POLISH NEUROSCIENCE SOCIETY

9th International Congress

The first FENS Featured Regional Meeting

WARSAW, POLAND

September 9-12, 2009



Warsaw University - the Old Library

Under the Honorary Patronage of the Rector of the Warsaw University

Organizer: Polish Neuroscience Society (PNS)

Co-organizers: Federation of European Neuroscience Societies (FENS), Committee of Neurobiology of Polish Academy of Sciences (CN PAS), Committee of Neurological Sciences of Polish Academy of Sciences (CNS PAS)

Partners: Nencki Institute of Experimental Biology PAS, Mossakowski Medical Research Centre PAS, Institute of Pharmacology PAS, International Institute of Molecular and Cell Biology

Chairperson: Jolanta Skangiel-Kramska - President of the Polish Neuroscience Society

Scientific Program Committee:

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FENS Office Berlin: Meino Gibson

Organizers of Brain Awareness Events:
History of Neuroscience in Poland – exhibition (In Polish and in English): Andrzej Wróbel
EDAB Open Lecture for Public (In Polish): Jolanta Skangiel-Kramska, Teresa Zalewska

Strategic sponsor: Kawa.ska & Leica

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NINTH INTERNATIONAL CONGRESS OF THE POLISH NEUROSCIENCE SOCIETY
First FENS Featured Regional Meeting

PROGRAMME
Wednesday, September 9

12.00 – 18.00 Registration

14.00 – 16.00 General Assembly of the Polish Neuroscience Society
location: Old Library, Warsaw University, Krakowskie Przedmieście 26/28

17.00 – 18.30 Opening Session
location: Old Library, Warsaw University, Krakowskie Przedmieście 26/28

Welcome Addresses (Jolanta Skangiel-Kramska, President of Polish Neuroscience Society and guests)
Opening Lecture
Sponsored by: Kawa.ska
Oswald Steward (Reeve-Irvine Research Center, University of California at Irvine, CA, USA)
Targeting of mRNA to postsynaptic sites on neuronal dendrites
Introduced by: Malgorzata Skup

19.30 Concert of Polish Ancient Music (Ars Nova Ensamble) and Get-Together Reception
location: Central Agricultural Library, Krakowskie Przedmieście 66

Thursday, September 10

08.30 – 09.20 **Plenary Lecture**
Pasko Rakic (Yale University School of Medicine, New Haven, CT, USA)
Making map of the mind: Molecular mechanisms of neuronal migration
Introduced by: Malgorzata Kossut

09.20 – 09.40 **Young Investigator Lecture** (PNS award)
Ksenia Meyza (Nencki Institute of Experimental Biology PAS, Warszawa, Poland)
Electrophysiological correlates of diverse emotional reactivity of Roman High - (RHA/Verh) and Roman Low Avoidance (RLA/Verh) rats
Introduced by: Elzbieta Pyza

09.40 – 10.00 Coffee break

10.00 – 11.30 **SYMPOSIA**
Symposium I: Learning-Dependent Brain Plasticity: Systems View

Organizer and Chair: Grzegorz Hess (Jagiellonian University and Institute of Pharmacology PAS, Krakow, Poland)

Carl Petersen (Mind Brain Institute, Lausanne, Switzerland)
Synaptic mechanisms of tactile sensory properties

Wioletta Waleszczyk (Nencki Institute of Experimental Biology PAS, Warszawa, Poland)
Reorganization of the visual cortex following monocular circumscribed retinal lesions: Update on Hebbian learning rules

Mathew Diamond (SISSA, Trieste, Italy)
What is stored in the hippocampus during a tactile categorization task?

Malgorzata Kossut (Nencki Institute of Experimental Biology PAS, Warszawa, Poland)
The learning brain – modulation of activation pattern

Symposium II: Using Neuroimaging to Understand Human Brain Function

Organizer and Chair: Anna Grabowska, Anna Nowicka (Nencki Institute of Experimental Biology PAS, Warszawa, Poland)

Piotr Bogorodzki (Institute of Electronics, Warszawa, Poland)
Inside BOLD – why neuronal activity modulates MR signal?

Greg Kroliczak (University of Oregon, Eugene, OR, USA)
Neural bases of praxis and target-directed actions

Stefan Heim (RWTH Aachen University, Jülich, Germany)
The neural correlates of language processing and its disorders over the life span

Artur Marchewka (Nencki Institute of Experimental Biology PAS, Warszawa, Poland)
Neural correlates of false recognition

9.40 – 11.40 **Workshop: Quantitative Analysis of Protein–Protein Interactions**
(Support contributed by Kawa.ska and Deutsche Forschungsgemeinschaft)

Organizer and chair: Jakub Wlodarczyk (Nencki Institute of Experimental Biology PAS Warszawa, Poland)

Erwin Neher (Max-Plank-Institute for Biophysical Chemistry, Göttingen, Germany)
Non-negative matrix factorization: A novel method to determine reference spectra of fluorescent dyes in a cellular environment

Diethelm Richter (University of Göttingen, Göttingen, Germany)
Quantitative measurements of cAMP concentration with a new Epac Based FRET-Sensor

Evgeni Ponimaskin (Medical School Hanover, Hanover, Germany)
Stimulation-dependent changes in oligomeric conformation of serotonin 5-HT_{1A} receptors

Leszek Kaczmarek, Jakub Wlodarczyk (Nencki Institute of Experimental Biology PAS, Warszawa, Poland)
Lux-FRET method for quantitative analysis of FRET signal

Grzegorz Wilczynski (Nencki Institute of Experimental Biology PAS, Warszawa, Poland)
High-resolution light-microscopic studies on synaptic extracellular proteolysis

11.30 – 12.00 Coffee break

12.00 –12.50 **Plenary Lecture**
Jerzy Lazarewicz (Mossakowski Medical Research Centre PAS, Warszawa, Poland)
Alternative methods of neuroprotection in brain ischemia
Introduced by: Krystyna Domanska-Janik

12.50 – 14.30 Lunch

14.30 – 16.30 **POSTER SESSION I**
location: Warsaw Academy of Fine Arts, Krakowskie Przedmieście 5

16.30 – 18. 00 **SYMPOSIA**

Symposium III: Learning-Dependent Brain plasticity: Synaptic View

Organizer and Chair: Malgorzata Kossut (Nencki Institute of Experimental Biology PAS, Warszawa, Poland)

Denise Manahan-Vaughan (Ruhr University, Bochum, Germany)
Regional and functional specificity of hippocampal synaptic plasticity in spatial memory formation

Krzysztof Tokarski (Institute of Pharmacology PAS, Kraków, Poland)
Sensory learning enhances GABAergic synaptic transmission in the barrel cortex of the mouse

Jerzy Mozrzymas (Wroclaw Medical University, Wrocław, Poland)
Impact of sensory learning on GABAergic tonic inhibition in mouse barrel cortex

Alison Barth (Carnegie-Mellon University, Pittsburgh, PA, USA)
Synaptic metaplasticity in somatosensory cortex

Symposium IV: Mechanisms, Functions and Modulation of Central Nervous System Inflammation

Organizer and Chair: Bozena Kaminska (Nencki Institute of Experimental Biology PAS, Warszawa, Poland)

Wolfgang J. Streit (University of Florida College of Medicine, Gainesville, Florida, USA)
Microglial senescence and degeneration in the pathogenesis of neurodegenerative disease

Anna Czlonkowska (Institute of Psychiatry and Neurology, Warszawa, Poland)
Manipulating inflammation and gliosis in neurologic diseases – a clinical perspective

Manuel B. Graeber (Imperial College, London, UK)
Glia and inflammation in the Parkinsonian substantia nigra

Barbara Przewlocka (Institute of Pharmacology PAS, Kraków, Poland)
Modulation of inflammation and neuropathic pain

Symposium V: Stress-Response Systems in the Pathogenesis and Prognosis of Cancer, Inflammation and Autoimmune Diseases

Organizer and Chair: Marta Kubera (Institute of Pharmacology PAS, Kraków, Poland)

Eadaoin Griffin (National University of Ireland, Maynooth, Ireland)
Psychological stress compromises the innate immune system: Implications for disease susceptibility

Danuta Lewandowska (University of Gdansk, Gdańsk, Poland)
Stress, emotion, and natural killer cell antitumor activity

Lisa Goehler (University of Virginia, Charlottesville, Virginia, VA, USA)
Role of stress on immunity and cancer development

Craig Stockmeier (University of Mississippi Medical Center, Jackson, MS, USA)
Cell pathology and plasticity in the hippocampus in major depressive disorder

18.00 – 18.10 Coffee break

18.10 – 18.30 **Young Investigator Lecture** (FENS stipend holder award)
Christian Wozny (MRC Laboratory of Molecular Biology, Cambridge, UK)
Looking at the top of the cortex: Neural connectivity in the superficial layers of the neocortex
Introduced by: Elzbieta Pyza

18.30 – 19.20 **Plenary Lecture**
Grazyna Rajkowska (University of Mississippi Medical Center, Center for Psychiatric Neuroscience, Jackson, MS, USA)
Glia, GABA and Glutamate: New key players in the pathology of depression
Introduced by: Irena Nalepa

19.30 – 20.15 Foundation for Polish Science presentation for FENS stipend holders

20.30 Welcome reception (sponsored by FENS)
location: Le Jardin Restaurant, Zamojski Palace, Foksal 2

Friday, September 11

08.30 – 09.20 **Plenary lecture**
Jerzy Samochowiec (Pomeranian Medical University, Szczecin, Poland)
Alcoholism: From neurobiology to new treatment approach
Introduced by: Adam Plaznik

09.20 – 09.40 **Young Investigator Lecture** (PNS award)
Lukasz Swiech (Institute of Molecular and Cell Biology, Warszawa, Poland)
TIPs for dendritogenesis: CLIP-170 in dendritic arbor development
Introduced by: Elzbieta Pyza

09.40 – 10.00 Coffee break

10.00 – 11.30 **SYMPOSIA**
Symposium VI: Genes and Behavior

Organizer and Chair: Artur H. Swiergiel (Institute of Biology, University of Gdansk, Gdańsk, Poland)

Grzegorz R. Juszczak (Institute of Genetics and Animal Breeding PAS, Jastrzebiec, Poland)
It is said "almost makes a difference": Are different strains of rodents the same or almost the same?

Dora Zelena (Group of Neuropsychopharmacology HAS and Semmelweis University, Budapest, Hungary)
Stress and adaptation in Brattleboro rats lacking arginine-vasopressin

Aet Alttoa (Department of Psychology, University of Tartu, Estonia)
Measurement of persistently expressed behavioural traits in outbred rats: Underlying gene expression patterns

Symposium VII: Stem Cells Based Therapeutic Approaches
(*Support contributed by Olympus Polska*)

Organizer and Chair: B. Lukomska, K. Domanska-Janik (Mossakowski Medical Research Centre PAS, Warszawa, Poland)

Marcin Jurga (Institute Novus Sanguis, Parc Technologique, St Priest, Lyon, France)
Artificial neural tissue from cord blood for tissue replacement therapy central nervous system

Kyung-Sun Kang (Adult Stem Cell Research Center, Seoul National University, Seoul, Korea)
Multi-functional therapeutic potentials of human umbilical cord blood-derived stem cell in neurodegenerative diseases

Klaus Reymann (Leibnitz Institute for Neurobiology, Magdeburg, Germany)
Stem cell transplantation in rodent stroke models

Symposium VIII: Cholinergic System in Neurodegereneration

Organizer and Chair: Andrzej Szutowicz (Medical University of Gdansk, Gdańsk, Poland)

Natalia Nalivaeva (University of Leeds, Leeds, UK)
Amyloid precursor protein and cholinesterase in brain function and neurodegeneration

Reinhardt Schliebs (University of Leipzig, Leipzig, Germany)
Cholinotoxicity of β-amyloid

Anthony Turner (University of Leeds, Leeds, UK)
Proteolytic cascades in the brain and their cholinergic regulation: Role in neurodegeneration

Andrzej Szutowicz (Medical University of Gdansk, Gdańsk, Poland)
Phenotype and energy-dependent cholinotoxicity

11.30 – 11.45 Coffee break

11.45 – 11.55 **FENS address**
Helmut Kettenmann – President of FENS

12.00 – 12.50 **Plenary lecture**
Morgan Sheng (Genentech Inc., South San Francisco, CA, USA)
Regulation of synaptic function by modification of postsynaptic density proteins
Introduced by: Jacek Jaworski

12.50 – 14.30 Lunch

14.30 – 16.30 **POSTER SESSION II**
location: Warsaw Academy of Fine Arts, Krakowskie Przedmiescie 5

16.30 – 18.00 **SYMPOSIA**
Symposium IX: Protein Toxicity and Molecular Mechanisms of Neurodegenerative Diseases

Organizer and Chair: Robert Strosznajder (Mossakowski Medical Research Centre PAS, Warszawa, Poland)

Shun-ichi Nakamura (Kobe University, Graduate School of Medicine, Kobe, Japan)
Sphingosine-1-phosphate in physiology and pathology in the brain

Michal Toborek (University of Kentucky, Lexington, KY, USA)
Role of caveolin-1 protein in signaling mechanism and in endothelial cell function and dysfunction

Agata Adamczyk (Mossakowski Medical Research Centre PAS, Warszawa, Poland)
Alpha synuclein and its neurotoxic fragment NAC in neurodegeneration

Martin Sadowski (New York University School of Medicine, New York, NY, USA)
Therapeutic approaches targeting Aβ cascade in Alzheimer's disease

Symposium X: Stroke-Induced Brain Reorganization

Organizer and Chair: Jolanta Skangiel-Kramska (Nencki Institute of Experimental Biology PAS, Warszawa, Poland)

Mathias Hoehn (Max-Planck Institute for Neurological Research, Köln, Germany)
Exploring the chances of stem cell based regeneration after stroke by MRI

Dorota Nowicka (Nencki Institute of Experimental Biology PAS, Warszawa, Poland)
How phothotrombotic stroke affects extracellular matrix?

Małgorzata Kossut (Nencki Institute of Experimental Biology PAS, Warszawa, Poland)
fMRI study of recovery from unilateral neglect

Otto Witte (Friedrich Schiller University, Jena, Germany)
Brain plasticity following stroke: Possible targets for therapeutic intervention

Symposium XI: Glutamatergic receptors as a target for future psychotropic drugs action

Organizer and Chair: Andrzej Pilc (Institute of Pharmacology PAS, Kraków, Poland)

Barbara Wroblewska (Georgetown University, Washington DC, USA)
Role of group II metabotropic glutamate receptors in schizophrenia models

Wojciech Danysz (Merz Pharmaceuticals, Frankfurt am Main, Germany)
Selected aspects of Group I mGlu receptor negative and positive modulators

Krystyna Ossowska (Institute of Pharmacology PAS, Kraków, Poland)
Involvement of mGlu receptors in ethiopathology of Parkinson's disease

Andrzej Pilc (Institute of Pharmacology PAS, Kraków, Poland)
Metabotropic glutamate receptor ligands as possible targets for treatment of anxiety and depression

18.00 – 18.15 Coffee break

18.15 – 18.45 **EJN Lecture** (EJN award)
Ji H. Ko (McGill University, Montréal, Canada)
Theta burst stimulation of dorsolateral prefrontal cortex reveals hemispheric asymmetry in striatal dopamine release during set-shifting task in human
Chair: Jean-Marc Fritschy (Institute of Pharmacology and Toxicology, University of Zurich, Zürich, Switzerland)

18.45 – 19.35 **Plenary Lecture**
Annamaria Vezzani ("Mario Negri" Institute for Pharmacological Research, Milano, Italy)
Cytokine-mediated cross-talk between glia and neurons: Neuropathological implications for epilepsy
Introduced by: Katarzyna Lukasiuk

20.30 **FENS Dinner** (by invitation only)
Location: "Belvedere" Restaurant, Royal Łazienki Park

Saturday, September 12

08.30 – 09.20 **Plenary lecture**
Bozena Kaminska (Nencki Institute of Experimental Biology PAS, Warszawa, Poland)
Exploring and manipulating function of microglia in brain pathologies
Introduced by: Ryszard Przewlocki

09.20 – 09.40 **Young Investigator Lecture** (FENS stipends holder award)
Piotr Walczak (Johns Hopkins University School of Medicine, Baltimore, MD, USA)
Guiding cell-based therapy for neurological diseases with noninvasive cellular imaging
Introduced by: Elzbieta Pyza

09.40 – 10.00 Coffee break

10.00 – 11.30 **SYMPOSIA**

Symposium XII: Interaction Between Antidepressant Drugs and Immunoendocrine Systems

Organizer and Chair: Boguslawa Budziszewska, Wladyslaw Lason (Institute of Pharmacology PAS, Kraków, Poland)

Peter Gass (Central Institute of Mental Health, Mannheim, Germany)
Mice with altered glucocorticoid receptor expression as a model for depression

Ewa Obuchowicz (Medical University of Silesia, Katowice, Poland)
Effects of antidepressant drugs on cytokines in experimental study

Francesco Roselli (Max-Planck Institute of Psychiatry, München, Germany)
Neuroplasticity in depression

Marta Kubera (Institute of Pharmacology PAS, Kraków, Poland)
Immunoendocrine changes in animal model of depression

Symposium XIII: Motor Units and Motoneurons

Organizer and Chair: Jan Celichowski (University School of Physical Education, Poznań, Poland)

Philip Gardiner (University of Mannitoba, Winnipeg, Canada)
Physiological adaptations in the rhytmic firing properties of alpha-motoneurons to changes in physical activity and aging

Claudio Orizio (University of Brescia, Brescia, Italy)
Motor units activity in EMG, MMG and force ripple: Three pictures for one subject

Piotr Krutki (University School of Physical Education, Poznań, Poland)
Contractions of motor units evoked by stimulation with pulses at variable interpulse intervals

Jan Celichowski (University School of Physical Education, Poznań, Poland)
Variability and plasticity of motor unit contractile properties

Symposium XIV: Advanced Methods of Brain Signal Analysis

Organizer and Chair: Katarzyna Blinowska (Warsaw University, Faculty of Physics, Warszawa, Poland)

Fabrizio De Vico Fallani (University of Rome La Sapienza, Rome, Italy)
The use of high resolution EEG methods for brain computer interface applications

Daniel Wojcik (Nencki Institute of Experimental Biology PAS, Warszawa, Poland)
Beyond the PSTH: Point process modeling of spike trains

Stilian Kalitzyn (Epilepsy Institute of The Netherlands – SEIN, Heemstede, The Netherlands)
Perturbing the epileptic brain, evoked responses and beyond

Maciej Kaminski (Faculty of Physics, Warsaw University, Warszawa, Poland)
Evaluation of dynamical information transfer in brain

11.30 – 12.00 Coffee break

12.00 – 12.30 **Young Investigator Lecture** (PNS award)
Luiza Wojcik (Mossakowski Medical Research Centre PAS, Warszawa, Poland)
Relationship between activity of matrix metalloproteinase -2 and -9 (MMP-2, MMP-9) and ischemia-induced neurogenesis in adult gerbil hippocampus
Introduced by: Elzbieta Pyza

12.30 – 13.20 **Plenary lecture**
(*Support contributed by EBBS*)
Mark Tuszynski (University of California, Los Angeles, CA, USA)
Growth factor gene delivery for Alzheimer's disease: From animal models to clinical trials
Introduced by: Julita Czarkowska-Bauch

13.30 Closing ceremony

BRAIN AWARENESS EVENTS

Saturday, September 12th

16.00 – 17.00 **Public lecture sponsored by EDAB** (In Polish)
Tomasz Gabryelewicz (Mossakowski Medical Research Centre PAS, Warszawa, Poland)
Demencja i depresja – czy starość musi tak wyglądać?
(Dementia and Depression – whether the senescence has to look like that?)
Introduced by: Teresa Zalewska

Septemeber 1st-12th

EXHIBITION "The History of Polish Neuroscience"
Location: Museum of the University of Warsaw, Pałac Kazimierzowski, Krakowskie Przedmiescie 26/28

during the Congress the posters on display will be presented by the authors at a **POSTER SESSION III** on Thursday and Friday, from 15.30 to 16.30.

