): Female rats were assigned to 3 groups: 1) control (C) – fed with a regular chow, 2) diet-induced obesity (DIO), 3) type 2 diabetes (DM2). Animals from groups 2 and 3 were fed with diet containing 50% of fat. All animals received diets for 11 weeks. To induce diabetes animals were injected with low doses of streptozotocin. To detect stage of the estrous cycle, vaginal smears were taken for two weeks. Hormonal and metabolic profiles were assessed and immunocytochemistry for KP, NKB and Dyn A were performed.

RESULTS: In contrast to males, there were no changes in the number of KP-, NKB- and Dyn A-ir cells in the ARC of diabetic females. However, similar to males, in obese females there were no significant differences in number of studied neurons compared to controls.

CONCLUSIONS: We have concluded that sex differences exist in response of studied neurons in diabetic, but not in obese animals. Further studies will explore a role of sex hormones (estrogen and progesterone) on the number of KNDy neurons in DIO and DM2 female rats.

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P1.89. GESTATIONAL PROFILE OF SUPPRESSOR OF CYTOKINE SIGNALING-3 (SOCS-3) TRANSCRIPTS IN OVINE BRAIN AND ANTERIOR PITUITARY

Małgorzata Szczęsna, Katarzyna Kirsz, Dorota A. Zięba

Faculty of Animal Sciences, Department of Animal Biotechnology, University of Agriculture in Krakow, Krakow, Poland

INTRODUCTION: Suppressors of cytokine signaling-3 (SOCS-3) are key negative regulators of the JAK/STAT signaling cascade – the most important pathway for leptin and prolactin signal transduction. Gestation which induces a state of specific leptin-resistance is an excellent model for examining changes in SOCS-3 signaling.

AIM(S): The aim of this study was to investigate the profile of SOCS-3 mRNA expression in different structures of the brain and in the anterior pituitary (AP) of pregnant sheep.

METHOD(S): Experiments were performed using 15 pregnant Polish Longwool ewes. At slaughter, AP and individual brain structures: mediobasal hypothalamus (MBH), arcuate nucleus (ARC), median eminence (ME), choroid plexus (CP), and pineal gland (PG) were isolated from non-pregnant ewes and ewes euthanized at 30, 60, 90, 120 d of pregnancy (3 ewes/group). Real-time PCR was used to measure SOCS-3 mRNA abundance.

RESULTS: Results showed that SOCS-3 transcript level increased in MBH at 30, 60 and 90 d of gestation in comparison with non-pregnant ewes ($P<0.05$). The greatest SOCS-3 transcript abundance was observed at 120 d of pregnancy in ARC and in AP. In ME, SOCS-3 expression significantly decreased ($P<0.05$) during early- and mid-pregnancy (at 30 and 60 d of gestation) but during late-pregnancy (120 d of gestation) it increased to a level comparable to that of non-pregnant ewes. In the CP, SOCS-3 mRNA expression in first half of pregnancy was similar to that observed in non-pregnant females, but increased markedly in the second half of pregnancy ($P<0.05$). Interestingly, SOCS-3 expression decreased throughout pregnancy in the PG ($P<0.05$).

CONCLUSIONS: The pattern of expression of SOCS-3 differs among brain locations and by stage of pregnancy within brain and AP locations and variation in SOCS-3 transcripts may be one of the factors in brain and AP that mediate homeorhetic adjustments in metabolism during gestation.

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P1.90. GHRELIN REGULATES NIGHTLY MELATONIN SECRETION FROM SHEEP PINEAL GLAND IN A TPH1-INDEPENDENT MANNER: AN IN VITRO STUDY

Katarzyna Kirsz, Małgorzata Szczęsna, Dorota A. Zięba

Agricultural University of Krakow, Department of Animal Biotechnology, Laboratory of Biotechnology and Genomics, Krakow, Poland

INTRODUCTION: Several studies suggest that ghrelin (GHRL) has neurobiological effects that extend beyond the control of food intake. Our previous results confirmed that GHRL modulates the secretory activity of the pineal gland (PG) through nocturnal melatonin (MEL) secretion in sheep, which are seasonally reproductive animals.

AIM(S): We investigated the effects of GHRL and seasons on the tryptophan 5-hydroxylase 1 (TPH1) expression in sheep PG. TPH1 is the rate-limiting enzyme in the biosynthesis of serotonin (MEL precursor).

METHOD(S): Glands were collected after sunset from 8 ewes during long-day season (LD; May) and from 8 ewes during short-day season (SD; November). The PG were transected into strips (n=72), with each equilibrated in 1.0 ml of DMEM for 60 min, followed by a 4-hr incubation in a gas-liquid interface in medium alone (control) or in medium containing GHRL (10 ng/ml). After each hr of incubation, selected explants were frozen in liquid nitrogen and stored at –80°C for Real-time PCR and ELISA. Sixty minutes

after GHRL treatment, 50 µl of medium was harvested and stored at -20°C until RIA for MEL.

RESULTS: The results showed that during the SD season, TPH1 transcript levels were higher (P<0.01) in the GHRL-treated PGs than in the controls. During the LD season, TPH1 mRNA levels were similar (P>0.05) in both groups. There were no effects (P>0.05) of GHRL on TPH1 protein concentrations compared with controls in both seasons. Within the GHRL group, the mean TPH1 protein concentration was greater (P<0.05) for PG explants collected from ewes during the SD season compared with the LD season. During the SD season, GHRL inhibited (P<0.001) MEL secretion from PG explants. However, during the LD season, no effect (P>0.05) of GHRL on MEL secretion was noted.

CONCLUSIONS: These findings indicate that GHRL regulates nightly MEL secretion during the SD season in a TPH1-independent manner.

FINANCIAL SUPPORT: Research supported by the Polish National Science Center (2012/05/B/NZ4/02408).

P1.91. CUTTING OFF BLUE LIGHT, CUTTING OFF RHYTHMICITY? EFFECTS OF SHORT WAVELENGTH LIGHT DEPRIVATION ON LOCOMOTOR ACTIVITY AND CORE BODY TEMPERATURE IN PIGMENTED RATS

Magdalena K. Smyk[1,2], Aleksandra Przytulska[2], Patrycja Orłowska-Feuer[1,2], Marian H. Lewandowski[2]

[1] Malopolska Centre of Biotechnology, Jagiellonian University Krakow, Krakow, Poland, [2] Department of Neurophysiology and Chronobiology, Chair of Animal Physiology, Institute of Zoology and Biomedical Research, Jagiellonian University Krakow, Krakow, Poland

INTRODUCTION: Melanopsin-positive intrinsically photosensitive retinal ganglion cells (max absorption: 480 nm) constitute the major retinal input to the brain structures engaged in circadian rhythmicity. The amount of blue light varies daily and seasonally, with maximal amount at dawn, dusk, and summer versus winter. Insufficient exposition to light in the winter is thought to be a major cause of circadian disturbances found in e.g. seasonal affective disorder. It is however not certain whether changes in the spectral composition of light contribute to the phenomenon.

AIM(S): Considering that melanopsin contribution to photoentrainment was established utilizing genetic knock-out models, the study aimed to investigate the effects of blocking the blue part of the white light spectrum on circadian rhythmicity in non-genetically modified, pigmented rats.

METHOD(S): Locomotor activity and core body temperature were simultaneously recorded in male, adult Long Evans rats (n=8). During 9 baseline days animals were kept in 12:12 light-dark cycle. Light was provided by halogen lamps emitting white light of full spectral composition (light intensity: 400 lux). During following 9 experimental days blue part of the spectrum was blocked by yellow filter (cut off at 525 nm). Locomotor activity, core body temperature and their circadian parameters were compared between light conditions.

RESULTS: After blue light blockage amplitude and robustness of the locomotor activity rhythm decreased. A significant reduction in the mean level of activity was observed in the dark phase. Moreover, the mesor of the rhythm of core body temperature decreased, core body temperature was reduced both in the light and the dark phase.

CONCLUSIONS: Blue light deprivation results in disturbances in circadian rhythmicity in non-genetically modified rats with fully functional retina. Observed changes resemble those found in depressed patients, suggesting that not only light intensity but also its spectral composition matters.

FINANCIAL SUPPORT: Supported by 2013/08/W/NZ3/00700.

P1.92. MEDIAL-SEPTAL CHOLINERGIC MEDIATION OF HIPPOCAMPAL THETA RHYTHM INDUCED BY VAGAL STIMULATION IN RATS

Paulina Kłos-Wojtczak[1], Adam Broncel[2], Renata Bocian[1], Jan Konopacki[1]

[1] Department of Neurobiology, University of Lodz, Lodz, Poland, [2] Medical Technology Center, Lodz, Poland

INTRODUCTION: Hippocampal (HPC) theta rhythm may be important for various phenomena, including attention and acquisition of sensory information. Two types of HPC theta (types I and II) exist based on pharmacological, behavioral, and electrophysiological properties. Both types occur in conscious animals, whereas only type II (atropine-sensitive) theta is present under anaesthesia. The circuit of HPC theta synchronization includes the medial septum-diagonal band of Broca (MSDB), with cholinergic and GABA-ergic neurons comprising the two main projections from MSDB to HPC. Just recently we have demonstrated that vagal nerve stimulation (VNS) induces HPC type II theta in urethanized rats.

AIM(S): The primary aim of the present study was to assess the effects of cholinergic MSDB inhibition on VNS induced HPC theta rhythm.

METHOD(S): Anesthetized rats were implanted with vagal bipolar cuff electrode. VNS parameters were constant: pulse duration (1.0 ms), train duration (10 s), frequency (10 Hz) and pulse intensity 8 mA. Monopolar tungsten electrodes were implanted into HPC in accordance to standard stereotactic technique. HPC field potential was analyzed of off-line using the Spike-2 software computing system (CED, Cambridge, UK). In a separate experiments cholinergic,

muscarinic antagonists, atropine sulphate, dicyclomine and gallamine (M1 and M2 antagonists respectively) were injected directly into MSDP.

RESULTS: It was demonstrated that atropine and dicyclomine abolished HPV theta rhythm induced by VNS. Gallamine, in contrast, did not block VNS induced HPC theta. The present data provide the first spectacular evidences for cholinergic MSDP mediation of VNS effect on HPC type II theta.

CONCLUSIONS: Since atropine sulphate does not discriminate between M1 and M2 cholinergic receptors two specific antagonist of muscarinic M1 and M2 receptor were applied: dicyclomine and gallamine. The present data suggest that M1 muscarinic-cholinergic receptors of MSDB determines the effect of VNS on HPC theta production.

P1.93. SOME TECHNICAL ISSUES OF VAGAL NERVE STIMULATION. THE APPROACH WITH USE OF MODEL OF HIPPOCAMPAL THETA RHYTHM

Renata Bocian[1], Paulina Kłos-Wojtczak[1], Adam Broncel[2], Jan Konopacki[1]

[1] The University of Lodz, Faculty of Biology and Environmental Protection, Department of Neurobiology, Lodz, Poland, [2] Medical Technology Centre, Lodz, Poland

INTRODUCTION: Electrical stimulation of the vagal nerve (VNS) has been used for years to treat patients with drug-resistant epilepsy. Although VNS was approved as an adjunctive therapy for reducing the frequency of seizures, the mechanisms through which VNS modulates activity in central nervous system are still poorly understood. Interestingly, this technique also remains under investigation as a specific treatment for several other psychiatric and neurological disorders: cognitive dysfunction in Alzheimer's disease, depression, schizophrenia, migraine and central inflammation. Since 1988 more than 100.000 patients were implanted with vagal nerve electrodes. However, not all epilepsy patients respond to VNS: 1/3 does not tolerate the cuff electrode implantation and 50% of remaining does not respond to VNS at all. Several studies on the effectiveness, safety, optimal stimulation, have been conducted.

AIM(S): In a present study we focused on type of electrode which are used for VNS. Specifically we compared the effect of VNS performed with use of bipolar tungsten electrode and bipolar platinum/iridium cuff electrode on hippocampal (HPC) field potential recorded in anesthetized rats.

METHOD(S): One group of rats were implanted with vagal bipolar cuff electrode. The second group of animals were positioned in a specifically designed holder and uninsulated bipolar tungsten electrode were used for VNS. Tungsten electrodes touched the vagal nerve surface only during stimulation. VNS intensity was tested in a range: 0.2–10.0 mA. Remaining parameters were constant: pulse duration (1.0 ms), train duration (10 s), frequency (10 Hz).

RESULTS: We demonstrated that the chronically implanted vagal cuff electrode decreased (vs tungsten electrode) the amplitude of HPC type II theta in 30%. The effect of different types of electrodes used for VNS was also noticed in the evaluation of HPC theta threshold.

CONCLUSIONS: The type of the stimulation electrode (cuff *vs.* tungsten) used determines the final effect of VNS on hippocampal field potential.

P1.94. INTRASEPTAL PROCAINE INJECTION ABOLISHES THETA RHYTHM INDUCED BY VAGAL NERVE STIMULATION

Adam Broncel[1], Renata Bocian[2], Paulina Kłos-Wojtczak[2], Jan Konopacki[2]

[1] Medical Technology Center, Lodz, Poland, [2] The University of Lodz, Faculty of Biology and Environmental Protection, Department of Neurobiology, Lodz, Poland

INTRODUCTION: Vagal nerve stimulation (VNS) is currently approved for treatment of both pharmacologically resistant seizures and severe refractory depression. In addition, VNS is used for treatment of Alzheimer disease, schizophrenia and central inflammation. Interestingly, VNS has also been demonstrated to enhance HPC-induced long-term potentiation (LTP) and improve memory in rats and humans. The above mentioned findings suggest the direct involvement of hippocampal formation (HPC) theta rhythm.

AIM(S): We have just recently documented for the first time the presence of HPC type II theta in response to the application of VNS. VNS-induced theta rhythm appeared in different experimental protocols and, depending on the current intensity, could occur directly during VNS (brief effect) or after vagal stimulation (delayed effect). The aim of the present study was to demonstrate that the effect of left VNS on HPC theta rhythm is mediated by medial septum.

METHOD(S): Anesthetized rats were implanted with vagal cuff electrodes and unilaterally with HPC recording tungsten electrode. The VNS (8 mA, 500 us, 10 s) were applied three times: before HPC injection of procaine (control), 10 min after and 60 min after. The EEG signals were analyzed off-line using the Spike-2 software computing system (Cambridge Electronic Design, Cambridge, UK).

RESULTS: We demonstrated that medial-septal procaine injection reversibly abolished HPC type II theta rhythm induced by VNS in anesthetized rats.

CONCLUSIONS: We believe that the present findings concerning type II theta rhythm open a new perspec-

tive into the study of vagal nerve involvement in central processes of sensory-motor integration, cognition and memory.

P1.95. HYPERPHAGIC OBESITY IN ADULT DICER CKO MICE: THE ROLE OF HYPOTHALAMIC AGRP/NPY NEURONS

Karolina Hajdukiewicz[1], Marzena Stefaniuk[2], Valery Grinievich[3], Witold Konopka[1]

[1] Laboratory of Animal Models, Neurobiology Center, Nencki Institute of Experimental Biology, Warsaw, Poland, [2] Laboratory of Neurobiology, Nencki Institute of Experimental Biology, Warsaw, Poland, [3] Schaller Research Group on Neuropeptides, German Cancer Research Center and University of Heidelberg, Heidelberg, Germany

INTRODUCTION: Obesity is a worldwide disease of complex etiology. The main appetite regulatory center is located within the brain, in the hypothalamus. A putative mechanism responsible for the obesity phenotype involves microRNA interplay between feeding regulatory elements of the hypothalamic AgRP/NPY-expressing, POMC-producing and probably other arcuate nucleus neurons. Dicer is a key enzyme in microRNA processing. In Dicer's absence, there is a pronounced lack of mature microRNAs and a disturbed regulation of translation.

AIM(S): We want to observe how massive, spatially and temporally defined, loss of microRNAs impacts metabolism and obesity outcome.

METHOD(S): We injected rAAV-coding Cre recombinase under the AgRP specific promoter into the arcuate nucleus of mice with a Cre- dependent Dicer sequence. As NPY is a known appetite stimulator, to determine whether NPY is the key player in this phenotype, we induced Dicer loss in NPY knockout (KO) mice via Tamoxifen IP injections.

RESULTS: Our preliminary data show that administration of AAV-AgRP- Cre construct leads to visible weight gain, correlated with increased food intake. This phenotypic effect is AAV-dose-dependent and is likely accompanied by an imbalance between anorexygenic and orexigenic neuropeptide levels. However, NPY KO mice with massive microRNA loss gradually put on weight, though with different kinetics as compared to Dicer CKO mice.

CONCLUSIONS: Our approach demonstrates that microRNA loss in a subpopulation of arcuate nucleus neurons has a very pronounced effect on the central regulation of metabolism, expressed by weight gain as well as hyperphagy. Moreover, it is likely the mechanism involves an extensive system of complex relationships because loss of single-gene coding of the main orexigenic neuropeptide (NPY) does not inhibit weight gain. Nevertheless, the exact mechanism underlying this phenomenon has not yet been elucidated.

POSTER SESSION 2

P2.1. MMP-9 CONTRIBUTES TO ALCOHOL-INDUCED SYNAPTIC PLASTICITY IN CENTRAL AMYGDALA

Anna Beroun, Marzena Stefaniuk, Leszek Kaczmarek

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

INTRODUCTION: Matrix metalloprotease 9 (MMP-9) is an extracellularly operating protease shown to play the key role in the morphological reorganization of dendritic spines as well as specific forms of learning and memory. Here we focus on a pathological form of plasticity - synaptic plasticity of alcohol addiction.

AIM(S): The aim of this study was to investigate the role of MMP-9 in functional and structural synaptic plasticity during development of alcohol addiction.

METHOD(S): Mice, housed in the IntelliCage system, were constantly monitored for alcohol consumption and motivation to reward. Spine morphology was evaluated by confocal imaging. We then performed whole-cell patch clamp recordings to test the strength of synapses as well as formation of silent synapses in the central amygdala.

RESULTS: MMP-9 KO mice display lower motivation towards ethanol compared to wild type mice (WT). Moreover, in central amygdala, chronic alcohol drinking produced alterations in dendritic spine shape of both WT and KO but interestingly, more pronounced changes were observed in WT high alcohol consumers. To test how altered structural plasticity affects functionality of synaptic connections, we performed electrophysiological analysis of the strength of glutamatergic synapses in central amygdala. We discovered that alcohol consumption elevates the number of silent synapses (neonatal-like, immature synapses considered as substrates for increased plasticity). MMP-9 KO mice, however, showed no such synaptic adaptations neither after alcohol drinking nor subsequent withdrawal.

CONCLUSIONS: These data suggest that MMP-9 is involved in synaptic plasticity associated with alcohol addiction. The change of spine morphology together with elevated silent synapse number might represent ongoing circuitry reorganization that primes neurons for enhanced learning, which might lead to compulsive ethanol use.

FINANCIAL SUPPORT: This study is supported by the National Science Centre grant Sonata (2015/19/D/NZ4/03701).

P2.2. TRANSCRANIAL DIRECT CURRENT STIMULATION (TDCS) APPLIED TO CEREBELLUM SUPPRESSES PENTYLENETETRAZOL-INDUCED KINDLED SEIZURES

Ihor Ostapenko, Vladislav Kirchev, Oxana Nenova, Tatyana Prybolovets, Katerina Bidnyuk, Leonid Godlevsky

Odessa National Medical University, Department of Biophysics, Informatics and Medical Devices, Odessa, Ukraine

INTRODUCTION: tDCS mapping of zones of brain with antiseizure potency might reveal their role in kindled seizures development.

AIM(S): To investigate the effects of cathode tDCS performed at different zones of brain upon generalized seizures induced in pentylenetetrazol (PTZ) – kindled rats.

METHOD(S): Kindling model of chronic generalized seizures were induced in rats via daily i.p. administration of PTZ (30 mg/kg) in male Wistar rats during three weeks. Only animals with generalized clonic – tonic fits as response to last three administrations of PTZ were used for further observation. tDCS were performed with cathode electrode with diameter of 7.5 mm which was located on the left side to bregma as a first type of position and at middle line to lambda as a second position. It was assumed that the first electrode location permitted to affect the frontal parts of cortex while the second one permitted to affect cerebellar cortex. The intensity of applied current was 0.7 mA, and tDCS was performed during 15 min. In 10 min from the moment of cessation of tDCS the PTZ was i.p. injected with a dosage of 30.0 mg/kg. Control group of PTZ-kindled animals was false stimulated.

RESULTS: Latency of seizures induced after tDCS of frontal cortical zone was not differ from such one in the control group. The generalized fits were protected in 5 out of 10 animals, while in the control group generalized fits were observed in all 10 animals ($P<0.05$). After tDCS at posterior zone latency of seizures exceeded such one in the control group by 35.0% ($P<0,05$) and generalized fits were not observed (10 rats), which was also significantly differ from group with tDCS at hemisphere. Besides, cerebellar stimulation prevented manifestations of postseizure depression.

CONCLUSIONS: Cathode tDCS applied to posterior zone of skull induced more pronounced antiseizure effects upon PTZ-kindled seizures when compared with tDCS of hemisphere. Such an effect might be explained by the involvement of cerebellar structures in the mechanisms of seizures suppression.

P2.3. OPTOGENETIC CONTROL OF DOPAMINERGIC NEURONS' ACTIVITY AND DOPAMINE RELEASE

Kamil Pradel[1,2], Tomasz Błasiak[1], Wojciech Solecki[2,3]

[1] Institute of Zoology, Jagiellonian University, Department of Neurophysiology and Chronobiology, Krakow, Poland, [2] Institute of Pharmacology Polish Academy of Sciences, Department of Molecular Neuropharmacology, Krakow, Poland, [3] Institute of Applied Psychology, Jagiellonian University, Department of Neurobiology and Neuropsychology, Krakow, Poland

INTRODUCTION: Dopaminergic (DA) neurons in the ventral tegmental area (VTA) are key players in regulating motivation and learning. Such control is mediated by DA innervation of other brain regions, such as the nucleus accumbens (NAc) and the prefrontal cortex (PFC).

AIM(S): With use of optogenetics we aimed to delineate if both electrophysiological activity of DA neurons and DA release in target brain structures follow the optogenetic light stimulation protocols. Additionally, our goal was to compare results obtained with these two approaches.

METHOD(S): To address these questions we used genetically modified rats expressing Cre recombinase gene under control of tyrosine hydroxylase gene – a marker of catecholaminergic neurons. Rats were stereotaxically injected into the VTA with adenoviral vectors carrying the Cre-dependent genes for channelrhodopsin-2 (ChR2) and yellow fluorescent protein. After proper expression of ChR2 in DA neurons *in vivo* electrophysiological or electrochemical experiments (single-unit recordings or fast-scan cyclic voltammetry, respectively) combined with optogenetic stimulation of the VTA were conducted.

RESULTS: We demonstrated that laser blue light (473 nm, 5–60 Hz) stimulation alters both the activity of ChR2-expressing TH-positive VTA neurons and DA release in target brain regions. We showed that both DA neuronal firing and DA release elevates not linearly with increasing frequencies of light stimulation. High stimulation frequencies (>20 Hz) decreases both fidelity and amplitude of action potentials, preventing further increase in DA release. Finally, we demonstrated differentiation in DA release within the mesocorticolimbic brain subregions, with higher light-evoked DA concentration in the NAc than in the medial PFC.

CONCLUSIONS: ChR2 enables selective control of DA neurons' activity and subsequent DA release in the target brain regions with high spatial but limited temporal resolution. We demonstrated that light-evoked DA release differ in mesolimbic brain regions.

FINANCIAL SUPPORT: This work was supported by the Polish National Science Center (Research grants UMO-2013/11/D/NZ4/02371 and UMO-2014/13/B/NZ4/00146).

P2.4. FUNCTIONAL INTERACTION OF EXCITATORY FOCI AND THEIR COMPLEXES IN THE CEREBRAL CORTEX

Oleksii A. Shandra[1], Natalia A. Kyrylenko[1,2], Oleksii O. Shandra[3]

[1] Department of Physiology of Odessa National Medical University, Odessa, Ukraine, [2] Department of Botany of I.I. Mechnikov Odessa National University, Odessa, Ukraine, [3] Virginia Tech Carilion Research Institute, Roanoke, USA

INTRODUCTION: Identification of the interaction between excitatory foci and their complexes in the cortex is important for understanding functional organization of the regions they arise from and help learn the pathophysiological mechanisms of various CNS pathologies development.

AIM(S): We aimed to identify the types and mechanisms of interactions between excitatory foci with variable intensity and their complexes in the neocortex.

METHOD(S): We used adult male cats and rats for applications of strychnine, penicillin, and acetylcholine with proserin to produce excitatory foci. In this experiment, we used different drug concentrations and application zones in the neocortex.

RESULTS: We established two main types of interactions: the determinant and the dominant. A focus of more powerful excitation may enhance and synchronize the activity in the weaker foci, integrate them in a single functional complex and define the pattern of its activity (determinant relations). Phenobarbital or surgical excision of the neocortical region with hyperactive foci can suppress determinant foci and destroy the complex. The hyperactive focus may also suppress the activity of previously created foci (dominant relationships). We observed the latter form of interaction in either transitional stage during complex formation or it had an independent meaning. The interaction between foci did not depend on their neurochemical background and localization in the cortex. In cortex isolé and preparations with mesencephalic sections of the stem, a more rapid formation of functional complexes occurred under the effect of the determinant complex.

CONCLUSIONS: These findings demonstrate two types of functional interaction both between separate foci and between their complexes. Complete elimination of the afferent and thalamic-cortical interactions does not hinder hypersynchronization of cortical neuronal elements or formation of functional complexes and generalization of seizure activity over the cortex.

P2.5. MATRIX METALLOPROTEINASE-9 (MMP-9) MEDIATES CHOLINERGIC-INDUCED PLASTICITY OF THE EXCITATORY INPUT TO HIPPOCAMPAL FAST SPIKING INTERNEURONS

Ahmad Salamian, Anna Beroun, Leszek Kaczmarek

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

INTRODUCTION: MMP-9 is an extracellular protease involved in modification of synaptic functions. Cholinergic receptors activation in the hippocampus, that is mostly mediated by projections from the medial septum, plays an important role in the hippocampal synaptic plasticity. Additionally, activation of muscarinic cholinergic receptors can be involved in synaptic transmission between parvalbumin positive cells, known as fast spiking inhibitory interneurons, and pyramidal cells.

AIM(S): The aim of this study was to evaluate the effect of MMP-9 on the cholinergic-induced synaptic plasticity.

METHOD(S): To induce synaptic plasticity, carbachol, a cholinergic agonist, triggering rhythmic activity, which causes a lasting synaptic enhancement, was applied to cultured hippocampal organotypic slices. MMP-9 activity was either genetically or pharmacologically blocked. Using whole-cell patch-clamp technique, AMPA receptor-mediated miniature excitatory postsynaptic currents (mEPSCs) were recorded. Enzymatic activity of MMP-9 was assessed by gelatin zymography.

RESULTS: One hour of carbachol treatment, followed by an overnight carbachol-free incubation, produced significant increase in frequency of mEPSCs. Treatment with either MMP-9 inhibitor I or genetic ablation of MMP-9 along with carbachol further enhanced frequency of mEPSCs. Recording mEPSCs from fast spiking GABAergic interneurons located at the stratum radiatum showed enhancement of mEPSCs frequency by carbachol. The increased excitatory inputs to those inhibitory interneurons were impaired while MMP-9 activity was inhibited. Evaluation of gelatinase activity in conditioned cultured medium indicated remarkable increase in the level of MMP-9 compared with control around 24 hours after carbachol treatment.

CONCLUSIONS: MMP-9 proteolytic activity can have a marked impact on the cholinergic-induced synaptic plasticity and transmission between pyramidal neurons and inhibitory interneurons.

FINANCIAL SUPPORT: ITN training network EU FP7 grant "EXTRABRAIN".

P2.6. ROLE OF DOPAMINE D1\D5 RECEPTORS IN MODULATING SYNAPTIC PLASTICITY AT BASAL AND APICAL DENDRITIC TREES OF PYRAMIDAL NEURONS IN MOUSE HIPPOCAMPUS

Olga Wójcicka[1], Jerzy W. Mozrzymas[1,2], Tomasz Wójtowicz[1]

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

INTRODUCTION: Deciphering cellular mechanisms of neuronal circuits plasticity remains of key importance in understanding learning and memory. In the hippocampus, CA1 pyramidal neurons receive different amount of excitatory and modulatory dopaminergic inputs to basal and apical dendritic trees. This layer-specific differential inputs may play a key role in encoding the respective memory traces. However the locus-specific mechanism of synaptic plasticity remains poorly understood.

AIM(S): The aim of the study was to identify to what extent dopamine receptors could regulate AMPARs and

NMDARs function in long-term synaptic plasticity within basal and apical dendrites of pyramidal neurons.

METHOD(S): We used combination of electrophysiological recordings (fEPSPs) and pharmacological treatment in acute hippocampal slices of adult P45-60 C57BL/6 male mice.

RESULTS: High frequency stimulation (HFS, 4×100 Hz every 10 s) of afferent fibers within basal dendrites (stratum oriens, SO) led to significantly larger long-term potentiation (LTP) of AMPARs-mediated fEPSPs (LTPAMPA) compared to apical dendrites (stratum radiatum, SR). When slices were incubated with dopamine D1/D5 receptors agonist (SKF-38393 hydrochloride), LTPAMPA was significantly upregulated at SO but not SR synapses. However, in both projections bath applied NMDARs-antagonist (APV) completely abolished LTPAMPA indicating that NMDAR are indispensable in both SR and SO. We next pharmacologically isolated NMDAR-mediated synaptic field-potentials and found that D1/D5Rs agonist potentiated these signals in SO but not SR. We next found that priming of D1/D5Rs with its agonist occluded further potentiation of NMDAR function upon HFS. Moreover, application of D1/D5Rs antagonist (SCH23390) prevented gain in NMDAR function at SO, an effect never observed at SR synapses.

CONCLUSIONS: Synaptic NMDARs located at basal and apical dendritic trees are differentially modulated by D1/D5Rs. Dopamine-mediated modulation of NMDARs function could locus-specific gain in AMPARs function at SO.

FINANCIAL SUPPORT: National Science Center SONATA/2014/13/D/NZ4/03045.

P2.7. THE EFFECT OF NOREPINEPHRINE ON THE RAT INTERGENICULATE LEAFLET NEURONS – AN IN VITRO ELECTROPHYSIOLOGICAL STUDY

Anna Sanetra, Katarzyna Palus, Łukasz Chrobok, Marian H. Lewandowski

Jagiellonian University, Departament of Neurophysiology and Chronobiology, Krakow, Poland

INTRODUCTION: The intergeniculate leaflet (IGL) is an important structure of the circadian timing system, involved in the integration of photic and non-photic information. The IGL receives input from the retina, as well as from many non-specific projections of the brainstem, which provide it with all the non-photic information needed for synchronization of circadian rhythmicity. One of the non-specific projections is the norepinephrine system, extending from the locus coeruleus throughout the brain. Norepinephrine in the central nervous system is involved in the mechanism of arousal, and therefore enhance cognitive processes such as attention and memory. However, how it regulates the circadian system via the IGL has not yet been described.

AIM(S): The aim of the study was to determine whether and how the norepinephrine (NE) can modulate the activity of single IGL neurons.

METHOD(S): The method used in this experiment was a whole-cell patch clamp on brain slices from 2/3-week-old Wistar rats. Norepinephrine and tetrodotoxin (TTX) were applied by bath perfusion. After each experiment, slices were immunostained to verify the localization of the recorded cell in the IGL.

RESULTS: Both depolarization and hyperpolarization of the IGL neurons were observed after NE application. In most cases the effect was postsynaptic, however, there were also a few cells which showed no direct response after norepinephrine application in the TTX solution.

CONCLUSIONS: Norepinephrine elicits different effects on IGL neuronal activity. The expression of different types of noradrenergic receptors in IGL cells' membrane could be a reason for such various types of responses. We believe that NE acts in the IGL as an activity modulator, which regulates its responses to other cues. To our knowledge, this is the first study analyzing the effect of norepinephrine on IGL neuronal activity.

FINANCIAL SUPPORT: This work is supported by grant obtained from the Institute of Zoology: DS/MND/WBiNoZ/IZ/29/2016.

P2.8. LOCAL ADMINISTRATION OF MUSCIMOL (GABAA RECEPTOR AGONIST) INTO THE MEDIAL MAMMILLARY NUCLEUS SUPPRESSES HIPPOCAMPAL THETA RHYTHM IN URETHANE-ANESTHETIZED RATS

Witold Żakowski, Łukasz Braszka, Piotr Zawistowski, Edyta Jurkowlaniec

University of Gdansk, Depertment of Animal and Human Physiology, Gdansk, Poland

INTRODUCTION: The mammillary body is a part of so-called extended hippocampal system and its importance for memory is well known. Our previous study has revealed that pharmacological inactivation of the medial mammillary nucleus (MM) attenuates theta rhythm activity in the hippocampus (HP) in urethane-anesthetized rats. In this study, we examined the involvement of GABAergic transmission in the MM in the regulation of hippocampal theta rhythm.

AIM(S): The aim of this study was to investigate whether local administration of GABAA receptor agonist (muscimol) into the MM affects theta rhythm in the HP in urethane-anesthetized rats.

METHOD(S): Male Wistar rats were implanted with unilateral recording electrodes into the dorsal HP (CA1) and unilateral injection cannula into the lateral part of the MM. Animals received microinjection of either muscimol (n=5)

or water (n=5). 1-min tail pinch stimulations were applied at 5- and 10-min intervals to evoke theta rhythm episodes in the HP. Changes in local field potential were assessed on the basis of percent change of total EEG signal power for 1-Hz bands.

RESULTS: We found that intra-MM muscimol injection suppressed sensory-elicited theta rhythm in the HP. The infusion decreased the EEG signal power most significantly in 3–4 Hz band, down to 19.1%, and in 7–8 Hz band, down to 48.6%, in comparison to the pre-injection conditions (100%). Simultaneously, the injection increased total power in delta frequency bands, up to 2415.6% in 0.5–1 Hz band. No significant changes in the hippocampal field activity were found after the water injections in the control water group.

CONCLUSIONS: Our study revealed for the first time that GABAergic transmission of the MM is significantly involved in electrophysiological activity of the HP in urethane-anesthetized rats. The obtained results also confirmed the importance of the mammillary body in regulating a theta-rhythm signaling in the extended hippocampal system.

FINANCIAL SUPPORT: This research was supported by the National Science Centre (DEC-2014/12/S/NZ3/00621).

P2.9. THE EFFECT OF TRANSFORMING GROWTH FACTOR β-1 ACTIVATION ON THE EXPRESSION OF NR1 SUBUNIT OF NMDA RECEPTOR IN A MURINE MODEL OF ACUTE LIVER FAILURE. ROLE OF THROMBOSPONDIN(S)

Mariusz Popek, Magdalena Zielińska

Mossakowski Medical Resarch Centre, Department of Neurotoxicology, Warsaw, Poland

INTRODUCTION: We have previously shown altered expression and/or intracellular distribution of selected synaptic proteins that may contribute to neurological dysfunction observed during acute liver failure (ALF) in mice. During ALF an increased level of TGF-β1 in the serum and brain was also observed. Moreover, neutralization of TGF-β1 appears to improve the neurological score of ALF mice.

AIM(S): We test the hypothesis that thrombospondin(s) – related activation of TGF-β1 may affect the expression of NR1, a major NMDA receptor subunit.

METHOD(S): We measured total and active TGF-β1 level in the brain and blood of C57Bl6 mice with ALF induced by single i.p. injection of azoxymethan (AOM; 100 mg/kg of b.w.) and after neutralization of TGF-β1 induced by single i.p. injection of ab-TGF- β1 (1 mg/kg) 2 h before AOM injection. In addition we analyzed the expression of thrombospondin-4 (Thrb4) and NR1 subunit in AOM brain homogenates and membrane/cytosolic fractions.

RESULTS: In ALF mice, active form to total TGF-β1 ratio was increased by ~35%,and ~30% in serum and rain cortex, respectively. Expression of Thrb4 was increased by ~30% in homogenates and by ~35% in cytosolic fraction. Both, AOM injection and TGF-β1 neutralization induced the increase of NR1 subunit in membrane fraction, by ~40%, and ~60% respectively.

CONCLUSIONS: The results indicate that in ALF mouse, neutralization of cytokine TGF-β1 may cause an increase in NR1 expression, the effect not potentiated by AOM. The increase in the expression of Thrb4 play a potential role in the activation of TGF-β1, as may be assumed from the increase in TGF-β1 ratio in the serum and the brain homogenates. The particular mechanism requires an additional research.

FINANCIAL SUPPORT: Supported by the grant Prelludium10 2015/19/N/NZ5/02249 and the Leading National Research Centre (KNOW).

P2.10. POSTNATAL DEVELOPMENT OF THETA-RELATED CELL ACTIVITY IN POSTERIOR HYPOTHALAMIC SLICES

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

Department of Neurobiology, University of Lodz, Lodz, Poland

INTRODUCTION: Hippocampal formation (HPC) theta rhythm is one of the best examples of neural synchrony in the mammalian brain. It is well-known that the pathway of theta generation originates in the pons, from where it projects to the posterior hypothalamic nuclei, and finally through the medial septal area reaches the HPC. Recent evidence shows that well-synchronized theta rhythm can also successfully be recorded locally from the posterior hypothalamic area (PHa) maintained *in vivo* and *in vitro*, specifically from the supramammillary nucleus (SuM) and the primary posterior hypothalamic nuclei (PH). Furthermore, the neuronal activity of the PH and SuM nuclei can be characterized according to the universal classification of theta-related cells which was earlier created for the hippocampus.

AIM(S): The purpose of the present study is to investigate theta-related cell activity in posterior hypothalamic slices taken from adolescent rats in order to examine the age at which theta rhythm and accompanying theta-related neuronal activity appears in rats' PHa.

METHOD(S): Forty-five experiments have been carried out using brain slices taken from 55 Wistar rats aged: 8–10 (A); 13–15 (B); 18–19 (C) and 22–24 (D) days. Each brain slice was perfused with 75 μM carbachol (cholinergic agonist) to induce theta rhythm and accompanying theta-related cell activity in the SuM and PH nuclei.

RESULTS: This study resulted in recording 16 theta-related neurons, 56 timing neurons, and 103 neurons classified as non-related to PHa theta among three experimental groups (B–D). There was no significant theta oscillations and accompanying cell discharges in slices taken from 8–10 days old rats.

CONCLUSIONS: Theta oscillations as well as theta-related neuronal activity can be observed in PHa slices delivered from rats not younger than 13–15 days.

FINANCIAL SUPPORT: Supported by NCN grant 2013/11/B/NZ4/04872.

P2.11. DIRECT ACTION OF NORADRENALINE ON THE MEDIAL PREFRONTAL CORTEX (MPFC) PYRAMIDAL NEURONS IN YOUNG RATS

Katarzyna Grzelka, Paweł J. Szulczyk

Medical University of Warsaw, Department of Physiology and Pathophysiology, Warsaw, Poland

INTRODUCTION: Noradrenaline (NA) and adrenergic receptors (α1, α2 and β) are crucial in regulating medial prefrontal cortex (mPFC) functions. Impaired modulation of the mPFC by NA has been implicated in many neuropsychiatric diseases, e.g. posttraumatic stress disorder, attention deficit hyperactivity disorder, depression. However, the mechanisms by which NA modulates mPFC neurons are not well understood.

AIM(S): The aim of this study was to investigate which adrenergic receptor subtype controls the resting membrane potential and holding currents in mPFC neurons and what are the cellular mechanisms underpinning the effects of NA.

METHOD(S): The resting membrane potential and holding currents were recorded in layer V mPFC pyramidal neurons. Gramicidin perforated-patch and classical whole-cell recordings were obtained from neurons in brain slices of young rats. Tested compounds were applied to the bath and/or to the solution in the recording pipette.

RESULTS: NA evoked depolarization of the membrane potential and the inward holding current. Stimulation of α1- and α2-receptors failed to evoke similar effects. Meanwhile, the nonselective β-receptor agonist as well as the selective β1-receptor agonist mimicked the effect of NA on holding currents. The NA-dependent inward current was considerably reduced by the selective β1-receptor antagonist. The β1-related inward current was significantly decreased in the presence of Cs^+ ions and the selective blocker of HCN channels – ZD7288. It was not affected by selective blockers of different signaling pathways known to be responsible for mediating the effects from β-receptors (e.g. adenylyl cyclase-PKA, PLC-PKC, protein tyrosine kinases).

CONCLUSIONS: We conclude that NA changes the membrane potential/holding currents of the mPFC pyramidal neurons acting via β1-receptors. The effects occur due to HCN channel activation and are not mediated by the classical signaling pathways.

FINANCIAL SUPPORT: Supported by National Science Centre, Poland, grant 2014/15/N/NZ4/04760 and FW5/PM2/16.

P2.12. LIPRIN-α-1 IS A NOVEL COMPONENT OF THE NEUROMUSCULAR JUNCTION AND INVOLVED IN THE ORGANIZATION OF THE POSTYSYNAPTIC MACHINERY

Krzysztof M. Bernadzki, Marcin Pęziński, Marta Gawor, Paula Mazurek, Paweł Niewiadomski, Jolanta Rędowicz, Tomasz J. Prószyński

Department of Cell Biology, Laboratory of Synaptogenesis, Nencki Institute of Experimental Biology Polish Academy of Sciences, Warsaw, Poland

INTRODUCTION: Neuromuscular junctions (NMJs) are specialized synapses formed between the motor neurons and skeletal muscles. The proper functioning of the NMJ is important for activities involving muscle contraction including breathing. Liprin-α-1 was previously identified in a biochemical screen as an interactor of α-dystrobrevin-1, a component of dystrophin-associated glycoprotein complex (DGC) responsible for the attachment of the postsynaptic machinery to the extracellular matrix. Liprin-α-1 has been shown to be an organizer of synapses in the central nervous system and our study presents an insight into the localization and function of this protein at the vertebrate NMJ.

AIM(S): To unravel the localization and function of liprin-α-1 at the vertebrate NMJ.

METHOD(S): We employed immunohistochemical, siRNA-mediated RNAi techniques and confocal microscopy techniques.

RESULTS: We show that liprin-α-1 localizes to the postsynaptic site of NMJ in a nerve-dependent manner, and its localization is maintained throughout postnatal development. Liprin-α-1 plays a crucial role in the formation of the postsynaptic machinery since liprin-α-1 depleted myotubes failed to cluster postsynaptic acetylcholine receptors (AChRs). We provide evidence that liprin-α-1 plays a role in the attachment of microtubule ends to the cell cortex, where they stabilize the postsynaptic machinery and promote clustering of AChRs. Consistently with this observation abrupt disruption of microtubules with in cultured myotubes did not affected preexisting AChR clusters, but abolished formation of the new assemblies.

CONCLUSIONS: Our results show that Liprin-α-1 is important component of the postsynaptic machinery involved in AChR clustering.

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P2.13. SPINAL BDNF OVEREXPRESSION LEADS TO OPPOSITE EFFECTS ON GLUTAMATERGIC AMPAR AND NMDAR THAN ON MUSCARINIC ACETYLCHOLINE RECEPTOR M2 TRANSCRIPT LEVEL IN RATS WITH COMPLETE SPINAL CORD TRANSECTION

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

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

INTRODUCTION: Complete spinal cord transection (SCT) disturbs the balance between inhibitory and excitatory inputs to motoneurons increasing their excitability. However SCT causes deficiency in excitatory cholinergic input to ankle extensor motoneurons, whereas brain-derived neurotrophic factor (BDNF) overexpression below the lesion site increases markers of spinal neurotransmission and improves locomotor performance. Because glutamatergic receptors (AMPAR, NMDAR) and muscarinic acetylcholine receptor M2 play a crucial role in motoneuron excitability, we investigated if SCT and BDNF affect their expression.

AIM(S): To disclose the impact of SCT and BDNF overexpression on levels of AMPAR, NMDAR and M2 mRNA transcripts 2 weeks after SCT.

METHOD(S): Total RNA was isolated from L1-2 and L3-6 spinal fragments after SCT followed by intraspinal injection of PBS (n=6) or AAV-BDNF (n=7). After cDNA transcription, AMPAR (subunits GluR1, GluR2), NMDAR (subunits NR1, NR2A, NR2B), and M2 expression were measured using qRT PCR.

RESULTS: In intact rats, GluR2 mRNA level was the highest, followed by NR2A/2B, while NR1 and M2 were the lowest. SCT tended to reduce levels of all mRNA transcripts, except for NR1 which tended to increase in L3-6. BDNF overexpression resulted in a significant increase of NR1 and tendency to increase of NR2A in both spinal fragments, while it led to a significant decrease of M2 in L1-2.

CONCLUSIONS: BDNF overexpression slightly upregulated mRNA levels of NMDAR after SCT, not preventing deficits of M2. If M2 mRNA decrease is reflected by M2 protein levels in motoneurons, reduced contribution of M2 in modulation of motoneuron excitability may be postulated.

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P2.14. A CELL ADHESION MOLECULE, DYSTROGLYCAN, IS IMPLICATED IN HOMEOSTATIC PLASTICITY

Izabela Figiel, Adam Krzystyniak, Anna Bartkowiak-Kaczmarek, Matylda Roszkowska, Monika Bijata, Jakub Włodarczyk

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

INTRODUCTION: A number of studies have demonstrated that regulated proteolysis of synaptically expressed cell adhesion molecules plays a fundamental role in the morphological reorganization of synapses underlying homeostatic plasticity. One of the major modulators of these processes is matrix metalloproteinase-9 (MMP-9), an extracellularly operating protease.

AIM(S): The main aim of our study is to investigate the subcellular localization of β-dystroglycan (β-DG), a well-known substrate of MMP-9, and its involvement in structural plasticity.

METHOD(S): We analyzed isolated mouse synaptosomes from P2 fraction with flow cytometry after immunostaining with antibodies against synaptosomal markers and β-DG. We also performed triple immunofluorescence labelling on primary hippocampal neurons. To study whether proteolytic cleavage of β-DG influences the dendritic spine shape we performed life imaging of MMP-9-treated primary hippocampal cultures, previously infected with lentiviral vector (LV) coding shRNA specifically silencing DG or LV carrying GFP. Furthermore, we investigated the correlation between β-DG localization and MMP-9 activity by using (FRET)-based MMP-9 activity biosensor.

RESULTS: We found out that β-DG is present on a small subset of synaptosomes that exhibit expression of both post-synaptic markers (psd-95 and gephyrin). Using immunofluorescence staining of primary neurons with pre-synaptic marker antibodies (v-GAT and v-GLUT) we confirmed β-DG localization at both inhibitory and excitatory synapses. We also found changes in the number of β-DG-containing synapses in response to chemically induced LTP (cLTP). Morphometric analysis of live-cell imaging experiments revealed that β-DG exerts an influence on dendritic spine structure. Moreover, the results concerning spatial location of MMP-9 activity and β-DG will be presented.

CONCLUSIONS: Our findings indicate β-DG involvement in synaptic structural plasticity.

FINANCIAL SUPPORT: This study has been supported by research grant 2015/19/B/NZ3/01376 from National Science Centre Poland.

P2.15. CHANGES OF GENES EXPRESSION IN THE NUCLEUS ACCUMBENS DURING NEUROPATHIC PAIN IN MOUSE BRAIN

Agnieszka Wawrzczak-Bargieła[1], Barbara Ziółkowska[1], Wiktor Bilecki[1], Ewelina Rojewska[2], Joanna Mika[2], Barbara Przewłocka[2], Ryszard Przewłocki[1]

[1] Department of Molecular Neuropharmacology, Institute of Pharmacology Polish Academy of Sciences, Krakow, Poland, [2] Department of Pharmacology of Pain, Institute of Pharmacology Polish Academy of Sciences, Krakow, Poland

INTRODUCTION: The nucleus accumbens (NAc), which is known to be an important component of the mesolimbic dopaminergic reward system also plays a role in pain, however the molecular mechanisms of this involvement are not known. In the present study we explored molecular pathways involved in the neuropathic pain. Understanding of this process would allow us brain mapping and find biomarkers for pain transmission.

AIM(S): The aim of this study was to investigate the alterations in genes expression after CCI in the NAc.

METHOD(S): Neuropathic pain was induced by applying a Chronic Constriction Injury (CCI) model in C57BL/6J mice. Two behavioral tests for neuropathic pain were used: the von Frey's test and the cold plate test. In our biochemical researches we used qRT-PCR.

RESULTS: We found that nerve injury produced a significant increase in the expression of opioid genes (PDYN, PENK), opioid kappa and delta receptors genes (KOR, DOR) and calcium/calmodulin - dependent protein kinase kinase 1 (CAMKK1) in the nucleus accumbens. Furthermore, we observed that neuropathic pain augmented the expression of stress – and inflammatory response genes coding for the glucocorticoid receptor (GR), FK506 binding protein5 (FKBP5), and interleukins IL1 beta and IL6 in the nucleus accumbens. Moreover, elevated levels of GFAP (astrocyte marker) but not C1q (microglia marker) mRNAs were detected.

CONCLUSIONS: Our results demonstrate that CCI produces lasting biochemical changes in the NAc.Taking into account the well-known roles of opioid systems in pain transmission and emotional processes, the observed changes in the expression of the opioid propeptides and receptors genes may contribute to changes in pain sensitivity and in affective response to nociceptive stimulation. Furhermore, increased expression of GFAP, GR, FKBP5, Il6 and Il1beta genes suggests that cellular stress and inflammatory processes are involved in this type of pain not only on the level of the spinal cord but also in the brain.

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P2.16. SOMATOSTATIN RECEPTOR 2 EXPRESSION MAP IN THE BARREL CORTEX

Anna Grabowska[1], Małgorzata Kossut[1,2]

[1] Laboratory of Neuroplasticity, Department of Molecular and Cellular Neurobiology, Nencki Institute of Experimental Biology Polish Academy of Scieces, Warsaw, Poland, [2] University of Social Sciences and Humanities, Warsaw, Poland

INTRODUCTION: Somatostatin (SST) is present in central and peripheral nervous system and it is a neurotransmitter or neuromodulator which can affect excitability and neuronal responses. In general SST is thought to inhibit excitatory synaptic transmission. Importantly, SST colocalizes with a gamma-aminobutyric acid (GABA) in inhibitory interneurons of cerebral cortex and hippocampus, and is widely used as a marker of interneurons. SST acts through a family of G protein-coupled receptors – somatostatin receptors (SSTRs) types 1–5. The SST-SSTRs system seems to be important in the development of cognitive processes like learning and memory. SSTR2 has been shown to be involved in the perceptual processes like anxiety and stress-induced behavior. Elucidating of SSTR2 distribution can significantly improve the knowledge about function of SST in the primary somatosensory cortex.

AIM(S): The aim of these studies is to reveal the characteristic expression pattern of SSTR2 distribution within the barrel cortex.

METHOD(S): Immunocytochemistry and immunofluorescent techniques on floating sections from wild type (C57BL/6) mice males were performed according to standard protocols using primary SSTR2 polyclonal antibody and were followed by Nissl or DAPI staining.

RESULTS: The SSTR2 scattered expression pattern was observed within the barrel cortex. There was a slight difference according to its distribution as less evident signals were observed in the hollows of the barrels and the stronger ones in the barrel walls. The puncta of SSTR2 were densely concentrated on a neural cell bodies and dendrites within the barrel cortex.

CONCLUSIONS: It is likely that the distribution of SSTR2 and action of SST-positive neurons together with GABA synthesis can via a pathway of disinhibition facilitate the learning-dependent plastic change.

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P2.17. EARLY CHANGES IN GENE EXPRESSION OF SPINAL PERINEURONAL NETS COMPONENTS IN RATS AFTER COMPLETE SPINAL CORD TRANSECTION

Kamil Grycz, Olga Gajewska-Woźniak, Benjun Ji, Julita Czarkowska-Bauch, Małgorzata Skup

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

INTRODUCTION: Perineuronal nets (PNNs), which restrict axonal regeneration in the glial scar and limit synaptic plasticity, are composed of chondroitin sulfate proteoglycans (CSPGs) and Crtl1/Hapln1 link protein essential for PNN formation. Spinal cord transection (SCT) leads to changes of various CSPG proteins differently distributed between 2nd–8th postlesion weeks. This raises the question if shortly after SCT when glial scar is formed, processes induced by tissue damage alter expression of genes coding for these proteins.

AIM(S): To characterize gene expression levels of the selected CSPGs (brevican, neurocan, aggrecan, phosphacan), and of Crtl1/Hapln1 in the spinal cord of the intact rats and to quantify their changes at the second week after SCT at low-thoracic segments.

METHOD(S): The CSPGs and Crtl1/Hapln1 gene transcripts were quantified in rats after complete SCT in fragments of the spinal cord: Th 9/10 (lesion site), its vicinity and in L1–L2. To quantify gene expression qRT-PCR was carried out and expression levels were presented relative to internal control gene (GAPDH) as the CT.

RESULTS: In intact rats mRNA level of brevican was the highest among all tested CSPGs and Crtl1/Hapln1. Its level exceeded that of neurocan by 5-fold and the rest of CSPGs by at least 10-fold. SCT caused significant, 4-fold increase of neurocan and 5-fold decrease of Hapln1 transcripts in the lesion site, comparing to controls, and did not affect phosphacan and brevican transcripts. SCT caused weaker effects in L1–L2 segments where only neurocan and brevican transcripts significantly increased (by 160% and 30% respectively) whereas Crtl1/Hapln-1 decreased by 40%.

CONCLUSIONS: Increased transcript levels of neurocan in the lesion site indicate stimulation of its gene expression in astrocytes. A decrease in Crtl1/Hapln1 transcript may point to potential disturbances in postlesion PNN formation.

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P2.18. MOUSE MONOCULAR VISUAL CLASSICAL CONDITIONING REDUCES SPONTANEOUS FIRING OF EXCITATORY LAYER 4 CORTICAL NEURONS – EX VIVO STUDIES ON BRAIN SLICES

Marek Bekisz, Ninad Shendye, Ida Raciborska, Andrzej Wróbel, Wioletta J. Waleszczyk

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

INTRODUCTION: It is accepted that learning induces plastic changes in adult neocortex. Our previous experiments on mice showed that classical conditioning in which monocular visual stimulation was paired with an electric shock to the tail enhanced GABA immunoreactivity within layer 4 of the monocular part of the primary visual cortex (V1), contralateral to the stimulated eye.

AIM(S): In the present study we investigated whether the same classical conditioning paradigm induced changes of neuronal excitability in this cortical area.

METHOD(S): We performed patch-clamp whole-cell recordings from *ex vivo* slices of mouse V1. Two experimental groups were used: mice that had 7-day visual classical conditioning and control animals. The slices were perfused with the modified artificial cerebrospinal fluid, the composition of which better mimics the brain interstitial fluid in situ and induces spontaneous activity in slices. The frequency of spontaneous action potentials was calculated as a general measure of neuronal excitability.

RESULTS: We found that layer 4 excitatory cells located in the monocular representation of the "trained" eye in V1 had lower frequency of spontaneous action potentials than neurons from the same cortical region of control animals.

CONCLUSIONS: Weaker spontaneous firing indicates decreased general neuronal excitability within layer 4 of the monocular representation of the "trained" eye in V1. Such effect could result from enhanced inhibitory processes in this cortical area.

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P2.19. REBOUND DEPOLARIZATION (RD) IN MEDIAL PREFRONTAL CORTEX (MPFC) PYRAMIDAL NEURONS

Przemysław N. Kurowski, Paweł J. Szulczyk

Medical University of Warsaw, Department of Physiology and Pathophysiology, Warsaw, Poland

INTRODUCTION: Rebound depolarization (RD) is an evoked membrane depolarization following the hyperpolarization of a neuron that converts an arriving inhibitory signal into cell excitation, which is subsequently transmitted to other neurons. RD is involved in the rhythmic discharges and oscillations of cortical neurons.

AIM(S): The aim of this study was to clarify the intrinsic mechanism responsible for RD in medial prefrontal cortex (mPFC) pyramidal neurons.

METHOD(S): Experiments were performed on layer V mPFC pyramidal neurons in slices obtained from 60-day-old rats. Recordings of membrane potential were performed in whole-cell current-clamp configuration in the absence of Ca^{2+} ions and in the presence of tetrodotoxin (TTX), glutamatergic and GABAergic blockers in extracellular solution.