PLENARY LECTURES

L1
TARGETING OF mRNA TO POSTSYNAPTIC SITES ON NEURONAL DENDRITES

Steward O.
Reeve-Irvine Research Center, University of California at Irvine, Irvine, CA, USA

A fundamental aspect of gene expression in neurons involves delivery of certain mRNAs to synaptic sites on dendrites, where they are locally translated at synapses. Local translation of mRNA plays a critical role in synaptic consolidation, the process through which early transient changes in synaptic efficacy become more enduring. Mechanisms of transport and localization have been revealed through studies of a unique immediate early gene (IEG) called *Arc* (activity-regulated cytoskeleton-associated protein), AKA *Arg 3.1. Arc* is strongly induced by intense neuronal activity, but is unique amongst known IEGs because its mRNA is rapidly delivered into dendrites. A remarkable feature of Arc is that newly-synthesized Arc mRNA localizes selectively at active synapses. Live cell imaging studies using a GFP-based labeling system reveal that exogenously expressed *Arc* constructs assemble into particles that are transported at rates up to 65 µm/minute, which would allow the delivery of an mRNA from the nucleus to synapses on distal dendrites within minutes. Transcripts that contain the 3'UTR of Arc localize in a highly selective manner at the base of dendritic spines, indicating that the signals mediating both dendritic transport and synaptic targeting are present in the 3'UTR of Arc mRNA. Docking of Arc mRNA at active synapses depends on NMDA receptor activation, a rapid polymerization of actin in spines, and local activation of MAP kinase. Continued strong activation of synapses after Arc mRNA is docked at synapses triggers Arc mRNA degradation; the mechanisms underlying this activity-dependent mRNA degradation remain to be identified. I will discuss how this mechanism might play a role in stabilizing changes in synapses induced by activity.

L2
MAKING MAP OF THE MIND: MOLECULAR MECHANISMS OF NEURONAL MIGRATION

Rakic P.
Department of Neurobiology & Kavli Institute for Neuroscience, Yale University School of Medicine, New Heaven, CT, USA

The identity, synaptic relationship and, ultimately, function of neurons is defined by their position. It is particularly evident in the cerebral cortex where neurons acquire their position by active migration before birth from multiple sites of origin and involve complex molecular events and cell–cell interactions. We have used a variety of *in vitro* and *in vivo* assays show how specific genes, encoding signaling and morphoregulatory molecules and their receptors cooperate in orchestrating various components of migration such as the mode of neuronal proliferation, phenotype determination, establishment of polarity, detachment from the local substrate and rate of migration to the proper position in the cortex. Interestingly, some of these genes and molecules are also implicated in the pathogenesis of cognitive disorders such as schizophrenia, mild mental retardation, childhood epilepsy and autism. In addition, we found that the rate of neuronal migration and precise inside-to-outside sequence of their deployment can be disrupted by exposure of embryo to various physical, chemical, and biological agents. Disruption or even slowing down of neuronal migration by either genetic or environmental factors results in gross heterotopias or in subtle abnormalities of neuronal positions that eventually affect the pattern of synaptic circuits and ultimately may cause variety of idiopathic disorders of highest cognitive function.

L3
ALTERNATIVE METHODS OF NEUROPROTECTION IN BRAIN ISCHEMIA

Lazarewicz J.
Department of Neurochemistry, Mossakowski Medical Research Centre PAS, Warszawa, Poland

Learning of cellular and molecular mechanisms of the ischemic neuronal damage led to development of pharmacological methods of neuroprotection targeted at these mechanisms. They were effective in animal stroke models, but none of them passed clinical trials. Thus the alternative experimental neuroprotective strategies emerge. Preclinical studies demonstrated that mild hypothermia significantly improves the outcome in the animal models of stroke and brain injury. This method of still unclear mechanism has been successfully used in specific clinical conditions and has been tested in several trials. It is hoped that moderate hypothermia soon may be introduced as an alternative method of stroke treatment. Ischemic preconditioning is a way of inducing tolerance to brain ischemia by preceding the injurious ischemia with the sub-lethal stressors like short ischemia, mild hypoxia, heating or pharmacological treatment. The exact mechanisms of activation and induction of brain tolerance to ischemia by preconditioning are not clear. Recently post-conditioning, i.e. neuroprotective effect of the post-treatment with sub-lethal stressors after injurious ischemia has been demonstrated, pointing to a therapeutic potential of such a treatment, still being tested at the preclinical level.

L4
GLIA AND GLUTAMATE: NEW KEY PLAYERS IN THE PATHOLOGY OF MAJOR DEPRESSION

Rajkowska G.
Department of Psychiatry and Human Behavior, University of Mississippi Medical Center, Jackson, USA

Postmortem studies in depression reveal age-dependent cell pathology in prefrontal cortex. Prominent reductions in glia and specifically, in astrocytes, are observed in younger depressed, whereas neuronal pathology is found in elderly with depression. As astrocytes regulate extracellular concentrations of glutamate (*via* glial glutamate transporters), an early deficit in astrocytes could lead to increases in extracellular glutamate and toxic damage to neurons as depression progresses. This is supported by postmortem studies of reduced expression of mRNA and protein for the glial glutamate transporter in younger depressed and by reductions in glutamatergic neurons in elderly depressed. Moreover, alterations in glutamate metabolism are reported in neuroimaging studies of depressed patients. Interestingly, agents increasing expression of glial glutamate transporters and/or altering glutamate neurotransmission show antidepressant activity. Our hypothesis that glial pathology is an initial stage of cellular and neurochemical changes in depression was confirmed by observations of glial/glutamate deficits in chronically stressed rodents. Pharmacologically-induced loss of astroglia, but not neurons in the rat prefrontal cortex will induce depressive-like behaviors. Moreover, treatment with riluzole (a modulator of glutamate

release) reverses stress-induced depressive-like behaviors and blocks glia impairments providing a link between dysfunction of glia and glutamate in depression.

L5
ALCOHOLISM: FROM NEUROBIOLOGY TO NEW TREATMENT APPROACHES
Samochowiec J.
Department of Psychiatry, Pomeranian Medical University, Szczecin, Poland

Great strides have been made in the last decade regarding the importance of both genetic and environmental contributors to the risk for heavy drinking, alcohol problems, and alcohol use disorders. This lecture reviews recent research findings regarding important endophenotypes contributing to these risks, (e.g. low response to alcohol, personality traits, sweet liking). Genetic findings will highlight several genes that appear to contribute to the level of response to alcohol, sweet liking and some personality traits predisposing to alcohol seeking behavior. This presentation will review ongoing steps to identify additional genes in several large population studies. The lecture will also briefly describe neurobiological background of drugs: (1) registered for alcohol treatment – disulfiram, naltrexon, acamprosate, (2) off label drugs used in alcohol dependence: topiramat and ondasetron and (3) new medication: nelmelfene. Also, the approach of using the existing alcohol typologies (proposed by Cloninger and Lesch) in treatment proposal will be presented using our own data. *Supported by MNiSW grant nr N N402 189335*

L6
REGULATION OF SYNAPTIC FUNCTION BY MODIFICATION OF POSTSYNAPTIC DENSITY PROTEINS
Sheng M.
Department of Brain and Cognitive Sciences, Massachusetts Institute of Technology, Cambridge, MA, USA; Department of Neuroscience, Genentech Inc, South San Francisco, CA, USA

Phosphorylation regulation of postsynaptic density proteins is likely to be a major means of regulating synaptic function. The PSD scaffold PSD-95, a powerful determinant of synaptic strength, is a case in point. Its precise role during synaptic plasticity (LTP *versus* LTD) has not been easy to interpret from overexpression, RNAi or knockout mice experiments. We found that the PSD scaffold PSD-95 is phosphorylated on multiple sites in cultured neurons and *in vivo*. Ser-295 phosphorylation, mediated by a Rac1-JNK1 MAP kinase pathway and countered by phosphatases PP1 or PP2A, promotes PSD-95 accumulation in synapses and is associated with LTP-inducing stimuli. More strikingly, LTD-inducing stimuli causes dephosphorylation of ser-295 rapidly and profoundly, correlating with activation of PP1. In addition, LTD was associated with phosphorylation of an N-terminal residue of PSD-95 by the protein kinase GSK3b. This site is also bidirectionally modulated by activity. A phospho-mimicking mutant of PSD-95 (S295D-PSD-95; which cannot be "dephosphorylated") impaired the internalization of AMPA receptors in cultured neurons and blocked the induction of LTD in cultured hippocampal slices. Our data indicate that dephosphorylation of PSD-95 on ser-295, and phosphorylation of the N-terminus of PSD-95, is required for mobilization of PSD-95 from the PSD, de-anchoring of AMPA receptors from the PSD for internalization, and hence induction of LTD.

L7
CYTOKINE-MEDIATED CROSS-TALK BETWEEN GLIA AND NEURONS: NEUROPATHOLOGICAL IMPLICATIONS FOR EPILEPSY
Vezzani A., Balosso S., Maroso M., Rizzi M., Noè F., Zardoni D., Frasca A., Ravizza T.
Department of Neuroscience, Mario Negri Institute for Pharmacological Research, Milan, Italy

Recent findings in experimental models and in the clinical setting highlight the possibility that inflammatory processes in the brain contribute to the etiopathogenesis of seizures and to the establishment of a chronic epileptic focus. Prototypical inflammatory cytokines such as IL-1β and TNF-α are overexpressed in epileptogenic brain areas, prominently by glia and to a lesser extent by neurons and endothelial cells of the blood brain barrier. Cytokine receptors are also upregulated, and the related intracellular signalling is activated, highlighting both autocrine and paracrine actions of cytokines in diseased brain. Cytokines can profoundly affect neuronal network excitability, and the recent demonstration of molecular and functional interactions between cytokines and classical neurotransmitters, such as glutamate and GABA, provides one mechanism by which cytokines affect neuronal activity. These interactions may result in increased tissue excitability leading to seizures and cell loss. These findings describe novel communications between glia and neurons which may contribute to pathological conditions (e.g. seizures, neurodegeneration) characterized by the activation of inflammatory processes, thus highlighting potential new targets for therapeutic intervention.

L8
EXPLORING AND MANIPULATING FUNCTION OF MICROGLIA IN BRAIN PATHOLOGIES
Kaminska B.
Department of Cell Biology, Nencki Institute of Experimental Biology PAS, Warszawa, Poland

Microglia are multifunctional immune cells of the brain executing various functions and rapidly responding to pathological insults. Brain injury, hypoxia, infection or aberrant protein accumulation may lead to chronic inflammation with a progressive shift in microglia function towards inflammatory phenotype and accumulation of immune cells. Under pathological conditions, the interplay of extrinsic signals directs microglia towards neuroprotective or detrimental phenotype. Molecular mechanisms of initiation, progression and termination of microglia-initiated inflammatory responses in the brain, in particular gene networks and signaling pathways are poorly understood. Characterization of the global transcriptome of microglia exposed to inflammatory or cytoprotective signals and analysis of signalling pathways revealed differences in expression of genes encoding cytokines/chemokines and transcription regulators. Identification of signal-

ling pathways contributing to discrete microglia phenotypes and discovery of transcription regulators which may serve as "master switches" for induction of an inflammatory phenotype, will allow to target specific functions of microglia. Therapeutic approaches targeting signal transduction in microglia will be discussed. A greater understanding of microglia functions coupled with advances in pharmacology and gene therapy will support development of functionally "engineered" microglia able to convey neuroprotection.

L9
GROWTH FACTOR GENE DELIVERY FOR ALZHEIMER'S DISEASE: FROM ANIMAL MODELS TO CLINICAL TRIALS

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Nervous system growth factors have extensive effects on neuronal function and survival. Nerve growth factor (NGF) prevents the death and stimulates the function of basal forebrain cholinergic neurons in correlational models of Alzheimer's disease (AD), leading to its translation to Phase 1 and 2 human clinical trials. Separately, Brain-Derived Neurotrophic Factor (BDNF) influences the survival and function of entorhinal cortical and hippocampal neurons in several animal models of AD, including transgenic mutant APP-expressing mice; aged rats and lesioned rats; and aged and lesioned primates. These beneficial effects appear to occur independent of alteration in beta amyloid load. We are currently examining the extended safety and tolerability of BDNF gene delivery to the entorhinal cortex in additional animal studies, potentially leading to specific targeting of short term memory loss in future human AD trials. This talk will review the history and current status of growth factor gene delivery in AD and other neurodegenerative disorders.

EJN-lecture
THETA BURST STIMULATION OF DORSOLATERAL PREFRONTAL CORTEX REVEALS HEMISPHERIC ASYMMETRY IN STRIATAL DOPAMINE RELEASE DURING SET-SHIFTING TASK IN HUMAN

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Previous neuroimaging studies have shown that executive processes requiring planning and set-shifting [e.g. Montreal card sorting task (MCST)] may engage the dorsolateral prefrontal cortex (DLPFC) while inducing dopamine (DA) release in the striatum. However, functional imaging studies can only provide neuronal correlates of cognitive performance and cannot establish a causal relation between observed brain activity and task performance. In order to investigate the contribution of the DLPFC during set-shifting and its effect on the striatal DAergic system, we applied continuous theta burst stimulation (cTBS) to left and right DLPFC. Our aim was to transiently disrupt its function and to measure MCST performance and striatal DA release during [11C]raclopride PET. cTBS of the left DLPFC impaired MCST performance and DA release in the ipsilateral caudate–anterior putamen and contralateral caudate, as compared to cTBS of the vertex (control). These effects were limited only to left DLPFC stimulation but not right DLPFC. This is the first study showing that cTBS, by disrupting left DLPFC function, may indirectly affect striatal DA release during performance of executive tasks. This cTBS-induced regional prefrontal effect and modulation of the frontostriatal network may be important for understanding the contribution of hemisphere laterality and its neural bases with regard to executive functions, as well as for revealing the neurochemical substrate underlying cognitive deficits.

EDAB lecture (in Polish)
DEMENTIA AND DEPRESSION – WHETHER THE SENESCENCE HAS TO LOOK LIKE THAT?

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Dementia is a neurological condition manifested by reduced cognitive and psychological functioning, altered behaviour and decreased autonomy for social and professional performance in activities of daily living. Dementia is one of the most important causes of disability in the elderly. The number of dementia patients is increasing, as the population is growing older. Recent epidemiological evidence suggested a 2001 prevalence of 24.3 million cases of dementia worldwide. Alzheimer's disease (AD) remains the most common cause of dementia, responsible for 60–70% of cases in Europe. There are 4.6 million new cases of dementia reported every year – that's one new case every 7 seconds. Patients with AD gradually lose the ability to function independently and interest in life and many experience changes in personality, social withdrawal, uninhibited behaviour, and psychotic symptoms such as delusions, hallucinations and aggressive behaviour. The associated likely impact on the health and quality of life of persons with AD, their families, and societal healthcare and welfare resources, have led many authors to describe the condition as a "ticking bomb". The depression prevalence in later life is 8 to 15%. Suicide in the elderly represents an immensely important and often overlooked medical problem. Based on its many distinct features, age-specific risk factors could be identified. Proper diagnosis and treatment of affective disorders – contributing greatly to the pathogenesis of suicide in the elderly – could lead to a major decline in the suicide rate in this population.

SYMPOSIUM I

Learning-Dependent Brain Plasticity: Systems View

SI.1
SYNAPTIC MECHANISMS OF SENSORY PERCEPTION

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A key goal of modern neuroscience is to understand the neural circuits and the synaptic mechanisms underlying simple forms of sensory perception and associative learning. Here, I will discuss our efforts to characterise sensory processing in the mouse barrel cortex, a brain region known to process tactile information relating to the whiskers on the snout. Each whisker is individually represented in the primary somatosensory neocortex by an anatomical unit termed a "barrel". The barrels are arranged in a stereotypical map, which allows recordings and manipulations to be targeted with remarkable precision. In this cortical region it may therefore be feasible to gain a quantitative understanding of neocortical function. As a mouse explores its environment, the whiskers are actively moved backwards and forwards as if searching the space for tactile input. Indeed sensory information in this pathway is usually actively acquired. It is therefore crucial to measure and manipulate cortical function in awake behaving mice. We have begun this process using whole-cell recordings, voltage-sensitive dye imaging, viral manipulations and two-photon microscopy. Through combining these techniques with behavioural training, our experiments provide new insight into sensory perception at the level of individual neurons and their synaptic connections.

SI.2
REORGANIZATION OF THE VISUAL CORTEX FOLLOWING MONOCULAR CIRCUMSCRIBED RETINAL LESIONS: UPDATE ON HEBBIAN LEARNING RULES

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Following circumscribed monocular retinal lesions most cells located in the lesion projection zone (LPZ) in the cat's primary visual cortices remain binocular but their receptive fields (RFs) revealed by stimulation *via* the lesioned eye, are displaced into proximity of the lesion. The process of topographic reorganization (adaptive learning) of the visual cortex, is based on spike timing-dependent plasticity rather than the classical Hebbian rules [Young et al. (2007) Nat Neurosci 10: 887–895]. The contrast sensitivities of the classical RFs of LPZ neurons revealed by stimulation *via* the lesioned eye are significantly lower, while the suppresive influences of their "silent", extra-classical RFs are weaker than those of their counterparts revealed by stimulation *via* non-lesioned eye. Other properties (e.g. orientation, spatial and temporal frequency preferences) however match well the properties of RFs revealed by stimulation *via* non-lesioned eye. These similarities are presumably visual experience-dependent, that is, they are related to the fact that in the post-lesion period (from adolescence to maturity), the neuronal activities originating from each retina are correlated by effectively synchronized exposure to the same visual stimuli. These results are consistent with the hypothesis that the ectopic RFs are based on enhancement of the synaptic efficacy of long-range, excitatory intracortical connections. *Financial support: Nencki Institute, Poland; NHMRC and ARC grants, Australia.*

SI.3
WHAT IS STORED IN THE HIPPOCAMPUS DURING A TACTILE CATEGORIZATION TASK?

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To understand how sensory experiences are stored in the brain, we examined neuronal firing in hippocampus during tactile behavior. Rats learned to associate stimulus texture with reward location; multiple textures were associated with the same reward location and thus formed a behavioral category. Rapid firing rate modulation carried texture identity information (10% of neurons), free from spatial and behavioral confounds; slow firing rate modulation carried behavioral category information (63% of neurons). Category information appeared during texture contact, simultaneous with an increase in theta power in the local field potential; it persisted or recurred during reward collection, when theta power was suppressed and "reward neurons" (8%) fired. Reward-triggered recurrence of category information could be a mechanism to link stimulus, action, and outcome when separated in time.

SI.4
THE LEARNING BRAIN – ACTIVATION DURING CLASSICAL CONDITIONING

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The neural bases of appetitive and aversive conditioning are different, and at various stages of learning may engage distinct cortical and subcortical networks. Using [^{14}C]2-deoxyglucose (2-DG) autoradiography we examined brain activation in mice during classical conditioning involving stimulation of whiskers on one side of the muzzle paired with an aversive or appetitive UCS. Both variants result in modification of cortical representations of vibrissae activated during the conditioning. Analysis of autoradiograms revealed that the nucleus basalis of Meynert (NBM) and ventral pallidum showed stronger labeling during appetitive training while the lateral hypothalamus (LH) and basolateral amygdala (BLA) were activated only by aversive learning. Apart from that, classicalconditioning with appetitive or aversive UCS increased 2-DG uptake in a similar set of brain structures – the posterior parietal cortex (PPC), cingulate (CG) and retrosplenial gyrus (RET), caudate nucleus (CPU) and nucleus accumbens (NA). Formation of sensory association, compared to pseudoconditioning, induces more activity in the subcortical sensory processing pathway (ventral postero-medial and posterior nuclei of the thalamus) but not in the barrel cortex. Also, conditioning contrasted with pseudoconditioning increases activity in structures important for cognitive and attentional functions (PPC, NA, CG, RET, CPU), which might provide the enhancing input necessary for memory trace formation.

SYMPOSIUM II

Using Neuroimaging to Understand Human Brain Function

SII.1
INSIDE BOLD – WHY NEURONAL ACTIVITY MODULATES MR SIGNAL?

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Although fMRI methods are well recognized as a powerful tool in neuroimaging, they still suffer from lack of repeatable quantitative measures of effect studied, especially in disease group studies, where pathologically changed brain morphologies or distortions caused by head movement arise. In such cases where typical statistical parametric mapping (SPM) approach based on general linear model (GLM) modeling fails, additional methods, giving complementary measures to standard statistical maps SPM may comprise an alternative approach. This subject covers two basic methodologies, which will be presented in this presentation: (1) A novel technique called Mean Regional Response (MRR) uses features derived from time-intensity curves in anatomically or functionally defined regions of interest (ROIs). Resulting features can be used for higher level analysis, like ANOVA or highlight differences between groups of subjects allowing inference about group separation; (2) Structural Equation Modeling (SEM) technique is focused on the extraction of connections between the active regions. Although SEM still do not allow arbitrary connection patterns, its use allows determination of time varying and bi-directional, asymmetric connections for groups of interest (patient vs. controls etc.). A methodological basics of BOLD modeling, which can be considered as a basis for most detection and classification methods, will be presented and illustrated with author's fMRI studies.

SII.2
NEURAL BASES OF PRAXIS AND TARGET-DIRECTED ACTIONS

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Neuroimaging evidence (Kroliczak and Frey 2009) indicates that, in right-handers, the left-hemispheric praxis representation network (PRN) constitutes a general domain neural substrate for the planning of meaningful actions, whether or not they involve objects. Less is known about the relationship between PRN and motor dominance in consistent left-handers. Nevertheless, more recent neuroimaging in healthy left-handed adults also shows that planning either transitive/tool use pantomimes or intransitive gestures is associated with increases of activation in the same cortical regions. In addition to the left parietal cortex, however, these increases tend to be more bilateral and involve the dorsal premotor and pre-supplementary motor cortices, as well as the rostral middle frontal gyri. These data suggest that despite reversed motor dominance and more bilateral PRN, planning any meaningful actions might be represented in a common network. The results will be discussed in the context of cortical areas mediating real, target-directed actions.

SII.3
THE NEURAL CORRELATES OF LANGUAGE PROCESSING AND ITS DISORDERS OVER THE LIFE SPAN

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I first present the results of a number of fMRI studies investigating the left-lateralised language network in the human brain. The focus will be here on phonological and lexical processing, which both recruit Broca's region. These data are summarised in a model which serves as framework for the subsequently presented data. Next, I explore commonalities and differences between primary school children and adults with respect to lexical and phonological processing. In the children, the same networks are involved, but are less specialised as compared to adults. Then, neurocognitive differences between dyslexic and normally reading children for lexical and phonological processing as well as for other cognitive functions relevant for reading are discussed. These functions include auditory and visuo-magnocellular processing, attention, and automatisation. Multivariate testing of these functions reveals different cognitive subtypes of dyslexia. Consequently, a new training approach is outlined. With respect to cognitive functions relevant for reading, fMRI reveals consistent right-hemispheric involvement for dyslexic children, which is regarded to reflect compensatory processes. Finally, a transfer is made to adult dyslexic processing and to linguistic difficulties in vascular and neurodegenerative aphasia in middle and older age. *This research is funded by BMBF 01GJ0613/01GJ0614/GJ01203907, DFG HE 5204/3-1, and JARA-BRAIN Excellence Initiative.*

SII.4
NEURAL CORRELATES OF FALSE RECOGNITION

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The neuronal basis of false recognition is still a subject of extensive debate. We used voxel based morphometry (VBM) to examine structural brain correlates of false and correct recognition processes. Since several studies indicate that emotional content facilitates false recognition we employed emotional stimuli taken from International Affective Picture System (IAPS). Behavioral measures, i.e., true and false recognition rates were used as covariants in VBM analyses. VBM results indicated that the true recognition correlated positively with grey-matter (GM) density in bilateral amygdala, anterior cingulate and middle temporal gyrus, i.e., brain regions, involved in the memory of emotional material, as revealed by fMRI results. False recognition correlated negatively with GM density in prefrontal areas (BA47 and BA9), which were implicated in false recognition by our previous fMRI study. Taken together, these results support the role of the prefrontal cortex in monitoring retrieval and limiting the probability of false recognition. Our VBM findings (1) point to the brain structures critical for correct and false recognition of emotional stimuli and (2) disclose structural differences between these processes.

Workshop

Quantitative Analysis of Protein–Protein Interactions

W1
NON-NEGATIVE MATRIX FACTORIZATION: A NOVEL METHOD TO DETERMINE REFERENCE SPECTRA OF FLUORESCENT DYES IN A CELLULAR ENVIRONMENT

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All spectrally resolved fluorescence-based methods depend critically on the availability of accurate reference spectra. Very often spectra in a cellular environment do not agree with those measured in a cuvette. Also, for a given measurement instrument-specific corrections have to be applied. We recently developed a method to extract reference spetra from standard fluorescence image stacks, such as those provided by a Zeiss Meta microscope. This method also allows one to separate spectral components of several simultaneously present dyes on a pixel by pixel basis. It should be ideal to complement lux-FRET measurements, providing reference spectra in the presence of autofluorescence.

W2
QUANTITATIVE MEASUREMENTS OF cAMP CONCENTRATION WITH A NEW EPAC BASED FRET-SENSOR

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FRET-based biosensors can be used for the analysis of intracellular signalling, including sensors for monitoring cyclic AMP. The concept is that the donor/acceptor emission ratio excited at the donor excitation wavelength can be used as a concentration dependent measure in donor/acceptor 1:1 tandem biosensors. General problem, however, is that this ratio varies not only with the changes in cAMP concentration, but also with the changes of the ionic environment or other factors affecting the folding probability. We are developing a novel Epac Based FRET-Sensor that is much less sensitive to changes in ion concentrations to obtain a reliable measure of the absolute cAMP concentrations under patho-/physiological conditions with high temporal and spatial resolution. Such data will be correlated with electrophysiological measurements obtained under pathological as well as physiological conditions.

W3
STIMULATION-DEPENDENT CHANGES IN OLIGOMERIC CONFORMATION OF SEROTONIN 5-HT_{1A} RECEPTORS

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An increasing number of G protein-coupled receptors (GPCRs) have been shown to form oligomeric units in the plasma membrane. In many cases there is evidence that this oligomerization can result in altered receptor pharmacology, desensitization and trafficking. These alterations can consequently result in different effects in intracellular signalling, thus diversifying the functional role of a GPCR. The serotonin (5-HT)1A receptor is a GPCR involved in multiple processes, among which, the regulation of neurogenesis, respiratory control, as well as depression and anxiety states have found the most interest. Biochemical experiments performed in N1E-115 neuroblastoma cells have suggested that 5-HT_{1A} receptors form homooligomers. Acceptor-photobleaching allows for the direct measurement of apparent FRET between the fluorophores of CFP- and YFP-tagged proteins, further suggesting some specific interaction between the receptors. Furthermore, with the use a novel FRET quantification method, we have also been able to discriminate between specific and random protein-protein interaction and to verify dynamics of homo- and hetero-oligomerization between 5-HT_{1A} and 5-HT_7 receptors.

W4
LUX-FRET METHOD FOR QUANTITIVE ANALYSIS OF FRET SIGNAL

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A novel method for spectral analysis of FRET-signals in living cells will be presented. The method allows for the determination of exact stoichiometry of interacting molecules as well as apparent FRET efficiency from two-wavelength measurement. Further considerations allow us to predict the influence of incomplete labeling of interacting partners. The method is based on information obtained from the full fluorescence emission spectra. In addition our analysis explicitly takes into account the contributions from simultaneously present free donors, acceptors and FRET-pairs, as well as the effects of non-functional fluorophores. The determination of apparent FRET efficiency in our method does not require, as in some other methods, acceptor photobleaching experiments, fluorescence lifetime measurements nor cell fixation. The method presented here can be directly applied to individual pixels of a fluorescence image in fluorescence microscopy image analysis.

W5
HIGH-RESOLUTION LIGHT-MICROSCOPIC STUDIES OF SYNAPTIC EXTRACELLULAR PROTEOLYSIS

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Proteolytic remodeling of perisynaptic environment, including extracellular matrix and adhesion molecules, is a novel important mechanism of synaptic plasticity. Among several enzymes participating in the phenomenon, the major role is played by matrix metalloproteinase 9 (MMP-9), a principal neuronal MMP. Recent evidence indicates MMP-9 being involved in long-term potentiation, learning and memory formation, as well as in pathological plasticity underlying epileptogenesis. Here, I present the results of the light-microscopic studies on MMP-9 localization at the synapses *in situ*, in both central and peripheral nervous systems. The data have been obtained using fluorescent cytochemical techniques, including immunoflorescence, *in situ* hybridization and in situ zymography, with the aid of deconvolution-enhanced confocal microscopy. The results underscore the potential of light-microscope-based molecular morphology for studying synaptic function *in situ*.

SYMPOSIUM III

Learning-Dependent Brain Plasticity: Synaptic View

SIII.1
SUBREGIONAL SPECIFIC ROLE OF HIPPOCAMPAL SYNAPTIC PLASTICITY IN SPATIAL MEMORY PROCESSING: SIGNIFICANCE OF β-ADRENERGIC RECEPTORS

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Neural mechanisms for spatial memory formation are believed to comprise an integration of processes mediated by hippocampal synaptic plasticity in the form of long-term potentiation (LTP) and long-term depression (LTD). Novel space consists of several types of information, but that may evoke differential responses in individual hippocampal subregions. In CA1, CA3 and dentate gyrus (DG) subregions and at multiple types of hippocampal synapses (perforant path-DG, mossy fiber-CA3, Schaffer collateral-CA1) exploration of a novel empty environment facilitates the expression of robust LTP (>24 h) in freely behaving animals. In contrast, LTD facilitation is subregion- and synapse- specific and dependent on the nature of the cues. In the CA1 region, partially concealed, small, contextual cues facilitate LTD. LTD in DG is facilitated by large directional cues. Arousal, mediated by activation of the noradrenergic (NA) system is a critical factor in information acquisition and may enhance the encoding of novel spatial information. We observed that learning-facilitated induction of LTD is inhibited by β-adrenoreceptor antagonist, and facilitation of LTD and the encoding of spatial memory is elicited when the locus coeruleus is activated. These results suggest that β-adrenoreceptors may facilitate encoding of spatial information through synaptic plasticity in the hippocampus and that NA released by the locus coeruleus during novel exploration, is a key factor in effective information acquisition.

SIII.2
SENSORY LEARNING ENHANCES GABAERGIC SYNAPTIC TRANSMISSION IN THE BARREL CORTEX OF THE MOUSE

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The barrel cortex is part of rodent primary somatosensory cortex, engaged in processing tactile information from the vibrissae. This part of the cortex contains layer IV cytoarchitectonic units – barrels, each corresponds to one of the contralateral vibrissae. Short-term, classical conditioning paradigm, consisting of three daily sessions of tactile stimulation of a row of vibrissae paired with electrical shock to the tail, induces expansion of the representation of stimulated vibrissae, pronounced in layer IV of adult mice barrel cortex. This effect has been accompanied by elevation in mRNA and protein level of GAD67, selective up-regulation of GABA and GAD67 in neurons within centres of the barrels receiving inputs from stimulated vibrissae, and an increase in the number of GAD-immunoreactive puncta. To investigate whether classical conditioning affects synaptic transmission in layer IV of the barrel cortex we recorded spontaneous inhibitory postsynaptic currents (sIPSCs) using *ex vivo* brain slices prepared from adult mice previously subjected to conditioning. We show that this associative learning paradigm results in a selective increase in the frequency of sIPSCs in layer IV excitatory neurons located within the barrel representing stimulated vibrissae, evident 24 h after the end of training. These data indicate that aversive training results in a selective and long-lasting enhancement of GABAergic transmission within the cortical representation of stimulated vibrissae.

SIII.3
IMPACT OF SENSORY LEARNING ON GABA-ERGIC TONIC INHIBITION IN MOUSE BARREL CORTEX

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Sensory learning is known to affect neuronal function, morphology and synaptic connectivity. It has been shown that various forms of classical conditioning involving whisker stimulation induce morphological changes in respective barrels (altered expression of GABAARs, NMDARs and GAD67). More recent studies have shown that sensory learning enhanced the inhibitory synaptic transmission (Tokarski et al. 2007). Our aim was to follow up the impact of sensory learning on GABAergic currents in the barrel cortex by addressing the issue of tonic GABAergic inhibition. We have characterized the tonic currents in naive mice for different cell types (Regular Spiking, Regular Spiking Non-Pyramidal and Fast Spiking) and found that the values of currents are correlated with the neuronal cell type. Interestingly, classical conditioning (tactile whisker stimulation associated with electric foot shock) affected the value of tonic current in the cell specific manner. To further explore the underlying mechanisms, we have used a specific "superagonist" (THIP) for δ-subunit containing GABAA receptors. Our data indicate that observed here impact of sensory learning on tonic currents results from altered responsiveness of δ-subunit containing receptors. In conclusion, we provide evidence that behavioral training does affect the tonic inhibition in the barrel cortex. *Supported by the Ministry of Science and Higher Education grants PBZ/MNiSW/07/2006/02 and N401 028 32/0664.*

SIII.4
A TRANSIENT SWITCH IN THE SIGN OF NMDAR-DEPENDENT SYNAPTIC CHANGE DURING EXPERIENCE-DEPENDENT PLASTICITY *IN VIVO*

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Sensory experience can augment excitatory synaptic strength. Removal of all but one whisker from both sides of the mouse

face (single-whisker experience; SWE) leads to an increase in synaptic strength at layer 4-2/3 synapses as well as elevated whisker-evoked firing *in vivo*. Although this potentiation requires NMDAR-activation in its early stages, NMDAR-activation subsequently shift the sign of synaptic change to favor synaptic depression. Thus, timed administration of NMDAR-antagonists can facilitate experience-dependent increases in excitatory synaptic strength. Here we show that the transition to NMDAR-dependent LTD is transient during ongoing experience-dependent synaptic plasticity in the barrel cortex. Systemic injection of NMDAR-antagonists within the first 12 h of SWE yields a robust enhancement of synaptic strength at layer 4-2/3 synapses. In contrast, later injection (after 36 h of SWE) does not alter synaptic strength compared to vehicle-injected control animals. Thus, as synaptic changes are consolidated by longer periods of whisker plasticity, the window for NMDAR-dependent synaptic depression closes and strengthened synapses become resilient to biochemical cascades responsible for weakening. Such a process may insure that spurious increases in synaptic strength are not permanently incorporated into cortical networks.

SYMPOSIUM IV

Mechanisms, Functions and Modulation of Central Nervous System Inflammation

SIV.1
MICROGLIAL SENESCENCE AND DEGENERATION IN THE PATHOGENESIS OF NEURODEGENERATIVE DISEASE

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The role of microglia in Alzheimer's disease (AD) pathogenesis remains unknown. Although many studies maintain that chronic microglial activation in the AD brain is detrimental and contributes to neurodegeneration, anti-inflammatory drugs show little promise for AD treatment or prevention. Studies provide support for the microglial dysfunction hypothesis, which postulates that the neurofibrillary degeneration of AD is the result of declining microglial neuroprotection resulting from aging-related microglial senescence and degeneration. I will report histopathological findings from humans covering the spectrum from none to severe AD pathology, including patients with Down syndrome, showing that degenerating neuronal structures positive for tau, invariably colocalized with severely dystrophic rather than with activated microglial cells. Using Braak staging of AD neuropathology, I will demonstrate that microglial dystrophy precedes the spread of tau pathology. Amyloid deposits devoid of tau-positive structures are found to be colocalized with non-activated, ramified microglia, suggesting that amyloid does not trigger microglial activation. The findings indicate that when microglial activation does occur in the absence of an identifiable acute CNS insult, it is likely to be the result of systemic infectious disease. These findings strongly argue against the belief that neuroinflammatory changes contribute to AD dementia. They may profoundly influence future treatment approaches.

SIV.2
MANIPULATING INFLAMMATION AND GLIOSIS IN NEUROLOGICAL DISEASES – A CLINICAL PERSPECTIVE

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In many neurological diseases, an inflammatory reaction in the brain is observed. In some of them, as in multiple sclerosis, the brain inflammation is a result of a systemic autoimmune response. In others, like the Parkinson's disease, Alzheimer's disease, brain injury and stroke, an inflammation and glial proliferation in the brain are a secondary phenomenon to neural degeneration or injury. The role of these reactions in the pathogenesis of neurological diseases is unclear. From one side they can contribute to greater neurological deficit. From the other side, inflammatory cells may release trophic factors and contribute to neuroprotection and neuroregenaration. Experimental works provide some evidences that pharmacological inhibition of brain inflammation and glial proliferation may be protective but also may be harmful. The influence of anti-inflammatory therapies on the progression of neurological diseases depends on a dose and time of the treatment after injury. Therefore, a clinical use of drugs influencing inflammation and glial proliferation is very difficult. Despite many anti-inflammatory therapies used in neurological diseases at present, there is no clinical evidence of their beneficial effects. Various immunomodulatory therapies have been tested in multiple sclerosis, but only some modest modifications of clinical course are observed. Similarly, in neurodegeneration like in the Parkinson's disease, non-steroidal anti-inflammatory drugs do not change disease progression.

SIV.3
GLIA AND INFLAMMATION IN THE PARKINSONIAN SUBSTANTIA NIGRA

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Microglia are the resident sensors of pathology in the central nervous system. Their main function is to control tissue damage and to engage in tissue repair processes. The exact role of microglial cells in neurodegenerative disorders is unknown. Based on their expression of some molecules found in established inflammatory disease states it has been suggested that microglia exert tissue destructive functions in Parkinson's disease (PD). We have indeed observed a correlation between α-synuclein (αSN) deposition and MHC class II-expressing microglia in the substantia nigra in PD but it remains unclear whether there is a pathogenetic link. In addition, in the absence of infiltrating T-cells which characterise chronic inflammatory or autoimmune conditions, e.g. CNS infections and multiple sclerosis, classical inflammation does not occur in PD. We therefore believe that "(micro)glial inflammation" represents a distinct process. This idea is supported by the up-regulation of CD163 by nigral microglia in PD suggesting a possible role of resident microglia in the down-regulation of inflammatory activity. Astrocytes in PD nigra strongly up-regulate their expression of the Hsp40

chaperone, DnaJB6 and they also increase their production of metallothioneins. They are thus in an activated state. The homocysteine-induced endoplasmic reticulum protein (Herp) may serve as a molecular link between neurodegeneration and the inflammation-like changes observed in PD.

SIV.4
MODULATION OF GLIA ACTIVATION IN NEUROPATHIC PAIN
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There is a growing body of evidence indicating that glial cells have causal role in the pathogenesis of pain hypersensitivity following nerve injury. We aimed to examine how chronic constriction injury (CCI) to the sciatic nerve influences glia activity markers, cytokine levels and expression of mGlu receptors in the spinal cord and dorsal root ganglia (DRG) as well as features of the neuropathic pain, such as allodynia and hyperalgesia in rats and mice. Our study showed that glial markers (C1q, GFAP), interleukins (IL-1β, IL-6) and mGlu receptor (mGlu5, mGlu3) mRNAs were strongly elevated ipsilaterally in the spinal cord after CCI. Microglial cells were more activated in the spinal cord in contrast to DRG where astrocytic activation prevailed. In the ipsilateral DRG, IL-1α, IL-6, and IL-10 mRNA levels were increased. Western blot analysis showed the presence of IL-1α protein in the DRG and down-regulation of these proteins after CCI. Minocycline and/or pentoxifylline administration reversed the injury-induced changes in glia markers and mGlu receptor mRNAs and protein levels, and significantly attenuated CCI-induced allodynia and hyperalgesia. The implication of glial activation paralleled with upregulation of mGlu receptors in neurodegenerative processes suggests that pharmacological targeting of spinal microglia in chronic pain may provide an effective therapy for controlling clinical pain syndromes. *Support: statutory funds of the Institute of Pharmacology, PAS.*

SYMPOSIUM V

Stress-Response Systems in the Pathogenesis and Prognosis of Cancer, Inflammation and Autoimmune Diseases

SV.1
STRESSOR EFFECTS ON IL-10 AND IFN-γ PRODUCTION ARE MEDIATED BY β-ADRENERGIC AND GLUCOCORTIOCID RECEPTORS AND ATTENUATED BY CHLORDIAZEPOXIDE
Curtin N.[1], Boyle N.[1], Griffin E.[1], Mills K.[2], Connor T.[1]
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Research indicates that psychological stress can modulate immune responses. Here we demonstrate that restraint stress suppresses interferon (IFN)-γ production and signalling in mice following *in vivo* lipopolysaccharide (LPS) treatment. Stress also suppressed production of the IFN-γ inducing cytokine interleukin (IL)-12 and increased production of the anti-inflammatory cytokine IL-10, which can inhibit both IL-12 and IFN-γ production. Studies with IL-10 knockout mice demonstrate that IL-10 does not mediate the suppressive effect of stress on IFN-γ production. Consistent with a role for glucocorticoids in the immunosuppressive actions of stress, the glucocorticoid receptor antagonist mifepristone blocked the stress-related suppression of IFN-γ. In contrast, the stress-induced increase in IL-10 was blocked by the beta-adrenergic receptor antagonist nadolol. Addition of IL-12 to LPS-stimulated spleen cells reversed the suppressive effect of stress and corticosterone on IFN-γ production, suggesting that reduced IL-12 production is a key event in stress-induced suppression of IFN-γ production. Finally, we demonstrate that treatment with the anxiolytic drug chlordiazepoxide prevents the ability of stress to increase IL-10 and suppress IFN-γ production.