RESULTS: The resting membrane potential in tested neurons was −67.3±0.95 mV (n=154). RD exhibited the following properties: evoked after prior cell hyperpolarization below −80 mV, a threshold of −63.8±1.3 mV, an amplitude of 33.2±1.7 mV above the resting membrane potential, a duration of 552.7±43.1 ms, and dependence on inward TTX-resistant Na^+ current. RD was abolished when pyramidal neurons were treated with Nav1.9 antibodies. RD was only evoked in the absence of Ca^{2+} in the extracellular solution or in the presence of Ca^{2+} together with a BK-type Ca^{2+}-dependent K^+ channel current blocker (paxilline, 10 μM) in the extracellular solution.

CONCLUSIONS: The obtained results suggest that hyperpolarization-dependent RD in layer V mPFC pyramidal neurons is evoked by the de-inactivation and subsequent activation of a voltage-dependent, low-threshold and TTX-resistant, inward Nav1.9-like Na^+ current. In the presence of Ca^{2+} ions in the extracellular solution, RD was suppressed due to the activation of BK channel currents.

FINANCIAL SUPPORT: The study was supported by National Science Centre (Poland) grant no: 2015/17/N/NZ4/02889.

P2.20. THE TRANSITORY FORCE DECREASE FOLLOWING HIGH-FREQUENCY STIMULATION BURST IN UNFUSED TETANI OF MOTOR UNITS

Joanna Grzesiak, Katarzyna Kryściak,
Hanna Drzymała-Celichowska, Jan Celichowski
Department of Neurobiology, University of Physical Education, Poznan, Poland

INTRODUCTION: The changes in force of motor units (MUs) following changes in activation pattern still are not fully understood, especially in relation to effects of decreasing rate of stimuli. It is known that at linearly decreasing stimulation frequency the force decrease is slower than expected when comparing to the constant stimulation frequency.

AIM(S): The aim of study was the explanation of recently observed surprising transitory force decrease resulting from a sudden decrease in stimulation frequency.

METHOD(S): The research was conducted on 6 adult female Wistar rats under pentobarbital anesthesia. 24 slow (S), 38 fast fatigable (FF) and 65 fast resistant (FR) MUs were isolated. Studied MUs were stimulated with 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. The tested low frequencies for fast MUs were 10, 20, 30, 40 and 50 Hz, and high frequencies amounted to 75, 90 and 150 Hz, whereas for slow motor units low frequencies were 10, 12.5, 15, 17.5, 20 and 25 Hz and high frequencies amounted to 50 and 75 Hz. Moreover, these trains of stimuli were tested at different levels of muscles stretching (30 mN, 100 mN, 200 mN) for all types of MUs.

RESULTS: Among the three MU types the studied force decrease was most frequent and the strongest for FR MUs. The highest noted decrease amounted to 36.5%. The greatest transitory force decreases were observed at muscle passive stretch of 100 mN. For MUs of the three types the force decrease was observed at middle-fused tetanic contractions (the fusion index 0.30–0.95).

CONCLUSIONS: The phenomenon most probably has biomechanical background and is conditioned by distribution of contracting muscle fibers in a deep part of muscle and slow adaptation of stretched collagen fibers to the lower force level of contracting muscle fibers at reduced stimulation frequency.

FINANCIAL SUPPORT: National Science Centre, Poland.

P2.21. ELECTROCHEMISTRY IN NEURONAL PLASTICITY STUDIES – A GLUTAMATE BIOSENSOR

Ewa Baczynska[1,2], Emilia Witkowska-Nery[2],
Martin Jonsson-Niedziółka[2], Jakub Włodarczyk[1]
[1] The Nencki Institute Polish Academy of Sciences, Department of Molecular and Cellular Neurobiology, Warsaw, Poland, [2] Institute of Physical Chemistry Polish Academy of Sciences, Department of Electrode Processes, Warsaw, Poland

INTRODUCTION: The majority of excitatory synapses in the mammalian brain are accommodated at the dendritic spines. One of the most characteristic features of dendritic spines is their morphological diversity which reflect their function. It has been already shown that the size of dendritic spine also correlates with the numbers of vesicles containing neurotransmitter released from presynaptic part of a synapse. The new, recently described morphological form of dendritic spines is the formation of spine-head protrusion (SHP). The studies have already shown that exogenous iontophoretic application of glutamate to medium of organotypic hippocampal culture induces the formation of SHPs. On this basis, it is hypothesized that the formation of SHPs occurs as a result of the neurotransmitter release to the extracellular matrix.

AIM(S): To determine the genesis of SHP formation in dissociated hippocampal culture.

METHOD(S): Development of an electrochemical glutamate biosensor which enables long-term and continuously measurements of endogenous glutamate concentration in neuronal culture.

RESULTS: Our results have shown that the formation of SHPs depends on proteolytic activity of matrix metallopro-

teinase 9 (MMP-9) and that SHPs are capable of creating new synaptic connections.

CONCLUSIONS: Due to the fact that we believe that SHP plays an important role in synaptic plasticity, underlying learning and memory processes, we are focused on developing biosensor which enables to verify theory of SHP formation.

P2.22. EPIDURALLY APPLIED DC EVOKES LONG-LASTING INCREASE IN AXONAL EXCITABILITY

Dominik Kaczmarek[1,2], Francesco Bolzoni[1,3], Ingela Hammar[1], Elzbieta Jankowska[1]

[1] Department of Neuroscience and Physiology, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden, [2] Department of Neurobiology and Department of Biochemistry, Poznan University of Physical Education, Poznan, Poland, [3] Human Physiology Section of the DEPT, Università degli Studi di Milano, Milano, Italy

INTRODUCTION: Epidural stimulation and trans-spinal direct current stimulation (tsDCS) are used in clinical practice for restoring motor functions or pain relief. However, the use of epidural stimulation is limited by low stimulus intensities tolerated by patients. Locally applied cathodal DC was recently demonstrated to increase the excitability of intraspinal preterminal axonal branches for more than one hour.

AIM(S): Our aim was to examine whether brief episodes of epidural DC combined with epidural stimulation evokes long-lasting increase in the excitability of myelinated axons within the dorsal columns.

METHOD(S): 17 adult rats of both sexes (Wistar, 2–6 months old, 200–450 g) were used in this study. In deeply anaesthetized animals, afferent volleys in sural and peroneal nerve were evoked by epidural stimuli via needle tungsten electrodes positioned in contact with the dura mater within the L1–L3 segments. The effects of cathodal DC (0.8–1.0 μA) on the excitability of skin and muscle sensory fibres were assessed by changes in antidromic compound action potentials. The areas of nerve volleys evoked before, during, and after DC polarization were measured within time windows of 0.3–1.4 ms from their onset.

RESULTS: The study revealed that cathodal DC applied via epidural electrodes resulted in a several-fold increases in the number of epidurally activated fibres. The volley area measured after 10 minutes increased by 411±97%, 733±251% and 502±94% following 15–30 s, 1 min and 2 or 5 min of DC application, respectively. Importantly, the increase in the excitability appeared within seconds and remained elevated for more than one hour.

CONCLUSIONS: Combining epidural stimulation and trans-spinal DC polarization may improve their clinical outcome. The differences in time course of DC evoked increases in the excitability of nerve fibres in the dorsal columns compared to previously reported effects in pre-terminal axonal branches suggest a new form of plasticity.

FINANCIAL SUPPORT: The study was supported by a grant from Stiftelsen Sigurd & Elsa Goljes Minne to Elzbieta Jankowska.

P2.23. ACTIVATION OF 5-HT7 RECEPTORS AFFECTS NEURONAL EXCITABILITY IN RAT CA1 HIPPOCAMPUS BY INHIBITING FAST INACTIVATING POTASSIUM CHANNELS

Joanna E. Sowa[1], Marcin Siwiec[1], Krzysztof Tokarski[1], Grzegorz Hess[1,2]

[1] Department of Physiology, Institute of Pharmacology Polish Academy of Sciences, Krakow, Poland, [2] Institute of Zoology and Biomedical Research, Jagiellonian University, Krakow, Poland

INTRODUCTION: Emerging evidence suggests the 5-HT7 receptor as a therapeutic target in stress-related disorders. Precise effects of the 5-HT7-mediated regulation of neuronal excitability remain to be elucidated. Preliminary recordings from rat CA1 piramidal neurons showed that 5-HT7 activation shortens the latency of the first spike in response to depolarization. Due to their rapid kinetics and fast recovery from inactivation, A-type potassium channels (KA) are prime candidates for mediating this effect.

AIM(S): The aim of our study was to assess whether the changes in neuronal excitability and response dynamics of CA1 pyramidal cells following the activation of 5-HT7 receptors are due to inhibition of A-type K+ channels.

METHOD(S): Whole-cell patch-clamp recordings were performed in current-clamp mode. Neurons were held at −65 mV and their excitability was assessed using depolarizing current pulses. To activate 5-HT7 receptors, 5-CT (250 nM) was applied along with WAY 100635 (2 μM), a 5-HT1A antagonist. Further recordings were performed in the presence of specific blockers of A-type and H-type channels.

RESULTS: Activation of 5-HT7 receptors increased the excitability of CA1 pyramidal cells as well as decreased the latency to 1st spike, and effect which was prevented by using a specific Kv4.3/Kv4.4 channel blocker. Blockade of HCN channels did not affect the decrease in spike latency.

CONCLUSIONS: Our data show that activation of 5-HT7 influences neuronal excitability in CA1 pyramidal cells partly by inhibiting fast-inactivating A-type potassium channels. These results help further explain the physiological role of the 5-HT7 receptor, hopefully leading to better understanding of its role in nervous system physiology and pathology.

FINANCIAL SUPPORT: This study was supported by the Ministry of Science and Higher Education (Warsaw, Poland) grant no 2016/21/B/NZ4/03618 and statutory founds from the Department of Physiology, Institute of Pharmacology Polish Academy of Sciences, Krakow, Poland. J.E.S ans M.S. are beneficiaries of the KNOW PhD scholarship sponsored by the Ministry of Science and Higher Education, Poland.

P2.24. ACUTE ACTIVATION OF 5-HT7 RECEPTORS AFFECTS ELECTROPHYSIOLOGICAL PROPERTIES AND SYNAPTIC PROCESSING OF RAT CA1 PYRAMIDAL CELLS BY INHIBITING FAST-INACTIVATING POTASSIUM CURRENTS

Marcin Siwiec[1], Joanna E. Sowa[1], Krzysztof Tokarski[1], Grzegorz Hess[1,2]

[1] Institute of Pharmacology Polish Academy of Sciences, Department of Physiology, Krakow, Poland, [2] Institute of Zoology and Biomedical Research, Jagiellonian University, Krakow, Poland

INTRODUCTION: Experimental evidence points to the 5-HT7 receptor as a potential therapeutic target for affective and neurodevelopmental disorders. The cellular/ionic mechanisms following the activation of the 5-HT7 receptor signaling pathway have not yet been fully characterized. Our preliminary recordings from hippocampal neurons have shown that 5-HT7 activation, in addition to increasing neural excitability, shortens action potential latency, which suggests involvement of voltage-gated potassium channels in the neural response to 5-HT7 activation.

AIM(S): The aim of our study was to directly investigate modulatory effects of 5-HT7 activation on voltage-gated potassium channels in rat CA1 pyramidal cells, as well as to examine the functional consequences of such effects on the hippocampal circuitry.

METHOD(S): We performed whole-cell voltage clamp recordings from rat CA1 pyramidal cells and tested the effects of 5-HT7 agonists on A-type and delayed rectifier potassium currents. To examine the influence of the 5-HT7-mediated channel modulation on synaptic transmission, we stimulated Schaffer collaterals and recorded evoked AMPA currents before and after 5-HT7 activation, as well as before and after blocking Kv4.3/Kv4.4 and/or HCN channel subunits.

RESULTS: Activation of 5-HT7 receptors markedly attenuated A-type potassium currents in CA1 pyramidal cells. Furthermore, 5-HT7 activation increased AMPA postsynaptic currents evoked by stimulation of Schaffer collaterals, and this effect was partially dependent on the inhibition of A-type potassium channels.

CONCLUSIONS: We found that 5-HT7 receptors can strongly influence neural activity by inhibiting A-type potassium currents, which affects both neural excitability and response dynamics, as well as CA3 -> CA1 synaptic transmission.

FINANCIAL SUPPORT: The study was supported by Ministry of Science and Higher Education (Warsaw, Poland) grant no 2016/21/B/NZ4/03618 and statutory funds from the Institute of Pharmacology Polish Academy of Sciences, Krakow, Poland. M.S. and J.E.S. are beneficiaries of the KNOW PhD scholarship sponsored by the Ministry of Science and Higher Education, Poland.

P2.25. POSSIBLE INVOLVEMENT OF KININ B1 RECEPTOR IN THE DEVELOPMENT OF EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS IN RATS

Karolina Podsiadło, Beata Dąbrowska-Bouta, Grzegorz Sulkowski, Lidia Strużyńska

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

INTRODUCTION: Experimental autoimmune encephalomyelitis (EAE) is the most commonly used animal model of multiple sclerosis (MS) which is neuroinflammatory demyelinating disease of autoimmune origin. Among inflammatory mediators, kinins are bioactive peptides critically involved in regulation of the inflammatory response and vascular permeability. These biological activities of kinins are mediated by B1 receptors through the release of pro-inflammatory cytokines.

AIM(S): Therefore, there are reasons to investigate the role of B1 receptor in the enhancement of the BBB permeability during development of EAE.

METHOD(S): Group of female Lewis rats was immunized by intradermal injection of 100 µl inoculum. The second group was injected i.p. with DALBK (B1R antagonist) after immunization. Control group was not immunized. Animals were sacrificed in different stages of the disease. Parts of brains were used for Western blotting analysis and measurement of inflammatory cytokines using RayBio Rat Cytokine Antibody Array (RayBiotech, Inc.). Immunohistochemical study on isolated fraction of microvessels was also performed. Gene expression was quantified by RT-PCR

RESULTS: We noticed the increased expression of B1R in rat brain and isolated fraction of microvessels in the symptomatic phase of EAE. Animals treated with DALBK exhibited improvement of neurological symptoms and decreased overexpression of B1R. We also noticed increased protein level of chemokines and proinflammatory cytokines. Using a confocal microscope, we observed lowered immunoreactivity of thight junc-

tions proteins (ZO-1, occludin, claudin 5) and pericytes markers (PDGFβR and angiopoietin-1) in microvessels' fraction obtained from EAE rats which increased after DALBK.

CONCLUSIONS: Administration of kinin B1 receptor antagonist (DALBK) significantly improved the condition of animals. Results show that B1R-mediated pro-inflammatory effect of kinins may be involved in pathomechanisms operating during EAE which may lead to enhanced permeability of microvessels.

P2.26. LOWERED OXYGEN CONCENTRATION AND CULTURE IN 3-DIMENSION STRUCTURE ENHANCE IMMUNOLOGICAL RESPONSE OF WJ-MSC TO PROINFLAMMATORY FACTORS

Anna Figiel-Dąbrowska, Wioletta Lech, Marzena Zychowicz, Krystyna Domanska-Janik, Anna Sarnowska

Stem Cell Bioengineering Unit, Mossakowski Medical Research Centre Polish Academy of Sciences, Warsaw, Poland

INTRODUCTION: MSC-based therapy is becoming more and more common treatment of various diseases, albeit still as an experimental approach. According to present-day literature, the therapeutic effects of transplanted cells would not be ascribed to their differentiation, trans-differentiation or repopulation but rather to their paracrine effect on damaged tissue. This way of treatment can be initiated and enhanced by local environmental mediators.

AIM(S): The aim of this study was to assess the influence of inflammation specific environment *in vitro* on secretory WJ-MSC properties and to evaluate the possibility of programmed and controlled induction and enhancement of anti-inflammatory cell properties in the context of further cell therapy.

METHOD(S): Our experiments were based on reconstruction *in vitro* the environment to which therapeutic cells (WJ-MSC) are usually transplanted. The inflammatory conditions that occur around the transplant were reproduced through TNFα and IFNγ stimulation. Tissue specific oxygen concentration (5%), 3-dimension transplant structure and chemical composition of the indirect transplanted cell surrounding as determined by additional scaffold ingredients (fibrin and platelet lysate) were also reconstituted.

RESULTS: Carried experiments have shown specific changes in the secreted cytokine pallet induced *in vitro* by the inflammation-like WJ-MSC surrounding. We have proved that environmental modifications cause changes in synthesis and secretion of the determined proteins. Both, the physioxia introduced in our *in vitro* experiments and WJ cells cultured in 3-dimensional structures enhanced cytoprotective paracrine properties of WJ-MSC. Additional reinforcing effect was observed when therapeutic cells were transplanted on platelet lysate – containing scaffolds.

CONCLUSIONS: Presented results indicate that by optimization of cell culture and transplantation conditions we could control and enhance cytokine-connected therapeutic properties of MSC.

FINANCIAL SUPPORT: The work was supported by National Centre for Research and Development grant No Strategmed 1/234261/2/NCBR/2014.

P2.27. TARGETING KEY SIGNALING FACTORS AS A WAY TO CONTROL MICROGLIAL ACTIVATION AND INDUCTION OF NEUROINFLAMMATION

Bogna Badyra[1], Marta Hereta[2], Anna Tejchman[1], Marcin Majka[1]

[1] Jagiellonian University Medical College, Department of Transplantation, Krakow, Poland, [2] Jagiellonian University, Institute of Zoology, Department of Endocrinology, Krakow, Poland

INTRODUCTION: Neuroinflammation is co-occurring phenomenon during pathological processes in the nervous system. Key player in this process is microglia. As sensing cells, microglia recognize any morbid changes and if needed, become activated. As moderate activation of microglia is beneficial, excessive one however, leads to more severe degeneration of tissue and inhibition of its endogenous regeneration. One way to prevent this situation is to modulate or inhibit microglia activation.

AIM(S): Aim of this study was to use gene silencing technique to influence microglial activation. By targeting key proteins – NF-κB, MyD-88 and TRIF, we intended to decrease inflammatory signaling network.

METHOD(S): To optimize gene silencing, we used stable murine microglia BV-2 cell line. To induce their activation, cells were exposed to lipopolysaccharide (LPS). After stimulation, cells were transfected with designed siRNA sequences targeting NF-κB, MyD-88 and TRIF. Efficacy of transfection was assessed by evaluating expression of NF-κB, MyD-88, TRIF as well as IL-1β, IL-6, TNF-α at mRNA (qPCR) and protein level (Western blot). Optimized sequences of siRNA were then used on primary microglia isolated from adult mice in same scheme.

RESULTS: Our results showed that siRNA can successfully inhibit activation of microglia *in vitro* after stimulation with LPS. Significant decrease was observed in expression of signaling proteins. However, depending on targeted factor, different decrease patterns were observed for IL-1β, IL-6 and TNF-α. Thus, mixture of siRNA was combined to achieve most successful effect. Importantly, no or slight effect on microglia activation was observed due to siRNA transfection alone.

CONCLUSIONS: Our results provide a new method to successfully limit microglia activation with siRNA technique. It may open new ways to modulate neuroinflammation and protect nervous system from severe degeneration. However, for effective use of such approach *in vivo*, optimization of delivery and stability of silencing molecules is needed.

P2.28. IMMUNOMODULATORY CAPACITY OF WHARTON'S JELLY MESENCHYMAL STEM CELLS AFTER STIMULATION IN VITRO: CYTOKINES AND GROWTH FACTORS TRANSCRIPTIONAL RESPONSE ANALYSIS

Wioletta Lech, Marzena Zychowicz, Anna Figiel-Dąbrowska, Krystyna Domanska-Janik, Leonora Buzanska

Stem Cell Bioengineering Unit, Mossakowski Medical Research Centre Polish Academy of Sciences, Warsaw, Poland

INTRODUCTION: Mesenchymal Stem Cells (MSC) possess ability to release cytokines and growth factors that suppress immune responses and stimulate tissue regeneration. Wharton's Jelly-derived MSC (WJ-MSC) in the addition to the strong adjuvant properties are characterized by low immunogenicity.

AIM(S): The aim of this study is to verify immunomodulatory properties of WJ-MSC after TNFα and IFNγ stimulation *in vitro*, by comparative analysis of the expression of cytokines and growth factors they produce.

METHOD(S): WJ-MSC isolated from human umbilical cords were cultured in closed system that provides a constant 5% oxygen concentration. We compared immunomodulatory properties of WJ-MSC in 2D or 3D structures (scaffolds) made in our laboratory. Both of those cell populations were cultured in medium with/without stimulate factors: TNF-α and IFN-γ. After stimulation, 2D and 3D cell cultures were characterized with quantitative RT-PCR for the expression of various cytokines and growth factors with non-stimulated 2D cells as an internal control. WJ-MSC grown in 3D were also characterized by live/dead cells presence which were labeled with calcein AM/ethidium homodimer.

RESULTS: The obtained results indicated increased expression of mRNA in ^{3}D structures *vs.* control ^{2}D cells for almost all analyzed cytokines: IL-6, TGF-β^{1}, BDNF, GDNF, EGF, bFGF. Moreover, TNF-α and IFN-γ stimulation causes even further increase of mRNA expression of those cytokines in ^{3}D cultures compared to non-stimulated ^{2}D control. In our scaffolds models, the intercellular connections which were labeled in live cells with calcein AM have been observed already after ^{24}h of culture and are visible also during the next days of analysis.

CONCLUSIONS: Finally we can conclude, that WJ-MSC produce immunomodulatory factors, and their expression can be modulated by stimulation with chosen cytokines and 3D microenvironment. Such properties of WJ-MSC are important for the potential therapeutic application in the treatment of the diseases of inflammatory and autoimmune origin.

FINANCIAL SUPPORT: The work was supported by National Centre for Research and Development grant No STRATEGMED1/234261/2/NCBR/2014.

P2.29. THE ROLE OF CD44 PROTEIN IN SYNAPSE PHAGOCYTOSIS BY ASTROCYTES IN VITRO

Agnieszka Chwedorowicz[1], Beata Kaza[2], Monika Zaręba-Koziol[3], Anna Konopka[1], Grzegorz M. Wilczyński[1], Joanna Dzwonek[1]

[1] Institute of Experimental Biology, Department of Neurophysiology, Laboratory of Molecular and Systemic Neuromorphology, Warsaw, Poland, [2] Institute of Experimental Biology, Neurobiology Center, Laboratory of Molecular Neurobiology, Warsaw, Poland, [3] Institute of Experimental Biology, Department of Molecular and Cellular Neurobiology, Laboratory of Cell Biophysics, Warsaw, Poland

INTRODUCTION: CD44 adhesion molecule is highly expressed in astrocytes but its role in these cells is unknown. Recently a novel function of astrocytes in the brain in synapse phagocytosis has been described but the molecular mechanisms underlying these processes remain largely unknown. Phagocytic receptors MEGF10 and MERTK have been shown to be involved in this phenomenon. However, CD44 was shown to act as phagocytic receptor in macrophages. We hypothesized that CD44 can regulate phagocytosis in astrocytes and can be involved in synapse elimination. To investigate the role of CD44 in astrocyte-mediated synapse pruning, we used an *in vitro* engulfment assay, where astrocytes were cultured in the presence of synaptoneurosomes. We investigated whether altered CD44 expression level in astrocytes influence the efficiency of engulfement of synaptoneurosomes conjugated with a pH-sensitive fluorescent dye.

AIM(S): The aim of our work was to determine whether CD44 protein regulates synapse phagocytosis by astrocytes *in vitro*.

METHOD(S): To check if CD44 regulates synapse phagocytosis by astrocytes we used primary astrocytes cultures *in vitro* that were transfected with specific CD44shRNA to knockdown CD44 expression or controls (empty pSuper). Astrocytes were cultured in the presence of synaptoneurosomes conjugated with pHrodo fluorescent dye. The amount of synaptoneurosomes engulfed by astrocytes were monitored in living cells with the use of confocal microscopy. Three-dimentional reconstructions of astrocytes and analysis of engulfed puncta were performed using Imaris software. Live imaging of transfected cells before and after incubation with fluorescently labaled synaptoneurosomes were performed.

RESULTS: We observed significantly higher number of engulfed puncta inside CD44 depleted cells comparing to controls.

CONCLUSIONS: Our results suggest that CD44 inhibits phagocytosis of synaptoneurosomes by astrocytes.

FINANCIAL SUPPORT: The work was supported by the National Science Center grant No 2015/17/B/NZ4/02540.

P2.30. ASSESSMENT OF PALMITOYLATION STATUS AND CYCLES ON PSD-95 IN NEURONS AND IN VIVO

Yuko Fukata[1,2], Norihiko Yokoi[1,2], Kenta Kobayashi[2,3], Masaki Fukata[1,2]

[1] Division of Membrane Physiology, Department of Molecular and Cellular Physiology, Okazaki, Japan, [2] Department of Physiological Sciences, School of Life Science, SOKENDAI, Okazaki, Japan, [3] Section of Viral Vector Development, National Institute for Physiological Sciences, National Institutes of Natural Sciences, Okazaki, Japan

INTRODUCTION: Precise synaptic function requires spatio-temporally regulated protein localization. Protein palmitoylation, a reversible lipid modification, represents one such mechanism. Although numerous synaptic palmitoylated proteins have been identified, the physiological importance of their palmitoylation remains incompletely understood due to the lack of quantitative information.

AIM(S): To determine the actual palmitoylation stoichiometry and state (for example, mono-, di-, tri-) of representative synaptic proteins in the rat brain, and to examine how dynamically the palmitoyl-turnover on proteins is regulated.

METHOD(S): We used recently developed acyl-PEGyl exchange gel-shift (APEGS) assay to profile palmitoylation stoichiometry of synaptic proteins and their dynamic changes, especially for PSD-95, in rat cultured hippocampal neurons and in rat brain.

RESULTS: We found that individual palmitoylated proteins have the distinct palmitoylation site occupancy and the kinetics in rat cultured hippocampal neurons. Unexpectedly, palmitate on synaptic proteins did not all turn over. Of particular importance however is uniquely robust and dynamic palmitoylation for a postsynaptic scaffold PSD-95. In young neurons the stoichiometry of PSD-95 palmitoylation was about 60% with the rapid palmitate cycling, whereas palmitate cycling on PSD-95 significantly decelerated accompanied by the increased stoichiometry in neurons and *in vivo*. Furthermore, we found that the sensitivity against recently discovered PSD-95 depalmitoylating enzyme, ABHD17, well correlated with the speed of palmitoyl cycling and cluster formation of PSD-95.

CONCLUSIONS: This study suggests that the palmitoylation stoichiometry and kinetics of PSD-95 could be tightly controlled in response to the physiological contexts during synapse development by the specific palmitoylating/depalmitoylating enzymes.

FINANCIAL SUPPORT: The Ministry of Education, Culture, Sports, Science and Technology – Japan (15H04279, 15H01299).

P2.31. KETOGENIC DIET AND NEURODEGENERATION OF DOPAMINERGIC NEURONS

Katarzyna Kuter, Łukasz Olech, Urszula Głowacka

Institute of Pharmacology Polish Academy of Sciences, Krakow, Poland

INTRODUCTION: Ketogenic diet (KD) changes energy metabolism by decreasing use of carbohydrates and substituting calories with fatty acids. Neuroprotection is attributed probably due to improved cellular energetics. It has been shown to be beneficial in epilepsy and recently also in neurodegenerative disorders as Parkinson's Disease (PD).

AIM(S): We studied effects of KD in an animal model representing early PD stages to see if KD would influence neuronal degeneration process.

METHOD(S): We prepared early PD rat model of selective, medium size nigrostriatal dopaminergic system degeneration by stereotaxic injection of 3 µg /3 µl 6-OHDA into medial forebrain bundle. Ketogenic diet (1% carbohydrates, 70% fat, 8% protein) was started 3 weeks before operation and continued to the end of experiment.

RESULTS: KD strongly increased ketone levels in plasma, striatum (STR) and substantia nigra (SN) and lesioning enhanced this effect in plasma 4 days and 2 weeks after operation. KD temporarily increased rats locomotor activity but didn't rescue neurons from 6-OHDA toxicity, as showed stereological neurons counting and dopamine (DA) levels. Although at the time when locomotor activity was enhanced decrease in DA turnover was observed in SN but not in STR. After 4 weeks this effect was reversed, behaviour normalised and DA turnover in SN increased. Interestingly, lesioned animals kept on KD showed increased levels of succinate in SN at all studied time-points.

CONCLUSIONS: We did not observe neuroprotective effect against 6-OHDA induced toxicity, although a modulatory effect in dopaminergic metabolism was detected along with some energetic changes.

FINANCIAL SUPPORT: The study was supported by the NCN grant 2012/05/B/NZ4/02599 and statutory funds of the Institute of Pharmacology Polish Academy of Sciences, Krakow, Poland.

P2.32. CD44 ADHESION MOLECULE INTERACTS WITH VESICULAR TRANSPORT REGULATORS IN NEURONS

Anna Skupień[1], Monika Zaręba-Kozioł[2], Joanna Dzwonek[1]

[1] Labolatory of Molecular and Systemic Neuromorpholgy, Department of Neurophysiology, Nencki Institute, Warsaw, Poland, [2] Labolatory of Cell Biophysics, Department of Molecular and Cellular Neurobiology, Nencki Institute, Warsaw, Poland

INTRODUCTION: The role of extracellular matrix and its cellular receptors in acquisition of proper dendrite morphology in neurons has remained enigmatic. Previously we have shown that CD44 adhesion molecule, the main hyaluronan receptor, plays a crucial inhibitory role in dendritic tree arborization. Additionally, our results clearly demonstrate that CD44 defines the structure of Golgi apparatus, suggesting that CD44 may regulate dendritic arbor development by modulating the Golgi apparatus morphology and positioning

AIM(S): The aim of our work is to find molecular partners of CD44 in neurons.

METHOD(S): Immunoprecipitation and subsequent mass spectrometry analysis were used to unravel new CD44-interacting proteins in cortical neurons cultured *in vitro*. Obtained results were validated by Western Blot analysis.

RESULTS: Mass spectrometry analysis pointed out several potential CD44-interacting partners in neurons. In the group of identified proteins we have distinguished many molecules involved in cellular vesicles transport. One of them is ERC2, the protein that belongs to Rab3-interacting molecule (RIM)-binding protein family. We confirmed the results obtained by mass spectrometry by immunoprecipitation and Western Blot methods. ERC2 protein was co-immunoprecipitated with anti-CD44 antibody, but not with the IgG control antibody.

CONCLUSIONS: We have shown for the first time, that CD44 interacts with ERC2 in neurons. These results suggest that the cellular mechanism underlying CD44-dependent modulation of dendritic tree development involves the regulation of cellular vesicular transport in neuronal cells.

FINANCIAL SUPPORT: 2014/15/N/NZ4/01912.

P2.33. CHANGES IN GNRH AND GNRH RECEPTOR BIOSYNTHESIS IN THE HYPOTHALAMIC-PITUITARY UNIT OF ANESTROUS EWES AFTER INFUSION OF GNRH INTO THE THIRD CEREBRAL VENTRICLE

Magdalena Łapot[1], Franciszek Przekop[2], Edyta Paruszewska[1], Bożena Antkowiak[1], Tadeusz Malewski[3], Magdalena Ciechanowska[1]

[1] Department of Pharmacology and Toxicology, The 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, [3] The Museum and Institute of Zoology Polish Academy of Sciences, Warsaw, Poland

INTRODUCTION: Physiological regulation of GnRH secretion in mammals is associated with complex interplay between excitatory and inhibitory neurotransmitter and neurohormone systems within the hypothalamus that mediate oestrogen signals to GnRH cells. Among numerous studies on the neuroendocrine processes controlling GnRH secretion only a few research have revealed that hypothalamic GnRH acting locally may also participate in the regulation of its own release, but the mechanisms involved remain poorly understood.

AIM(S): This study was designed to evaluate the effect of prolonged intermittent infusion of small doses of exogenous GnRH on GnRH and GnRH receptor (GnRHR) biosynthesis in the hypothalamus, GnRHR expression in the anterior pituitary gland (AP), and on LH secretion in sexually inactive sheep.

METHOD(S): Studies were conducted on 3–4-year-old Polish Merino ewes during the middle of the anestrous season. Infusions of exogenous GnRH (0.2 μg GnRH per animal daily) were performed into the third cerebral ventricle for 3 consecutive days. The control for GnRH-treated group was animals infused with equivalent to GnRH volume of Ringer's solution. The measurement of GnRH and GnRHR levels was performed using an enzyme-linked immunosorbent assay (ELISA). Plasma LH concentration was analysed by a double-antibody radioimmunoassay (RIA).

RESULTS: The results of this study demonstrate that prolonged infusion of small doses of GnRH into the third cerebral ventricle of anestrous ewes increased drastically GnRH and GnRHR levels in the hypothalamus, but decreased GnRHR expression in the AP and also reduced LH secretion.

CONCLUSIONS: The study indicates the existence of ultrashort loop feedback mechanism of GnRH release from the hypothalamus in which hypothalamic GnRHR participate. Decreased expression of GnRH in the AP and diminished LH secretion in GnRH-treated ewes provide indirect evidence for suppressive effect of exogenous GnRH on GnRH release from hypothalamic nerve terminals.

FINANCIAL SUPPORT: This work was supported by grant National Science Center Poland No UMO-2012/05/B/NZ4/02443.

P2.34. MOLECULAR MECHANISMS PARTICIPATING IN INHIBITION OF GNRH/LH SECRETION INDUCED BY CRH

Magdalena Ciechanowska[1], Magdalena Łapot[1], Edyta Paruszewska[1], Marek Brytan[1], Franciszek Przekop[2]

[1] Department of Pharmacology and Toxicology, The 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

INTRODUCTION: It is generally accepted that corticotropin-releasing hormone (CRH) is the central media-

tor of stress-activated changes in the pituitary-adrenal axis because it results in the release of adrenocorticotropic hormone (ACTH) and finally increases the levels of cortisol. In some situations CRH also inhibits the release of GnRH and it has been proposed as a mediator of the anti-reproductive effects of stress.

AIM(S): This study aimed to explain how prolonged activation or inhibition of CRH-ergic activity affected molecular processes governing GnRH/LH secretion in follicular-phase sheep.

METHOD(S): The study included two experimental approaches: first, we investigated the effect of CRH or CRH antagonist (α-helical CRH 9-41; CRH-A) on GnRH and GnRH receptor (GnRHR) biosynthesis in the hypothalamus and on GnRHR in the anterior pituitary gland (AP) using an immunoassay (ELISA). This analysis was supplemented by radioimmunoassay (RIA) method for LH; second, we used Real-time PCR to analyse the influence of CRH and CRH-A on the levels of kisspeptin (Kiss 1) mRNA in the preoptic area (POA) and ventromedial hypothalamus including arcuate nucleus (VMH/ARC).

RESULTS: Our results show that stimulation or inhibition of CRH receptors significantly decreased or increased GnRH biosynthesis in the hypothalamus, respectively, and led to different responses in the expression of GnRHR. CRH increased GnRHR abundance in the POA, but decreased it in the hypothalamus and in the AP. Blockade of CRH receptors had the opposite effect on the level of post-translational product of GnRHR gene. In addition, administration of CRH decreased plasma LH concentration and Kiss1 mRNA in the POA and VMH/ARC, while CRH-A exerted an opposite action.

CONCLUSIONS: The study demonstrates that CRH-ergic neurotransmission is involved in the regulatory pathways of GnRH and GnRHR biosynthesis in the hypothalamic-pituitary unit of follicular-phase sheep conceivably via mechanisms in which Kiss 1 participate.

FINANCIAL SUPPORT: This work was supported by grant National Science Center Poland No UMO-2012/05/B/NZ4/02443.

P2.35. THE INFLUENCE OF SOMAN ON THE EXPRESSION OF TP53 GENE IN THE RAT BRAIN; DISTANT EFFECT

Marek Brytan[1], Marek Kowalczyk[1,2], Magdalena Łapot[1], Edyta Paruszewska[1], Bożena Antkowiak[1], Franciszek Przekop[3], Magdalena Ciechanowska[1]

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

INTRODUCTION: The organophosphorus compound soman (GD), an irreversible inhibitor of cholinesterases, produces seizure activity and related brain damage. Studies using various biochemical markers of programmed cell death indicate apoptotic rather than necrotic mechanism of GD-induced acute cell damage in the brain. One of the most important links between the proliferation and cell death machinery is the tumor suppressor p53, which as a guardian of the genome and the element promoting apoptosis makes it a prime target for a prognostic factor.

AIM(S): The aim of this study was to examine distant effects of poisoning with a small, repeated dose of GD on the expression of mRNA encoding p53 protein in the rat brain.

METHOD(S): The study was performed on maternal generation (F0) and on first filial generation (F1) of Wistar rats. Low clinically asymptomatic dose of GD (0.2×LD50) was administered by subcutaneous repeated injections, first in pregnancy and subsequently during the lactation period. Six months after the end of poisoning the animals were euthanised and brain structures (hippocampus, cerebellum and piriform cortex) were isolated aseptically for evaluation of p53 mRNA. To determine p53 transcript levels Real-Time PCR with SYBR Green dye was applied.

RESULTS: GD action resulted in a significant increase of p53 transcript in the cerebellum and in the piriform cortex of both F0 and F1 females as well as in F1 males. The significant elevation of p53 mRNA level in the hippocampus was observed only in F1 females.

CONCLUSIONS: The study demonstrates that GD causes distant changes in the expression of p53 mRNA in the rat brain. Increased expression of p53 mRNA provides indirect evidence that GD-induced distant disorders may include DNA damage and cell cycle disturbances leading to cell dysfunction and their elimination via apoptosis.

FINANCIAL SUPPORT: This work was supported by Polish Ministry of Science and Higher Education No O R00 0042 08, "Soldier as a precise weapon – individual sets and kits".

P2.36. IN VITRO MODEL OF RAT PERINATAL HYPOXIA-ISCHEMIA. HOW TEMPORAL OXYGEN-GLUCOSE DEPRIVATION AFFECTS MATURATION OF OLIGODENDROCYTE PROGENITOR CELLS

Justyna Janowska, Joanna Sypecka

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

INTRODUCTION: The frequent reason of brain damage in newborns is perinatal hypoxia-ischemia. It may cause the central nervous system hypomyelination, which often results in the long-term neurobehavioral disabilities. Oligodendrocytes, responsible for myelination, differentiate from oligodendrocyte progenitor cells (OPCs) in developing brain.

AIM(S): In our study we investigated how oligodendrocyte progenitor cells respond to oxygen-glucose deprivation (OGD) in the *in vitro* model of neonatal asphyxia.

METHOD(S): OPCs were obtained from rat primary glial cultures. 24 h after seeding, the cells were exposed to short OGD procedure. Its effect on the cell proliferation was measured by immunolabeling the dividing cells for Ki67 marker and BrdU incorporation. Cell viability was evaluated with AlamarBlue testing. Impact of OGD on OPC differentiation was assessed by making microscopic examination of immunocytochemically labeled cells with antibodies against NG2 for OPCs, GalC for immature oligodendrocytes, MBP for myelinating cells. Expression of characteristic myelin proteins was additionally verified by ELISA analysis.

RESULTS: The obtained data revealed that OGD stimulated proliferation of oligodendrocyte progenitor cells, which was shown in 2% higher number of Ki67-positive cells 1 day after OGD and 17% increase in the amount of BrdU- positive cells versus control 3 days after injury. Results of AlamarBlue assay also indicated the twofold higher viability of OGD-affected cells versus control cells 6 days after injury. Microscopic analysis, confirmed by quantitative ELISA measurement, indicated a significant inhibition of oligodendrocyte maturation after injury.

CONCLUSIONS: Hypoxic-ischemic insult, reflected *in vitro* by OGD procedure, alters OPCs differentiation process causing the CNS hypomyelination. In our *in vitro* studies we showed that this may be associated with the enhanced proliferation of oligodendrocyte progenitors as a compensative mechanism for the injury and the disrupted maturation process.

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P2.37. MOUSE SPECIFIC EXPRESSION OF THE NOVEL SPLICE VARIANT OF PCP2/L7

Jarosław J. Barski[1,2], Marta Przybyła[1,2], Marta Nowacka-Chmielewska[1,3]

[1] Department of Experimental Medicine, School of Medicine in Katowice, Katowice, Poland, [2] Department of Physiology, School of Medicine in Katowice, Medical University of Silesia, Katowice, Poland, [3] Laboratory of Molecular Biology, Faculty of Physiotherapy, The Jerzy Kukuczka Academy of Physical Education, Katowice, Poland

INTRODUCTION: Pcp2/L7 is a member of the GoLoco protein family with a very cell-specific expression in cerebellar Purkinje cells and retinal bipolar neurons. Its precise functional role remains still unclear. Sparse studies indicated its possible role as a guanine nucleotide dissociation inhibitor or guanine nucleotide exchange factor. Studies on genomic structure of pcp2 gene revealed some alternative splice variants expressed in Purkinje cells and retinal bipolar neurons.

AIM(S): Here we attempted to shed some light on the conservation of a novel pcp2 splice variant in closely related laboratory rodents: mouse, rat and hamster.

METHOD(S): PCR: Both splice variants were amplified with the primers L7sense and L7anti, which yielded two reaction products of 371 and 312 bp. The novel splice variant including exon 3B was detected using the primers L7sense (as above) and L73Aanti, for a product size of 274 bp. Quantitative real-time PCR (qPCR) analysis: To validate the results obtained by RT-PCR, we conducted an additional qPCR experiment to investigate the expression of Pcp2 transcripts with greater accuracy. The region of interest was amplified from cDNA resulting in a product size of 90 bp.Relative gene expression levels were calculated with the 2-ΔΔCt method.

RESULTS: In our approach we were able to confirm expression of the novel longer transcript in mouse, however PCR amplification on cDNA from rat and hamster did not reveal the long splice variant with additional exon 3B*.

CONCLUSIONS: Obtained data indicate, that the novel splicing variant of pcp2 is mouse-specific and is expressed only in Purkinje cells and retinal bipolar neurons in this species. In course of the evolution it appeared probably in that species as result of a spontaneous mutation. Herewith we suggest a very specific and not known yet function of pcp2 in the mouse eye and/or Purkinje cells.

FINANCIAL SUPPORT: Research supported by the Ministry of Science and Higher Education core grant #KNW-2-011/D/4/N.

P2.38. EXPRESSION OF CALRETININ AND CALBINDIN IN THE CINGULATE CORTEX OF THE GUINEA PIG

Beata Hermanowicz-Sobieraj, Krystyna Bogus-Nowakowska, Maciej Równiak, Anna Robak

Department of Comparative Anatomy, Faculty of Biology and Biotechnology, University of Warmia and Mazury in Olsztyn, Olsztyn, Poland

INTRODUCTION: The cingulate cortex (CC), a part of the limbic cortex, is one of the major components of the Papez circuit. Mammalian cerebral cortex contains excitatory pyramidal neurons (70–80%), which use glutamate as neurotransmiter, and interneurons (20–30%), mostly inhibitory, using GABA as principal neurotransmiter. The maintenance a balance between these neurotransmitters is essential for proper functioning of neurons. GABAergic neurons deficit is often related to neurodegenerative disorders. Markers for GABAergic neurons are calcium-binding proteins: calretinin (CR) and calbindin (CB), which may act as calcium sensors as well as both fast and slow calcium buffers.

AIM(S): The aim of the study was to describe the distribution of CR and CB and compare expression of both CaBPs

at transcriptional and final product levels in the cingulate cortex of the adult guinea pig.

METHOD(S): Genes expression of CR and CB was measured on mRNA by quantitative real-time PCR (qPCR) analysis. Total RNA was isolated using Total RNA Mini and then was reverse transcribed to cDNA using Maxima cDNA Synthesis Kit. qPCR was conducted using SYBR® Green JumpStartTMTaqReadyMixTM. To visualize CR and CB immunoreactivity frozen sections were undergone for routine single immunofluorescence labelling, using solution of antibodies raised against CR and CB.

RESULTS: The immunohistochemical study indicates the presence of CR and CB in the whole CC. The number of CB-positive perikarya was lower than the CR ones. CR-positive perikarya in comparison to CB-positive, were more numerous in the superficial than in the deep layers of the CC. The qPCR analysis showed that the mRNA expession for CR was higher than for CB.

CONCLUSIONS: The CB and CR mRNA expression level revealed by qPCR correlate with their protein abundance level revealed by immunohistochemistry. Calretinin expression was higher than calbindin at both levels.

FINANCIAL SUPPORT: Co-financing the scientific studies for young scientists or PhD students at the Faculty of Biology and Biotechnology, University of Warmia and Mazury in Olsztyn (ID:12.620.026-300).

P2.39. TRAFFICKING OF SLC6A14 – AN AMINO ACID TRANSPORTER B(0,+) TO PLASMA MEMBRANE

Vasylyna Kovalchuk[1], Dominika Jurkiewicz[1], Barbara Juraszek[1], Łukasz Samluk[1], Sonja Sucic[2], Michael Freissmuth[2], Katarzyna A. Nałęcz[1]

[1] Department of Molecular and Cellular Neurobiology, Nencki Institute of Experimental Biology of Polish Academy of Sciences, Warsaw, Poland, [2] Center for Physiology and Pharmacology, Institute of Pharmacology, Medical University of Vienna, Vienna, Austria

INTRODUCTION: The solute carrier 6 (SLC6) family of genes codes transporters for neurotransmitters, amino acids, osmolytes and energy metabolites. In the brain SLC6 transporters play an important role in the reuptake of GABA, serotonin, dopamine, noradrenaline, glycine and proline. SLC6A14 is a Na/Cl-dependent amino acid transporter ATB(0,+) – specific towards neutral and cationic amino acids. It is present in astrocytes and in the blood-brain barrier. In the first step of trafficking to plasma membrane – ER exit, the neurotransmitter transporters interact with specific isoforms of SEC24 proteins, the components of Coatomer II (COPII).

AIM(S): To verify, which proteins are involved in trafficking of ATB(0,+) to the cell surface from ER.

METHOD(S): Trafficking of rat SLC6A14 to plasma membrane was studied in heterologous expression system.

RESULTS: Immunofluorescence analysis showed that SLC6A14 appears in the plasma membrane after 48 h, with the majority of the transporter not leaving ER. This observation was confirmed by biotinylation of surface proteins and analysis of SLC6A14 glycosylation status in order to distinguish core glycosylation (taking place in ER) from full glycosylation (taking place in Golgi apparatus). Trafficking was attenuated after co-transfection with the dominant negative mutants of Sar1 GTPase – the first protein involved in COPII formation. Further studies demonstrated that out of four SEC24 proteins, exclusively SEC24C co-localized and interacted with SLC6A14, a result confirmed in the proximity ligation assay.

CONCLUSIONS: These observations confirm a hypothesis that lysine in position +2 towards an ER export signal is crucial for specific interaction of SLC6 transporters with SEC24C.

FINANCIAL SUPPORT: This work has been supported by the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement no 665735 (Bio4Med) and by the funding from Polish Ministry of Science and Higher Education within 2016–2020 funds for the implementation of international projects (agreement no 3548/H2020/COFUND2016/2).

P2.40. ELEVATED INTRAOCULAR PRESSURE IMPAIRS DISTRIBUTION OF RNA-BINDING PROTEIN HUR IN GLAUCOMATOUS RAT RETINA

Joanna Lewin-Kowalik[1], Adrian Smędowski[1,2], Xiaonan Liu[3], Lucia Podracka[4], Anna Trzeciecka[2], Marita Pietrucha-Dutczak[1], Markku Varjosalo[3], Kai Kaarniranta[2,5], Marialaura Amadio[6]

[1] Department of Physiology, Medical University of Silesia, School of Medicine in Katowice, Katowice, Poland, [2] Department of Ophthalmology, University of Eastern Finland, Kuopio, Finland, [3] Institute of Biotechnology, University of Helsinki, Helsinki, Finland, [4] School of Pharmacy, University of Eastern Finland, Kuopio, Finland, [5] Department of Ophthalmology, Kuopio University Hospital, Kuopio, Finland, [6] Department of Drug Sciences, Section of Pharmacology, University of Pavia, Pavia, Italy

INTRODUCTION: Glaucoma is a serious social problem as it may result in blindness. Most often, it is related to increased intraocular pressure, but the exact biologic mechanism is not known yet. RNA-binding proteins may be one of the pathogenetic factors for this disease.

AIM(S): To evaluate impact of increased intraocular pressure (IOP) on HuR protein expression in retina and optic nerve in rat glaucoma model.

METHOD(S): IOP was increased unilaterally using modified rat bead model. Fellow eye was used as a healthy control. Animals were sacrificed 1 day, 1-, 4-, 6- or 8-weeks after beads injection. Retinas and optic nerves were collected and processed for Western blot (WB) analysis, mass spectrometry (MS), PCR and immunostainings.

RESULTS: The loss of retinal ganglion cells (RGCs) was at the level of 36% after 8-weeks of IOP elevation. The presence of HuR protein and its transcipt was confirmed in retinas and optic nerves using WB, MS and PCR analysis. Additionally, Gene Ontology enrichment analysis revealed that the most significant alterations in glaucoma retinas were linked to the molecular function of binding proteins. In fractionated WB of retinal homogenates, the level of cytoplasmic fraction of HuR was decreased approximately 3-times when compared with healthy tissue ($p<0.05$). This decrease was accompanied by alteration of the cytoplasmic level of HuR-regulated proteins, i.e. Hsp70 decrease in retina and p53 decrease in optic nerve. Stereological analysis of retinas revealed that some RGCs have lost visible HuR expression. Immunostaining of retinal and optic nerves cross sections showed decreased staining for HuR within RGCs and increased within optic nerve glia, with nuclear polarisation of HuR expression in glaucoma samples.

CONCLUSIONS: Increased intraocular pressure results in alteration of RNA-binding protein HuR within retina and, subsequently, decreased expression of HuR-dependent stress-response regulatory proteins. This alterations might contribute to the development of glaucomatous degeneration.

P2.41. THE ROLE OF ADAPTOR COMPLEX AP2 IN FORMATION OF DENDRITIC ARBORS OF HIPPOCAMPAL NEURONS

Alicja Kościelny, Anna Malik, Ewa Liszewska, Justyna Zmorzyńska, Aleksandra Tempes, Bartosz Tarkowski, Jacek Jaworski

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

INTRODUCTION: The proper dendritic branching is a highly regulated process. Among its regulators are membrane proteins internalized via clathrin-mediated endocytosis. AP2 adaptor complex is a key player in this process, but its role in mammalian dendritogenesis has not yet been tested.

AIM(S): The aim of this study was to find how AP2 complex contributes to shaping dendritic tree of developing hippocampal neurons.

METHOD(S): To study role of AP2 complex in dendritic arborization we used primary hippocampal neurons expressing AP2b1 (b-adaptin) shRNA alone or in combination of functional rescue constructs (i.a. GluA2, S6K1ca). The effect was also tested *in vivo* by lentiviral injections to newborn rats. Upon b-adaptin knockdown, we tested GluA2 trafficking via internalization assay and GluA2 level by Western blot and immunochemistry. GluA2 degradation and mTOR dependent biosynthesis were investigated by e.g. cycloheximide or rapamycin treatment.

RESULTS: We showed that knockdown of b-adaptin led to reduction in dendritic arbors of developing hippocampal neurons *in vitro* and *in vivo*. The knockdown of AP2 also led to decreased level of GluA2, what is a result of impaired mTOR dependent GluA2 biosynthesis. However, the overexpression of functional GluA2 or restoration of mTOR activity rescued this effect.

CONCLUSIONS: AP2 adaptor complex regulates the dendritogenesis of mammalian neurons via mTOR dependent GluA2 biosynthesis.

FINANCIAL SUPPORT: This work has been financed by National Research Centre grant no. 2011/03/B/NZ3/01970.

P2.42. NEW EVIDENCE FOR A ROLE OF MGLUR7 IN ASTROCYTE SURVIVAL: POSSIBLE IMPLICATIONS FOR NEUROPROTECTION

Danuta Jantas[1], Tomasz Lech[2], Sławomir Gołda[3], Andrzej Pilc[2], Władysław Lasoń[1]

[1] Department of Experimental Neuroendocrinology, Institute of Pharmacology Polish Academy of Sciences, Krakow, Poland, [2] Department of Neurobiology, Institute of Pharmacology Polish Academy of Sciences, Krakow, Poland, [3] Department of Molecular Neuropharmacology, Institute of Pharmacology Polish Academy of Sciences, Krakow, Poland

INTRODUCTION: A specific activation of metabotropic glutamatergic receptor subtype 7 (mGluR7) by its allosteric agonist AMN082 has been shown to protect neuronal cells against various detrimental factors. It is well established that some of subtypes of mGluRs (e.g., mGluR5 or mGluR3) engage glia cells to more efficiently protect neurons against various harmful stimuli.

AIM(S): We aimed to study the role of mGluR7 in glia and neuronal cell survival.

METHOD(S): We used primary cortical glia cell cultures and cerebellar granule neurons (CGNs)from mGluR7+/+ and mGluR7-/- C57Bl/6J mice which were exposed to various cell damaging factors (staurosporine (St), doxorubicin (Dox)and low potassium (LP)). MTT reduction, LDH release and caspase-3 activity biochemical assays were used for assessment of cell damage. The mRNA expression level of various subtypes of mGluRs was measured by qPCR.

RESULTS: We showed the expression of mGluR7 in glia cell cultures and demonstrated the higher toxicity of St and Dox in mGluR7-/- glia cells when compared to wild type one. Moreover, we found a partial protection mediated by AMN082 against St and Dox in mGluR7+/+ glia cells. However, we did not find any differences in vulnerability of CGNs derived from mGluR7+/+ and mGluR7-/- animals to the cell damaging action of LP, St or Dox under standard treatment. Intriguingly, when we primed both types of CGNs by culturing them overnight in LP medium, we found significant higher toxic action of St and Dox in mGluR7-/- CGNs. Finally, we confirmed neuroprotective properties of AMN082 in CGNs and showed that this effect is stimuli- and development-dependent.

CONCLUSIONS: Our data obtained in isolated glia and neuronal cellular models showed a protective potential of mGluR7-specific agonist AMN082 and pro-survival role of mGluR7 in glia cells which together with its already known direct role on neuronal cells could suggest its higher efficacy under *in vivo* conditions.

FINANCIAL SUPPORT: The study was supported by statutory funds for Institute of Pharmacology PAS and grant No NN405611638 from the Ministry of Science and Higher Education, Warsaw, Poland.

P2.43. DEVELOPMENTAL CHANGES OF CALBINDIN IMMUNOREACTIVITY IN THE PREOPTIC AREA OF THE MALE GUINEA PIG (CAVIA PORCELLUS)

Krystyna Bogus-Nowakowska, Beata Hermanowicz-Sobieraj, Maciej Równiak, Anna Robak

Department of Comparative Anatomy, Faculty of Biology and Biotechnology, University of Warmia and Mazury in Olsztyn, Olsztyn, Poland

INTRODUCTION: Calbindin (CB) is one of the members of the EF-hand family of calcium-binding proteins which are involved in controlling intracellular calcium ion homeostasis. It may act as Ca2+ "fast" buffers and also as Ca2+ sensors. Intracellular calcium ions play an important role in immature and mature neurons. During early stages of development, calcium ions are involved e.g. in neuronal differentiation and plasticity, migration of neurons, or extension of neuronal processes. The preoptic area (POA) is a key structure which takes part in many autonomic functions (for example thermoregulation, thirst or hunger) as well as in reproduction and maternal behaviour, especially for pup retrieval as well as the onset of parental behaviour in females and males.

AIM(S): The aim of the study was to examine the distribution of CB expression during the development of the preoptic area in the guinea pig by means of immunohistochemistry.

METHOD(S): Brains from fetal stages (E50, E60), newborns (P0) and postnatal stages (P10, P20, P40, P100) were fixed in 4% paraformaldehyde in phosphate buffer and then cryoprotected. Frozen sections were processed for two immunohistochemical methods: an immunoenzymatic and immunofluorescence.

RESULTS: Calbindin was highly expressed in the preoptic area of the male guinea pig, especially in the periventricular region. CB- immunoreactive (-ir) perikarya, fibers and punctate structures were observed at each examined stages. CB-ir perikarya were the most numerous at E50 and the least numerous at P100. The CB-ir neurons had oval, rounded or polygonal perikarya and some of them had processes of various length which emerged from perikarya. CB-ir fibers differed according to lengths.

CONCLUSIONS: The highest expression of CB in the preoptic area at E50 coincides well with major developmental events (i.e. eyes opening) which in the guinea pig occur just before E50 stage.