SV.2
STRESS, EMOTION, AND NATURAL KILLER CELL ANTITUMOR ACTIVITY
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Neoplasia is one of the paradigmatic psychoneuroimmunology (PNI) disorders, and several studies showcased clinical associations between behavioral and emotional states and cancer and between those states and the immune system. If clinically relevant PNI effects on cancer were to be found, they would likely depend on selection of appropriate parameters: tumors susceptible to identified immunologic functions; immune effects acting at appropriate points in the natural history of neoplastic disease, and effective immunomodulatory intervention. Animal studies suggest that natural killer (NK) cells are important for cancer control, especially with respect to dissemination and growth of metastasis. Surgical and psychological stress have been reported to suppress NK activity (NKCC) in animals and humans and this suppression was shown to compromise animal resistance to tumor progression. Studies

published by Ben-Eliyahu showed powerful model of stress-related NK-dependent metastasis. Metastatic progression in their MADB106 mammary adenocarcinoma model is related to surgical stress, swim stress, hypothermia or social confrontation and stress hormone-induced suppression of NKCC that is not dependent on physical dissemination of the primary tumor. Moreover, perioperative suppression of NKCC in the immediate postoperative period may contribute in part to the risk of subsequent emergence of metastasis, and perioperative interventios that reduce this risk may improve patient outcome.

SV.3
NEURAL PATHWAYS SIGNALING CANCER AND INFLAMMATION: INTERFACE WITH STRESS-RESPONSIVE BRAIN REGIONS
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Challenges to the body's host defense, such as infections, cancer, or inflammation serve as physiological stressors. For the body to respond appropriately, the brain must be alerted to the challenge, and initiate appropriate host defense responses. To this end, viscerosensory pathways detect immune- or pathogen-related signals, and convey this information to stress response systems in the brain. The viscerosensory pathways from the body involve both neural and humoral mechanisms. Of the neural pathways, the vagus is most important for signaling the brain. In addition, spinal nerves signal pain and inflammation in a parallel pathway. Immune and cancer related signals activate "danger pathways" originating in the caudal brainstem that drive neuroendocrine (HPA) responses to stress *via* their terminations in the PVN. These pathways involve primarily catecholaminergic neurons in the ventrolateral medulla and rostral nucleus of the solitary tract. A separate pathway from area postrema targets the parabrachial nucleus of the pons, which in turn influences other stress-sensitive forebrain regions including the paraventricular thalamus, the central amygdala and bed nucleus of the stria terminalis. The medial prefrontal, anterior cingulate, and insular cortex also contribute to the neural network that responds to different categories of stressors, such as host defense. Together these brain regions co-ordinate physiological and behavioral adjustments to homeostatic challenges.

SV.4
CELL LOSS AND IMPAIRED NEUROPLASTICITY IN PREFRONTAL CORTEX AND HIPPOCAMPUS IN MAJOR DEPRESSIVE DISORDER (MDD)
Stockmeier C.[1], Mahajan G.[1], Kooiman H.[1], Austin M.[1], Szewczyk B.[2], Iyo A.[1], May W.[1], Rajkowska G.[1]
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Studies in *postmortem* brain reveal changes in the density and size of neurons and glia in cerebral cortex and the hippocampus. Molecular mechanisms may underlie these changes and volume decreases in depression. *In situ* hybridization and immunohistochemistry were used to estimate expression of mRNA for tyrosine kinase B (TrkB), length of axons expressing the serotonin transporter (SERT) and expression of polysialylated neural cell adhesion molecule (PSA-NCAM) in the hippocampus of subjects with MDD and control subjects. In MDD, there was a significant decrease in expression of mRNA for TrkB, the receptor for brain-derived neurotrophic factor, in CA2/3, and in the mean total length of axons expressing SERT-immunoreactivity (IR) in CA1. Area fraction of PSA-NCAM-IR was significantly increased in the hilus only in MDD with an antidepressant prescription.Decreased expression of TrkB receptor mRNA may affect cell survival and synaptic plasticity in depression. The decrease in length of axons expressing the serotonin transporter suggests a decrease in neuropil or in serotonergic innervation of CA1 in depression. The role of PSA-NCAM in establishing synaptic contacts and regulating neurite growth may be increased in subjects treated for depression. Cellular changes at the microscopic level plus neuroimaging changes detected *in vivo* provides an integration of clinical and basic research for disentangling the pathophysiology of depression.

SYMPOSIUM VI

Genes and Behavior

SVI.1
IT IS SAID THAT "ALMOST MAKES DIFFERENCE": ARE DIFFERENT STRAINS OF MICE THE SAME?
Juszczak G.
Department of Animal Behavior, Institute of Genetics and Animal Breeding, Jastrzębiec, Poland

Both inbred (A/J, AKR, Balb/c, C3H, C57BL/6, DBA/2, FVB) and outbred (Swiss Webster, CD-1) strains of mice are commonly used in *in vivo* and *in vitro* experiments. Behavioral and pharmacological comparisons of different strains of mice revealed high variability in a number of behavioral parameters and in sensitivity to pharmacological treatments. Between-strain differences were observed in sensitivity to antidepressant drugs, morphine, MK-801, kainate and amphetamine. There are both highly sensitive strains and strains resistant to action of drugs. Less often are observed opposite reactions to pharmacological treatments. The genetic background is, therefore, an important factor contributing to variability of results obtained in different laboratories using different strains of mice. This problem is important not only in behavioral pharmacology but also in *in vitro* experiments and in genetic engineering.

SVI.2
STRESS AND ADAPTATION IN BRATTLEBORO RATS LACKING ARGININE-VASOPRESSIN
Zelena D.[1], Tímár J.[2], Bagdy G.[3]
[1] Behavior and Stress Studies, Institute of Experimental Medicine HAS, Budapest, Hungary; [2] Department of Pharmacology and Pharmacotherapy, [3] Department of Pharmacodynamic, Semmelweis University, Budapest, Hungary

Vasopressin (AVP) has fundamental role in the regulation of the hypothalamo-pituitary-adrenal axis, the main target of stress and adaptation. Its role is especially important during chronic load, which can induce anxiety and depression. The

importance of AVP in stress and related mood disorders were studied in spontaneously mutated AVP-deficient Brattleboro rats (di/di). AVP was confirmed to be a prominent regulator of the adrenocorticotrop hormone secretion in wide range of stresses. Accompanied corticosterone elevation was reduced in di/di rats just occasionally and remained elevated longer. Endogenous AVP acts as a paracrine signal to facilitate the return of plasma corticosterone to basal levels. Behavioral studies confirmed that di/di animals were less anxious in the defensive withdrawal test and revealed lower depressive indeces during forced swimming. Thus, AVP-antagonists are promising targets for drug development.

SVI.3
MEASUREMENT OF PERSISTENTLY EXPRESSED BEHAVIOURAL TRAITS IN OUTBRED RATS: UNDERLYING GENE EXPRESSION PATTERNS
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Affective disorders are often accompanied by changes in anxiety and motivation to engage in active behaviours. We have developed an exploration-based behavioural test which allows distinguishing between rats belonging to clusters with persistently high anxiety/low motivation to explore the environment and low anxiety/high motivation to explore (LE- and HE-rats, respectively). The LE-rats are consistently more anxious and use passive coping strategies in several tests of affective disorders, and have concordant deviations in the monoaminergic neurochemistry and brain oxidative metabolism. Genome-wide microarray analysis of gene expression in three brain regions known to be involved in mood disorders (raphe, hippocampus, and the frontal cortex) revealed significant alterations in the expression of several neurotransmission-related genes expected to be relevant to affect (e.g. downregulation of *Gabra1*, *Gabra4*, *Gabrg1*, *Gabrg2*, *Glrb*, *Htr1a*, *Htr2c*, *Tph2*, and upregulation of *Gria3*, *Grin2a*, and *Cnr1*) in the LE- vs. HE-animals. Also, overrepresentation of differentially expressed genes involved in the Wnt signalling pathway in all three brain regions, and the MAPK signalling, long-term potentiation and long-term depression pathways in the hippocampus and the frontal cortex suggests that the behavioural differences between rats with persistently high or low exploratory activity may be related to differences in synaptic plasticity in the raphe nuclei as well as its projection areas.

SYMPOSIUM VII

Stem Cells Based Therapeutic Approaches

SVII.1
ARTIFICIAL NEURAL TISSUE FROM CORD BLOOD FOR TISSUE REPLACEMENT THERAPY OF CENTRAL NERVOUS SYSTEM
Jurga M., Ali H., Forraz N., McGuckin C.
Department of Neurobiology, Cell Therapy Research Institute, CTI-Lyon, France

Tissue engineering gives new tools for stem cell-based regenerative medicine and *in vitro* toxicology. Among different sources of stem cells human cord and cord blood are safe, clinically and ethically approved and easily obtainable sources of pluripotent stem cells able to differentiate into many tissues originated from 3 germ layers. Recently we showed that functional artificial neural tissue can be generated from pluripotent stem cells derived from freshly isolated human cord and cord blood stem cells in fully-defined culture conditions. We showed that cell starving and precise sequential introduction of single growth factors and morphogenes resulted in differentiation of pluripotent stem cells towards mature neurons in scaffold-based three-dimensional environment. The pluripotent stem cells decreased expression of the key pluripotency transcription factors: Oct4A, Sox2, Nanog, c-Myc, and acquired phenotypes of neuroblasts (Nestin+/GFAP+) followed by generation of mature neuronal networks (NeuN+, PSD95+, TUJ1+, S100beta). Finally we showed that cell–cell interactions within the 3D environment of artificial neural tissue were crucial for functional, electrical and calcium, activity of neural networks *in vitro*. We conclude that tissue-engineering approach is important for generation of functional neural tissue *in vitro* from cord and cord blood stem cells and therefore might be useful for future therapies of central nervous system.

SVII.2
FUNCTIONAL NEUROGENIC POTENCIES OF HUMAN UMBILICAL CORD BLOOD-DERIVED MULTIPOTENT STEM CELLS THROUGH MULTIPLE MECHANISMS IN MICE WITH NEURODEGERATIVE DISEASES
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Stem cell transplantation in neurodegenerative diseases including Alzheimer's disease has been reported. In this study, we hypothesized that human umbilical cord blood-derived multipotent stem cells (hUCB-MSCs) have the ability to differentiate into functional neurons in the brain. To test this hypothesis, we verified this assumption in a mouse model of Niemann-Pick type C (NPC1-/-) disease using hUCB-MSCs. hUCB-MSCs were transplanted into the hippocampus of asymptomatic mice with NPC1-/- disease. The transplantation resulted in the recovery of motor function and dramatically prolonged the survival of the NPC1-/- mice. Interestingly, many of the transplanted hUCB-MSCs showed microtubule associated protein 2-positive cells with electrophysiological function in the brains of the NPC1-/- mice. To examine if the hUCB-MSCs affect endogenous cell

survival in the brain, we performed western blotting using mice brains. These results showed that the hUCB-MSCs can activate the Phosphoinositide 3-kinase-Akt-glycogen synthase kinase 3 beta ser9 and JAK2-Stat3 pathways for neuronal survival and regeneration in the brain by secreting functional factors. We concluded that hUCB-MSCs could contribute to extending the life of the NPC1-/-mice with the recovery of motor function by the differentiation into functional active neurons and cell survival signal cascade. Therefore, it is suggested that hUCB-MSCs may be applicable in a broad range of neurodegenerative diseases.

SVII.3
EMBRYONIC AND ADULT STEM CELLS FOR TREATMENT OF STROKE
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Grafted stem cells may confer beneficial effects to the recipient brain in a twofold manner: (1) they produce a cocktail of trophic factors and thus promote endogenous repair mechanisms and also act neuroprotective for endangered neurons and (2) they differentiate into neurons and glial cells and replace dead cells. We have transplanted pre-differentiated mouse EGFP+ embryonic stem cells 7 days after experimental stroke into rats. After 4 weeks cells had been differentiated into neurons and astrocytes. We detected several sub-types of neurons by means of neurotransmitter expression and electric activity. However, cells displayed limited migratory activity and cell survival declined substantially after 12 weeks. Bone marrow stromal cells (MSC) have been described to be neuroprotective. We found that MSC are neuroprotective to organotypic hippocampal slice cultures subjected to an oxygen-glucose deprivation (OGD) even without having direct cell–cell contact. This effect was increased if MSC were treated by complete serum deprivation before delivery to OGD treated slices. Interestingly, OGD damaged slices stimulated the MSC to produce certain growth factors like bFGF and NGF. A complete serum-deprivation induces the generation of a subpopulation of small-sized cells expressing nestin, GFAP, Sox-2 and Oct4 and by this resembling both neural and embryonic stem stem cells. It remains open whether this sub-population has the potential to differentiate into neuroectodermal cells.

SYMPOSIUM VIII

Cholinergic System in Neurodegereneration

SVIII.1
AMYLOID PRECURSOR PROTEIN AND CHOLINESTERASES IN BRAIN FUNCTION AND NEURODEGENERATION
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Amyloid precursor protein (APP) and acetylcholinesterase (AChE) are membrane-bound proteins possessing a variety of roles in brain development and functioning. While APP is mostly known as a protein producing amyloid β peptide (Aβ) both AChE and butyrylcholinesterase (BuChE) are generally linked to cholinergic neurotransmission. All three proteins have soluble forms which, despite their different origin, are believed to play a role in neurogenesis, synaptogenesis and neuroprotection. There is evidence that APP and AChE can form complexes in the membrane and both AChE and BuChE are found in tight association with Aβ in senile plaques characteristic for Alzheimer's disease. Under certain circumstances AChE is shed from the neuronal membrane by an enzymatic process similar to that involved in the non-amyloidogenic shedding of APP. Our data demonstrated that the content of membrane bound and soluble forms of APP, AChE and BuChE significantly increase in various brain structures of rats during the first month of postnatal life suggesting their role in brain development and synaptogenesis. The levels of soluble and membrane-bound forms of these proteins were affected by prenatal hypoxia with subsequent developmental deficit observed in these animals. The molecular mechanisms of the APP and AChE shedding and their involvement in the pathogenesis of AD will also be discussed. *Supported: the UK MRC, RAS "Fundamental sciences to medicine".*

SVIII.2
CHOLINOTOXICITY OF β-AMYLOID
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Many Alzheimer patients demonstrate also cerebral amyloid angiopathy and microvascular degeneration. The upregulation of the angiogenic vascular endothelial growth factor (VEGF) in brains of Alzheimer patients in close relationship to β-amyloid plaques, suggests a link of VEGF action and processing of the amyloid precursor protein (APP). As the brain vascular system underlies a functional control by basal forebrain cholinergic terminals, the question arises of whether there is a relationship between damage of brain capillaries, VEGF upregulation, and cortical cholinergic denervation. Tg2576 mice, that express the Swedish double mutation of human amyloid precursor protein and progressively develop β-amyloid deposits, were used to study age- and β-amyloid related changes in cerebral cortical microvessels, VEGF level, and associated cholinergic terminals. Treatment of brain slices with VEGF affected the formation of soluble β-amyloid, while the overproduction of β-amyloid led to significant decrease of cholinergic fibre density in cortical regions of aged transgenic mice. The data further sug-

gest that changes in cholinergic innervation of microvessels may contribute to pathological alterations of the cerebrovascular system. *Supported by IZKF, University Leipzig, and Alzheimer Forschungsinitiative (AFI).*

SVIII.3
PROTEOLYTIC CASCADES IN THE BRAIN AND THEIR CHOLINERGIC REGULATION: ROLE IN NEURODEGENERATION

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Progressive dysfunction of cholinergic neurotransmission in selected brain regions contributes to the cognitive and behavioural disturbances in Alzheimer's disease although the underlying mechanisms remain unclear. The amyloid β-peptide (Aβ) is a major player in Alzheimer progression and its levels are modulated by the cholinergic system. The level of Aβ in the brain represents a dynamic equilibrium state as a result of its biosynthesis from the amyloid precursor protein (APP) by β- and γ-secretases, its alternative processing by α-secretases and its degradation by a team of amyloid-degrading enzymes. The first and rate-limiting step in amyloid synthesis, mediated by the membrane-bound β-secretase occurs in lipid raft domains and is modulated by other interacting proteins, e.g. the prion protein. Alternatively, and by far the major metabolic route for APP, is its processing in non-raft domains, to release the soluble extracellular domain, sAPPα, in a process mediated by a cohort of ADAMs zinc metalloproteases and regulated through cholinergic signalling pathways. We have used a variety of biochemical and genetic techniques to identify the proteases involved in these processes, their regulation and their subcellular localizations. Manipulation of cholinergic signalling could provide one strategy for reducing amyloid burden alongside direct targeting of the amyloid-forming and -degrading enzymes. *This work was supported by the UK Medical Research Council.*

SVIII.4
PHENOTYPE AND ENERGY DEPENDENT CHOLINOTOXICITY

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Cognitive deficits in Alzheimer's disease (AD) are caused by preferential loss of septal cholinergic neurons. We postulate that utilization of acetyl-CoA for ACh synthesis creates its deficit for energy production in cholinergic neurons. Therefore, we investigated whether higher expression of the cholinergic phenotype could make these neurons more susceptible to putative AD pathogens. Chronic exposure of nondifferentiated (NC) and differentiated (DC) cholinergic septal neuroblastoma SN56 cells to amyloid-β, NO excess and Al inhibited pyruvate dehydrogenase (PDH) and other oxidative enzymes activities in, with similar potencies. However, they caused much greater decrease of acetyl-CoA content, cholinergic functions and viability in DC than in NC, respectively. On the other hand, both in NC and DC, Zn caused acute inhibition of PDH, aconitase and NADP isocitrate dehydrogenase, with similar K_i values of about 0.058, 0.010 and 0.005 mM, respectively. Unexpectedly, Zn-K_i for ketoglutarate dehydrogenase (KDH) in DC was 0.0005 mM, whereas in NC 0.0040 mM, respectively. Significant correlations were found between acetyl-CoA in mitochondria and mortality as well as between cytoplasmic acetyl-CoA and expression of the cholinergic phenotype in SN56 cells. We postulate that these two partially independent pools of acetyl-CoA affect survival and transmitter functions of cholinergic neurons, respectively. *Supported by MNiSW grants 2P05A 11030, NN401139933 and AMG project St-57.*

SYMPOSIUM IX

Protein Toxicity and Molecular Mechanisms of Neurodegenerative Diseases

SIX.1
REGULATION OF NEUROTRANSMITTER SECRETION BY SPHINGOSINE-1-PHOSPHATE

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Neuronal activity greatly influences the formation and stabilization of synapses. Although receptors for sphingosine-1-phosphate (S1P), a lipid mediator regulating diverse cellular processes, are abundant in CNS, neuron-specific functions of S1P remain largely undefined. Here we present novel actions of S1P using primary hippocampal neurons as a model system, i.e. S1P triggers neurotransmitter secretion in a depolarization-independent manner. Sphingosine kinase 1 (SK1), a key enzyme for S1P production, was enriched in functional puncta of hippocampal neurons. Silencing SK1 expression by siRNA resulted in strong inhibition of depolarization-evoked glutamate secretion. FRET analysis demonstrated that S1P1 receptor at the puncta was activated during depolarization and that depolarization-induced S1P1 receptor activation was inhibited in SK1-knockdown cells. Importantly, exogenously added S1P at nanomolar concentration by itself elicited glutamate secretion from hippocampal cells even when Na^+-channel was blocked by tetrodotoxin, suggesting that S1P acts on presynaptic membranes. These findings indicate that S1P, through its autocrine action, facilitates spontaneous glutamate secretion. We are now confirming these findings using electrophysiological approaches and also studying the role of S1P in memory formation and learning using knockout mice.