P2.44. SIRT1 GENE EXPRESSION LEVEL AND ITS INFLUENCE ON THE PROGRESSION OF PRIMARY OPEN-ANGLE GLAUCOMA IN A POLISH POPULATION

Mateusz Siwak[1], Alicja Nowak[1], Marcin Maślankiewicz[1], Krystian Romaszko[1], Jerzy Szaflik[2,3], Jacek Szaflik[2,3], Ireneusz Majsterek[1]

[1] Department of Clinical Chemistry and Biochemistry, Medical University of Lodz, Lodz, Poland, [2] Department of Ophthalmology, Medical University of Warsaw, Warsaw, Poland, [3] SPKSO Ophthalmic Hospital, Warsaw, Poland

INTRODUCTION: Primary open-angle glaucoma (POAG) is an eye disease, which characterized by impairment of retinal neurons resulted in a changes in optic nerve head, damage and death retinal ganglion cells by apoptosis, which lead to loss of vision. Therefore, the scientists were pointed out some resemblance of pathomechanism of neurodegenerative diseases and glaucoma. The number of animal studies have been conducted to examine the role of sirtuins in ocular aging. Upregulation of SIRT1 has been shown to have an important protective effect against various ocular diseases, such as cataract, retinal degeneration, optic neuritis and uveitis.

AIM(S): Assessment of the relationship between SIRT1 gene expression level in patients with POAG and the control group. Additionally, analyze the effect of SIRT1 expression level on the progression of POAG depending on clinical parameters.

METHOD(S): The study included 34 patients of POAG and 31 control subjects. RNA was extracted from peripheral blood, digested with DNase and converted to cDNA. The SIRT1 expression levels were measured by QPCR method. The non-parametric Mann-Whitney U test was applied to determine the levels of mRNA expression in blood of POAG patients and healthy subjects. The non-parametrical statistical tests (ANOVA with *post hoc* Tukey's HSD test) were applied to compare level of mRNA expression with clinical parameters of POAG patients.

RESULTS: The results shown no statistically significant differences between SIRT1 mRNA levels of POAG patients and controls ($p>0.05$). However, there was significant association of the SIRT1 expression level with progression of POAG based on RA value (Rim Area), $p=0.012$. We observed increase of SIRT1 expression level with the early stage of glaucoma, which confirms the protective role of sirtuin in the development of glaucoma.

CONCLUSIONS: In conclusion, our study showed a statistically significant association of SIRT1 genes with progression of POAG in a Polish population.

FINANCIAL SUPPORT: This work was supported by Umed in Lodz Grants no 500/5-108-05/500-41.

P2.45. MICROGLIAL CELLS IN THE DORSAL RAPHE NUCLEUS PLAY A POTENTIAL ROLE IN BOTH SUICIDE FACILITATION AND PREVENTION IN AFFECTIVE DISORDERS

Ralf Brisch[1], Johann Steiner[2], Christian Mawrin[3], Marta Krzyzanowska[1], Zbigniew Jankowski[1], Tomasz Gos[1]

[1] Department of Forensic Medicine, Medical University of Gdansk, Gdansk, Poland, [2] Department of Psychiatry, Otto-von-Guericke-University of Magdeburg, Magdeburg, Germany, [3] Institute of Neuropathology, Otto-von-Guericke-University of Magdeburg, Magdeburg, Germany

INTRODUCTION: An involvement of the central serotonergic system has been reported in the pathogenesis of suicide. The dorsal raphe nucleus (DRN) is the main source of serotonergic innervation of forebrain limbic structures disturbed in suicidal behavior, in which an abnormal reaction of microglial cells seems to play an important role.

AIM(S): In our present study, the densities of microglial cells immunostained for the HLA-DR antigen were evaluated in the DRN.

METHOD(S): These analyses were carried out on paraffin-embedded brains from 24 suicidal patients and 21 non-suicidal patients; among them 27 depressed patients (15 major depressive disorder and 12 bipolar disorder), 18 schizophrenia patients (9 residual, 9 paranoid), and 22 matched healthy control subjects.

RESULTS: Only the non-suicidal depressed subgroup revealed a significantly lower microglial reaction. This means that we found a decreased density of HLA-DR immunopositive microglia compared with those of both depressed suicide victims and healthy control subjects. This effect was not related to antidepressant or antipsychotic medication, as the former correlated positively with microglial density in non-suicidal depressed patients, and the latter had no effect.

CONCLUSIONS: Moreover, the comparison of these results with previously published data from our workgroup in the same cohort suggested a positive impact of microglia on ribosomal DNA transcription in DRN neurons in the non-suicidal depressed subgroup, but not in depressed suicidal cases. Therefore, the interaction between microglia and neurons in the DRN may be potentially involved in opposite ways regarding suicide facilitation and prevention in the tested subgroups of depressed patients.

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P2.46. IRREGULAR NUCLEAR SHAPES OF NEURAL STEM CELLS IN ADULT MURINE DENTATE GYRUS

Aneta W. Grymanowska[1], Ana Martin[2], Krzysztof H. Olszyński[1], Grzegorz M. Wilczyński[2], Adriana Magalska[2], Robert K. Filipkowski[1]

[1] Mossakowski Medical Research Centre Polish Academy of Sciences, Warsaw, Poland, [2] Nencki Institute of Experimental Biology Polish Academy of Sciences, Warsaw, Poland

INTRODUCTION: In adult hippocampal neurogenesis, stem cells and their derivative progenitors are generated. They differentiate into neurons as they migrate from the subgranular zone to the granule cell layer of the dentate gyrus, maintaining homeostatic tissue regeneration and supporting brain plasticity. Depending on the stage of the neurogenesis, different subpopulations of cells of the neurogenic lineage can be distinguished, i.e. neural stem cells (NSC, type 1), intermediate progenitor cells (type 2a and type 2b), neuroblasts (type 3) and granule neurons (type 4). Little is known about the architecture of nuclei in these cells, while the cell nucleus is known to be highly organized with numerous functional and structural domains as well as dynamic organization of chromatin governed by epigenetic mechanisms which were shown to respond to external signals.

AIM(S): We aimed to distinguish type 1 through 4 cells and investigate their nuclear shape.

METHOD(S): Transgenic Nestin-GFP mice were used. Cell types were identified with immunohistochemistry and morphological features: type 1 (GFP+, one long neural process), type 2a (GFP+), type 2b (GFP+/DCX+), type 3 (DCX+), type 4 (NeuN+). Confocal microscopy was used to collect z-stack files of the nuclei of different cell types.

RESULTS: We observed irregularity in shape of the nuclei in type 1 cells (NSC) with the presence of nuclear envelope invaginations. When selected layers were analyzed, NSC nuclei turned out to have reduced circularity, roundness and solidity when compared with other cell types.

CONCLUSIONS: The irregularity observed and nuclear envelope invaginations seem to be characteristics of the "stemness" as the shape of the nucleus becomes more regular with successive stages of neurogenesis. The biological significance of the observed phenomenon is not yet clear and further studies are necessary to better understand the process of adult neurogenesis at the nuclear level.

FINANCIAL SUPPORT: The work was supported by National Science Centre, Poland, grant no. 2014/14/M/NZ4/00561.

P2.47. KAINIC ACID CAUSES MTOR ACTIVATION AND TRANSLOCATION FROM CYTOSOL TO THE NUCLEUS – LIVE IMAGING STUDY

Matylda Macias, Aleksandra Janusz-Kamińska, Bartosz Tarkowski, Agata Gozdz, Jacek Jaworski

Laboratory of Molecular and Cellular Neurobiology, International Institute of Molecular and Cell Biology, Warsaw, Poland

INTRODUCTION: The mechanistic target of rapamycin (mTOR) is a protein kinase, which integrates eukaryotic cell growth, metabolism and external stimuli. Most research link mTOR with control of protein translation but recent studies revealed additional mTOR function in cell nucleus. Previously, we showed that phosphorylation of mTOR (Ser2448; P-mTOR) is upregulated in neuronal nucleus upon kainate (KA) induced status epilepticus. Whether other stimuli have the same effect on nuclear mTOR phosphorylation and if increased nuclear import of mTOR contributes to this phenomenon remained unknown. Also it was not known if nuclear transport of other proteins affects mTOR signaling.

AIM(S): To analyze effects of neuronal activity on nuclear translocation of mTOR and its nuclear activity. To analyze importance of nuclear transport for mTOR signaling.

METHOD(S): Cultured hippocampal neurons were treated with: KA, BDNF; NMDA and chemical LTP (cLTP) protocol or TTX. mTOR activity was measured with FRET method. mTOR nuclear translocation was assessed using FRAP. Nuclear import was blocked with importazole. Immunofluorescence of P-S6 protein was used as a marker of mTOR activity.

RESULTS: We found that KA, BDNF, NMDA and cLTP caused nuclear upregulation of P-mTOR. However, TTX or cLTD had no effect. FRAP and FRET revealed that mTOR activity due to KA treatment is first observed in cytosol and then in nucleus, where mTOR is translocated upon treatment. Blocking nuclear import silenced mTOR activity in response to KA and inhibited P-mTOR upregulation in the nucleus.

CONCLUSIONS: Our experiments showed that increased neuronal activity upregulates nuclear P-mTOR and increases nuclear activity of mTOR due to nuclear translocation of the kinase.

FINANCIAL SUPPORT: The research was supported by PNSC grants no. 2012/05/B/NZ3/00429 and 2012/07/E/NZ3/00503.

P2.48. CRE/LOX TRANSGENIC MICE AS A TOOL TO STUDY CD44 FUNCTION IN THE BRAIN

Patrycja Kruk, Gabriela Olech-Kochańczyk, Grzegorz M. Wilczyński, Joanna Dzwonek

Department of Neurophysiology, Laboratory of Molecular and Systemic Neuromorphology, Nencki Institute of Experimental Biology Polish Academy of Sciences, Warsaw, Poland

INTRODUCTION: The role of CD44 protein in astrocytes in physiological and pathological conditions in the brain remains largely unknown. To study its function the transgenic animal models were used. The CD44 knock-out mice are commercially available, however the compensatory effect of ICAM-1 molecule for the CD44 deficiency has been previously described. For that reason, we decided to create conditional knockout mice where we can control the time of gene silencing during animal development by tamoxifen (TAM) administration. Moreover, we generated a conditional overexpression mouse line in which the transgene overexpression is also initiated by TAM. By using CreERT2 fusion protein driven by GFAP (glial fibrillary acidic protein) promoter, we can achieve inducible astrocyte-specific CD44 knock-out/overexpression line in which CD44 gene becomes altered in astrocytes of the adult brain upon the tamoxifen-driven activation of Cre recombinase, at the chosen time point. This gives us an ability to change the CD44 expression after the mice reach adulthood.

AIM(S): The aim of our work is to validate two conditional double transgenic mouse lines created with the use of Cre/lox system to study the function of CD44 protein in astrocytes.

METHOD(S): For the comparison of Cre/lox activation efficacy, mice were injected with TAM either every 12 hours (10 mg/ml) or every 24 hours (20 mg/ml) for the duration of 5 constitutive days. Then, the effect of the transgene activation was validated using western blot and immunohistochemistry techniques.

RESULTS: Validation studies confirm CD44 overexpression model works. CD44 overexpression can be seen in all hippocampus, cerebral cortex and cerebellum. Immunohistochemistry staining shows increased level of CD44 in astrocytes of cerebral cortex and hippocampus, especially in the molecular layer of dentate gyrus.

CONCLUSIONS: Described inducible CD44 transgenic mouse lines are the first animal models that can help scientists study the yet undiscovered function of CD44 protein in astrocytes.

P2.49. ANGIOTENSIN CONVERTING ENZYME INHIBITORS AND ANGIOTENSIN II TYPE 1 RECEPTOR BLOCKERS AFFECT KYNURENIC ACID PRODUCTION IN RAT BRAIN CORTEX IN VITRO

Izabela Zakrocka[1], Katarzyna M. Targowska-Duda[2], Artur Wnorowski[2], Tomasz Kocki[1], Krzysztof Jóźwiak[2], Waldemar A. Turski[1]

[1] Department of Experimental and Clinical Pharmacology, Medical University of Lublin, Lublin, Poland, [2] Department of Biopharmacy, Medical University of Lublin, Lublin, Poland

INTRODUCTION: Angiotensin converting enzyme inhibitors (ACE-Is) and angiotensin II type 1 receptor blockers (ARBs) possess antihypertensive and neuroprotective properties. Kynurenic acid (KYNA), a broad spectrum glutamate antagonist is produced from L-kynurenine by kynurenine aminotransferases (KAT), mainly by KAT I and KAT II isoforms.

AIM(S): The goal of our study was to analyze the effect of ACE-Is and ARBs on KYNA synthesis and KATs activity in rat brain cortex *in vitro*.

METHOD(S): The influence of ACE-Is (lisinopril, perindopril and ramipril) and ARBs (irbesartan, losartan, telmisartan) on KYNA production and KATs activity was examined. Slices and homogenates of rat brain cortex were incubated for 2 hours in the presence of L-kynurenine and examined ACE-I or ARB. KYNA was separated by HPLC and quantified fluorometrically. Moreover, the molecular docking of ARBs to KAT II and the analysis of KAT II coding genes expression in human and rat cerebral cortex were performed.

RESULTS: ACE-Is differently influenced KYNA synthesis and KATs activity in rat brain cortex *in vitro*. All examined ARBs decreased KYNA production and both KAT I and KAT II activity in rat brain cortex *in vitro*. Molecular docking showed that analyzed ARBs can bind to an active site of KAT II.

CONCLUSIONS: Our study indicates that ACE-Is and ARBs may change KYNA production in rat brain cortex *in vitro*. ARBs inhibitory effect on KATs activity and KYNA synthesis suggest that ARBs may have beneficial effect in schizophrenia or dementia.

FINANCIAL SUPPORT: This study was supported by National Science Centre (NCN) grant PRELUDIUM 4, No UMO-2012/07/N/NZ4/02088.

P2.50. CROSS-TALK BETWEEN STIM PROTEINS AND IONOTROPIC RECEPTORS IN NEURONS

Joanna Gruszczynska-Biegala, Maria Sladowska, Filip Maciag, Jacek Kuźnicki

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

INTRODUCTION: Depletion of Ca^{2+} in endoplasmic reticulum (ER) is sensed by STIM proteins, which then activate influx of these ions via Orai channels from the extracellular environment. This process is called the Store-Operated Calcium Entry (SOCE), and its role in non-excitatory cells is to refill ER with Ca^{2+}. In neurons, however, SOCE is also used for signaling and seems to involve several types of channels.

AIM(S): The aim of this study is to determine which ionotropic receptors (IR) react with STIM proteins and are involved in calcium influx during SOCE.

METHOD(S): In cultured cortical neurons we recorded single-cell Ca^{2+} levels using Fura-2AM. SOCE was measured after depletion of intracellular Ca^{2+} stores by thapsigargin and subsequent incubation of cells in 2 mM Ca^{2+} media. To investigate the involvement of IR in SOCE, we applied antagonists of these receptors such as CNQX and NBQX (AMPAR), MK-801, memantine and D-AP5 (NMDAR). To determine the effects of SOCE on IR agonist-induced Ca^{2+} entry we applied SOCE inhibitors (ML9, SKF96365). The co-immunoprecipitation assay was used to detect the interaction between STIMs and IR. Electrophysiology experiments are also being performed.

RESULTS: We report that ML-9 and SKF reduced AMPA- and NMDA-induced Ca^{2+} influx. In addition, SOCE was decreased by CNQX, NBQX, D-AP5, memantine, but no significant effect was observed in the presence of MK801. Physical association of endogenous STIM proteins with endogenous GluA1 or GluA2 subunits of AMPAR and N2B subunit of NMDAR we detected by immunoprecipitation. Application of SOCE inhibitor, SKF96365, had no effect on the amplitude of AMPA-mediated miniature excitatory postsynaptic currents (mEPSCs).

CONCLUSIONS: Ca^{2+} measurements using specific inhibitors and immunoprecipitation experiments indicate that STIM proteins might participate in neuronal signaling by the interaction with ionotropic receptors such as AMPA and NMDA.

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P2.51. TNF-α CHANGES EXPRESSION OF KIDNEY TYPE GLUTAMINASE IN RAT CORTICAL ASTROCYTES IN STAT3 DEPENDENT MANNER

Krzysztof Milewski, Małgorzata Bogacińska-Karaś, Magdalena Zielińska

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

INTRODUCTION: Pro-inflammatory cytokines are strongly involved in the pathogenesis of hepatic encephalopathy (HE) associated with both acute and chronic liver failure. Elevation of IL-1, Il-6 and especially TNF-α in blood strictly correlates with severity of observed symptoms in HE patients. On the other hand also a significant relationship is observed between TNF- α and received the most attention -ammonia theory in the pathogenesis of HE. In astrocytes, excess of formed glutamine (Gln) as a product of ammonia detoxification is further metabolized by mitochondrial glutaminase (GLS), which is thought to play a central role in the generation of excitotoxic glutamate (Glu). So far reported data suggest that TNF-α increased the expression of neuronal and microglial GLS.

AIM(S): The aim of this study was to analyze the expression of gls1 in rat astrocytes subjected to two key pathogenic factors operating in HE: ammonia and pro-inflammatory factors: TNF-α, IL-1, Il-6.

METHOD(S): In the following study we measured the gene expression and protein level of gls1 isoformes (KGA

and GAC) in rat cortical astrocytes treated by 48 h with TNF-α (50 ng/ml), 10n IL-6 (10 ng/ml), IL-1 (10 ng/ml) and/or 5 mM ammonium chloride (ammonia). We also used inhibitors of nuclear factor kappa B – Bay-11, STAT-1 – flutarabine and STAT-3 (STA-21).

RESULTS: TNF-α specifically increased the expression of KGA, but not GAC, at both mRNA and protein level. Used interleukines nor ammonia did not affect gls1 expression. Observed phenomena were significantly abolished by STAT-3 inhibition.

CONCLUSIONS: Our findings demonstrate that TNF-α -induced up-regulation of GLS in cultured astrocytes, which may contribute to the response to an inflammatory stimulus associated with HE. The response appears to be triggered at the level of transcription of the KGA isoform and may involve a signaling pathway associated with STAT3. Our findings underscore the potential role of inflammatory cytokines in the derangement of astrocytic Glu metabolism associated with HE.

FINANCIAL SUPPORT: Supported by the National Science Centre (NCN) grant no. 2014/15/N/NZ5/03634.

P2.52. TRPC3 AND TRPV1 CHANNELS ARE ENGAGED IN MEMORY CONSOLIDATION IN PASSIVE AVOIDANCE TASK IN ONE-DAY OLD CHICKS

Andrzej Krolik, Apolonia Ziembowicz, Elżbieta Salińska

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

INTRODUCTION: The stimulation of both ionotropic and metabotropic glutamate receptors and influx of calcium ions (Ca2+) into neurons is a crucial step in intracellular cascade of memory formation. Recently the existence of additional mechanism involved in intracellular Ca2+ increase, triggered by internal signals like increase of Ca2+ within the cell and activation of G protein coupled receptors, was demonstrated. This mechanism involves transient receptor potential (TRP) channels.

AIM(S): The aim of our study was to investigate the participation of TRP channels in intracellular mechanisms engaged in memory consolidation

METHOD(S): The model of passive avoidance task in one day old chicks was used. Chicks were injected with non-specific TRP channels antagonist SKF96365 or with specific antibodies against chosen TRP channels: TRPC3, TRPC5, TRPV1 and TRPV3. The injections were made into brain region connected with memory formation – intermediate medial mesopallium – immediately after training (time choice based on previous experiments) and animals were tested 2 h and 24 h after training.

RESULTS: The injection of SKF96365 and anti-TRPC3 antibody immediately after training resulted in strong task amnesia when tested 2 h and 24 h later. Injection of anti-TRPV1 and anti-TRPV3 antibody resulted in less manifested amnesia, whereas application of anti-TRPC5 antibody did not produce significant amnesia.

CONCLUSIONS: Our results show that inhibition of TRPC3, TRPV1 and TRPV3 channels significantly disturbed memory of the task indicating on an involvement of these channels in memory formation.

FINANCIAL SUPPORT: G1136-2014/15/B/NZ4/04487.

P2.53. A RELATIONSHIP BETWEEN GLUTATHIONE DEFICIENCY DURING THE EARLY POSTNATAL LIFE AND SCHIZOPHRENIA-LIKE BEHAVIOR IN ADULTHOOD OF SPRAGUE-DAWLEY RATS

Elżbieta Lorenc-Koci[1], Magdalena Górny[2], Kinga Kamińska[1], Zofia Rogóż[1]

[1] *Institute of Pharmacology Polish Academy of Sciences, Krakow, Poland,*
[2] *Jagiellonian University Medical College, Krakow, Poland*

INTRODUCTION: A growing body of evidence implicates glutathione (GSH) deficiency and dysregulation of redox state in the pathophysiology of schizophrenia.

AIM(S): The aim of the present study was to examine the effects of buthionine-S,R-sulfoximine (BSO)-mediated inhibition of glutamate cysteine ligase (GCL; an enzyme contributing to the GSH synthesis), during the early postnatal life on the GSH and cysteine (Cys) levels in the liver and kidney of 16-days old Sprague-Dawley rats and on the rat performance in the behavioral tests that were evaluated in the adulthood.

METHOD(S): Male Sprague-Dawley pups between the postnatal day p5 and p16 were treated sc with the selective inhibitor of GCL, compound BSO (3.8 mmol/kg) and the dopamine reuptake inhibitor GBR 12909 (5 mg/kg every second day), alone or in combination. Biochemical parameters, i.e. GSH and cysteine (Cys) levels were determined in the rat liver and kidney 4h after the last doses of the drugs. Other groups of rats treated with BSO and GBR 12909 were examined during adulthood in the social interaction test (p42, p60, p90), novel object recognition test (p43, p61, p91) and in the open field test (p44, p63, p93).

RESULTS: BSO alone or especially with GBR 12909 significantly decreased GSH level measured 4h after its last doses both in the liver and kidney. However, Cys concentration was distinctly reduced only in the kidney. Treatment with BSO and GBR 12909 induced deficits in both parameters of the social interaction test (number and time of interactions) assessed in the defined time points in adulthood. Such treatment also evoked a decrease in

the memory retention in the novel object recognition test and an increase in exploratory activity in the open field test.

CONCLUSIONS: Our results suggest that the inhibition of GSH synthesis and the dopamine reuptake during the early postnatal development induces in rats long-term behavioral changes that correspond to negative and positive symptoms of schizophrenia.

FINANCIAL SUPPORT: Partially supported by statutory funds of the Institute of Pharmacology Polish Academy of Sciences, Krakow, Poland.

P2.54. MATERNAL HIGH FAT DIET PROVOKES DEPRESSIVE-LIKE BEHAVIOR IN OFFSPRING RATS AND VULNERABILITY TO COCAINE ADDICTION

Dawid Gawliński, Małgorzata Frankowska, Klaudia Kluska, Małgorzata Filip

Laboratory of Drug Addiction Pharmacology, Institute of Pharmacology Polish Academy of Sciences, Krakow, Poland

INTRODUCTION: The epidemiological and animal studies underline that composition of maternal diet (e.g., high fat diet, HFD) is associated with an increased susceptibility to several health problems in the adult offspring, including risk of a cluster of behavioral disorders such as depression.

AIM(S): The aim of this study was to investigate the effect of maternal HFD on the offspring phenotype assessed in locomotor activity study, forced swim test (FST) and cocaine self-administration.

METHOD(S): Wistar rat dams were maintained ad libitum either on a special HFD (35% crude fat) or standard rodent chow during gestation and lactation (21 days). At postnatal day (PND) 27, the male and female litters were separated from their mother and were switched to a standard diet. Locomotor activity was recorded for 120 min at PND 28 and 63. At PND 34 the FST was performed. Moreover, at PND 63 male rats were introduced into two different cocaine self-administration protocols: 1) stable cocaine dose (0.5 mg/kg/inf.) with increasing schedule of reinforcement fixed ratio (FR) 1 to 5 or 2) increasing cocaine doses (0.25–1 mg/kg/inf.) and stable FR1. Following 10 extinction days, male rats were tested for the response reinstatement of drug-seeking behavior induced by cocaine-priming dose (10 mg/kg, i.p.) and cue (tone+light).

RESULTS: HFD group exhibited depressive-like phenotype, characterized by increased immobility time and decreased climbing in both tested time points what was not affected by the changes in basal locomotor activity. The HFD rats displayed an attenuation of the cocaine-associated lever presses and cocaine intake during the acquisition/maintenance of cocaine self-administration and lower response to relapse behavior evoked by cocaine priming or the drug-associated conditioned stimulus.

CONCLUSIONS: Our data suggest the influence of maternal HFD on the offspring's behavior, however underlying molecular mechanism requires further investigations.

FINANCIAL SUPPORT: Supported by research grant UMO-2016/21/B/N24/00203 from the NCN (Poland).

P2.55. ANTIDEPRESSANTS ENHANCED THE ANTIPSYCHOTIC-LIKE EFFECT OF ARIPIPRAZOLE IN THE TESTS USED FOR EVALUAION OF SOME POSITIVE AND COGNITIVE SYMPTOMS OF SCHIZOPHRENIA IN MICE

Zofia Rogóż[1,2], Elżbieta Lorenc-Koci[2]

[1] *The Podhale State Higher Vocational School, Nowy Targ, Poland,*

[2] *Institute of Pharmacology Polish Academy of Sciences, Krakow, Poland*

INTRODUCTION: Schizophrenia is a devastating psychiatric disorder that impairs mental and social functioning and affects approximately 1% of the world's population. It is known that in contrast to pharmacotherapy with typical antipsychotics, atypical antipsychotics alleviate not only the positive symptoms of schizophrenia but also the cognitive ones, however those effects are small and the mechanisms of this action are still unknown. A few clinical reports have suggested that antidepressants (ADs) are able to augment the activity of atypical antipsychotics.

AIM(S): In the present study, we aimed to evaluate the effect of ADs escitalopram (ESC) or mirtazapine (MIR) and aripiprazole (ARI, an atypical antipsychotic drug) given separately or jointly, on the MK-801-induced positive and cognitive symptoms of schizophrenia in mice.

METHOD(S): The experiments were conducted on male Albino Swiss mice (25–27 g). ADs and ARI were given 30 min before MK-801 injection. Locomotor hyperactivity induced by MK-801 (0.3 mg/kg) was measured for 30 min, starting 30 min after MK-801 administration. In the novel object recognition test, MK-801 (0.2 mg/kg) was given 30 min before the first introductory session. Memory retention was evaluated for 5 min, starting 90 min after the introductory session.

RESULTS: ARI (0.3 mg/kg) decreased locomotor hyperactivity induced by MK-801 (0.3 mg/kg). Co-treatment with an inactive dose of ARI (0.01 mg/kg) and ESC (5 or 10 mg/kg) or MIR (2.5 and 5 mg/kg) inhibited the effect of MK-801. Moreover, MK-801 (0.2 mg/kg) decreased the memory retention. ARI (0.3 mg/kg) reversed that effect. Co-treatment with an inactive dose of ARI (0.03 mg/kg) and ESC (5 and 10 mg/kg) or MIR (2.5 and 5 mg/kg) abolished the deficit of object recognition memory induced by MK-801.

CONCLUSIONS: The obtained results suggest that ADs may enhance the antipsychotic-like effect of ARI in the ani-

mal tests used for evaluation of some positive and cognitive symptoms of schizophrenia.

FINANCIAL SUPPORT: This study was financially supported by statutory funds of the Institute of Pharmacology Polish Academy of Sciences, Krakow, Poland.

P2.56. IMPACT OF ENVIRONMENTAL ENRICHMENT ON ANXIETY AND LEARNING IN THE RAT MODEL OF EPILEPSY INDUCED BY ELECTRICAL STIMULATION OF THE AMYGDALA

Aleksandra Stępniak, Karolina Nizińska, Katarzyna Łukasiuk, Kinga Szydłowska

Department of Molecular and Cellular Neurobiology, Institute of Experimental Biology, Warsaw, Poland.

INTRODUCTION: Environment plays influential role in the development of many brain disorders, however its role in modulation of epilepsy phenotype has not been studied in details.

AIM(S): The aim of this study was to investigate whether environmental enrichment impacts anxiety and learning in experimental model of epilepsy.

METHOD(S): Male Sprague-Dawley rats were allocated to either environmentally enriched (EE; n=13) or standard housing condition (SH; n=13). Epilepsy was induced by SE (Status epilepticus) evoked by electrical stimulation of the amygdala (25 min, 100 ms train of 1 ms, 60 Hz bipolar pulses, 400 μA, every 0.5 s). Following tests were conducted to assess the behavior of animals: behavioral hyperexcitability, open field, new object recognition, elevated plus maze, social interactions, and Morris water maze. Blood was withdrawn on days 7 and 29 after stimulation and on the day of perfusion to assess cortisol levels.