SIX.2
HIV-1 AND AMYLOID BETA PEPTIDE INTERACTIONS AT THE BLOOD–BRAIN BARRIER LEVEL

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An increase in the older population infected with HIV-1 is an emerging development in HIV-1 epidemiology. Aging is connected with increased deposition of amyloid beta peptide (amyloid beta) in the brain. In the current study, we propose that amyloid beta and HIV-1 can potentiate their toxic effects at the blood–brain barrier (BBB)

level. To address this notion, we employed an *in vitro* model of human brain microvascular endothelial cells (HBMEC) directly exposed to HIV-1 or co-cultured with HIV-1 infected human monocytes. Exposure of HBMEC to amyloid beta (1-40) in the presence of HIV-1 resulted in a markedly increased amyloid beta binding/entry into HBMEC. We then hypothesized that HIV-1 may either increase binding/entry of externally added amyloid beta or elevate the amount of endogenously produced amyloid beta. The receptor for advanced glycation end products (RAGE) is known to be involved in the transport of amyloid beta across the BBB into the brain. RAGE immunoreactivity was stronger and RAGE protein levels were elevated in HBMEC exposed to HIV-1 as compared to control. In contrast, exposure to HIV-1 decreased expression of lipoprotein receptor related protein-1 (LRP1) which is the main receptor that transports amyloid beta from the brain to blood. These results indicate that HIV-1 can decrease the ability of the BBB to transport amyloid beta from the brain and thus predispose the brain to increased amyloid beta accumulation. *Supported by MH072567, MH63022, and NS39254.*

SIX.3
ALPHA SYNUCLEIN AND ITS NEUROTOXIC FRAGMENT NAC IN NEURODEGENERATION
Adamczyk A., Kazmierczak A., Strosznajder J.
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Alfa-Synuclein (ASN), a small (14 kDa) presynaptic protein abundant in the brain play significant role in pathogenesis of Parkinson's disease (PD) and is implicated in the other neurodegenerative disorders. The central domain of ASN, the non-amyloid β component of Alzheimer's disease amyloid (NAC) could be responsible for its toxicity. Our study showed the significant role of ASN/NAC in disturbance of dopamine transporter (DAT) and voltage-dependent calcium channels (VDCC) function in the brain. ASN-evoked disregulation of Ca^{2+} homeostasis leads to activation of neuronal nitric oxide synthase (nNOS). Our data indicated NO-dependent modification of several proteins involved in decision of cell life/death including caspase-3 and poly(ADP-ribose) polymerase 1 (PARP-1). The further study carried out on cells in culture presented ASN/NO dependent mitochondria failure and indicated that NO pool liberated by ASN activates caspase-3 that leads to PARP-1 degradation. Inhibitor of NOS (NNLA), caspase-3 (Z-DEVD-FMK) and a mitochondrial permeability transition pore blocker, cyclosporine A protected cells against ASN evoked cell death. Our results indicate that ASN/NAC leads to NO mediated mitochondria dysfunction and caspase-dependent programmed cell death. *Supported by the Ministry of Science and Higher Education Grant 2PO5A4129 and Scientific Network 28/E-32/BWSN-0053/2008.*

SIX.4
THERAPEUTIC APPROACHES TARGETING Aβ CASCADE IN ALZHEIMER'S DISEASE
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Alzheimer's disease (AD) is the most common cause of dementia, affecting nearly five million patients in the USA and 20 million worldwide. The β-amyloid (Aβ) cascade hypothesis maintains that accumulation of Aβ peptide is a critical event in the early pathogenesis of AD. This lecture will review key steps of Aβ metabolism and their disturbances leading to build-up of Aβ in the AD brain. Development of therapeutic approaches targeting Aβ cascade will be subsequently discussed. They include passive and active immunization against Aβ, inhibitors of β and γ secretases, RAGE inhibitors, β-sheet breakers and approaches targeting the apolipoprotein E (apoE). ApoE is a critical factor promoting Aβ deposition in the brain and affecting its clearance. The magnitude of the apoE/Aβ interaction is isoform specific, providing an explanation for the linkage between the apoE4 allele and an increased risk of sporadic AD. Our laboratory has demonstrated that blocking the apoE/Aβ binding with synthetic peptide-Aβ12-28P, which mimics the apoE binding site on Aβ, reduces the burden of vascular and parenchymal Aβ deposits in AD transgenic mice and prevents them from developing a memory deficit. Ongoing research pursues development of peptidomimetic derivatives of Aβ12-28P with improved therapeutic efficacy and biostability with the aim to obtain a lead therapeutic compound for clinical investigations. *Support: Dorothy D. Eweson Lectureship on the Advances in Aging Research, grants AG24847, AG31221.*

SYMPOSIUM X

Stroke-Induced Brain Reorganization

SX.1
EXPLORING THE CHANCES OF STEM CELL BASED REGENERATION AFTER STROKE BY MRI
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Stem cell based regeneration after stroke is a concept of investigating the chronic phase, following stroke and stem cell implantation. Therapeutic success is measured assessing functional deficit and improvement with functional brain activation studies by fMRI. We demonstrate an animal fMRI protocol to exclude spontaneous recovery as a confounding factor of therapeutic improvements. Stem cell implantation into the ischemic hemisphere of those animals without spontaneous recovery shows functional improvement beginning approximately ten weeks after implantation. This functional improvement is confirmed in electrophysiological recordings of somatosensory evoked potentials and supported by sensorymotor behavioral test results. Further experiments on transgenic mice allow to monitor neurogenesis of new cells migrating from the subventricular zone to the ischemic territory. For this new approach, a multimodal molecular imaging approach is used, combining optical and magnetic resonance imaging techniques *in vivo.*

SX.2
HOW PHOTOTHROMBOTIC STROKE AFFECTS EXTRACELLULAR MATRIX?
Nowicka D., Karetko M., Aleksy M., Skangiel-Kramska J.
Department of Molecular and Cellular Neuroscience, Nencki Institute of Experimental Biology PAS, Warszawa, Poland

Perineuronal net (PN), a lattice-like structure surrounding certain neurons, represents a special form of extracellular matrix of the brain. The role of this structure remains elusive. It has

been suggested to regulate neuronal plasticity, accumulate trophic molecules and protect neurons from damaging factors. Here we studied whether PN-enwrapped neurons are protected against ischemic injury. To this aim we performed cortical photothrombotic stroke in rats and investigated PNs and their co-localization with Fluoro Jade C (FJ), a marker of rapid cell death. We found that in the ischemic core 4 h after the stroke still PNs were observable, none of them around FJ-positive neurons. No PNs were detected after 24 h. In the peri-infarct area, a huge loss of PNs was observed 24 h after stroke, followed by a partial restoration 30 days post-infarct. Similar effect was found in remote cortical areas, except that in this case the restoration was almost complete already 7 days after stroke. No FJ staining was observed outside ischemic core. These results suggest that in areas that are not directly damaged, ischemic insult disrupts PNs but the neurons survive this disruption. *Supported by MNiSW Grant N3030300832/0474.*

SX.3
fMRI STUDY OF RECOVERY FROM UNILATERAL NEGLECT
Seniow J[1]., Polanowska K.[1], Marchewka A.[2], Jednoróg K.[2], Członkowska A.[1,3], Grabowska A.[2], Królicki L. [3], Kossut M.[2]
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Abstract not received

SX.4
BRAIN PLASTICITY FOLLOWING STROKE: POSSIBLE TARGETS FOR THERAPEUTIC INTERVENTION
Witte O.
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Abstract not received

SYMPOSIUM XI

Glutamatergic Receptors as a Target for Future Psychotropic Drugs Action

SXI.1
DIFFERENTIAL ROLES OF mGluR2 AND mGluR3 IN THE BRAIN
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Group II metabotropic glutamate receptors (mGluR2 and mGluR3) are negatively coupled to adenylate cyclase and cAMP transduction system. They decrease cAMP through a pertussis toxin-sensitive G-protein coupled mechanism. Recently we have shown that mGluR3 expressed in rat cerebellar granule cells and astrocytes also may be coupled through a G-protein mechanism to a cGMP transduction pathway. To test the hypothesis that mGluR2 and mGluR3 share a coupling to the cGMP pathway, their cDNA were transfected into C6 glioma cells, characterized by robust cGMP response, and their coupling to cAMP and cGMP transduction pathways was characterized. Consistent with data from neurons and astrocytes, mGluR3 stably expressed in C6 cells responded to agonists with reductions in cGMP levels. In contrast, the mGluR2 receptor was negatively coupled to cAMP in C6 cells but not to cGMP, indicating the functional difference between these two receptors. The coupling to cGMP signal transduction depends on the cell type – it is pertussis toxin-sensitive in the astrocytes and C6 mGluR3 cell line, while it is not in the cerebellar granule cells. In all investigated cell types the coupling was not mediated by the interactions with phosphodiesterase (IBMX-insensitive). Evidence that human mGluR3 (GRM3 gene) may be a susceptibility gene for schizophrenia and prospective therapeutic potential of group II agonists may speed up the development of selective agonists or positive allosteric modulators for these two receptors

SXI.2
SELECTED ASPECTS OF GROUP I mGlu RECEPTOR NEGATIVE AND POSITIVE MODULATORS
Danysz W.
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Several mGluR5 modulators have became available showing acceptable pharmacokinetics and selectivity. There are negative allosteric modulators such as MTEP, MPEP, or fenobam (NAMs) or positive allosteric modulators (PAMs) such as CDPPB or ADX47273. We performed a verification of the therapeutic potential of these modulators using various behavioural methods. mGluR5 NAMs produced analgesic effects in several pain models such as formalin, and Freund adjuvant model of inflammatory pain. Additionally they very strongly attenuated L-DOPA induced dyskinesia and produced anxiolytic activity in some models of anxiety such as fear potentiated startle, context freezing. However, efficacy was not better then respective indication reference agents. mGluR5 PAMs are expected to have antipsychotic-like activity and improve learning. These expectations can be in our hands only partially fulfilled for schizophrenia (positive effects in amphetamine-induced hyperactvitivity, apomorphine-induced prepulse inhibition, or conditioned avoidance response inhibition). No clear positive effect was observed in models of cognition. Recently, several clinical trials with metabotropic glutamate receptor type 5 negative

modulators showed positive effects in indications such as migraine, reflux and L-DOPA-induced dyskinesia. This regards ADX 10059 from Addex and AFQ056 from Novartis indicating that their introduction to clinical practice may be just a matter of short time.

SXI.3
CAN LIGANDS OF METABOTROPIC GLUTAMATE RECEPTORS BECOME ANTIPARKINSONIAN DRUGS?
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Symptoms of Parkinson's disease (akinesia, muscle rigidity and tremor) result from degeneration of dopaminergic nigrostriatal pathway. Furthermore, a shift of dopaminergic-glutamatergic balance towards overactivation of glutamatergic systems exists in this disease. Therefore, drugs which inhibit glutamatergic transmission were expected to have antiparkinsonian impact. Our studies showed that the blockade of the group I mGluRs (mGluR1 and 5) in the striatum decreased catalepsy and muscle rigidity in the haloperidol-induced animal models of parkinsonism, which seemed to be due to inhibition of the indirect striopallidal pathway. On the contrary, systemic injections of mGluR5 antagonists did not influence the tacrine-induced tremor and diminished functioning of the nigrostriatal pathway. Moreover, the mGluR1 antagonist strongly enhanced the tremor induced by harmaline. Agonists of groups II and III mGluRs exerted antiparkinsonian-like effects which differed depending on their brain targets. Agonists of the group II seemed to act at the level of the subthalamo-nigral glutamatergic synapses, but not in the striatum, whereas an antiakinetic effect of agonists of group III was strong in the globus pallidus and striatum, but weak or none in the substantia nigra. The above results seem to suggest that because of a wide distribution of mGluRs in the brain, ligands of these receptors may exhibit limited efficiency as antiparkinsonian drugs in humans.

SXI.4
METABOTROPIC GLUTAMATE RECEPTOR LIGANDS AS A POSSIBLE TARGETS FOR TREATMENT OF ANXIETY AND DEPRESSION
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Preclinical data indicated that antagonists of group I mGlu receptors, particularly antagonists of mGlu5 receptors, produced both anxiolytic-like and antidepressant-like effects. Clinical data also demonstrated that mGlu5 receptor antagonist, fenobam, was an active anxiolytic drug. The anxiolytic effects exerted by mGlu5 receptor antagonists are profound, comparable to or stronger than those of benzodiazepines. Among all mGlu receptor ligands, group II mGlu receptor agonists seem to be drugs with promising therapeutic potential and good safety profile. Animal studies showed anxiolytic-like effects of group II mGlu receptor agonists. Currently, group II mGlu receptor agonists are in phase III clinical trials for potential treatment of anxiety disorders. On the other hand, data have been accumulated indicating that antagonists of group II mGlu receptors have an antidepressant potential. Group III mGlu receptor ligands represent the least investigated group of mGlu receptors. The preclinical data, however, indicate that a selective agonist of $mGlu_4$ and Glu_8 receptors- ACPT-I produced anxiolytic but not antidepressant effects after peripheral administration, while the selective positive allosteric modulator of Glu_7 receptors, AMN082 produced both anxiolytic and antidepressant-like. The data show that the effects of group III mGlu receptor ligands may differ dependently of their receptor subtype specificity

SYMPOSIUM XII

Interaction Between Antidepressant Drugs and Immunoendocrine Systems

SXII.1
MICE WITH ALTERED GLUCOCORTICOID RECEPTOR EXPRESSION AS A MODEL FOR DEPRESSION
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Impaired glucocorticoid receptor (GR) signaling is a postulated mechanism for the pathogenesis of major depression. Since *in vivo* expression and functional studies of GR are not feasible in humans, we have generated mouse strains that over- or underexpress GR: (1) GR heterozygous mice ($GR^{+/-}$) with a 50% GR gene dose reduction; and (2) GR transgenic mice (YGR) with a 100% gene dose elevation. $GR^{+/-}$ mice exhibit normal baseline behaviors, but demonstrate after stress exposure increased helplessness, a behavioral correlate of depression in mice. Similar to depressed patients, $GR^{+/-}$ mice have a disinhibited HPA system and a pathological DEX/CRH test. Thus, they represent a murine depression model with good face and construct validity. YGR mice, in contrast, show reduced helplessness after stress exposure, and an improved HPA system feedback regulation. Therefore they are a model for a stress-resistant strain. These models can be used to study plasticity changes underlying the pathogenesis of depressive disorders. As first potential molecular correlate we identified a downregulation of BDNF in the hippocampus of $GR^{+/-}$ mice. Translational approaches, i.e. how to use these models specifically for clinically relevant questions, will be discussed.

SXII.2
EFFECTS OF ANTIDEPRESSANTS ON CYTOKINES IN EXPERIMENTAL STUDIES
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Cytokines play an important role as immunotransmitters and coordinate the activity of the endocrine, immune and neurotransmitter systems. The altered cytokine plasma levels have been found in some groups of depressed patients. Administration of proinflammatory cytokines (IL-1β, TNF-α) or unspecific stimulator of their synthesis – lipopolysaccharide produces in lab animals "sickness and depressive-like behavior". These behavioral effects are attenuated by pretreatment with some antidepressants. Results of the investigation carried out on transgenic mice support suggestion that proinflammatory cytokines may be involved in pathogenesis of depressive symptoms. In majority of *in vivo* and *ex vivo* studies it has been observed that chronic administration of some antidepressants shifts peripheral balance between IL-1β, TNF-α and antiinflam-

matory cytokine – IL-10 towards the latter. Antiinflammatory and neuroprotective effects of various antidepressive drugs were confirmed using cultured brain cells. Data from these *in vitro* studies indicate that antidepressants suppress IL-1β, TNF-α gene expression likely due to blockade of NFκ-B and p38 MAPK pathways. It is suggested that changes in cytokine networks might be of significance for antidepressive and/or analgesic action of antidepressants and may justify their use in therapy of neurological diseases accompanied by elevated levels of proinflammatory cytokines.

SXII.3
NEUROPLASTICITY IN DEPRESSION
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Depression may be viewed as a disorder of networks that interconnect different brain areas to which predominant roles in the regulation of individual behavioural domains (mood, anxiety, cognition and reward) that contribute to the depressive state have been ascribed. Using an animal model of depression (chronic unpredictable stress) as well as primary neuronal cell cultures, our studies have focused on volumetric and cell number changes (including those resulting from neurogenesis and apoptosis) in the prefrontal cortex and hippocampus, as well as on patterns of dendritic arborization and signs of synaptic plasticity that parallel the manifestation of depressive-like behaviours. They have also begun to cast light on some of the cellular mechanisms underlying these changes; in this respect, insights into the requirements for maintaining synaptic structural and functional integrity have been gained from work with Alzheimer's disease-related proteins and peptides. Besides identifying potential targets for antidepressant drugs, our studies add support to the network hypothesis of depression.

SXII.4
THE EFFECT OF ANTIDEPRESSANT DRUGS ON IMMUNOENDOCRINE CHANGES IN ANIMAL MODELS OF DEPRESSION
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Exacerbated glucocorticoids and cytokines action are essential factor in the pathogenesis of depression, and the effects of antidepressant drugs on these parameters are poorly recognized.We investigated the effect of antidepressant drugs on the HPA axis activity in prenatally stressed S-D rats and on cell-mediated immunity in Wistar rats and C57BL/6 mice subjected to chronic mild stress (CMS) model of depression. The activity of HPA axis was estimated by measuring the level of glucocorticoid receptors (GR) and activity of some kinases which are known to influence GR action. Adult rats subjected to prenatal stress displayed prolonged immobility in the Porsolt test and in open-field test, elevated corticosterone level, increased GR level in the hippocampus but not in frontal cortex. They also showed decreased FKBP51 in the frontal cortex, but not in hippocampus, decreased the active, phosphorylated form of the JNK1 and 2 kinase in the hippocampus and the active form of p38-MAPK in the frontal cortex. Chronic imipramine, fluoxetine, mirtazapine or tianeptine administration normalized most of these parameters. In CMS model of depression anti-anhedonic effect of imipramine was accompanied by decreased proliferative activity of splenocytes and their ability to produce pro-inflammatory cytokines in rats. In desipramine treated mice subjected to CMS increased ability of T cells to produce negative immunoregulator IL-10 and decreased the cytotoxic activity of NK cells were observed.

SYMPOSIUM XIII

Motor Units and Motoneurons

SXIII.1
PHYSIOLOGICAL ADAPTATIONS IN THE RHYTHMIC FIRING PROPERTIES OF ALPHA-MOTONEURONES TO CHANGES IN PHYSICAL ACTIVITY AND AGING
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Our previous research has revealed that hindlimb α-motoneurones respond to increases and decreases in the activity of the organism, as well as aging, by alterations in biophysical properties measured in anesthetized rats using intracellular recording techniques. Properties that change include resting membrane potential (RMP), voltage threshold (Vth), afterhyperpolarization (AHP) amplitude, rates of antidromic spike development, and rheobase. Rhythmic firing properties that also show changes include minimum and maximum firing frequencies, frequency/current slopes, and the degree of late adaptation. Thus, generally speaking, "excitability" of motoneurones increases and decreases in various models of increased and decreased chronic activity, respectively, and decreases during aging, with implications for motor unit recruitment during voluntary movement. Single-cell modelling of ionic currents has suggested that these chronic adaptations most likely involve changes in the transient sodium and DR potassium ion channels, and perhaps channels associated with the AHP as well as leak currents. Results from this research program have implications for treating the neuromuscular system compromised by disease, trauma, age, and even "chronic sedentarism".

SXIII.2
MOTOR UNITS ACTIVITY IN EMG, MMG AND FORCE RIPPLE: THREE PICTURES FOR ONE SUBJECT
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Mechanomyogram (MMG) detects the muscle surface oscillations due to the mechanical activity of the motor units (MU). Even during static contractions MMG presents macro components (due to the gross dimensional changes of the muscle-tendon unit from rest to activity) and micro components (the summation of the dimensional changes of the fibres of each recruited motor unit). The single MU mechanical contribution to MMG (MUMC) was defined by Gordon and Holbourn a the ''mechanical counterpart'' of the MU action

potential. During voluntary contraction MUMC and MU twitch (MUT) can be extracted from MMG and force ripple signal by means of the EMG driven spike triggered averaging technique. In small hand muscles during 5% of the maximal voluntary isometric contraction (MVC) the values of MUMCs and MUTs were around 11 mm/s² and 7 mN, respectively. Already at these low effort levels the summation in MMG of single MUMCs of the recruited MUs is not linear. This may be due to the long duration of the MUMCs and to the changes in the muscle transverse and longitudinal compliance to mechanical stress determined by the overall MUs activity. On this basis the meaning of the MMG and force ripple oscillations (when larger of the peak to peak noise of the signals during rest and of the MUCMs and MUTs reported for single MUs) with respect to the MUs driving program has to be deeply investigated. This could be done by a cross analysis with the EMG which may partly disclose the central nervous system MUs activation strategy.

SXIII.3
CONTRACTIONS OF MOTOR UNITS EVOKED BY STIMULATION WITH PULSES AT VARIABLE INTERPULSE INTERVALS
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The rate of motoneuronal firing is a major factor regulating the force of motor units (MUs). During voluntary activity of a muscle, its MUs generate tetanic contractions which are characterized by variable force and fusion degree. This study aimed at analysis of force changes during tetani evoked at random stimulation patterns and their mathematical decomposition into responses to individual pulses. It was demonstrated that longer interpulse intervals and lower initial levels of force result in higher force increase during next components of tetanic contractions, and that random stimulation pattern produces higher output of MUs (and higher economy of contraction) than the constant frequency during relatively weak contractions. The decomposition revealed considerable variability in twitch responses to successive pulses. Their basic parameters can be predicted with high accuracy on the basis of the force level at which the next contraction begins. The physiological significance of successive action potentials generated by active motoneurones appears to show considerable variation, so the application of constant frequency patterns during experimental analysis of functionally isolated MUs can lead to several conclusions that do not correspond to activity of MUs during natural voluntary movements. This concerns parameters of successive components of tetanic contractions: the contraction time, the instantaneous force and its variability, the effectiveness and economy of the MU's contraction.

SXIII.4
VARIABILITY AND PLASTICITY OF MOTOR UNIT CONTRACTILE PROPERTIES
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The motor unit contractile properties respond to changes in activity and in motor innervation of muscles. The contractions of isolated motor units evoked by the electrical stimulation of very thin filaments of the ventral root were studied in anesthetized animals. Changes in the distribution of the three basic physiological types of motor units (fast fatigable, fast resistant and slow) in the rat medial gastrocnemius muscle and plasticity of their contractile properties as: contraction and relaxation time, force and fatigability were studied in ageing process, as effects of total and partial spinal cord injury, locomotor and vibration training. Moreover, distribution and variability of motor unit properties as well as innervation of skeletal muscles in male and female muscles were investigated. The comparison of motor unit properties in rat and cat muscle revealed considerable differences in twitch time and force parameters, distribution of motor unit types in muscles of the two species whereas the fatigability, ability to potentiate the force, sag and tetanic phenomenon revealed numerous similarities.

SYMPOSIUM XIV

Advanced Methods of Brain Signal Analysis

SXIV.1
ESTIMATION OF THE TIME-VARYING CORTICAL CONNECTIVITY CHANGES DURING THE ATTEMPT OF FOOT MOVEMENTS BY SPINAL CORD INJURED AND HEALTHY SUBJECTS
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In this study we estimated time-varying cortical connectivity patterns from a group of Spinal Cord Injured (SCI) patients during the attempt to move a paralyzed limb. These data were compared with the time-varying connectivity patterns estimated in a control group during the real execution of the movement by using time-varying Partial Directed Coherence. Connectivity was estimated from high resolution EEG recordings with the use of realistic head modelling and the linear inverse estimation of the cortical activity in a series of Regions of Interest of the cortex (ROIs). The experimental evidences obtained support the conclusion that the SCI population involved a larger cortical network than those generated by the healthy subjects during the task performance. Such network differs for the involvement of the parietal cortices, which increases in strength near to the movement imagination onset for the SCI when compared to the normal population. Such details about the temporal evolution of the connectivity patterns cannot be obtained with the application of the standard estimators of connectivity.

SXIV.2
BEYOND THE PSTH: POINT PROCESS MODELING OF SPIKE TRAINS
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A prerequisite for a quantitative theory of neural coding is adequate description of spike trains. Fifty years ago it was understood that the probability to generate a spike at a given time from the stimulus onset – the post-stimulus time histogram (PSTH) – brings in useful information adding to the mean number of spikes in the trial. Today there is a growing consensus that one must go beyond the PSTH building more complex point process models of neural activity which can account,

for basic physiological properties of spike firing, e.g. for the refractory properties or for adaptation mechanisms of the cell. We shall present some basic concepts of the point process theory in the context of the spike trains and present a simple method of estimation of a class of second order processes for stimulus-evoked activity. We will illustrate the results with an analysis of sample data from the cat superior colliculus. *Supported by grants N401 146 31/3239 and 46/N-COST/2007/0.*

SXIV.3
BRAIN STIMULATION AND EPILEPSY: PROBING THE AUTONOMY OF THE NEURAL SYSTEM
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A decreased ability of the brain to retain sufficiently its autonomous dynamics during stimulation can be indicative for pathological neurological conditions, such as epilepsy. Sensory as well as direct brain stimulation can be therefore valuable supplementary tools for identification of suspected epileptic disorders and even for localization of epileptic tissue in the brain. While most stimulation-based approaches rely on spectral or amplitude analysis we argue that important information is contained in the phase clustering index (PCI) derived from the evoked responses to relatively high-frequencies stimulation (>10 Hz) . We present examples where visual sensitivity can be detected by phase-related features of the visual evoked potentials. We also present analysis from intracranial recordings and direct electric stimulation in patients undergoing pre-operative video/EEG. Our results support the hypothesis that the patient's brain occupies states of increased probability of an epileptic transition prior to seizure. Detection of these states can be relevant as an early warning paradigm. We were also able to find a correlate between the same features of the electrically evoked responses and the location of the seizure onset in cases of localization related epilepsies. We argue that stimulation allows revealing features that are sometimes undetectable by passive observation techniques.

SXIV.4
MULTICHANNEL DATA ANALYSIS – INVESTIGATION OF CONNECTIVITY IN THE BRAIN
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In everyday practice of neurobiology research multivariate datasets are analyzed. This concerns EEG, ECoG, MEG data but also can be extended to fMRI and other recordings as well. Multichannel data require specific approach in order to fully explore information they contain. The issues and problems arising during analysis of such datasets will be discussed. With special emphasis relations between signals which describe influences between investigated structures will be discussed. The concept of Granger causality will be introduced and examples of estimators of causal influence of signals, especially the Directed Transfer Function, and time-varying DTF (which describes dynamical properties of transmissions between channels of the process) will be presented. The performance of the selected estimators will be shown on simulated examples as well as on real EEG data.

POSTER SESSION I

TI: Development and Adult Neurogenesis

TI.01
SPATIAL MEMORY AND DIFFERENTIATION OF ADULT HIPPOCAMPAL NEURONS: ROLE OF TIMING
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In the dentate gyrus adult neurogenesis plays a critical role in hippocampus-dependent spatial learning. However, how new neurons become functionally integrated into spatial circuits and contribute to learning and memory remains yet unknown. To study this issue, we used a mouse model in which the differentiation of adult-generated dentate gyrus neurons can be anticipated by conditionally expressing the pro-differentiative gene *PC3* (*Tis21*/*BTG2*) in nestin-positive progenitor cells. This strategy selectively changes the timing of differentiation of newly generated neurons without affecting their number. New, adult-generated dentate gyrus progenitors, in which the *PC3* transgene was expressed, showed accelerated differentiation, reduced dendritic arborization and spine density. The genetic manipulation affected different hippocampus-dependent learning and memory tasks and selectively reduced synaptic plasticity in the dentate gyrus. Morphological and functional analyses of hippocampal neurons at different stages of differentiation, following transgene activation within defined time-windows, revealed that the new, adult-generated neurons up to 3–4 weeks of age are required not only to acquire new spatial information but also to use previously consolidated memories. Thus, the correct unwinding of these key memory functions is critically dependent on the correct timing of the initial stages of neuron maturation and connection to existing circuits.

TI.02
INTERACTION OF SONIC HEDGEHOG SIGNALING WITH PACAP IN CEREBELLAR GRANULE CELL PROGENITORS
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Granule cells of the cerebellum arise from a secondary germinal zone, the external granule layer (EGL), where granule cell progenitors (GCPs) proliferate in response to stimulation with Sonic hedgehog (Shh). Deregulation of Shh signaling in the cerebellum results in medulloblastoma (MB) in both mice and humans. Pituitary adenylate cyclase-activating polypeptide (PACAP), a ubiquitous and multifunctional polypeptide, is produced by the Purkinje cells of the cerebellum and by GCPs during development and binds to specific PAC1 receptors on GCPs. It has been shown that (1) PACAP signaling prevents apoptosis of GCPs exposed to mutiple insults, (2) it is capable of counteracting the proliferative effect of Shh on these cells and (3) its deletion in ptc1+/- mice significantly increases MB incidence. In the current study we present evidence suggesting that PACAP antagonizes Shh signaling in GCPs through activation of

protein kinase A. Our results also imply that PKA activation is regulated by Shh- and PACAP-activated signaling pathways independently of each other. We also show through microarray analysis of cultured GCPs that PACAP and Shh activate distinct sets of genes in these cells and that virtually all Shh-induced changes in gene expression can be efficiently inhibited by 10 nM PACAP.

TI.03

THE EXPRESSION OF ERYTHROPOIETIN AND ITS REGULATION DURING THE POSTNATAL DEVELOPMENT OF THE RAT RETINA

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In the present study, we examined developing postnatal and adult rat retinas by immunohistochemistry and western blot in order to identify the temporal and spatial onset of the expression of EPO and its presumptive regulating factors (HIF1-α and HIF2-α). In the adult the EPO was expressed in the photoreceptor inner segments, in the inner nuclear, ganglion (GCL), and both plexiform layers. At birth, we found strong immunoreactivities in the GCL and in the inner part of the neuroblast layer and weak staining in the outer part of the neuroblast layer. During the following days the immunoreactivity of the ganglion cells and presumptive amacrine cells became more prominent. From the middle of the first week strongly stained horizontal cells were observed at the level of the separating OPL. The WB analysis resulted high protein levels in the developing retina samples and low protein amounts in the adult. Both hypoxia-induced factors showed similar expression pattern, being expressed in the inner retinal layers. At birth, only the HIF1-α was detectable. The HIF2-α was not detectable until the second half of the second postnatal week and its expression remained weak. The early appearance and distribution of the erythropoietin correlates in time with the main apoptotic processes of the postanatal rat retina. Since the EPO exerts neuroprotective properties in neural tissue, we hypothesize that it acts as a regulating factor during the retinal development.

TI.04

EFFECT OF OLFACTORY STIMULATION ON NEUROGENESIS IN THE ROSTRAL MIGRATORY STREAM

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The subventricular zone retains the capacity to generate new neurons from stem cells. The newly generated cells migrate along the rostral migratory stream (RMS) to the olfactory bulb (OB) where they differentiate into interneurons. The OB is the first relay on the olfactory sensory pathway. We have previously shown that the number of proliferating cells in the RMS of young rats depends on their age. The aim of this study was to investigate if olfactory stimulation (OS) can influence the number of proliferating cells in the RMS of young rats. Since it is known that nitric oxide have antiproliferative action we have also studied occurrence of nitrergic NADPH-d positive cells in the vicinity of proliferating cells. We used 15 natural or synthetic odorants to stimulate olfaction. The rats were olfactory stimulated from the day of their birth (postnatal day 0 – P0) for 2 weeks (P14 group) or for 3 weeks (P21 and P28 groups). Control rats were alike divided into three groups according to the age. To label proliferating cells in the RMS, the rats were injected with proliferation marker – bromdeoxyuridine (BrdU). Brain sections were processed for BrdU immunohistochemistry and NADPH-d histochemistry. We found that OS significantly increased the number of proliferating cells in the RMS of young rats and concurrently number of nitrergic cells decreased. These results indicate positive effect of OS on postnatal neurogenesis in the RMS. *Support: VEGA grants 2/0147/09; 2/0058/08.*

TI.05

NEUROGENESIS AND OLFACTORY-GUIDED BEHAVIOR IN ADULT LABORATORY OPOSSUMS (*MONODELPHIS DOMESTICA*)

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Activation of the receptor 5-HT_{1A} increases the rate of neurogenesis in the subventricular zone (SVZ) and its blocking reduces it. Cells generated in the SVZ settle in the olfactory bulb as its interneurons. We investigated in laboratory opossums if changes in the rate of SVZ neurogenesis influence olfactory detection of food and discrimination of conspecific odors. Four groups of one year old opossums were tested. The first group was injected i.p. for 7 days with saline, the second group with 5-HT_{1A} receptor agonist 8-OH-DPAT, the third group with partial agonist buspirone and the fourth group with the antagonist, WAY100635. BrdU was injected as a marker of newly generated cells. One month later opossums had to locate crickets hidden in the litter. Pretreatment with WAY100635 significantly extended time needed for location of the hidden food in comparison to other groups. Next opossums were exposed for two days to samples of urine of two other opossums. Time of investigation of samples decreased with experience. Opossums injected with buspirone or WAY100635 investigated probes for a longer time and buspirone-treated approached them more frequently. There were no preferences for investigation of new odors. *Post mortem* investigation of the numbers of BrdU-labeled cells showed predictable differences. These results indicate that reduction of the rate of neurogenesis hampers olfactory detection of food, but detection of odors of conspecifics is influenced in more complex way.

TI.06

NEURAL XENOTRANSPLANTATION: THE FATE OF HUMAN UMBILICAL CORD BLOOD NEURAL STEM CELLS (HUCB-NSC) AND HOST RESPONSE TO BRAIN INJURY AND CELL GRAFT

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Neural stem cells (NSC) are potentially a promising treatment strategy for neurological diseases. There is a proof that intrac-

erebral grafting of NSC can have restorative effects. Nevertheless, a major obstacle is the induction of a host-derived immune response followed by graft injection. The goal of the study was to analyze the fate of HUCB-NSC transplanted in injured rat brain. Methods: 2×10^4 HUCB-NSC were tx into corpus callosum of a focal brain injury induced by OUA injection (1 µl/50 nmol) into striatum of adult Wistar rats. After 1, 3, 7 and 14 days brains were removed and analyzed immunocytochemically. Results. One day after HUCB tx, most cells remained in the injection site with few cells migrated to the lesion area. Concomitantly, infiltration of ED1+ and CD15+ cells with occasional appearance of CD5+ and CD45+ cells was seen. At 3rd day some HUCB-NSC in graft core expressed neuronal (NF-200) or astrocytic (GFAP) markers. Migration of tx cells into the lesion was observed. At 7th day HUCB-NSC were found close to injured area. By 14 days, no viable HUCB-NSC have been noticed with cellular debris in graft core. Limited number of ED1+, CD15+, CD5+ and CD45+ cells were found most likely due to HUCB-NSC rejection. Conclusions. Massive loss of transplanted HUCB-NSC was probably due to post traumatic inflammation and acute immune reaction of the host in term of cross-species grafting paradigm. *Supported by MSHE grant N401 014235 and Fondation Jerome Lejeune scientific grant.*

TI.07

SPINAL CORD INJURY OR ENHANCED PHYSICAL ACTIVITY STIMULATE DIVISION OF EPENDYMAL CELLS IN RAT SPINAL CORD

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Ependymal cells (EC) in the spinal cord central canal (CC) are believed to be responsible for the postnatal neurogenesis following pathological or stimulatory conditions. In the present study we have analyzed the proliferation of the CC EC in adult rats processed to spinal cord injury (SCI) or enhanced physical activity. To label dividing cells, a daily injection of Bromodeoxyuridine (BrdU) was administered over a 14 days. Quantification of BrdU positive EC was performed by using stereological principles of systematic random sampling and optical Dissector software. The number of BrdU labeled EC increased gradually with the time of survival after both paradigms, SCI or increased physical activity. In the SCI group we have found 8.2-fold (7 days) and 11.3-fold (14 days) increase of proliferating EC in the rostro-caudal regions. Furthermore, the cervical spinal cord segments revealed 2 to 3-fold increase of EC for both time-points analyzed. In the second group subjected to enhanced physical activity by running wheel, we have observed 1.8 fold increase of dividing EC in the lumbar and 3.2 fold increase in the cervical spinal cord segments at 7 days, but no significant progression at 14 days. This data shows, that SCI or enhanced physical activity in adult rats induces an endogenous EC response leading to their increased proliferation, which may be beneficial for recovery of motor function. *Supported by: APVV 51-002105, VEGA 2-0019-08, VEGA 1-0674-09, VEGA 1/4223/07, APVV SK-CZ-0045-07.*

TI.08

GUIDING CELL-BASED THERAPY FOR NEUROLOGICAL DISEASES WITH NONINVASIVE CELLULAR IMAGING

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Taking advantage of recent developments in molecular biology and imaging, we have developed a system for monitoring status of transplanted cells non-invasively. We use high-resolution MRI to acquire information about the position of grafted cells and reporter gene-based bioluminescent imaging (BLI) to monitor their survival and differentiation. Here, we report on two different approaches for the *in vivo* imaging of glial precursors cells (GRPs). Approach I: Monitoring of the targeted, intravascular cerebral delivery of GRPs. Cells were engineered to express VLA-4 integrin (to enhance vascular adhesion) and were labeled with the MR contrast agent Feridex. Recipient rats were injected i.p. with lipopolysaccharide, a known inducer of endothelial VCAM-1 expression, and the cells were infused into the carotid artery. MRI demonstrated extensive hypointense regions, indicating successful targeting. Approach II: Monitoring of the survival and differentiation of intracerebrally injected GRPs. Cells were engineered to express luciferase under the control of a constitutive or the cell-type-specific promoters and were injected into the brain of immunodeficient or immunocompetent mice. BLI demonstrated that transplanted GRPs survived for extended periods of time in immunodeficient animals, while, in immunocompetent animals, rejection was initiated two weeks after grafting. With cell type-specific promoters, we were able to visualize the process of glial differentiation *in vivo.*

TI.09

RELATIONSHIP BETWEEN EXTRACELLULAR MATRIX COMPONENTS AND MMPS ACTIVITY DURING DEVELOPMENT OF NEURAL STEM CELLS FROM UMBILICAL CORD BLOOD (HUCB-NSC)

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Accumulating evidence indicates that extracellular matrix components and metalloproteinases play a pivotal role in the development of stem cells. The aim of our study was to check if the presence of laminin, fibronectin, and collagen affect MMPs activity (MMP-9 and MMP-2) which in turn might enhance cell proliferation and differentiation. The cells of HUCB-NSC were cultured without serum for two weeks on extracellular matrix components-coated plates. On 4th, 8th, and 14th day we performed proliferation assay, and determination of MMPs activity (*in situ* zymography) followed by immunocytochemistry with specific neural markers. Our results show that among all of the investigated ECM components fibronectin stimulated most intensively cell proliferation and differentiation, especially toward neurons. We also observed the increase of MMPs activity (~20% increase in the 2-weeks culture) in the presence of fibronectin. To confirm the influence of MMPs on developmental processes of neural stem cells we used inhibitors of MMPs – GM6001, and doxycycline. We found that the inhibition of MMPs by GM6001 decreased cell proliferation (~30%) and differentiation into neurons (~20%). As it was shown in

the present study the fibronectin occured to be the potent factor in promoting cell proliferation and differentiation and support the idea that MMPs may contribute to the mechanism involved in the development of neural stem cells. *Supported by MSRHE grants: 1266/P01/2006/31 and N40101832 /0296.*

TI.10
HUCB-NSCS GROWTH CONTROL BY BIOFUNCTIONALIZED SURFACES

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Cell growth platforms with biofunctionalized surfaces were fabricated to control and direct HUCB-NSC fate decisions. Two different nano/micro techniques: microcontact printing and piezoelectric non-contact spotting were used to allocate biomolecules (poly-L-lysine and fibronectin) on cell-repellent, non adhesive substrate. Such methods allow controlling the spatial distribution and content of the biomolecules on the microarray and governing cell adhesion in unspecific (electrostatic) or specific (receptor-mediated) manner. Patterning of biomolecules in different conditions on the single growth platforms enables to influence and compare stem cells developmental processes (proliferation /differentiation) at variable environments. To reflect/mimic stem cell niche we applied functional domains containing ECM protein spotted together with the small signaling molecules (notch, wnt, shh). Such approach enable directing of neural stem cell developmental program by inducing intracellular molecular pathways leading to either self renewing- or differentiating- (neuronal or astrocytic) state. We characterized active biofunctionalized domains on fabricated microarrays by applying two methods of surface analysis: ellipsometry measurement and surface plasmon resonance system. This type of bioengineered cell growth platforms can be used for screening the mechanisms governing neural stem cell fate decisions and adverse reactions upon environmental stimuli. *Grant No 0141/B/ P01/2008/35.*

TI.11
THE PHENOTYPE OF NEWLY GENERATED CELLS IN THE ADULTS' BRAIN OF SOME SPECIES OF RHINOLOPHIDAE

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In the brain of adult mammals neurogenesis continues in two regions: the dentate gyrus (DG) and subventricular zone of the lateral ventricles (SVZ). Here we present data concerning neurogenesis in brains of *Rhinolophus (Rh) ferrumequinum*, *Rh euryale*, *Rh mehely* and *Rh hipposideros.* These bats were captured on permission of the Ministry of Environment of Armenia. After capture all bats were injected with bromodeoxyuridine (BrdU, 300 mg/kg). Seven to nine days later animals were perfused transcardially with 4% paraformaldehyde in narcosis. We used immunohistochemical double-labeling to characterize the phenotype of newly generated cells. Colocalization of BrdU with NeuN (marker of mature neurons), glial fibrillary acidic protein (GFAP, astrocytic marker) and 2`,3`cyclic nucleotide phosphatase (CNP, oligodendrocytic marker) was examined using confocal microscope. We found that in both neurogenic regions the rate of neurogenesis was highest in *Rhinolophus ferrumequinum* and lowest in *Rh mehely.* Double-immunolabeling showed that in all neurogenic regions of the bats' brain the neuronal phenotype dominated among newly generated cells, while proportion of astrocytes was low. BrdU colocalized with CNP in only a few cells in the SVZ of *Rh ferrumequinum*. We suggest that the rate of neurogenesis in different species may depend on the bats' ecology.