RESULTS: Environmental enrichment significantly reduced anxiety levels. We observed, reduced mobility in the open field test (EE=2.6±3.3; SH=179.1±107.8 s; p<0,0001), decrease in total distance travelled in the social interactions test (EE=1210.2±574.4; SH=2937.0±711.3 cm; p<0,0001) or decreased touch-response test in the behavioral hyperexcitability test (score: EE=2.1±1.1; SH=3.6±1.8; p<0,0001). SH animals showed impaired spatial memory and learning compared to EE animals. Rats from EE group spent more time near platform (EE=25.5±4.7; SH=21.5±5.0 s; p<0,05) in Morris Water Maze test. Moreover, SH rats showed hyperactivity and thigmotaxis. Blood analysis demonstrated that SH rats had significantly higher level of cortisol (EE=0.4±0.7; SH=1.1±0.6 μg/dl; p<0,01) compared to EE rats.

CONCLUSIONS: The present study indicates that environmental enrichment had beneficial effects on anxiety and learning and memory, which may be caused by lower stress hormone levels.

FINANCIAL SUPPORT: This work was supported by the FP7-HEALTH project 602102 (EPITARGET) and Polish Ministry of Science and Education grant W19/7. PR/2014.

P2.57. BEHAVIORAL CHARACTERISTIC AS A BIOMARKER OF DEVELOPMENT AND PHENOTYPE OF EPILEPSY IN THE TEMPORAL LOBE EPILEPSY INDUCED BY ELECTRICAL STIMULATION OF THE AMYGDALA IN RATS

Karolina Nizińska, Kinga Szydłowska, Katarzyna Łukasiuk

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

INTRODUCTION: Non-invasive biomarkers of epileptogenesis and epilepsy in experimental models would allow fast and easy evaluation of disease development and impact of treatments aiming at modification of disease development or progress.

AIM(S): The project was conducted to test the hypothesis that there are differences in the results of behavioral tests depending on 1) the time to develop epilepsy, measured by the length of the latency to the first spontaneous seizure, and 2) the intensity of epilepsy, measured by the number of seizures.

METHOD(S): Animals were kept in enriched environment and were subjected to the handling procedure. Epilepsy was induced by status epilepticus using electrical stimulation of the amygdala (25 min, 100-ms train of 1-ms biphasic square-wave pluses, 400 μA, 60 Hz, delivered every 0.5 s). EEG was used to classify rats into animals groups with short (<20 days, n=7) and long (>20 days, n=8) latency and into groups with low (SE_L: 62±64.5, n=7) and high (SE_H: 456±185, n=8) seizure number. We applied following behavioral tests: behavioral hyperexcitability, open field, novel object exploration, elevated plus maze and Morris water maze.

RESULTS: We observed decreased learning abilities of epileptic animals compared to control (SE: 14.07±9.5, CTRL: 28.36±21.5, p<0.001) in 4 training day of Morris water maze. We observed no difference in the stress level, activity and learning between groups with short and long latency in all behavioral tests and between groups with low and high seizures number. However, we observed increased stress level, measured by number of imputes to the closed arms, in group with high number of seizures in elevated plus maze carried out in 26 week compared to animals with low seizures number (SE_H: 35±13.6, SE_L: 15.14±8.9, p<0.01).

CONCLUSIONS: Increased stress level in elevated plus maze might become convenient biomarker of epilepsy phenotype.

FINANCIAL SUPPORT: This work was supported by the FP7-HEALTH project 602102 (EPITARGET) and Polish Ministry of Science and Education grant W19/7.PR/2014.

P2.58. DIFFERENCES IN BEHAVIOR AND BRAIN PLASTICITY OF LABORATORY MICE DIVERGENTLY SELECTED FOR HIGH AND LOW METABOLIC RATE

Anna Goncerzewicz[1], Tomasz Górkiewicz[1], Jakub Dzik[1], Ewelina Knapska[1], Marek Konarzewski[2]

[1] Department of Neurophysiology, Institute of Experimental Biology, Warsaw, Poland, [2] University of Białystok, Institute of Biology, Białystok, Poland

INTRODUCTION: Encephalization, i.e., the amount of brain mass related to an animal's total body mass is increased in homeotherms comparing to ectotherms. A larger brain offers behavioral advantages, but also means energy expenditures that are an order of magnitude higher than in ectotherms. What are the benefits of larger, energetically expensive brains that allowed them to evolve? The 'Expensive Tissue' hypothesis links evolution of enlarged brain to increased cognitive skills that improve foraging performance.

AIM(S): We aim at testing the ET hypothesis using two lines of mice bred for low and high basal metabolic rate (BMR).

METHOD(S): Low (L-BMR) and high (H-BMR) lines of Swiss Webster mice were selected reaching 40% between-line difference in BMR. The weight of their internal organs, including the brain, their cognitive abilities and neural plasticity were measured. The cognitive abilities of the mice were tested in IntelliCage system which allows for assessment of learning of individual mice living in the social group. To test the brain plasticity-related differences between the lines we used a model of neural plasticity, CA3-CA1 hippocampal long-term potentiation (LTP).

RESULTS: The weight of internal organs differed, with H-BMR mice organs being heavier. We found increased exploration of the environment in H-BMR mice, which also showed higher motivation to obtain the reward and faster learning of the reward's position. In line with learning results, we found that LTP was induced at significantly higher level in H-BMR mice, suggesting higher neural plasticity in this line.

CONCLUSIONS: Together, our results suggest that higher BMR is associated with more efficient exploration of the environment, higher motivation and better place learning. Increased cognitive skills, probably mediated by enhanced neuroplasticity, allow for improved foraging performance, in line with the ET hypothesis.

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P2.59. EMOTIONAL CONTAGION IN FVB MICE

Tomasz Nikolaev, Ksenia Meyza

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

INTRODUCTION: Lately, we can observe a steady increase in the number of autism spectrum disorder (ASD) diagnoses every year. ASD is often connected with empathy impairments, a phenomenon thought to be limited only to humans, yet the simplest form of empathy, emotional contagion, can be examined also in rodents.

AIM(S): In our previous study we have shown that C57BL/6 mice are capable of transferring the emotional information, but Keum and colleagues (2015) reported that strain is a major factor influencing empathic fear responses. They showed that FVB mice are not capable of Observational Fear Learning. In the current study, we tested if exposure to a stressed Demonstrator in the safe environment of the home cage (providing remote information about the stressor) elicits emotional contagion in the FVB mice.

METHOD(S): Between subject transfer of emotional information paradigm was used, in which mice are housed in pairs for three weeks, one of them labelled as a Demonstrator, and the other as an Observer. In the test session, the Demonstrator is subjected to a series of aversive stimuli outside of the home cage, while the Observer remains there undisturbed. Then, the Demonstrator is returned to the home cage, where it can freely interact with the Observer. The following interactions are recorded and then analysed using BehaView software.

RESULTS: Here we report that FVB mice display emotional contagion in the between-subject transfer of emotional information paradigm. Observers exposed to stressed Demonstrators show increased social behaviours towards the Demonstrators. Results are similar to these obtained on C57BL/6 mice, albeit the behavioural response was slightly different.

CONCLUSIONS: Confirmation of empathic abilities in the FVB strain allows for further studies of genetic influence on empathic responsivity. FVB mice lacking Fmr1 gene, encoding the fragile X mental retardation protein, are considered a strong animal model for autism spectrum disorder (ASD).

FINANCIAL SUPPORT: This study was supported by NCN Sonata BIS grant no. 2015/18/E/NZ4/00600 to Ksenia Meyza.

P2.60. EVALUATION OF THE EFFECTS OF NEW ANTIEPILEPTIC DRUG, AMPA RECEPTOR ANTAGONIST – PERAMPANEL ON NEUROTOXICITY OF DEXAMETHASONE – BEHAVIORAL STUDY

Zofia Danilczuk, Hanna Koszel

Department of Experimental and Clinical Pharmacology, Medical University of Lublin, Lublin, Poland

INTRODUCTION: The long-term treatment with glucocorticoids (GCs) and other preparations, e.g. dexamethasone (DEX – a synthetic GCs receptor agonist) or prolonged stress and elevated levels of endogenous corticosteroids are frequently associated with psychosis as well as with cognitive deficits, such as the impairment of memory and learning. GCs potentiate stress or ischemia-induced accumulation of excitatory amino acids (EAA) in the extracellular space of hippocampus. The antagonism of glutamate receptors may potentially play a role in the safe therapy with glucocorticoids.

AIM(S): The purpose of this study was to investigate the effect of perampanel (Fycompa – a new antiepileptic drug, non-competitive α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) receptor antagonist) in animal model of dexamethasone-induced neurotoxicity.

METHOD(S): The experiments were carried out on male Albino Swiss mice (25-30 g). Perampanel, at the dose of 4, 6, 10 or 12 mg/kg/day, was administered ip, 30 min before DEX (16 mg/kg/day, ip), for 14 days. The long-term memory acquisition, the motor performance, the locomotor activity, as well as the body weight and the lethality were evaluated after 14 days of drugs administration.

RESULTS: The results of the study have shown that DEX evoked deterioration of all parameters in behavioral tests. At the doses of 4 or 6 mg/kg/day, perampanel administered in mice treated with DEX, had no significant effect on the parameters of the motor performance and the locomotor activity tests, although it degraded the long-term memory acquisition. However, when administered at the dose of 10 mg/kg/day (but not at the dose of 12 mg/kg/day), perampanel slightly improved acquisition of memory, but it had no impact on other behavioral parameters in mice subjected to DEX for 14 days.

CONCLUSIONS: The above findings suggest that treatment with perampanel at higher doses (10 mg/kg/day) could prevent the neurotoxic effects induced by DEX, but further study needs to be carried out to explain this effect.

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P2.61. SCOPOLAMINE INCREASES PERSEVERATION IN MICE SUBJECTED TO THE DETOUR TEST

Grzegorz R. Juszczak[1], Rafał Stryjek[2]

[1] Department of Animal Behavior, Institute of Genetics and Animal Breeding Polish Academy of Sciences, Jastrzebiec, Poland, [2] Institute of Psychology Polish Academy of Sciences, Warsaw, Poland

INTRODUCTION: Perseveration is defined as a tendency to respond persistently to a particular stimulus, even after the response has become inappropriate. Increased perseveration is associated with aging in humans and leads to impaired ability to cope with problems. Aging is also associated with progressing dysfunction of cholinergic system, which is associated with various cognitive processes.

AIM(S): Therefore, we tested an effect of anticholinergic drug on the level of perseveration in mice subjected to the water escape detour test.

METHOD(S): Animals were first trained to use a visible platform and next were injected with saline or scopolamine (1 or 5 mg/kg, i.p.). Thirty minutes after the injection mice were tested with transparent barrier separating them from the escape platform.

RESULTS: During initial detour trials all mice displayed behavior characterized by persistent returning to the place located in front of the transparent barrier. In control animals, this behavior was gradually replaced by swimming around the barrier. In contrast, mice treated with scopolamine continued to repeat the pattern of swimming toward the central part of transparent barrier separating them from the platform. This perseverative behavior was dose dependent and was significant at the end of detour training.

CONCLUSIONS: These data show that dysfunction of cholinergic system leads to increased perseveration.

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P2.62. NS398 (COX-2 INHIBITOR) POTENTIATES THE ANTIDEPRESSANT-LIKE EFFECTS OF MTEP (MGLUR5 ANTAGONIST): INVOLVEMENT OF 5-HT SYSTEM

Katarzyna Stachowicz[1], Magdalena Sowa-Kućma[1], Tomasz Lenda[2], Paulina Misztak[1,3], Patrycja Pańczyszyn-Trzewik[1], Gabriel Nowak[1,3], Andrzej Pilc[1]

[1] Department of Neurobiology, Institute of Pharmacology Polish Academy of Sciences, Krakow, Poland, [2] Department of Neuropsychopharmacology, Institute of Pharmacology Polish Academy of Sciences, Krakow, Poland, [3] Department of Pharmacobiology Krakow, Jagiellonian University Medical College, Krakow, Poland

INTRODUCTION: Compounds acting via metabotropic glutamate (mGlu) receptors, exhibit antidepressant activity. Moreover, development of depressive-like behavior in mice is accompanied by elevated level of prostaglandins. In our earlier study, augmentation of antidepressant-like effects of MTEP by NS398 was presented.

AIM(S): The aim of this research was to verify the involvement of serotoninergic(5-HT) system in this interaction.

METHOD(S): C57Bl/6J male mice were co-treated with MTEP(1 mg/kg; i.p.) and NS398 (3 mg/kg; i.p.) for 7 or 14 days. 24 h after last injection, hippocampus(Hp) and

prefrontal cortex(pFC) were collected. The tissue 5-HT and 5-HIAA levels were measured using P680 HPLC system(Dionex, Sunnyvale, CA, USA). Data presented as the mean ±SEM, using one-way ANOVA(n=7–9, Newman-Keuls test), p<0.05 was considered as statistically significant.

RESULTS: 14 days co-administration of MTEP with NS398 resulted in statistical significant increase (by 48%) of 5-HT level in pFC [p<0.0001], comparing to the 5-HT level observed after 7 days of administration. Similar picture (increase by 47%) was observed in pFC in 5-HIAA level [p<0.01]. Quite different picture of changes was observed in Hp, as 5-HT level was significantly decreased (by 36%) between 7 and 14 days of co-administration of both MTEP with NS398 [p<0.01]. 5-HT:5-HIAA turnover in pFC and Hp, comparing 7 *vs.* 14 days of co-treatment MTEP with NS398, showed no significant changes[ns].

CONCLUSIONS: Our findings revealed that, chronic co-treatment MTEP with NS398 affects 5-HT level in examined brain structures of mice. Observed effect was without changes in 5-HT:5-HIAA turnover, between 7 and 14 days of administration, in pFC and HP of C57Bl/6J mice. This kind of modulation of 5-HT system maybe interesting in the field of psychopharmacology. Further studies are necessary to determine the precise mechanism of interaction of mentioned pathways.

FINANCIAL SUPPORT: Study supported by grant UMO-2014/13/D/NZ7/00292.

P2.63. ELECTRICAL ACTIVITY OF THE BASOLATERAL AMYGDALA AND NUCLEUS ACCUMBENS DURING CLASSICAL FEAR CONDITIONING AFTER MK801 AND CLOZAPINE ADMINISTRATION

Karolina Rejniak, Maciej Olszewski, Stefan Kasicki

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

INTRODUCTION: Dysfunction of the brain glutamatergic system is supposed to be one of fundamental causes underlying the schizophrenia. Administration of NMDA receptor antagonist (eg. MK801) leads to occurrence of various symptoms of that disease, inter alia, disruption of cognitive functions.

AIM(S): The main aim of our study was to verify whether emotional/cognitive processes disturbed by local administration of MK801 into the basolateral amygdala (BLA) were associated with changes in electrical activity of two limbic structures: BLA and nucleus accumbens (NAc).

METHOD(S): Experiments were done according to a classical fear conditioning paradigm. During consecutive sessions we recorded Local Field Potentials (LFPs) and videotaped the behavior of animals. Three experiments were performed: A) control (no pharmacological intervention), B) with MK801 infusion into BLA and C) with MK801 infusion and clozapine injection (i.p.). In experiments B and C animals were divided into two groups: drugs were administered 1) before acquisition sessions or 2) before first three extinction sessions. Power spectra were calculated for frequency bands: delta, theta, beta 1, beta 2, low gamma and high gamma during freezing and non-freezing behaviour for two time segments: during and after conditioned stimulus presentation.

RESULTS: Analysis of data revealed that in experiment (BII) MK801 infusion disrupted extinction process. In experiment (CII) clozapine did not influence the delayed extinction of conditioned response. Most differences between band powers were found for theta, beta 1 and 2 bands, with increase in power in consecutive sessions in both experiments (A, B). In experiment C the greatest power was observed in sessions with clozapine.

CONCLUSIONS: The same behavior (freezing) was associated by various patterns of differences between power spectra. The increasing power of theta, beta 1 and 2 bands in experiments A and B may indicate increasing level of attention.

FINANCIAL SUPPORT: Funding: the National Center of Science (Poland) DEC-2011/03/B/NZ4/03768.

P2.64. THE IMPACT OF SELF-ESTEEM ON NEURAL BASIS OF SELF-REFLECTION: EVENT-RELATED POTENTIAL (ERP) STUDY

Maria Nowicka[1], Michał Wójcik[1,2], Ilona Kotlewska[1], Michał Bola[1], Anna Nowicka[1]

[1] Department of Neurophysiology, Nencki Institute of Experimental Biology, Warsaw, Poland, [2] SWPS University of Social Sciences and Humanities, Faculty in Wroclaw, Wroclaw, Poland

INTRODUCTION: Throughout the history of philosophy and psychology, the question of the self has been one of the most salient problems. In the contemporary social and cognitive neuroscience, there is an ongoing debate on brain correlates associated with processing of the self. Electrophysiological studies in this field consistently reveal higher amplitudes of early and late ERP components associated with processing of self-related information *vs.* information referring to other people (i.e. a self-preference effect). The question, however, arises whether this preference is influenced by personal characteristics of participants.

AIM(S): The aim of the current ERP study was to investigate whether the self-esteem modulates neural correlates of self-related information processing. Specifically, we were interested whether the level of self-esteem would exert significant influence on amplitudes of late positive ERP component appearing approximately 600 ms after stimuli onset (i.e. LPC) recorded in the self *vs.* other reflection task.

METHOD(S): Low self-esteem (LSE) and high (HSE) self-esteem groups of participants (20 in each group)

were tasked with judging whether a given adjective was suitable to describe/characterize one's own person (the self), a close-other and famous person. Yes/no responses were given by pressing one of two buttons on a response pad. EEG was continuously recorded from 62 scalp sites using a 128-channel amplifier (Quick Amp), and BrainVisionRecorder® software.

RESULTS: In general, the process of reflection on the self was associated with significantly higher mean LPC amplitudes in comparison to control conditions (targets of reflection: a close-other and a famous person), indicating a strong self-preference effect. Importantly, this effect was much stronger in the HSE group.

CONCLUSIONS: Explicit self-esteem modulates electrophysiological correlates of self-related information processing.

FINANCIAL SUPPORT: This work was funded by the National Science Centre [2015/19/B/HS6/01258].

P2.65. ANALYSIS OF MORPHINE-INDUCED GENE EXPRESSION IN THE MOUSE HIPPOCAMPUS

Justyna Barut, Urszula Skupio, Łucja Marut, Magdalena Tertil, Sławomir Gołda, Ryszard Przewłocki

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

INTRODUCTION: Exposure to drugs of abuse initiates molecular alterations in the central nervous system that lead to an increased overall tenderness to addiction with subsequent drug exposures. These drug-induced alterations employ changes in gene expression, which may underlie the behavioral aberrancy that define a state of addiction.

AIM(S): To identify the specific transcriptional alterations in different stages of morphine addiction in the hippocampus (Hip), brain region which play a role in the acquisition and extinction of memories associated with drug seeking behavior.

METHOD(S): C57BL/6J male mice were injected twice daily for 3 weeks with morphine (increasing doses, 20-100 mg/kg i.p.). Animals were observed for spontaneous signs of withdrawal and behavior was measured in first and third week of abstinence. Morphine induced gene expression in the Hip was analyzed, using the qPCR technology.

RESULTS: 24 h after chronic treatment we have observed spontaneous withdrawal syndrome and the peak of corticosterone levels in blood. Morphine-abstinent mice exhibited a variety of depression-like behaviors and cognitive deficits. Analyzes of Hip transcriptional responses to morphine indicated that most of genes regulated by morphine injection are GR-dependent, with a number of them being astrocyte-specific (Gjb6, Plin4, Slc1a3, Gfap, Gja1). Analyzed genes clustered into few co-expressed groups, i.a. GR-dependent (Fkbp5, Tsc22d3, Zbtb16, Plin4) and activity-dependent (Fos, Fosl2) both upregulated in single and chronic exposure to morphine. Interestingly, 3-weeks abstinent mice didn't exhibit any significant difference in transcription, but single dose of morphine (relapse) trigger sensitization of expression of some interesting genes (camk1g, Fosl2, Arc).

CONCLUSIONS: Our results reveal that morphine induces drug-specific transcriptional signatures in the Hip. Stress systems in Hip may modify the reward circuit through GR-dependent molecular pathways and this mechanism may be a fundamental for addiction therapy research.

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P2.66. THE ROLE OF THE CENTROMEDIAL SUBDIVISION OF THE HUMAN AMYGDALA IN SIGNALING SURPRISE DURING REINFORCEMENT LEARNING

Emilia Kolada[1], Krzysztof Bielski[1], Pamela Sobczak[2], Marek Wypych[3], Artur Marchewka[3], Iwona Szatkowska[1]

[1] Laboratory of Psychophysiology, Nencki Institute of Experimental Biology Polish Academy of Sciences, Warsaw, Poland, [2] Institute of Psychology, The Faculty of Christian Philosophy, Cardinal Stefan Wyszynski University, Warsaw, Poland, [3] Laboratory of Brain Imaging, Nencki Institute of Experimental Biology Polish Academy of Sciences, Warsaw, Poland

INTRODUCTION: Surprise (i.e., errors in outcome prediction) drives reinforcement learning. Animal studies point to the critical role of the centromedial subdivision (CMA) of the amygdala in signaling reinforcement-related surprise. Little is known as to the role of the CMA in this process in humans, as the problem of functional organization of the human amygdala was undertaken by only few studies.

AIM(S): The goal of this study was to investigate the role of the human amygdala subdivisions in signalling surprise during reinforcement learning.

METHOD(S): We used a Pavlovian conditioning task. The task was composed of two trial types: aversive and neutral, in which small amounts of aversive (0.4 M NaCl) and neutral (25 mM KCl and 2.5 mM NaHCO3) gustatory stimuli (liquids) were provided to participants. In the beginning of each trial, participants were presented with two visual cues: one associated with a high probability (on 70% of occasions) and the other with a low probability (on 30% of occasions) of obtaining gustatory stimulus. After a few seconds, one of visual cues disappeared and the subjects' task was to indicate whether the remaining cue forerun liquid delivery. In an fMRI study, we compared the amygdala activity during: 1) reinforcement-related surprise (unexpected *vs.* expected delivery of aversive stimuli), and 2) surprise

not related with the reinforcement (unexpected *vs.* expected delivery of neutral stimuli).

RESULTS: We found the right CMA activation during reinforcement-related surprise, whereas surprise not related with the reinforcement did not activate any amygdala subdivision.

CONCLUSIONS: The results showed selective involvement of the CMA in signalling reinforcement-related surprise in humans. Moreover, they prove that investigation of the amygdala at the level of distinct subdivisions using fMRI in humans should be valuable direction for future studies. This work was supported by a grant from the Polish National Science Centre based on decision number DEC-2014/15/B/HS6/03658.

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P2.67. TWO DIFFERENT CLASSES OF 22-KHZ ULTRASONIC VOCALIZATIONS DURING SEXUAL INTERACTIONS, CORRELATION WITH RAT BEHAVIORAL STATE

Michał Biały[1], Martyna Podobińska[1], Jarosław J. Barski[2], Wiktor Bogacki-Rychlik[1], Elżbieta Sułkowska-Sajdel[1]

[1] Department of Experimental and Clinical Physiology, Laboratory of Centre for Preclinical Research, Medical University of Warsaw, Warsaw, Poland,
[2] Department for Experimental Medicine, Medical University of Silesia, Katowice, Poland

INTRODUCTION: Low frequency ultrasonic vocalizations, commonly referred as 22-kHz, occur in different situations including both aversive as well as positive emotional states in rats. Our previous research suggests that following ejaculation, long, lasting low frequency vocalizations reflect a positive emotional state – relaxation after high arousal state. It has been found that there is another type of 22-kHz vocalizations, which occurs during sexual interactions – at the time of first separated-noncontact test.

AIM(S): The aim of our study was to investigate the spectral analysis of low frequency ultrasonic vocalizations when male is prevented from a direct contact with estrous female and compare such calls to postejaculatory vocalizations.

METHOD(S): The subjects were Long–Evans males (N=9) and female (N=9) rats 4.5 months old at the start of the experiment. The clear Plexiglas test chamber (50×25×30 cm) was used for copulatory and barrier noncontact (NC) tests. For NC tests, the chamber was bisected. Behaviors were recorded using Noldus Ethovision system simultaneously, on the same computer, with ultrasounds recording using Metris Sonotract system and analyzed manually.

RESULTS: We found that about half of males prevented from a direct contact with an estrous female vocalized at frequency below 30-kHz. These vocalizations usually initiated with short signal at higher 45-kHz frequency and then transited to lower, below 30-kHz frequency. Usually these vocalizations coexist with sniffing of a hole or exploration activities – rearing or sniffing cage. The high activity state observed in separated males during low frequency vocalization is in contrast to male immobility observed during postejaculatory period when male emit extremely flat 22-kHz ultrasonic vocalizations.

CONCLUSIONS: A pattern of low frequency vocalizations in rats during frustration differs significantly from that observed during relaxation state after ejaculation and correlates to the different type of behavioral activity.

FINANCIAL SUPPORT: This work was supported by the Medical University of Warsaw (grant 1MA/N/170 and mini Grant for Wiktor Bogacki-Rychlik.

P2.68. EFFECTS OF ETHANOL, Δ(9)-TETRAHYDROCANNABINOL, OR THEIR COMBINATION ON THE SHORT-TIME SPATIAL MEMORY AND COGNITIVE FLEXIBILITY IN ADOLESCENT AND ADULT MALE RATS IN THE BARNES MAZE TEST

Ewa Gibula-Bruzda, Ewa Kedzierska, Marta Marszałek-Grabska, Joanna Filarowska, Jolanta H. Kotlinska, Sylwia Talarek, Joanna Listos

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

INTRODUCTION: During the past few years it has become clear that both ethanol and cannabinoids affect adolescents and adults differently. For example, both Δ9-tetrahydrocannabinol (THC) and ethanol disrupt spatial learning more potently in adolescents than adults. The fact that both ethanol and THC impair learning and learning-related hippocampal function more potently in adolescents than in adults is obviously of great importance. But, particularly among teens, ethanol and marijuana are often used in combination. Although there have been a number of studies of the combined effects of ethanol and THC, developmental comparisons are conspicuously absent. This is of particular concern given that early misuse of these substances has been linked to an increased likelihood of later substance use and related behavioral problems.

AIM(S): The aim of our study was to reveal whether ethanol and/or THC induced greater spatial memory impairment in adolescent than adult male rats using the Barnes maze test when compared to these drugs alone.

METHOD(S): Adolescent rats (postnatal day 30) were submitted into four groups, each of them received injection of: 0.9% NaCl, 1.5 g/kg ethanol, 1.0 mg/kg THC or 1.5 g/kg ethanol+1.0 mg/kg THC on 30, 33, 36 and 39 postnatal day. 24 hours after last injection, half of treated animals from each group were tested in the Barnes

maze test. The remaining animals were tested at on postnatal day 70.

RESULTS: The results show that there was an age effect on spatial memory in Barnes maze test after the ethanol+THC challenge. Specifically, adolescent animals showed more significant deficits in the short-time spatial memory (probe trial) or cognitive flexibility (reversal learning) than adults.

CONCLUSIONS: These novel findings clearly indicate that further understanding of this age–drug interaction is crucial to elucidating the influence that adolescent ethanol+THC use may have on repeated drug use and abuse later in life.

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P2.69. THE IMPACT OF TREATMENT AND PHYSICAL TRAINING ON MOTOR SKILLS AND LEARNING IN THE CHRONIC MPTP-TREATED-MOUSE MODEL OF PARKINSONISM

Radoslaw Folcik[1], Ewelina Palasz[1], Anna Gasiorowska[1,2], Wiktor Niewiadomski[2], Grazyna Niewiadomska[1]

[1] Laboratory of Preclinical Studies in Neurodegenerative Diseases, Nencki Institute of Experimental Biology Polish Academy of Sciences, Warsaw, Poland, [2] Department of Applied Physiology, Mossakowski Medical Research Centre Polish Academy of Sciences, Warsaw, Poland

INTRODUCTION: Motor impairment is fundamental feature of Parkinson's disease (PD). There are several reports on the beneficial effect of physical training on the PD symptoms reduction, however the mechanisms underlying this improvement are not known The selection of an appropriate animal model is crucial to demonstrate positive effect of physical effort on motor function, because even despite extensive loss of dopaminergic neurons the detection and quantification of motor impairment is difficult.

AIM(S): The purpose of the study was to examine the efficacy of physical training in reversing the expected motor impairment in chronic MPTP mice model of parkinsonism.

METHOD(S): C57BL/6 mice were treated for five weeks with 12,5 mg/kg MPTP in combination with 250 mg/kg probenecid. Mice were subdivided into: 1) control sedentary; 2) control trained (10 weeks); 3) MPTP sedentary (non-exercised with PD); 4) early trained MPTP (10 weeks: before, during, and after the induction of PD), and 5) late trained MPTP (10 weeks, started after the induction of PD). To assess motor performance rotarod, open field and inverted horizontal grid tests were performed before MPTP treatment, after the completion of intoxication and when the training was finished.

RESULTS: MPTP did not impair motor function. We observed improvement of motor performance in rotarod and open field test in MPTP, early trained mice. Some enhancement of motor skills in rotarod test was observed also for MPTP non-exercised mice. In horizontal grid test the only parameter significantly influenced by MPTP treatment was the total number of touches and we did not observe the impact of physical training on the reduction of this parameter.

CONCLUSIONS: We did not observe the impact of MPTP and physical training alone on motor performance in mice model of parkinsonism. However, there has been a certain improvement in some of the motor parameters in both groups of MPTP treated mice, which performed physical training.

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P2.70. PREFRONTAL CORTEX SINGLE UNIT ACTIVITY DURING EXTINCTION OF CONDITIONED FEAR

Weronika Szadzinska[1], Daniel Jercog[2], Ewelina Knapska[1], Cyril Herry[2]

[1] Nencki Institute of Experimental Biology Polish Academy of Sciences, Warsaw, Poland, [2] Neurocentre Magendie, INSERM U1215, Bordeaux, France Université de Bordeaux, Bordeaux, France

INTRODUCTION: In fear extinction, a model of exposure-based therapy, a tone-conditioned stimulus previously paired with a footshock is presented repeatedly in the absence of the aversive outcome, resulting in fear reduction. It is well documented that the dorsal (including the prelimbic area, PL) and the ventral (the infralimbic area, IL) regions of the medial prefrontal cortex (mPFC) differentially regulate conditioned fear responses. The PL stimulation increases, whereas the IL stimulation decreases fear expression. In addition, the IL is critical for consolidation of extinction memories. Little is known, however, how different parts of the prefrontal cortex interact with each other and how their activity changes in the course of the extinction training.

AIM(S): We aimed at detailed description of neural activity changes within the PL and IL during fear extinction.

METHOD(S): We performed single unit recordings simultaneously in the PL and IL, during the habituation and two sessions of fear extinction in freely moving mice. Recorded neurons were divided into excitatory pyramidal cells and interneurons. The neuronal responses to the conditioned stimuli were analyzed and the activity of significantly responsive neurons was averaged.

RESULTS: We found patterns of the single unit activity that differed along the dorso-ventral axis of the mPFC. The averaged IL activity followed the behavior during the extinction session, while the PL pronouncedly showed inhibition during the fear expression at the beginning of the extinction session but not at later times.

CONCLUSIONS: The results suggest different involvement of the PL and IL during the acquisition of extinction association, with the PL being mostly active during the high fear state, while the IL being active throughout the entire extinction session. The analysis of the mPFC activity suggests that its ventral region is mostly involved in the change of the association value from the perceiving conditioned stimuli as a threatful to the point of perceiving it as safe.

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P2.71. MANIPULATION OF CREB RELATED GENE EXPRESSION IN NEURONS INFLUENCES ADULT NEUROGENESIS AND MORRIS WATER MAZE STRATEGIES

Artur Janusz[1], Agata Klejman[1], Anna Kiryk-Jaśkiewicz[1], Adam Gorlewicz[1], Katarzyna Biegańska[1], Paulina Jedynak[2], Leszek Kaczmarek[1], Witold Konopka[1]

[1] Nencki Institute of Experimental Biology Polish Academy of Sciences, Warsaw, Poland, [2] Department of Biomedical and Neuromotor Sciences, University of Bologna, Bologna, Italy

INTRODUCTION: Active reorganization of extracellular matrix in the brain allows for growth of neuronal dendrites and axons which guarantees successful incorporation of new born neurons into neuronal network during adult neurogenesis in the hippocampus. Activity of surrounding neurons may affect adult neurogenesis.

AIM(S): In order to test whether manipulation of CREB dependent gene expression in neurons and hence their activity will influence adult neurogenesis we have developed the Syn-Flag-ICER II transgenic rat line. The ICER (Inducible cAMP Early Repressor) is effective endogenous repressor of CREB/CREM/ATF transcription factors family.

METHOD(S): BrdU labeling to asses a level of adult neurogenesis in the hippocampus qPCR for changes in transcription of CREB/CREM and related genes gelatin zymography to measure MMP9 activity Morris Water Maze spatial learning tests Patch Clamp

RESULTS: ICER II overexpressing rats showed diminished hippocampal neurogenesis. We have observed a reduced number of mature BrdU positive cells in granular zone of hippocampus of transgenic rats, in comparison to control group. We have observed also that neurons of dentate gyrus demonstrate increased excitability. Paradoxically, we have detected increased levels of mRNA for CREB or CREM factors. Also CREB dependent miR-132 expression was upregulated in transgenic rats, which regulates expression of MMP-9 – extracellular matrix metalloproteinase. We have found the decreased activity of MMP9 in ICER overexpressing rats. Morris Water Maze tests didn't show overall differences in rats learning and memory capabilities, however male ICER rats chose more often imprecise strategies to find hidden platform than control males.

CONCLUSIONS: Obtained results indicate that CREB dependent gene expression in neurons regulates a set of genes e.g. miR-132 that may in turn regulate translation of proteins involved in remodeling of extracellular matrix and affect adult neurogenesis, what changes discrete aspects of animal cognitive behavior.

P2.72. EFFECT OF SPRINT INTERVAL EXERCISES ON PERIPHERAL LEVEL OF NEUROPROTECTIVE PROTEINS AND HUMAN COGNITIVE ABILITIES

Sylwester Kujach[1,3], Robert Olek[2], Rafał Kubiak[1], Maciej Chroboczek[1], Irena Majkutewicz[3], Radosław Laskowski[1]

[1] Faculty of Physical Education, Department of Physiology, Gdansk University of Physical Education and Sport, Gdansk, Poland, [2] Faculty of Kinesiology and Rehabilitation, Department of Bioenergetics and Nutrition, Gdansk University of Physical Education and Sport, Gdansk, Poland, [3] Department of Animal and Human Physiology, University of Gdansk, Faculty of Biology, Gdansk, Poland

INTRODUCTION: Overwhelming amount of scientific evidence suggest that regular physical activity is effective in the prevention of chronic diseases. Most adults do not meet even the minimum guidelines for regular physical efforts. Numerous studies have shown that the main reason for the absence of regular exercise is lack of time. Interval training is a potent, time-efficient therapeutic intervention that is more effective than continuous exercise. Certain evidence indicates that Sprint Interval Exercises (SIE) may result in rapid phenotypic changes in both the cardiovascular system and in the skeletal muscle. However, studies evaluating the effects of SIE on cognitive functions are limited.

AIM(S): The aim of this study was to investigate whether SIE affects the peripheral level of selected neuroprotective proteins (BDNF, IGF-1, VEGF) as well as modulate human cognition.

METHOD(S): The study involved Gdansk University of Physical Education and Sport students. Subjects were divided in two groups: Sprint Interval Exercise and Control group. To evaluate serum concentrations of BDNF, IGF-1 and VEGF the ELISA method was applied. Cognitive testing include: Stroop interference test, Adult Intelligence Scale Wechsler WAIS-R – Repeat numbers subtest and Trail Making Test part A and B.

RESULTS: SIE contributed to a significant, transient increase of three neurotrophins BDNF, IGF-1, VEGF. Obtained results of cognitive functions indicated that acute SIE significantly improved selected human cognitive abilities performance.

CONCLUSIONS: The results indicate that proposed SIE can induce positive changes in neuroprotective proteins improving human cognition. Given the growing number of

people with cognitive impairment around the world, there is a recognized need for further research, explaining how specific exercise can influence the improvement of these functions. Such studies can be viewed as another important step towards the development of non-pharmacological therapeutic strategies in improving human cognitive function.

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P2.73. REPEATED INTRINSIC SIGNAL OPTICAL IMAGING (ISOI) FROM THE MICE PRIMARY SOMATOSENSORY CORTEX

Anna Posłuszny[1], Renata Zakrzewska[1], Małgorzata Kossut[1,2]

[1] Department of Molecular and Cellular Neurobiology, Nencki Institute of Experimental Biology Polish Academy of Sciences, Warsaw, Poland,
[2] Department of Psychology, University of Social Sciences and Humanities (SWPS), Warsaw, Poland

INTRODUCTION: A noninvasive method of mapping cortical activation in the same animal before and after behavioral training for assessing experience-dependent cortical plasticity is of great value. Previously, we mapped changes in cortical representation of vibrissae involved in behavioral training with 2-deoxyglucose (2-DG). We wanted to replicate the 2-DG results with ISOI.

AIM(S): The aim of the experiments was to find a protocol of vibrissae stimulation for the ISOI from the barrel cortex, which could provide constant signal in temporally separated recordings and enable visualization of plastic changes.

METHOD(S): Two subsequent ISOI were performed with 6 days interval. Conditioning consisted of 3 daily sessions of 40 trials of manual stimulation of row B vibrissae on one side of the snout coupled with a mild tail shock. 24 hours after the last conditioning session the second ISOI was performed. Two protocols of vibrissae stimulation during ISOI were tested. In the first protocol 5-ms deflections of B1 vibrissa were applied for 1 s with frequency of 5 Hz (this protocol was used in earlier studies of experience-dependent cortical plasticity seen after whisker deprivation). In the second protocol 5-ms deflections of B1 vibrissa were applied for 6 s with frequency of 10 Hz. Additionally, after second ISOI a 2-DG mapping was performed in order to confirm plasticity in the somatosensory cortex. Using 2-DG method a comparison was performed between left (involved during conditioning) and right (not involved) vibrissae representation.

RESULTS: The paired ISOI using the first protocol (1 s/5 Hz) of vibrissae stimulation revealed no change of the B1 vibrissa representation after conditioning, a result that is inconsistent with 2-DG maps. ISOI in which the second vibrissae stimulation protocol (6 s/10 Hz) was applied showed expansion of cortical representation of vibrissa stimulated during conditioning.

CONCLUSIONS: Different protocols are suitable for different types of experience-dependent cortical plasticity.

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P2.74. ULTRASONIC AND CARDIOVASCULAR RESPONSES TO ULTRASONIC SOUNDS AND VOCALIZATIONS

Robert K. Filipkowski, Rafał Polowy, Monika Małż, Olga Gawryś, Krzysztof H. Olszyński

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

INTRODUCTION: Ultrasonic vocalizations (USV) of adult rats are thought to be means of social communication and are divided into two categories, 55 kHz and 22 kHz, signaling, respectively, appetitive and aversive states. These states are also known for changes in the heart rate (HR). The autonomous system has a role in both USV shaping and HR response. A common signaling pathway via the vagus nerve connects the laryngeal muscles and the heart. This causes an overlap in HR parameters and many behavioral reactions (the polivagal theory).

AIM(S): The aim of this study was to investigate USV emissions and HR changes in rats evoked by USV presentation.

METHOD(S): Ten weeks old Wistar male rats were housed in pairs or separately for 4 weeks. Telemetry transmitters for HR acquisition were implanted in the peritoneum with the detector placed in the aorta. Rats were exposed to five 10-s sets of sounds (counterbalanced): 55-kHz, 22-kHz USV (both natural, collected from other animals), 55-kHz, 22-kHz tones, 22-kHz uninterrupted tone (all three artificial, software-generated) separated with 5 min silence intervals. HR and USV emitted were registered.

RESULTS: Rats of both groups responded with USV mostly and more often to 55-kHz tones and vocalizations than during presentation of 22-kHz sounds. The responses were, almost exclusively, within 55 kHz range. In general, single-housed rats vocalized more often than pair-housed ones but the effect was not strong. Also, HR changes were more pronounced following presentation of natural USV. During 55-kHz USV presentation, there was an elevation of HR in single-housed animals, while in pair-housed animals, this elevation was preceded by a transient HR drop. During 22-kHz USV presentation, a decrease in HR in both groups was observed, although it was more clear in paired-housed rats.

CONCLUSIONS: Social context may have an impact on HR levels and USV emissions in response to ultrasounds presentations. However, it does not seem to influence the distinction between artificial and natural USV.

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P2.75. TO HAVE OR NOT TO HAVE A BREAK. NEURAL CONNECTIVITY PATTERNS DURING TASK-TO-REST TRANSITIONS IN HEALTHY SUBJECTS AND PATIENTS WITH OBSESSIVE-COMPULSIVE DISORDER (OCD)

Bartosz Zurowski[1], Andreas Kordon[1], Fritz Hohagen[1], Sarah J. Buetof[2]

[1] *Center for Integrative Psychiatry, University of Luebeck, Germany,*
[2] *Department of Neurophysiology and Pathophysiology, University Medical Center Hamburg-Eppendorf, Germany*

INTRODUCTION: The investigation of resting state connectivity typically provides a static view on neuronal integration that does not capture dynamic transitions between rest and task-related network connectivity underlying real life functioning – particularly in neuropsychiatric disorders characterized by cognitive inflexibility and difficulties to disengage from cognitive processing.

AIM(S): The aim of the present study was to characterize network connectivity differences between OCD patients and healthy controls. Using fcMRI, we explored whether engagement in a perceptual decision task has differential impact on subsequent topographical and timely aspects of neural connectivity.

METHOD(S): fMRI data from 18 OCD and 14 healthy subjects was obtained using a 3T Siemens scanner before (fixation1), during, and after (fixation2) a luminance-based perceptual decision task. For analysis SPM8 and CONN Toolbox were used. To test for differences in spontaneous and task-induced fcMRI study groups were contrasted in fixation1 and the decision block, respectively. In a second analysis, fixation1 was compared to fixation2 to determine the impact of the decision task. In addition, the fixation2 period was segmented into three time windows which were then contrasted separately with fixation1 and with each other.

RESULTS: Healthy controls had stronger connections between the orbitofrontal cortex and superior and medial frontal gyri during fixation1 and during the decision task. The segmentation of fixation 2 into 3 time windows revealed that only the 1. window differed significantly from fixation1 in controls. In contrast, the fcMRI discrepancy in OCD was significant across the entire period of fixation2.

CONCLUSIONS: Healthy subjects and OCD patients show differences between pre-task and post-task resting state network connectivity, which is prolonged in OCD patients, however. The investigation of dynamic rather than static network connectivity is strongly encouraged both in healthy and neuropsychiatric populations.

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P2.76. C-FOS DRIVEN MODULATION OF APPETITIVE BEHAVIOR IN THE CENTRAL NUCELUS OF AMYGDALA

Tomasz Lebitko[1,2], Hubert Madej[1], Tomasz Jaworski[1], Anna Beroun[1], Kacper Kondrakiewicz[2], Ewelina Knapska[2], Leszek Kaczmarek[1]

[1] *Department of Molecular and Cellular Neurobiology, Nencki Institute of Experimental Biology Polish Academy of Sciences, Warsaw, Poland,*
[2] *Department of Neurophysiology, Nencki Institute of Experimental Biology Polish Academy of Sciences, Warsaw, Poland*

INTRODUCTION: The central nucleus of the amygdala (CeA) has primarily been studied as a structure involved in processing of aversive behaviors, whereas its role in appetitively-motivated learning is less understood. The published data show involvement of the basolateral amygdala (BL), which sends projections to the CeA, in encoding sensory-specific features during appetitive learning. In contrast, the CeA was implicated in modulation of incentive motivation to pursue an associated external reward. Previously we reported that after appetitive, but not aversive learning, expression of c-Fos, a protein closely linked to synaptic plasticity, is significantly increased in the CeA.

AIM(S): We aimed at testing the hypothesis that appetitive learning depends on c-Fos expressing neural circuits in the CeA.

METHOD(S): We first compared c-Fos expression pattern in the amygdala following place preference and place avoidance training and examined inputs from the BL on the activated CeA neurons. Then we used c-fos-driven targeting of channelrhodopsin and trained the animals in an operant conditioning task, in which they learned to associate auditory stimulus with food reinforcement. To further test the role of c-fos-expressing neurons in appetitive learning, we locally blocked behaviorally-induced c-fos expression using a shRNA.

RESULTS: The c-Fos expression in the CeA was significantly higher following place preference than place avoidance training, with over 90% of the c-Fos positive cells receiving projections from the BL. Optogenetic stimulation of the neurons increased bar-pressing responses but only when the conditioned stimulus was present. Blocking c-fos expression resulted in impairment of appetitively but not aversively motivated discrimination learning and decreased motivation to seek reward.

CONCLUSIONS: The results reveal that c-fos expression in the CeA neurons is necessary for appetitively but not aversively motivated learning, modulating of incentive motivation but not reward consumption.

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P2.77. NEURAL CIRCUITS IN THE CENTRAL AMYGDALA MEDIATE SOCIALLY TRANSFERRED FEAR

Karolina Rokosz, Adam Hamed, Kacper Kondrakiewicz, Ewelina Knapska

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

INTRODUCTION: Social complex behavior, like empathy, emerge over phylogeny from various precursors. One of the simplest is emotional contagion, i.e. sharing emotional states between individuals. Receiving signals of a potential danger may increase chances of survival, thus emotional contagion plays an important role in learning about external environment. The phenomenon is well described at the behavioral level, but the neural circuits necessary for sharing emotions are unknown. We designed a rat model of fear contagion and showed that a brief social interaction with a fearful cage mate promotes risk assessment behavior and activates the central amygdala (CeA) in an otherwise naïve rat.

AIM(S): The purpose of this project was to elucidate the role of the CeA circuits involved in socially shared fear.

METHOD(S): To investigate the functional outputs of the activated CeA neurons, we mapped neural circuits downstream from the CeA combining anterograde tracing with an imaging of activated neurons in transgenic "Venus" rats. To test the function of CeA "social fear" neurons, we optogenetically stimulated or inhibited subpopulation of CeA neurons activated by social interaction using c-fos-driven targeting of channelrhodopsin and halorhodopsin.

RESULTS: In rats that socially shared fear of their partners, we observed strong activation of structures involved in anxiety and motor functions. Most of the activated cells received projections from the CeA. Optogenetic activation of the "social fear" neurons in a social context led to behavioral pattern resembling the one observed during social interaction with a fearful partner. Activation of neurons in non-social context induced exploration and risk assessment behavior (active fear). Inhibition of them had the opposite effects.

CONCLUSIONS: The results suggest that the CeA neurons involved in socially transferred fear mediate active fear responses and anxiety-related behaviors in both social and non-social conditions.

P2.78. BODY ENERGY STATUS IN MOTIVATIONAL STUDIES

Bartosz Zglinicki, Witold Konopka

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

INTRODUCTION: Motivational experiments allow for assessing the inner drive of the subject to carry out a particular task. For this purpose the Skinner box is widely used. It allows the animal to acquire a conditional response, which can be used to measure the level of motivation. Adipose tissue is involved in maintaining body energy status. It secrets hormone leptin, which signals the brain about current fat storage. Thus, low level of plasma leptin elicits hunger and other responses of the organism related e.g. fertility.

AIM(S): In the study we sought to establish a link between plasma leptin level and the level of motivation demonstrated by the animal.

METHOD(S): Mice were trained in Skinner box to successfully acquire a conditional response. In addition all animals were calorie restricted (CR) prior to training, to improve performance. Afterwards mice were divided into 3 groups: group fasted for 6 h, group fasted for 24 h and group with CR. Last group of 6 was used as a control, where animals were fed ad libitum. Test was performed on all mice, in which overall lever press was counted during 20 minutes in the Skinner box. Second panel of animals was used to measure the actual plasma leptin level. Animals were sacrificed, blood plasma collected and leptin measurement was carried out using ELISA method.

RESULTS: The highest level of motivation was observed in CR only group. Similar result was observed in a group that was fasted for 24 h. Surprisingly the group fasted for 6h only did not show significant differences from control animals. Motivation and plasma leptin level are negatively correlated in our study, with CR animals showing the lowest concentration of hormone.

CONCLUSIONS: We demonstrated that calorie restriction is the most crucial factor in controlling motivation level of the animal, compared to short lasting starvation periods. This might be explained by strongly decreasing level of leptin hormone in the animal, when CR is introduced.

P2.79. UP-REGULATION OF MTOR SIGNALING PATHWAY IN FOREBRAIN NEURONS LEADS TO IMPROVEMENT OF COGNITIVE FUNCTIONS IN PTEN KNOCK-OUT MICE

Natalia Chwin, Anna Kiryk, Bartosz Zglinicki, Witold Konopka

Nencki Institute of Experimental Biology Polish Academy of Science, Neurobiology Centre, Warsaw, Poland

INTRODUCTION: PI3K-Akt-mTOR pathway plays important role in long-term synaptic plasticity and mem-

ory formation. In our studies describing the Dicer1 gene knock-out model we have shown that improved learning and memory phenotype in these mice was related to up-regulation of the PI3K-Akt-mTOR pathway. In order to prove that the PI3K-Akt-mTOR pathway is crucial for observed phenotype we have generated Pten gene knock-out model in which this up-regulation is achieved by elevation of intracellular levels of phosphatidylinositide 3 in neurons. To examine cognitive functions in Pten model, we have used the IntelliCage system.

AIM(S): The purpose of our research was to define the cognitive functions in Pten/CaMKCreERT2 mouse model.

METHOD(S): Mouse model: We used Pten/CaMKCreERT2 mouse model in which up-regulation of mTOR activity was generated by mutation of Pten gene restricted to forebrain neurons induced by tamoxifen. Behavioral tests: Following induction of the mutation, Pten mutant mice and controls were tested in learning and memory test in the IntelliCage: a fully automated system for the behavioral assessment of mice that live in social groups. We measured spatial learning with appetitive reinforcement in place preference learning task.

RESULTS: Life span: Long-term of PI3K-Akt-mTOR pathway activity in the brain led to increased mutant mice mortality. Pten mutants were able to survive no longer than 13 weeks after the induction of mutation. Place learning in IntelliCage: In the IntelliCage, housing and testing occur in the same cage that is a familiar environment, thus creating a unique opportunity to test behavior for a long-term period in relatively low-stress conditions without handling or social isolation. Using the system, we were able to discover the better performance of Pten mutants in place learning task. Moreover, improved memory was detectable even 24 hours before the death.

CONCLUSIONS: Pten/CaMKCreERT2 mice show enhanced memory of rewarded place compare to control littermates. In mutants show decreased life span.

P2.80. NEW INSIGHT INTO KERNEL CURRENT SOURCE DENSITY RECONSTRUCTION

Marta Kowalska, Michał B. Czerwiński, Chaitanya H. Chintaluri, Daniel K. Wójcik

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

INTRODUCTION: The low-frequency part of extracellular potential, called the Local Field Potential (LFP), is a useful measure of neural systems activity. However, a direct interpretation of LFP is problematic as it is not a local measure – each electrode may record activity observed millimeters away from source. Estimation of current source density (CSD), the volume density of net transmembrane currents, has become a convenient way to deal with this problem.

AIM(S): The aim of the study is to investigate the properties of kCSD method to develop a procedure which will facilitate optimal usage of the presented method in complicated experimental scenarios, for complex measurement setups etc.

METHOD(S): In the study we use kCSD method which estimates the sources in a family of allowed CSD distributions of dimensionality larger than the number of measurements. To identify the parameters of the method leading to optimal source estimation, a statistical technique of cross-validation is used. We perform this study using Python programming language with several types of known (model) reference data and different electrodes setups. We employ singular value decomposition (SVD) method to study the internal properties of kCSD reconstruction.

RESULTS: To examine the influence of the measurement setup on the reconstruction capability of the kCSD method we performed simulated study. We present error maps of CSD estimation which give us valuable insight into kCSD reconstruction quality.

CONCLUSIONS: The quality of CSD estimation significantly depends on the measurement setup. This study enables the researchers to check how much they can trust the obtained kCSD reconstruction for a given setup and specific collection of recordings.

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P2.81. KERNEL CURRENT SOURCE DENSITY AND CONNECTIVITY ESTIMATION METHODS FOR THE ANALYSIS OF LOCAL FIELD POTENTIALS

Władysław Średniawa[1,2], Jan Jabłonka[2], Maria Sadowska[2], Piotr Bębas[2], Daniel K. Wójcik[1]

[1] *Department of Neurophysiology, Nencki Institute of Experimental Biology Polish Academy of Sciences, Warsaw, Poland,* [2] *Instytut Zoologii, Wydział Biologii, University of Warsaw, Warsaw, Poland*

INTRODUCTION: Extracellular potentials, such as the Local Field Potentials (LFPs), are routinely measured in numerous electrophysiological experiments. LFP can carry valuable information about the electric properties of the tissue, however analysis of the recorded signal is usually a complex task. Apart from basic preparation, such as bandpass filtering and artifact removal, many other analytic methods have been proposed for the LFP study. Here we discuss methods for estimation of electric sources and sinks in brain tissue (Current Source Density, CSD) and methods to estimate connectivity in small networks, and their utility in analysis of cortical recordings in rats.

AIM(S): Comparison of the effective connectivity and the structure of sinks and sources in cortical columns during whisker stimulations.

METHOD(S): Analytic methods: kernel Current Source Density and Modular Connectivity Factorization (MCF) applied to LFP recordings and simulated data from cortical column. Experimental methods: Simultaneous multielectrode *in vivo* recordings from both hemispheres of the rats brain.

RESULTS: Preliminary studies show different distribution of the current sources in contralateral to ipsilateral hemisphere during whisker stimulation in rats. Comparison of the hemispheres from deprived rats shows an extension of the whisker representation in the barrel cortex receptive field.

CONCLUSIONS: KCSD method shows significant differences in current sources localization in contralateral to ipsilateral hemisphere. Modular Connectivity Factorization method applied to LFP recordings from simulated data separates cortical column layers into interpretable modules. Physiological interpretation of the results needs further validation on the cortical column model.

FINANCIAL SUPPORT: Instytut Biologii Doświadczalnej im. Marcelego Nenckiego Polska Akademia Nauk, Warsaw, Poland, Uniwersytet Warszawski, Warsaw, Poland.

P2.82. GLOBAL FIELD POWER: TIME-FREQUENCY DOMAIN APPROACH

Joanna Duda[1], Kamil Imbir[2], Jarosław Żygierewicz[1]

[1] Division of Biomedical Physics, Institute of Experimental Physics, University of Warsaw, Warsaw, Poland, [2] Faculty of Psychology, University of Warsaw, Warsaw, Poland

INTRODUCTION: Classic approach of Global Field Power (GFP) is defined as a function of time, where the GFP maxima are used to determine the latencies of evoked potential components. The GFP corresponds to the spatial standard deviation, and it quantifies the amount of activity at each time point in the field (which consider signal from all electrodes simultaneously). Its results in a reference-independent descriptor of the potential field. This study shows an extension of this method to time-frequency domain.

AIM(S): The main aim of this study was to determine time windows for chosen frequency bands suitable for further statistical analysis. The criterion for selection of the time windows should rely on stability of topography of band power.

METHOD(S): The frequency enabled GFP relies on estimation of instantaneous band-power and evaluation of its spatial standard deviation. Estimator of band power was obtained by band-pass filtering, followed by rectification of the signal and smoothing of the output. Analogously to the classical GFP, high value of obtained measure indicates spatial variability of power distribution. Changes in its level indicate time-periods of stable topography of power.

RESULTS: The method was applied to data recorded in a psychological experiment related to cognitive processing under different emotional conditions elicited by words. Data were analysed in four frequency bands, specific to EEG signal: delta, theta, alpha and beta. Analysis of time course of the GFP in these bands allowed to indicate time periods of stable topography of power.

CONCLUSIONS: This study shows that frequency enabled GFP may be used as a simple and intuitive tool for selecting time-frequency regions of interest, suitable for further statistical analysis.