TI.12
TRANSPLANTATION OF NNC1 CELLS AFTER EXPERIMENTAL STROKE

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Transplantation of stem cells is currently investigated as an option for treatment of stroke. In this study we transplanted adult human cells NNC1 derived from brain biopsy after experimental stroke in rats. Transient ischemia in rats was performed by the filament model of middle cerebral artery occlusion (fMCAO). About 3×10^5 NNC1 cells were transplanted near to the necrotic area 7 days after fMCAO and the brain of treated rats was investigated 4 weeks after transplantation. Rats were immunosuppressed by daily injections of cyclosporine A, prednisolone and azathioprine. Grafted cells were localized by anti-human specific antibodies HuNu and MTC02. Cells appeared to be located dispersed, however there was almost no cell-migration. The number of localized NNC1 cells 4 weeks after transplantation was rather limited indicating a reduced survival rate which was probably caused by host *versus* graft rejection processes. The latter is reflected by activation of Ox42 and ED1 positive microglia/macrophages. Also, we detected CD8 and TCR positive T-cells infiltrating to the necrotic area. Immunocytochemical analysis revealed expression of nestin and βIII-tubulin by grafted NNC1. Interestingly, some βIII tubulin expressing NNC1 were found to be located in blood vessels. In conclusion, both the survival and the capacity to neuronal differentiation of grafted NNC1 cells are limited.

TI.13
ROBUST *IN VITRO* MIGRATION OF HUMAN UMBILICAL CORD BLOOD NEURAL STEM CELLS (HUCB-NSC) TOWARD INFARCTED BRAIN TISSUE

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Potentially therapeutic neural stem cell line from human cord blood (HUCB-NSC) has been established in our laboratory. Reaching appropriate target by transplanted cells is a prerequisite for success of cell therapy for stroke. The question arises what is the migration potential of HUCB-NSC towards infarcted brain tissue. The migration of HUCB-NSC towards rat tissue homogenates from healthy brain (THHB) and ouabain-induced focal brain injury (THIB) obtained 6 h, 48 h and 7 days after insult was studied *in vitro* using transwells.

Additionally the migratory activities of HUCB-NSC was checked in the presence of migration inducing proteins IGF-1 (200 ng/ml) and SDF-1 (10 ng/ml) dissolved in culture medium. Immunocytochemical analysis of migration-related receptors (CXCR-4, IGF-1R) on HUCB-NSC was performed. Results: Immunohistochemistry of HUCB-NSC unveiled expression of CXCR-4, IGF-1R. HUCB-NSC revealed robust migration toward THIB in comparison to THHB, which was most pronounced in the presence of 48 h postinfarct brain tissue (900 vs. 300 cells/well, $P<0.05$). The presence of IGF-1 and SDF-1 in medium increased significantly HUCB-NSC migration but the effect was much weaker in comparison to injured brain tissue. The ability of robust *in vitro* migration of HUCB-NSC towards infarcted rat brain tissue has been confirmed. Neither IGF-1 nor SDF-1 seems to play a pivotal role in this lesion-induced migration of HUCB-NSC. *Supported by MSHE grant: N N401 332636.*

TI.14
CLIP-170 AND IQGAP1 IN DENDRITIC ARBOR DEVELOPMENT
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The precise control of the microtubule polymerization dynamics as well as strict actin organization are both crucial for formation of neuronal dendritic arbor and require microtubule and actin binding proteins activity. Cytoplasmic linker protein 170 (CLIP-170), one of microtubule plus-end binding proteins, regulates microtubule dynamics at plus-end during polymerization, by promoting rescue-phase in its phosphorylation status dependent manner. We show evidence that mammalian target of rapamycin (mTOR), is one of kinases capable of regulating CLIP-170 activity and both, mTOR and CLIP-170 are crucial for proper dendritic arbor development of hippocampal neurons. Furthermore, we identified in neurons several proteins, which bound to CLIP-170 when mTOR is active, including IQGAP1, a known partner of CLIP-170 and regulator of the actin dynamics. Taken together our data strongly suggest that CLIP-170 activity during dendritogenesis can be regulated by mTOR at the level of CLIP-170 protein-protein interactions. Moreover, obtained results, showing mTOR dependent interaction of CLIP-170 with IQGAP imply that mTOR can coordinate tubulin and actin cytoskeleton organization. *Supported by Polish Ministry of Science and Higher Education Research Grant 2P04A01530 and Polish-Norwegian Research Found grant PNRF-96-AI-1/07.*

TI.15
DYNAMIC RELATIONSHIPS OF CONVENTIONAL AND NEURON-SPECIFIC ENDOSOMAL REGULATORS IN DENDRITES
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The unique functions and morphology of neurons require a specialized endosomal system that recycles, sorts, and targets proteins. The neuronal endosome is unique in that it is polarized into somatodendritic and axonal domains, has neuronal-specific regulators, and is modulated by generic regulators that confer neuronal-specific properties. NEEP21, a neuronal-specific early endosomal regulator, and EEA1, a generic early endosomal regulator, are polarized to somatodendritic domain and bind syntaxin13 (stx13), which occupies early/recycling endosomes in somatodendritic and axonal domains. NEEP21 and stx13 regulate AMPA receptor recycling; NEEP21 regulates trafficking of L1 cell adhesion molecule. It is not known how these endosomal regulators function together to transport cargo in neurons. Due to the dynamic nature of the endosomal system, live imaging is required to understand the relationships and dynamics of different compartments. We carried out live imaging of GFP/RFP-tagged regulators in cultured hippocampal neurons. NEEP21 occupies stationary (containing L1) and dynamic (devoid of L1) round compartments. EEA1-containing round and static compartments do not significantly colocalize with NEEP21, but often are adjacent to it. Stx13 resides in dynamic, elongated carriers and in round stationary compartments where it partially colocalizes with EEA1 and NEEP21. We interfered with function of stx13 using expression of dominant-negative construct and found that it affects L1 trafficking.

TI.16
RNA-BINDING PROTEINS IN DENDRITOGENESIS
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Proper development of dendritic arbor is a precisely orchestrated process in which translation is shown to play pivotal role. RNA binding proteins such as ZBP1 (beta-actin zipcode binding protein 1), Staufen1 and Staufen2 bind to and transport their target mRNAs, enabling their local translation in dendrites and taking part in regulation of this process. ZBP1 knockdown with short interfering RNAs in developing rat hippocampal neurons *in vitro*, resulted in decrease in total number of dendrites and dendritic tree complexity, which was reversed both with siRNA-resistant ZBP1 rescue mutant and jasplakinolide treatment that prevents depolymerisation of actin cytoskeleton. In contrast, mature neurons with stable dendritic arbors, did not lose dendrites upon ZBP1 depletion, which suggests ZBP1 plays role in formation of new dendrites as opposed to maintenance of mature ones. We also show that both Staufen1 and Staufen2 knockdown in developing neurons results in impoverishment in dendritic arbors complexity and decrease in total number of dendrites. RNA-binding proteins can be subject to phosphorylation, which modulate their activity, and one possibility for a common regulator of their role in dendritogenesis would be Src kinase, which we show to phosphorylate ZBP1 and Staufen1. Taken together, presented data demonstrate that mRNA transport and possibly local protein synthesis play a prominent role in development of dendritic arbor. *Ministry of Science and Higher Education Grant NN301314733*

TI.17
FUNCTIONS OF TGF-β2 AND GDNF IN THE DEVELOPMENT OF THE MOUSE NERVOUS SYSTEM: EVIDENCE FROM DOUBLE MUTANT MICE
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A major area of investigations in neuroscience is directed at understanding factors that participate in neuronal survival and

death. Therefore they are good candidates to be responsible for different neurodegenerative diseases such as Alzheimer's, and Parkinson's diseases. Many recent advancements in our laboratory have revealed that growth factors acting in synergy can regulate neuronal survival much more effectively than individual factors alone. Impairment of neuronal survival is the cause of a considerable number of neurodegenerative diseases. TGF-β has been shown to act in synergy with neurotrophic factors, most strikingly in combination with GDNF. Since no information was available about the overall significance of TGF-β/GDNF cooperation and/or synergism for nervous system development, the present study was undertaken to test the paradigm of the developmental requirement of growth factor synergism and cooperation. Double knockout mice that lack both TGF-β2 and GDNF were generated. Neuron populations analyzed included all those in which biological effects of either growth factor had been documented before. Finally, an understanding of the role of TGF-β/GDNF synergism *in vivo* through the analysis of the nervous system development of the corresponding double mutant mice can provide significant basic and clinical insight into mechanisms that may lead to overcoming neuron loss and verify potential targets for neurological diseases.

TI.18
A QUANTITATIVE STUDY OF THE GLIAL CELL RESPONSE TO BRAIN INJURY AFTER INTRACEREBRAL AND INTRAVENOUS TRANSPLANTATION OF BONE MARROW STROMAL CELLS

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Experimental studies have shown that bone marrow stromal cells (BMSCs) promote functional recovery after traumatic brain injury. The beneficial effects of BMSCs therapy were demonstrated after intravenous, intraarterial and intracerebral transplantation of these cells. A reasonable explanation for the benefit provided by BMSCs is that these cells stimulate regenerative changes in the damaged brain, including glial remodeling. The aim of the present study was to determine and compare the time course of the astrocyte and microglial/macrophage cell reaction to cerebral cortex injury after intracerebral and intravenous administration of BMSCs. Female Wistar rats were subjected to cerebral cortex injury followed by the injection of BMSCs or PBS directly to the injury site or to the tail vein. Using histology and immunohistochemistry, the distribution of astrocytes and microglia/macrophages was analyzed in the injured cerebral cortex. BMSCs treatment affected glial cell response to brain injury. The effects of BMSCs activity were dependent on the site of their administration. Intracerebral transplantation of BMSCs showed significant effect on the astrocyte response whereas intravenous route of BMSCs administration increased especially the microglia/macrophages number in the injured brain. The results suggest that implanted cells can enhance the restorative processes in the injured brain through the stimulation and modulation of the cellular response to injury.

TI.19
FUNCTIONAL AND MORPHOLOGICAL ANALYSIS OF DRG NEURONS UNDER L-ARGININE APPLICATION AFTER PNS INJURY

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Over the past 30 years, the role of nitric oxide (NO) in biology has evolved from being recognized as an environmental pollutant to an endogenously produced substance involved in cell communication and signal transduction. NO can play a double role after injury of the PNS and CNS in accordance with its concentration, time of synthesis and type of NO-synthase participating in its production. Three isoforms of NOS have been reported – nNOS, eNOS and iNOS. They are reaching greatest expressions during the 2nd week, and the dynamic expression of individual isoforms is different. Since the protective or toxic effects of NO depend on its concentration, the cell type it is expressed, it is useful to apply donors or inhibitors of NOS in restrict time points after injury. It was established previously that increasing expression of iNOS results in death of neurons but nNOS plays neuroprotective role after PNS lesion. It is plausible that producing of NO with participation of nNOS provide the amount of NO which serves as a neuroprotective signal for neurons after injury. In this study, we studied the expression of caspase-3 in different population of DRG neurons using sciatic nerve transection with the purpose to explore the potential functional link between locally applied donor of NO and the ability of sensory neurons to survive. We observed better functional recovery in animals treated with L-arginine 7 days after nerve injury and decreasing of caspase-3 expression in DRGs L4-L5.

TII: Neural Excitability, Synapses and Glia: Cellular Mechanisms

TII.01
ANTIDEPRESSANT-LIKE FEATURES OF MICE WITH TRANSGENIC ACTIVATION OF Ras IN BRAIN NEURONS

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Brain-derived neurotrophic factor (BDNF) is implicated in clinical depression. Thus, BDNF expression is enhanced by antidepressants while stress exposure and depression decreases it. A major BDNF intracellular signalling pathway is the Ras-extracellular signal-regulated kinase (ERK) cascade. Here, we test its possible contribution on antidepressant activity by utilizing a synRas transgenic mouse model expressing constitutively activated human Ha-Ras in differentiated neurons [Heumann et al. (2000) J Cell Biol 151: 1537]. Immunoblotting analysis in hippocampus and cortex revealed that chronic fluoxetine administration to C57Bl/6 mice led to an increased activation of endogenous Ras and activating ERK1/2 phosphorylation. SynRas mice exhibited chronically increased levels of activated Ras and activating ERK1/2 phosphorylation in the cortex and hippocampus. This was clearly associated with an antidepressant-like behaviour in four different animal models of depression. Furthermore, restraint stress-induced corticosterone response was attenuated in synRas mice, throughout stress and recovery time period. Because adult neurogenesis may play a role in depression we assessed the proliferation of hippocampal progenitors. Here we show that proliferation was not correlated with the antidepressant activity in synRas mice. Taken together, an antidepressant state was established in a genetic model of enhanced neuronal Ras signalling without correlation to hippocampal precursor cell proliferation.

TII.02
HIPPOCALCIN SIGNALING IN SPINES OF HIPPOCAMPAL NEURONS

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Hippocalcin (HPCA) is a Ca^{2+}-binding protein, and its Ca^{2+}-dependent activation in hippocampal neurons is one of the necessary steps involved in many signal transduction mechanisms. In this work we have examined if synaptic glutamate receptor activation can result in HPCA signaling in spines of hippocampal neurons. Spontaneous and evoked synaptic activity induced excitatory postsynaptic potentials (EPSPs) in the hippocampal neurons leading to AP bursts and HPCA-YFP translocation to many sites in a neuronal dendritic tree including dendritic spines. In neurons clamped at −40 mV, episodes of presynaptic activity resulted in EPSCs associated with HPCA translocation mainly to dendritic spines while no translocation was observed at −70 mV . These results indicate that synaptic NMDAR activation is necessary for HPCA-YFP translocation. T- and R- rather than L-types of voltage activated Ca^{2+} channels also contribute to the observed translocation. FRET measurements between HPCA tagged by Yellow and Cyan Fluorescent Proteins have shown that the translocation was due to HPCA-FPs insertion in patches of spine membrane resulting in decrease of protein concentration in the cytosol of spines and diffusion of new HPCA-FP molecules from the dendritic trunk. Thus, we have shown that hippocalcin may signal as a coincident detector in spines of hippocampal neurons by means of its robust insertion in spine plasma membrane.

TII.03
EFFECTS OF CHRONIC FLUOXETINE TREATMENT ON CATALEPSY, IMMUNE RESPONSE AND BRAIN SEROTONIN SYSTEM IN MICE GENETICALLY PREDISPOSED TO CATALEPSY

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ASC/Icg (Antidepressant Sensitive Catalepsy) mouse strain selected for high predisposition to pinch-induced catalepsy is characterized by depressive-like behavior and impaired immune response. Chronic treatment with SSRI fluoxetine attenuated catalepsy manifestation and normalized a decreased number of rosette-forming cells (RFC) in spleen in ASC mice. Chronic fluoxetine administration had no effect on catalepsy and RFC number in mice of parental cataleptic CBA/Lac strain. Fluoxetine failed to alter $5\text{-}HT_{1A}$ receptor functional activity in mice of both strains and diminished $5\text{-}HT_{2A}$ receptor functional activity in CBA but not in ASC mice. No effect on cortical $5\text{-}HT_{1A}$ and $5\text{-}HT_{2A}$ receptor mRNA levels and on $5\text{-}HT_{1A}$ receptor, tph2 (tryptophan hydroxylase-2) and SERT (serotonin transporter) mesencephalic gene expression was observed in ASC mice. The data evidenced the validity of ASC mouse strain as prospective model for research of psychoneuroimmune mechanisms of antidepressant effects and development of depressive-like state.

TII.04
LONG-TERM POTENTIATION IN HIPPOCAMPAL BASAL DENDRITES: ROLE OF DIFFERENT MODULATORY SYSTEMS

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Long-term potentiation (LTP) and long-term depression (LTD) are considered to be cellular models suitable for studying the synaptic changes that likely occur during learning and memory. LTP has distinct phases, a transient protein synthesis-independent stage (early-LTP) followed under distinct circumstances by a late, long-lasting and protein synthesis-dependent stage (late-LTP). In hippocampal CA1 neurons, LTP in either the apical or basal dendrites differ in their molecular requirement during induction as well as

the setting of the tag, for example CaM kinase II mediates the setting of the tag in stratum radiatum but in basal dendrites (stratum oriens) the setting of the tag is mediated by either protein kinase A or protein kinase M zeta (Sajikumar et al. 2007). It has been reported that the late-LTP in the CA1 stratum radiatum requires the synergistic activation of different neurotransmitters during its induction (Frey 1997, Frey and Morris 1998). From this background we now investigated whether the LTP in stratum oriens requires similar or other synergistic interactions of different modulatory systems when compared with the stratum radiatum. Our preliminary studies using different selective antagonists of distinct modulatory transmitters systems revealed that late-LTP in basal dendrites is different with respect to its requirements for its induction when compared with late-LTP in apical CA1-dendrites.

TII.05
NEURON TYPE RECONFIGURATION OF GABA-ERGIC TONIC INHIBITION IN MOUSE BARREL CORTEX INDUCED BY SENSORY LEARNING
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Pairing tactile stimulation of a row of whiskers with a tail shock results in expansion of the cortical representation of stimulated vibrissae accompanied by increase of the GABAergic markers. Using the same paradigm, we examined exstrasynaptic $GABA_A$ receptors-mediated tonic inhibition. Whole-cell patch-clamp recordings were performed in layer IV neurons and tonic current was assessed by application of the $GABA_A$ receptors blocker-picrotoxin (PTX) or by 4,5,6,7-tetrahydroisoxazolo[5,4-c]pyridin-3-ol (THIP, a superagonist at delta-subunit containing $GABA_A$ receptors) in *ex vivo* slices of the barrel cortex in adult mice. PTX and THIP produced greater shifts in baseline holding current in excitatory cells within the cortical representation of "trained" vibrissae, visible 24 hours after the end of training. Recordings from layer IV fast spiking neurons showed that the training induced smaller baseline shifts after the application of both substances. Regular spiking non-piramidal neurons had similar shifts in baseline holding current in both control and trained mice. These data indicate that associative learning paradigm results in a neuron type reconfiguration in tonic inhibition of layer IV neurons situated within the barrel representing stimulated vibrissae. This reconfiguration can be caused by changes in the level of delta-subunit containing $GABA_A$ receptors. *Supported by the Ministry of Science and Higher Education grants PBZ/MNiSW/07/2006/02 and N401 028 32/0664.*

TII.06
GLUTAMATE TRANSPORTERS IN RAT BRAIN EXPOSED TO POLYCHLORINATED BIPHENYLS AND POLIBROMINATED FLAME RETARDANTS
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Polychlorinated biphenyl (PCB) congeners and polibrominated flame retardants (BFRs) are environmentally occurring toxins that exhibit a broad range of adverse biological effects including neurotoxicity. Due to their stability and lipophilic character, they persist in the environment and accumulate in brain of animals and humans contributing to induction of neurological disorders. The mechanisms by which PCBs and BFRs cause neurotoxic effects are still not completely understood. However, it is postulated that glutamate excitotoxicity may be involved. The main glutamate transporters GLT-1 and GLAST are membrane-bound proteins localized in glial cells. The clearance of synaptically released glutamate by these proteins protects neurons from excitotoxicity. The aim of the study was to establish whether the chronic exposure to these substances may influence the expression and activity of main glutamate transporters in rat brain. Aroclor 1254 (PCBs) and tetrabromobisphenol A (BFRs) were administered by oral gavage, which resembles the human exposure through the food chain, for two weeks. Significant changes in the expression of glutamate transporters were observed, especially in the case of GLT-1. The effect was more pronounced for Aroclor 1254. The results suggest that GLT-1 is a molecular target of this toxin that may, almost partially, contribute to PCBs-induced excitotoxicity. *This study was supported by grant nr NN401024635 from Polish Ministry of Science and Higher Education.*

TII.07
BRAIN LEVEL OF FKBP-51, A GLUCOCORTICOID RECEPTOR COCHAPERONE, IS DECREASED IN A NEURODEVELOPMENTAL ANIMAL MODEL OF SCHIZOPHRENIA
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Recent studies suggest that dysregulation of the HPA axis activity and disturbance in glucocorticoid receptor (GR) action are involved in the pathogenesis of schizophrenia. The hyperfunction of GR can result from alterations in GR phosphorylation status or numbers of its cochaperones. The most important GR cochaperone FKBP-51, is known to inhibit GR transcriptional activity. The aim of the present work was to investigate the concentration of the immunophilin FKBP-51 in the hippocampus and frontal cortex in a neurodevelopmental animal model of schizophrenia. This model is based on administration of lipopolysaccharide to pregnant rats (in the second and third week of pregnancy). Amount of FKBP-51 was measured by Western blot method. In order to verify the above model, exploration, efficacy of sensorimotor gating and performance in the social interaction test were determined. Prenatal LPS treatment induced behavioral disturbances typical of schizophrenia, like sensorimotor gating deficit, higher exploratory activity and changes in social interaction test in the adult offspring. Furthermore, the level of the immunophilin FKBP51 was lower in both female and male offspring. These results suggest that multiple administrations of LPS to pregnant rats evoke GR hyperfunction in adult offspring by decreasing the concentration of FKBP-51, a protein which is known to inhibit GR function. *This study was partially supported by the grant N40101231/0174 from the MSHE, Warsaw, Poland.*

TII.08
NUCLEAR β-CATENIN IS CONSTITUTIVELY PRESENT IN THALAMIC NEURONS
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Wnt signaling is one of the most important mechanisms engaged in embryonic development. In the adult organism it is present in regenerating tissues. The key mediator in the signaling is β-catenin, which translocates to the nucleus and triggers transcription of the Lef1/Tcf target genes. Recently, expression of some of the Wnt pathway components has been observed in the thalamus of the adult brain. We show that β-catenin and Lef1 are constitutively present in nuclei of thalamic neurons *in vivo*. We also demonstrate that β-catenin accumulates in about 40% of thalamic cells *in vitro*, without any prior stimulation. This phenomenon does not depend on soluble factors produced by glia cells or cortical neurons, because nor glia cells co-culture neither cortical conditioned medium affects the number of β-catenin positive cells. These observations support a new idea that Wnt/β-catenin signaling is an intrinsic feature of thalamic neurons, independent on thalamo-cortical interaction what was proposed before. Whether the nuclear localization of β-catenin in thalamic neurons is a consequence of autocrine Wnt stimulation or a specific regulation inside the cells remains to be determined.