P2.83. NEW MEASURES OF SOCIAL INTERACTIONS FOR ECOHAB CAGE

Jan Mąka[1,2], Alicja Puścian[1], Ewelina Knapska[1], Daniel K. Wójcik[1]

[1] Department of Neurophysiology, Nencki Institute of Experimental Biology Polish Academy of Sciences, Warsaw, Poland, [2] Faculty of Physics, University of Warsaw, Warsaw, Poland

INTRODUCTION: Eco-HAB is an open source system for automated measurements and analysis of social preferences and in-cohort sociability in mice. It requires no contact between a human experimenter and tested animals. In Eco-HAB, group-housed mice live in a spacious, four-compartment, resembling natural burrows. It allows an assessment of the tendency of mice to voluntarily spend time together in ethologically relevant mouse group sizes. Results are obtained faster, with less manpower needed and without confounding factors.

AIM(S): The aim of the of this study is to develop measures for the EcoHAB system, which could well describe social relations in a group of mice. We test the proposed measures in experiments with four FX WT and three FX KO groups. We expected that FX KO mice would have disturbed social skills comparing to FX WT.

METHOD(S): We developed a dedicated workflow for analysis of social interactions based on analysis of the decision patterns. For each pair of mice, one mouse is a leader, the other is a follower. After the leader changes the room, the follower's reaction in a 3-second window is analysed. If the follower acts on the leader's movement and follows it, the pattern is classified as "following"; otherwise it is "evasion". Lack of follower's reaction is ignored. The numbers of interactions for each pair and distribution of the patterns were obtained. To characterize the relations between the mice in selected time windows we used binomial model. We also studied changes of these relation in time and their distribution in mice groups.

RESULTS: Our study proved that FX KO mice have significantly less interactions within a pair than FX WT.

What's more, FX WT are following each other more often and the character of interaction is more stable.

CONCLUSIONS: EcoHAB is a good environment for conducting advanced analysis of mice social interactions. Proposed measures show significant difference between WT and KO group and are a promising tool to study social interactions.

P2.84. TO LFP OR TO CSD? – THAT IS THE QUESTION

Michał B. Czerwiński, Marta Kowalska, Chaitanya H. Chintaluri, Daniel K. Wójcik

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

INTRODUCTION: Current Source Density (CSD) is spatially smoothed transmembrane activity of the neurons. Local Field Potential (LFP) is the electric potential generated by ionic currents in the neural tissue and it is directly related to the CSD. LFP is relatively easily accessible experimentally but due to the long range of electric field, it is difficult to interpret. CSD needs to be calculated but it reflects the local neural activity directly. Since the currents directly reflect neuronal computations, using electric potentials (LFP's) to infer performed computation may lead to misinterpretations.

AIM(S): 1) Discuss challenges arising in multielectrode LFP and CSD analysis, in particular case where direct analysis of LFPs can lead to misinterpretation. 2) Show that the kernel Current Source Density reconstruction method (kCSD) gives a better insight into the underlying phenomena than the observed potentials, and to show the limits and uncertainties yielded by the method. 3) Present the kCSD-python toolbox for CSD analysis.

METHOD(S): All of the modeling and computations was done in Python and tested on model data. Potentials were calculated using assumed physical models of tissue. The kCSD library in Python is available at: https://github.com/Neuroinflab/kCSD-python.

RESULTS: We show examples where the LFP's can 'hide' more complex underlying CSD patterns and how the kCSD can reconstruct those sources, depending on the number and configuration of recording electrodes.

CONCLUSIONS: Complex CSD patterns studied at the resolution of few electrodes can be obscured if only direct LFP analysis is used. The kCSD method can help to recover them. The main limiting factors are the number of recording electrodes and their configuration.

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P2.85. MODELING ARTIFACT IN THE LIVING NEURAL NETWORKS STIMULATING SYSTEM

Karolina Kołodziej, Paweł Hottowy

Department of Particle Interactions and Detection Techniques, AGH University of Science and Technology, Krakow, Poland

INTRODUCTION: Electrical stimulation of neurons results in large artifacts that makes recording of the stimulated activity difficult. In particular, detection of low-latency spikes from directly activated neurons at the stimulating electrodes remains virtually impossible.

AIM(S): We tested a new idea for artifact reduction, based on an optimized correction pulse (CP) applied to the stimulating electrode instantly after the stimulation pulse (SP). While being generated, the CP would induce the exact opposite of the artifact initiated by the SP. The signal distortion would be minimized, allowing for detection of the neuronal response during application of the CP.

METHOD(S): Based on realistic model of the electrode impedance we estimated the shape of the stimulation artifact and calculated the optimal shape of the CP. We analyzed the hardware limitations of the stimulation circuit and its impact on the reduced artifact amplitude. We also considered the effects of the impedance model inaccuracy, as the real-life experiment will be based on impedance measurements with limited precision. We analyzed the artifact level at the output of the recording amplifier to take into account its filtering properties.

RESULTS: We analyzed the artifact reduction procedure for typical symmetric biphasic SP and impedance model for 5-micron platinium electrode. Even 2 microampere pulse without the CP generated the artifact that saturated the amplifier for at least 300 microseconds following the SP. Simulations confirmed that the optimal CP reduced the artifact almost completely. More importantly, even when electrode impedance was given with 5% error, the artifact was reduced more than 10 times for the first 300 microseconds after the SP, compared with the SP without correction.

CONCLUSIONS: Numerical simulations suggest that our method will allow for reliable spikes recording even on the stimulating electrode. The experimental validation will take advantage of the novel stimulation/recording system currently being developed in our laboratory.

FINANCIAL SUPPORT: This work was supported by Polish National Science Centre grant DEC-2013/10/M/NZ4/00268.

P2.86. COMPARISON OF ORIGINAL AND MODIFIED CANOLTY METHOD FOR ASSESSMENT OF PHASE TO AMPLITUDE CROSS-FREQUENCY COUPLING

Gabriela Bernatowicz, Jarosław Żygierewicz

Division of Biomedical Physics, Institute of Experimental Physics, Faculty of Physics, University of Warsaw, Warsaw, Poland

INTRODUCTION: Recent studies indicate that coupling between low- and high-frequency brain rhythms provides valuable information on cognitive processing in humans. One of the approaches to study these couplings is based on analysis of time-frequency representations of the ECoG or EEG signals aligned to a given phase in the low-frequency band. The method proposed by Canolty (2006) is based on time-frequency representations obtained by bandpass filtering of the signal. Each band is normalized by means of the z-transform.

AIM(S): We propose a development of Canolty's method. The purpose of this study was to test the properties and efficacy of the enhanced method, and to compare the results to those obtained with the original Canolty's method.

METHOD(S): The proposed method relies on time--frequency representation of signal's energy density derived from continuous wavelet transform, and normalization of each frequency relative to its average value in the baseline period (analogously to Event Related Synchronization/Desynchronization analysis). Moreover, we proposed the use of cluster-based statistic to identify statistically significant effects. Both methods were tested on simulated signal. The simulations consist of a low-frequency sine (in the range of theta rhythm frequencies) with superimposed spindles of high-frequency (from the gamma band range) and white noise. For each method we determined the signal-to-noise ratio range where methods give reliable results, for selected ratios of gamma to theta amplitude.

RESULTS: The proposed method turned out to be more sensitive and more specific. It identifies coupling only in the correct theta frequency, whereas original method localizes coupling also in neighboring frequency bands. The modified method is also more robust to higher noise levels.

CONCLUSIONS: The findings suggest that the enhanced method have sufficient sensitivity to measure the theta-gamma coupling as measured by high quality EEG or ECoG.

P2.87. ENABLING CELLULAR-RESOLUTION CONNECTOMIC ANALYSIS OF THE PRIMATE CORTEX: SHARING DATA ON CORTICO-CORTICAL CONNECTIVITY THROUGH AN OPEN ACCESS WEB PLATFORM

Piotr Majka[1,2,3], Jonathan Chan[2], Bai Shi[2], Ianina Hutler Wolkowicz[2], Natalia Jermakow[1], Marcello Rosa[2,3]

[1] Nencki Institute of Experimental Biology Polish Academy of Sciences, Warsaw, Poland, [2] Department of Physiology, Monash University, Melbourne, Australia, [3] Australian Research Council Centre of Excellence for Integrative Brain Function, Canberra, Australia

INTRODUCTION: Processes such as perception, action and cognition are determined by the connectivity between different neuronal groups. Understanding the principles of this network is a core objective of present-day neuroscience. Several animal models are used to investigate this relationship between structure and function, among them marmosets, which recently came to prominence. They are small monkeys (300–400 g) but their brain retains all defining features of the primate brain.

AIM(S): The aim is to create a publicly available, the world's most comprehensive repository of the afferent cortico-cortical connectivity of any primate species, enabling a new level of analysis and modelling. The connectome will be publicly available on-line making it possible to flexibly access all the data via a graphical front-end or via an application programming interface.

METHOD(S): The already available body of data comprises results of over 100 monosynaptic retrograde tracer injections in marmosets. The brains were cut in 40 µm sections. The sections were plotted using an epifluorescence microscope, and stained for Nissl substance. To map individual injections into the atlas space, a previously established pipeline was used.

RESULTS: The current version of the portal is available at http://marmoset.braincircuits.org. It allows one to access unprocessed experimental data, mostly injections in dorsal prefrontal cortex, parietal and occipital lobes. Additionally, the locations of individual cells are expressed in atlas-based stereotaxic coordinates which allows one to perform either area-based or parcellation-free connectivity analyses.

CONCLUSIONS: The release of open access connectomes is known for triggering numerous follow-up modelling and theoretical studies. In a longer perspective, the unique nature of data in our project will help to understand how the highly complex network of neuronal connections enable brain functions in primates, and, in general, in mammals.

FINANCIAL SUPPORT: The project is supported by the Australian Research Council grant (DP140101968) and International Neuroinformatics Coordinating Facility Seed Funding grant.

P2.88. NEW APPROACH FOR STUDYING LAMINA X NEURONS OF THE SPINAL CORD

Volodymyr Krotov[1], Anastasia Tokhtamysh[2], Andrii Dromaretskii[1], Evhen Sheremet[1], Pavel Belan[1], Nana Voitenko[1]

[1] Bogomoletz Instutue of Physiology, Kyiv, Ukraine, [2] Taras Shevchenko National Universityof Kyiv, Kyiv, Ukraine

INTRODUCTION: The area around the central canal (or lamina X) is the most enigmatic lamina of the spinal cord. It is established that somatosensory integration, visceral nociception, autonomic regulation and modulation of motoneuron output are mediated by lamina X neurons, nevertheless their electrophysiological properties and functional connectivity are largely unknown.

AIM(S): Electrophysiological investigations of lamina X neurons are hampered primarily by technical challenges in the preparation procedures, so we aimed to develop a reliable technique for functional studies of the neurons around the central canal.

METHOD(S): Our approach relies on the method of oblique LED illumination for cell visualization in thick blocks of tissue, developed by Prof. Safronov and colleagues.

RESULTS: We have developed simple, fast (5–10 min) and reliable lamina X preparation technique, that preserves whole spinal cord architecture including dorsal and ventral roots. In combination with oblique LED illumination, allowing cell visualization in thick blocks of tissue, our preparation enables visually guided patch clamp of lamina X neurons coupled with simultaneous stimulation of either afferent or efferent fibers with suction electrode. Our preparation also permits the usage of fluorescent approaches which might additionally boost the relevance of lamina X studies. First, injections of retrograde dye Fluorogold (or its analogues) into the peritoneum or lateral thalamus selectively label sympathetic preganglionic or projection neurons respectively, giving the possibility to work with these specific neuronal populations. Second, calcium transients might be recorded after loading the cells with either cell-impermeable (through the patch pipette) or cell-permeable (AM ester) Fura 2 calcium dye.

CONCLUSIONS: We introduce a new methodology that combines electrophysiological and fluorescent approaches for the research of lamina X neuron functioning in physiological and pathological conditions.

FINANCIAL SUPPORT: Supported by IBRO.

P2.89. MODULAR MICROELECTRONIC SYSTEM FOR IN VIVO ELECTRICAL STIMULATION AND RECORDING AT UP TO 512 ELECTRODES

Małgorzata Szypulska[1], Piotr Wiącek[1], Andrzej Skoczeń[1], Tomasz Fiutowski[1], Imran Ahmed[1], Mariusz Jędraczka[1], Joanna Dusik[1], Michał Dwużnik[1], Ewa Kublik[2], Władysław Dąbrowski[1], Paweł Hottowy[1]

[1] Faculty of Physics and Applied Computer Science, AGH University of Science and Technology, Krakow, Poland, [2] Department of Neurophysiology, Nencki Institute of Experimental Biology, Warsaw, Poland

INTRODUCTION: Multielectrode silicon probes can record neuronal signals with combination of spatial and temporal resolution that other recording techniques cannot provide. Here we propose a novel microelectronic system that combines this functionality with advanced electrical stimulation.

AIM(S): We designed a modular system for multielectrode electrical stimulation and recording in the brain of a living animal. It can be combined with any silicon probe used for brain research. It can generate complex sequences of stimulation pulses and simultaneously record at up to 512 electrodes. It can use up to 4 silicon probes in parallel, providing bidirectional communication with populations of neurons simultaneously in several brain areas.

METHOD(S): The system is based on a dedicated multichannel CMOS chip. The chip includes 64 channels, digital circuitry for real-time communication with the control computer and a multiplexer that sends amplified signals from 64 electrodes into a single output line. The amplifier gain can be changed from 110 to 550. The low cut-off frequency is set between 200 mHz and 3 Hz, the anti-aliasing filter is set at 7 kHz and the sampling rate is 40 kHz. The stimulation signal is controlled independently for each channel with 12-bit resolution and refresh rate of 40 kHz. Each amplifier can be disconnected from the electrode for the duration of the stimulation pulse for the artifact reduction. Up to 8 chips can be controlled in parallel with dedicated LabView software.

RESULTS: Base version of the system was produced and tested with positive results. The final system is in the integration phase. We plan the first experiments to take place in the fall 2017 at the Nencki Institute for Experimental Biology.

CONCLUSIONS: The reported system can generate complex sequences of stimulation pulses and record neuronal signals with very low artifacts at 512 electrodes, making it a powerful tool for mapping of the functional connections between brain circuits.

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P2.90. REALLY REPRODUCIBLE BEHAVIOURAL PAPER

Jakub M. Dzik[1], Alicja Puścian[2], Zofia Harda[3], Katarzyna Radwańska[3], Szymon Łęski[1]

[1] Laboratory of Neuroinformatics, Department of Neurophysiology, Nencki Institute of Experimental Biology Polish Academy of Sciences, Warsaw, Poland, [2] Laboratory of Emotions Neurobiology, Department of Neurophysiology, Nencki Institute of Experimental Biology Polish Academy of Sciences, Warsaw, Poland, [3] Laboratory of Molecular Basis of Behavior, Department of Molecular and Cellular Neurobiology, Nencki Institute of Experimental Biology Polish Academy of Sciences, Warsaw, Poland

INTRODUCTION: The reproducibility of behavioural tests has been improved by the introduction of a number of automated experimental systems. One of such systems is IntelliCage™, which allows for sophisticated experimental designs. Despite the improved reproducibility of experiments, reported results may be rendered irreproducible due to errors introduced by manual data analysis and not standardized reporting of analysis methods. The efficiency of manual analysis is also an issue.

AIM(S): Our aim was to facilitate development of automated workflows for reproducible analysis of data yielded by the IntelliCage™ system.

METHOD(S): We developed an open source Python library (PyMICE – RRID:nlx_158570) providing IntelliCage™ data as collection of data structures. We have described the library and presented some examples of its use in a paper. According to the literate programming paradigm, the paper was composed of Python and LaTeX snippets. Pweave tool has been used to weave the paper.

RESULTS: All analyses contained in our paper "PyMICE – a Python library for analysis of IntelliCage data" (accepted by Behavior Research Methods) are fully reproducible. The source code of the paper (https://github.com/Neuroinflab/PyMICE_SM) does not contain any plots. Instead, they may be easily reproduced by the reader. Also, the correctness of performed analyses may be easily verified.

CONCLUSIONS: We propose PyMICE as a common platform for implementing and sharing automated analysis workflows for IntelliCage™ data. The library is a user-friendly tool for analysis of behavioural data in an automated workflow. Such workflow is an unambiguous, formal specification of the performed analysis. The analysis itself may be easily reproduced by simply reapplying the workflow to the same data. Such workflow may be used to perform exactly the same analysis for multiple datasets, e.g. when the same protocol is applied to multiple groups of animals. This is a very common case, as most of experiments have at least one experimental and one control group.

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P2.91. MIR-132 REGULATES MMP-9 PROTEIN LEVELS IN VIVO BY TARGETING ITS 3'UTR REGION

Bozena Kuzniewska[1], Olga Gewartowska[2], Magdalena Jasinska[1], Jakub Gruchota[2], Karolina Rejmak[1], Andrzej Dziembowski[2], Magdalena Dziembowska[1]

[1] Laboratory of Molecular Basis of Synaptic Plasticity, Centre of New Technologies, University of Warsaw, Warsaw, Poland, [2] Laboratory of RNA Biology and Functional Genomics, Institute of Biochemistry and Biophysics Polish Academy of Sciences, Warsaw, Poland

INTRODUCTION: MicroRNAs (miRNAs) are small non-coding RNAs that bind to target sites in mRNAs, leading to translational repression. MiRNAs are present in dendrites and synapses where they are believed to fine-tune the local expression of synaptic proteins. MiR-132 is a neuronal activity-regulated microRNA that controls the morphology of dendritic spines and synaptic transmission. Similar activities have recently been attributed to matrix metalloproteinase-9 (MMP-9), an extrasynaptic protease. Our previous studies show that miR-132 can directly regulate Mmp-9 mRNA by targeting its 3'UTR in cultured primary neurons.

AIM(S): In the current study, we aimed at verification whether miR-132 regulates the expression of Mmp-9 *in vivo* in the mouse brain.

METHOD(S): To determine whether miR-132 binds to the 3'UTR of Mmp-9 mRNA, the luciferase reporter assay using the coding sequence of firefly luciferase fused with the 3'UTR of Mmp-9 mRNA. Next, CRISP-Cas9 technology was used, in order to introduce mutations in putative binding site for miR-132 in 3'UTR of Mmp-9 locus in mice. Subsequently, gelatin zymography was used to evaluate the levels of MMP-9 protein in different brain regions of mutant and control mice.

RESULTS: Overexpression of miR-132 in cortical neurons significantly reduced the luciferase activity of MMP-9 3'UTR reporter. Importantly, miR-132 failed to regulate the mutated MMP-9 3'UTR luciferase reporter, confirming the functionality of the predicted sequence within the 3'UTR of MMP-9. Mutation in 3'UTR region of MMP-9 targeted by miR-132 in mice, resulted in higher MMP-9 protein levels in different brain regions of mutant mice as compared to controls.

CONCLUSIONS: We show, that miR-132 binds to the 3'UTR of Mmp-9 mRNA in primary cortical neurons. Moreover, we developed a new mouse model to study miR-132 – Mmp-9 interaction *in vivo*. Our data suggest, that miR-132 targets 3'UTR of Mmp-9 mRNA *in vivo* and can regulate MMP-9 protein in mouse brain.

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P2.92. HUMAN INDUCED PLURIPOTENT STEM CELL (HIPSCS) BASED HUNTINGTON'S DISEASE (HD) NEURONS AND THEIR GENETIC CORRECTION USING PERSONALIZED RNA-GUIDED DESIGNER NUCLEASES (CAS9) MEDIATED GENOME EDITING

Aleksandra Golonko[1], Agnieszka Pollak[2], Małgorzata Rydzanicz[3], Rafał Płoski[3], Paweł Lisowski[4,5]

[1] Department of Biotechnology, Faculty of Civil and Environmental Engineering, Białystok University of Technology, Białystok, Poland, [2] Department of Genetics, Institute of Physiology and Pathology of Hearing, Warsaw, Poland, [3] Department of Medical Genetics, Warsaw Medical University, Warsaw, Poland, [4] Department of Molecular Biology, Institute of Genetics and Animal Breeding Polish Academy of Sciences, Jastrzebiec, Magdalenka, Poland, [5] iPS Cell-Based Disease Modeling Group, Max-Delbrück-Center for Molecular Medicine (MDC) in the Helmholtz Association, Berlin, Germany

INTRODUCTION: Huntington's Disease (HD) is an neurodegenerative disorder, caused by a mutation in the CAG repeat tract of the huntingtin gene HTT, characterized by a progressive loss of neurons in the striatum and pheno-

typic features that are common in other neurodegenerative diseases.

AIM(S): The aim was to 1) test personalized, precise genome editing technology based on high fidelity Cas9 variants in somatic gene therapy of trinucleotide repeat degenerative disorder such as HD, 2) and to test whether the N-terminal truncated protein is able to support normal neuronal development, 3) dissect the impact of the mutation on neuronal development.

METHOD(S): Patient specific hiPSCs were generated using an integration-free method. hiPSC were edited by improved fidelity Cas9-sgRNA expression vectors located upstream and downstream of the CAG repeats in Exon 1, HDR repair templates with different numbers of CAG repeats. Gene edited iPS clones were characterized for the potential modification at predicted off-target sites. The lines were subjected for the functional studies with high-content screening.

RESULTS: hiPSCs editing resulted into different products that underwent the non-homologous end joining (NHEJ), precisely corrected clones by homologous recombination (HR) and NHEJ mediated excision of the Q/P repeat region by reannealing of the DSB resulted into an in-frame Htt coding region lacking the N-terminal Q/P repeat.

CONCLUSIONS: 1) HD and corrected isogenic hiPSCs can be differentiated into excitable, synaptically active neurons; 2) Study demonstrates associated phenotypic abnormalities; 3) Personalized high fidelity Cas9 variants showed an improved specificity profile, suitable for somatic gene therapy; 4) Study shows the importance of isogenic controls for modeling and personalized gene therapy using patient specific hiPSCs.

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P2.93. INFLUENCE OF DIFFERENT OPTICAL STIMULATION PARAMETERS ON STRENGTH OF DEPOLARIZATION IN CHR2-TRANSFECTED NEURONS

Aleksandra Składowska[1], Marek Bekisz[1], Magdalena Majkowska[1], Martyna Łempicka[1,2], Ewa Kublik[1]

[1] Department of Neurophysiology, Nencki Institute of Experimental Biology Polish Academy of Sciences, Warsaw, Poland, [2] Faculty of Physics, Warsaw University of Technology, Warsaw, Poland

INTRODUCTION: To use optogenetics in well control manner it is necessary to characterize the relation between the light power and resulting opening of light gated channels.

AIM(S): To this end we tested the dependence of membrane depolarization on the parameters of light stimulation in channelrhodopsin-transfected neurones in rat's central nucleus of amygdala (CeA).

METHOD(S): Under general anesthesia rats were were injected with AAV-hSyn-ChR2-EYFP viral vector introducing ChR2 to CeA. During *in vitro* patch-clamp recording on brain slices, we measured the membrane depolarization evoked by a blue light emitted from LED source. Cells were stimulated with trains of light impulses with varying: 1) light power; 2) duration of light impulse; 3) frequency of light impulses.

RESULTS: 1) Train of 2 ms light impulses delivered at 20 Hz, a driving current varying from 0.1 to 1 A: relation between light power and membrane depolarization can be approximated by a logarithmic function: 2.2 ln(x)+13 (at the resting potential kept at –50 mV) and 8 ln(x)+28 (at –60 mV). The dependency of the latency of first action potential on the light intensity can be approximated by a power function: 1.2 ×^(–1.3); 2) Train of light impulses of varying duration (range 2–20 ms) at 20 Hz, current 1 A and resting membrane potential kept at –50 mV: the relation between light impulse duration and resulting membrane depolarization can be approximated by a logarithmic function 3 ln(x)+4; 3) Train of 2 ms light impulses with a current set at 1 A and a frequency varying in a range 20–200 Hz (resting potential kept at –50 mV): relation between light impulse frequency and resulting membrane depolarization can be approximated by a logarithmic function: 2.5 ln(x)+3.5.

CONCLUSIONS: Our results offers the guideline allowing to estimate expected depolarizing effects of light stimulation on the ChR2-transfected neuronal population in central nucleus of amygdala in rats.

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P2.94. SPATIAL DISTRIBUTION OF LIGHT EVOKED CURRENTS IN CHR2-TRANSFECTED RAT'S CORTEX

Ewa Kublik[1], Aleksandra Składowska[1], Michał B. Czerwiński[1], Michał Pasierski[1,2], Daniel K. Wójcik[1]

[1] Department of Neurophysiology, Nencki Institute of Experimental Biology Polish Academy of Sciences, Warsaw, Poland, [2] Second Faculty of Medicine, Medical University of Warsaw, Warsaw, Poland

INTRODUCTION: Optogenetics allows to stimulate selected neuronal populations with high temporal resolution but the spatio-temporal extent of resulting effects is not well characterized.

AIM(S): Experiment were aimed to evaluate spatial distribution of the potentials and currents evoked by light impulses in the channelrhodopsin-transfected rat cortex.

METHOD(S): Rats were injected with viral vector introducing ChR2 into large portion of somatosensory cortex. 2–3 weeks later we performed acute *in vivo* experiments recording multichannel local field potentials evoked (EP) by a blue light delivered either to the cortical surface (surf-stim) or into the cortex (deep-stim). We analyzed spa-

tio-temporal patterns of EPs and their 2-D current source density (CSD) profiles (kernel CSD method, https://github.com/Neuroinflab/kCSD-python).

RESULTS: Our preliminary results indicated that light evoked potentials consisted of early waves, resulting from opening ChR2 channels, overlapping with later components related to the synaptic spread of activity within cortical network. As expected, largest EPs were recorded close to the fiber tip, in layer 2–3 with surf-stim and layer 5 with deep-stim. Longer impulses (10 ver 1 ms) evoked around 20% stronger responses. Up to 600–800 μm from a light source EPs sustained ~50% of max amplitude. However, CSD analysis indicated that after surf-stim the early current sink (1–2 ms) was restricted to ~400 μm in layer 2–3. Later, postsynaptic sink developed at 5–8 ms in layer 5. Later components had wider lateral spread across few columns with clear reflection of cortical layering. After intra-cortical light delivery activity seemed to spread within, not across the cortical columns.

CONCLUSIONS: For well controlled use of optogenetics it is not enough to ensure light beam of sufficient strength. The localization of the fiber tip can have specific impact on the activity developing within local neuronal network.

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P2.95. THE ROLE OF HIPPOCAMPAL PML IN TRANSGENIC MOUSE CIRCADIAN RHYTHM

Gabriela Olech-Kochańczyk[1], Anna Kiryk-Jaśkiewicz[1], Andrii Domanskyi[2], Witold Konopka[1], Grzegorz M. Wilczyński[1]

[1] Nencki Instiute of Experimental Biology Polish Academy of Sciences, Warsaw, Poland, [2] Institute of Biotechnology, University of Helsinki, Helsinki, Finland

INTRODUCTION: Circadian clock is an evolutionarily well-conserved mechanisms in higher organisms. PML protein implicated in many important biological processes (response to DNA damage, cell division control) influences also circadian rhythm by regulating nuclear localization of Per2, a significant positive regulator of the clock transcriptional mechanisms.

AIM(S): Our aim is to explain how overexpression or knock-out of PML affect the oscillations of levels of proteins involved in circadian rhythm in hippocampus.

METHOD(S): We generated transgenic mouse models with overexpression or knock-out of PML gene that are induced in specific time and location in brain. The project consists of two tasks: 1) generation of transgenic animals with overexpression of PML gene using AAV vectors encoding PML and mCherry reporter gene, and 2) generation of transgenic animals with knock-out of PML gene using AAV vectors encoding PML gene-targeting gRNAs and Cas9 (CRISPR/Cas9 system).

RESULTS: We generated AAV vectors with PML-mCherry and microinjected them into the hippocampus of mice that were subject to behavioral tests (in the IntelliCage system). AAV PML-mCherry mice showed impairment in circadian activity 45 days after surgery and displayed proper spatial learning of cage corners with appetitive reinforcement and slower re-learning process. Next, we designed gRNAs for PML exon I and II and generated plasmid vectors containing gRNAs for the PML gene and tdTomato reporter. Using both these vectors and vectors containing Cas9 endonuclease fused with GFP reporter we transfected NIH 3T3 cells to induce PML gene knock-out *in vitro*. Next, using T7E1 endonuclease we confirmed that knock-out of PML works. Afterwards, we generated AAV vectors encoding PML gene-targeting gRNAs and Cas9 endonuclease. Both vectors were injected into the hippocampus.

CONCLUSIONS: Thus far research on PML function in the circadian rhythm was usually performed *in vitro*. Our tools enable us to study the role of PML in mammalian molecular clock mechanisms *in vivo*.

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