TII.09
EXPRESSION REGULATION OF A NOVEL Ca^{2+} BINDING PROTEIN CALMYRIN 2 (Cib2) INDICATES ITS FUNCTION IN NMDA RECEPTOR MEDIATED Ca^{2+} SIGNALING
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Calmyrin 2 (CaMy2, Cib2) represents a novel calmyrin subfamily of Ca^{2+} binding proteins that coordinate Ca^{2+} in the EF-hand motives. CaMy2 mRNA was recently detected in brain, but nothing is known about CaMy2 protein localization and properties in the brain. We have cloned CaMy2 from rat brain, demonstrated CaMy2 Ca^{2+}-sensor properties and determined its neuronal pattern of rat brain expression. CaMy2 protein is expressed mainly in hippocampal neurons and localizes together with Golgi apparatus and dendrite markers. Our studies in primary cultures of hippocampal neurons demonstrate that expression of CaMy2 protein is induced upon neuronal activation with pharmacological agents that stimulate Ca^{2+} influx through such types of Ca^{2+} channels as glutamate excitatory receptors and voltage-operated Ca^{2+} channels. In addition, increase in CaMy2 protein level was induced by soluble amyloid β and BDNF. However, most prominent increase in CaMy2 protein (7-fold), and mRNA (2-fold) occurs upon stimulation of NMDA receptor (NMDAR). The induction is blocked by translation inhibitors, specific antagonists of NMDAR, the Ca^{2+}-chelator BAPTA, and inhibitors of ERK1/2 and PKC, kinases transmitting NMDAR-linked Ca^{2+} signal. Our results show that CaMy2 level is controlled by NMDAR and Ca^{2+} and suggest CaMy2 role in Ca^{2+} signaling underlying NMDAR activation. *This work was supported by the Polish Ministerial Research grant N301 109 32/3854.*

TII.10
IMMUNOHISTOCHEMICAL ANALYSIS OF NEUROPEPTIDES DISTRIBUTION IN *SPODOPTERA EXIGUA* AND *PYRRHOCORIS APTERUS* BRAIN
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The corazonin (Crz), was originally purified as a cardioacceleratory neuropeptide in *P. americana*. Following studies indicated that Crz induces dark colorization and morphometric changes in locust; reduces the spanning rate of silk in *B. mori*; is implicated in circadian clock. The pigment dispersing hormone/pigment dispersing factor (PDH/PDF), primary was described in crustaceans and in following studies in insects. Researchers have shown its role in circadian organization of insects as a important output factor of circadian clock. CCAP (crustacean cardioactive peptide), has a broad range of activity including: cardioacceleratory effect; affecting muscle and gut functions; induces release of AKH. Mapping of Crz-, PDF-, CCAP- containing neurons provides an important information concerning sites of release and action of these peptides. Here we employed immunohistochemical methods to confirm the presence of above mentioned neuropeptides and to investigate their distribution in the brain of *Spodoptera exigua* (Lepidoptera) and *Pyrrhocoris apterus* (Hemiptera). Using [His7]-Crz antibody we were able to detect immunoreactive signal in both species in dorsolateral (DL) neurosecretory cells projecting ipsilateraly to the corpora cardiaca. Additional immunoreative somata were observed in pars intercerebralis (PIC). PDF-like immunoreativity was observed in optic lobes, DL and much weaker in PIC. CCAP-ir was detected in OL and PIC.

TII.11
GENERATION OF TRANSGENIC MICE WITH SELECTIVE ABLATION OF NMDA RECEPTORS IN NORADRENERGIC NEURONS
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The brain's noradrenergic system system provides essential modulation to neuronal activity, with well established roles in control of arousal and selective attention, as well as memory, learning and response to stress. We have generated a transgenic mouse, $NR1^{DbhCre}$, with ablation of the essential NMDA receptor subunit NR1 (*Grin1*) in noradrenergic cells. Since no functional NMDA receptors may be formed in the absence of NR1, and their expression is restricted to the central nervous system, the $NR1^{DbhCre}$ mice have impaired glutamate-dependent plasticity in the central noradrenergic neurons, without observable alterations in the sympathetic system or the hypothalamus-pituitary-adrenal axis. Transgenic animals were born at expected ratios and developed normally, displaying no obvious impairments. The general anatomy of the noradrenergic system in the mutant mice was normal, no loss of cells was observed and noradrenaline content in the prefrontal cortex was not altered. Interestingly, preliminary electrophysiological analysis indicates

that loss of functional NMDA receptors attenuates the spontaneous activity in current-clamped locus coeruleus noradrenergic neurons manually held at –50 mV potential. In summary the *NR1*^{DbhCre} transgenic mice are a novel model for the study of the roles of the noradrenergic system in the central nervous system.

TII.12
THE EFFECT OF BIOGENIC AMINES SEROTONIN, DOPAMINE, OCTOPAMINE AND TYRAMINE ON AGGRESSIVE BEHAVIOUR OF WORKERS OF THE RED WOOD ANT *FORMICA POLYCTENA*

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We investigated the effect of abdominal injections of 4 biogenic amines – serotonin (5-HT), dopamine (DA), octopamine (OA) and tyramine (TA) – on behaviour of workers of the red wood ant *Formica polyctena* in 4 types of aggression tests. The ants were observed during a 10 min encounter with a nestmate, a conspecific ant from an alien colony, a worker of another ant species (*Formica fusca),* and a small larva of the house cricket (*Acheta domesticus*). DA administration had the most important effect on ant aggressive behaviour. DA injections exerted a stimulatory effect on threats directed to *F. fusca* and to larvae of *A. domesticus* and on biting behaviour directed to larvae of *A. domesticus.* TA suppressed the frequency and the duration of biting behaviour directed to these larvae*,* although that effect was significant only when the effects of TA were compared with those of DA and 5-HT. TA also reduced self-grooming behaviour, but only in tests with alien conspecifics. Other effects of biogenic amine administration included a stimulatory effect of OA on other active behaviour displayed by the ants during the tests with *A. domesticus,* inhibitory effects of 5-HT, OA and TA on allogrooming of a nestmate, and lenghtening of the latency to the first antennal contact with a nestmate observed in TA and 5-HT-treated ants. The most important effects of biogenic amine administration were observed in the case of interactions of the ants with crickets. *Support: grant MNiSW N N303 3075 33.*

TII.13
CALCIUM-BINDING PROTEINS IN THE HIPPOCAMPAL FORMATION OF THE GUINEA PIG

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Calcium ions (Ca^2) are known as a second messenger in neurons. However, the concentration of Ca^2 should be maintained at an optimal level in view of their high toxicity. Calcium-binding proteins (CaBPs) bind Ca^2 with high affinity. The amount of the CaBPs is age-related and varies during brain development. The aim of this study was to analyze the distribution of CaBPs, parvalbumin (PV) and calbindin D-28k (CB), in the hippocampal formation (the dentate gyrus – DG and hippocampus proper – HP). The studies were carried out on the brains of newborn (P0) and 80-day-old (P80) guinea pigs. Labelling immunofluorescence was performed on 10 µm-thick frozen sections. PV- and CB-immunoreactive (PV-IR, CB-IR) structures were identified with antibodies against PV (1:2000, code P3088) and CB (1:2000, code C9848). Both studied parts are composed of three cellular layers. In the dentate gyrus, PV-IR cells (elongated and oval-shaped) were labelled in the hilus. In the middle (granular) layer only PV-IR dots surrounding negative cells were observed, whereas in the third (molecular) layer no immunoreactive structures were seen. In the hippocampus proper PV-IR neurons (triangular, piriform and oval-shaped) were found in each sector (CA1–CA3) of the pyramidal layer, the same as in the oriens and molecular layers. CB-IR neurons (oval-shaped, densely arranged) were observed mainly in the granular and pyramidal layers. The distribution of the studied calcium-binding proteins was similar in P0 and P80.

TII.14
REGULATION OF SERUM RESPONSE FACTOR-DRIVEN TRANSCRIPTION BY MKL2 (MEGAKARYOBLASTIC LEUKEMIA 2) IN RESPONSE TO BDNF

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SRF-mediated transcription contributes to brain plasticity. However, it is unclear which of the several SRF co-activators participates in transcriptional events underlying the formation and modifications of neuronal circuitries. We investigated the role of SRF co-activator, MKL2, in regulation of SRF-driven transcription in neurons. MKL2 expression was observed in newborn cortical or hippocampal neurons in culture as well as in adult rat forebrain. *In-situ* hybridization showed the presence of MKL2 mRNA in all fields of the hippocampus, especially in dentate gyrus. Neither overexpression nor inhibition of MKL2 by shRNA caused apoptotic cell death in neurons. Overexpression of MKL2 in primary cortical neurons enhanced SRF-driven transcription elevated by BDNF stimulation. In addition, inhibition of MKL2 reduced BDNF activation of SRF-driven transcription, on classical SRE promoters. MKL2 is a less potent activator of SRF-mediated transcription than recently studied member from the same family of co-activators MKL1. Interestingly, MKL2 is working as a partial inhibitor of BDNF activated MKL1-dependent transcription in case of promoters with isolated SRF binding sites. These results suggest that MKL2 contributes to BDNF-mediated regulation of SRF-driven gene expression. Particular mode of regulation depends on the presence of a second co-activator MKL1 or the type of SRF binding sites within the regulatory regions of respective genes. *FNP Homing - MF EOG to KK, RO1 (NS047341-01) to MH.*

TII.15
ADMINISTRATION OF CLENBUTEROL IMPROVES MORPHOLOGY OF CHOLINERGIC NEURONS AND COGNITIVE PROCESSES IN AGED RATS
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A deficit in the cholinergic system of the basal forebrain (BF) is thought to contribute to the development of cognitive symptoms of dementia. Forebrain cholinergic neurons are highly dependent on nerve growth factor (NGF) for phenotype maintenance. Attempts to prevent age-associated cholinergic vulnerability and deterioration therefore represent a crucial point for pharmacotherapy in the elderly. The pharmacological induction of endogenous NGF synthesis in the brain could be an elegant way to overcome application problems. Therefore, the present experiment was undertaken to determine the influence of prolonged pharmacological stimulation of NGF biosynthesis on learning and memory in aged rats. To address these issues we used young (4-month-old) and aged (28-month-old) rats in which we stimulated endogenous NGF biosynthesis by treatment with clenbuterol (β2-adrenergic receptors agonist). The cognitive behavior of the young and aged rats was assessed in the long-lasting "Non-Matching to Position Test". Our data suggest that in aged rats, clenbuterol positively affects cognitive processes related to formation of associations established in recognition memory and discrimination tasks. In addition, NGF significantly improved morphological parameters of BF cholinergic cells in aged rats. These data suggest that endogenous NGF induction restores the age-related decline of the cholinergic system activity in rats, resulting in improvement of water maze performance in old animals.

TII.16
ANXIETY, DEPRESSIVE, COGNITIVE AND MOTIVATIONAL PHENOTYPE IN MICE WITH SELECTIVE ABLATION OF NMDA RECEPTORS IN NORADRENERGIC NEURONS
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The noradrenergic system of the brain has been postulated to mediate arousal, selective attention, as well as anxiety, depression and learning and memory. Here we report the behavioral phenotype of transgenic mouse (*NR1*^{DbhCre}) with selective ablation of the NMDA receptor subunit NR1 in noradrenergic cells. We focused on behaviors modulated by the noradrenergic system, in particular anxiety-like behaviors in the light/dark box, and open field as well as depressive-like behaviors in forced swim test. Furthermore, memory performance was measured using elevated plus maze spatial learning (EPMSL), Y maze, right/left discrimination, habituation to open filed and novel object recognition and motivation-dependent behaviors was studied using food pellet self-administration paradigm. While no alterations were found in anxiety- and depressive-like behaviors in *NR1*^{DbhCre} mice, the transgenic animals displayed impaired object recognition memory, but increased performance in right/left discrimination test. In addition, the *NR1*^{DbhCre} mice displayed higher food reward sensitivity as evidenced by faster acquisition of food self-administration and higher breaking point in progressive ratio schedule of reinforcement. In conclusion, the ablation of NMDA receptors in noradrenergic neurons in mice produce impairment of recognition memory and alteration of motivational systems.

TII.17
DISTRIBUTION OF CAPACITATIVE CALCIUM ENTRY ACTIVATOR STIM1 IN THE MOUSE BRAIN
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Capacitative calcium entry (CCE) is a significant component of calcium homeostasis in non-excitable cells. In neurons, an increasing number of evidence points to CCE as an important event in neuronal physiology and pathology. STIM1 is an endoplasmic reticulum (ER) residing protein, where it serves as a calcium sensor. Low level of calcium in ER drives STIM1 oligomerize and interact with plasma membrane protein Orai1, hence leading to calcium entry. Previously, we showed STIM1 expression in neurons, where it exhibits the same mode of action as described for non-excitable cells. Here we present description of STIM1 distribution in the mouse brain. The highest STIM1 immunoreactivity was observed in Purkinje neurons and their dendrites. Very high immunoreactivity was found in the basal ganglia. High immunoreactivity was present in the hippocampal formation, in the piriform cortex, also in infragranular layers and layer V pyramidal neurons in the neocortex. In amygdala most nuclei showed strong immunoreactivity, only the basolateral complex was weakly stained. The thalamus was very weakly stained, and the hypothalamus showed immunoreactivity of both cells and neuropil. Observed differences in STIM1 distribution in the brain indicates that various brain structures depends on CCE to different extend. Prominent dissimilarity of STIM1 immunoreactivity between amygdaloid nuclei is, in our opinion, the most interesting in light of anatomical and functional organization of the amygdala.

TII.18
PROTEIN SUMOYLATION IN SYNAPTOSOMES, EFFECTS ON GLUTAMATE RELEASE AND PRESYNAPTIC Ca^{2+} INFUX
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SUMO-1 is a protein with similar structure to ubiquitin. SUMOylation modifies proteins structure effecting their molecular mechanisms. SUMO-1 is present in neurons and in the synapse. Here we are investigating whether SUMO-1 is a presynaptic protein and whether SUMOylation modulates glutamate release. Ultrasynaptic separation approach was used to investigate the distribution of SUMO-1 in peri-, post- and presynaptic compartments in synaptosomes. Presynaptic protein SUMOylation is augmented by KCl or AMPA stimulus and is decreased by kainate. NMDA has no influence presynaptic protein SUMOylation level. Synaptosomes glutamate release was measured. Recom-

binant SUMO-1 and SENP-1, a SUMO-specific proteases which cleave SUMO from its substrates, where used with their mutated recombinant variant in synaptosomes as entrapped proteins. KCl stimulated glutamate release is increased when synaptosomes contain SENP-1 but is decreased when synaptosomes contain full-length SUMO-1. It was observed decrease in kainate evoked glutamate release from synaptosomes preloaded with SENP-1 but a marked increase in release from synaptosomes preloaded with SUMO-1. Intracellular Ca^{2+} concentration was also measured. Wild-type SENP-1 increased Ca^{2+} influx evoked by KCl or AMPA and decreased it following a kainate challenge. Conjugatable SUMO-1(GG), on the other hand, reduced Ca^{2+} influx evoked by KCl or AMPA and increased the levels of Ca^{2+} influx elicited by kainate

TII.19
THE EFFECT OF PROLONGED ZOLPIDEM TREATMENT ON $GABA_A$ RECEPTORS IN PRIMARY CULTURE OF RAT CEREBELAR GRANULE NEURONS
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The aim of this study was to further explore the mechanisms leading to adaptive changes in $GABA_A$ receptors following their prolonged exposure to hypnotic zolpidem, a positive allosteric modulator of $GABA_A$ receptors. Imidazopyridine zolpidem is the most widely prescribed non-benzodiazepine hypnotic, with preferential, although not exclusive, binding for receptors containing α1 subunit. It was suggested that drugs with high selectivity for α1 containing receptors produce upon repeated treatment less tolerance and dependence than classical benzodiazepines. As an extension of our previous work, we treated cerebellar neuronal cells isolated from 8-days old rats with 10 μM zolpidem during 48 h. Radioligand binding studies and RT-PCR analysis were preformed under conditions previously described [Pericic et al. (2007) Naunyn Schmiedebergs Arch Pharmacol 375: 177–187]. Results demonstrate that prolonged treatment of these cells with zolpidem induced changes neither in $GABA_A$ receptor number nor in expression of α1 subunit mRNA. As evidenced by a decreased ability of GABA to stimulate [3H]flunitrazepam binding, chronic exposure of these cells to zolpidem produced the functional uncoupling between GABA and benzodiazepine binding sites on $GABA_A$ receptor complex. If this mechanism is responsible for the development of tolerance following chronic administration of classic benzodiazepines, than one might can expect that chronic zolpidem might also induce tolerance.

TII.20
MODULATION OF NEURONAL MEMBRANE PROPERTIES BY THE COXSACKIEVIRUS-ADENOVIRUS RECEPTOR
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The Coxackievirus and Adenovirus receptor (CAR) was originally identified as a cell surface protein enabling both viruses to interact with cells. Besides this pathological role as a virus receptor, the physiological function of CAR in the CNS is largely unknown. CAR is a member of the Ig superfamily composed of two Ig-like domains, a transmembrane stretch and a cytoplasmic segment. CAR is expressed early in development on neurons and becomes restricted to synapse-rich layers at more advanced stages. Application of the fiber knob of the adenovirus, which binds to CAR, resulted in longer neurites in comparison to the untreated cell cultures. Furthermore, the formation of cell aggregates was reduced by the fiber knob. By using whole-cell patch clamp technique we analyzed the effect of the fiber knob on passive membrane properties and synaptic activity. The fiber knob reduces the membrane resistance (Rm) of cultured neurons. Consistently, in CAR deficient neurons Rm was significantly higher compared to wild type neurons, and application of fiber knob had no effect on Rm on CAR-deficient neurons. Thus, CAR may influence Rm *via* membrane proteins with a membrane conductance (e.g. ion channels, gap junction). Our model is, that CAR deficient neurons may not express or express some not functional ion channel or transport proteins in the membrane. On the other side, binding of the fiber knob to CAR might induce activation – opening of such transport protein.

TII.21
CULTURE CONDITIONS SEVERELY ALTER THE PROPORTIONS BETWEEN GABA- AND GLUTAMATE-EVOKED CURRENTS IN HIPPOCAMPAL PYRAMIDAL NEURONS
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Cultured hippocampal cells are commonly used as a convenient model but non-physiological conditions (impaired homeostasis, lack of glial cells etc.) persisting over long period of time raise concerns. Acute brain slices are believed to better maintain the physiological features but are often problematic for technical reasons (e.g. complicated access to neurons, attenuated spread of pharmaceutics). Non-physiological conditions in cell cultures during maturation of GABAergic and glutamatergic systems may alter expression of $GABA_A$ and glutamate receptors affecting thus the excitation to inhibition balance. Proportion between GABA and glutamate receptors may be evaluated by determining ratio between amplitudes of current responses to saturating agonist concentrations. We have recorded current responses to ultrafast applications of saturating GABA and glutamate concentrations in hippocampal cell cultures (9–15DIV) and in brain slices from CA1 pyramidal neurons of P19–P23 rats. For cell cultures, GABAergic and glutamergic currents ratio was 2.34 ± 0.45 (n=12) while for the brain slices it was only 0.24 ± 0.02 (n=5). These results provide evidence that non-physiological conditions in cell cultures may cause a dramatic change in expression pattern of $GABA_A$ and glutamate receptors. The underlying mechanisms are not known, but we may hypothesize that a homeostatic modulation due accumulation of neurotransmitters in culture medium could be involved.
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TII.22
MATRIX METALLOPROTEINASE-9 INCREASES NMDA RECEPTOR LATERAL DIFFUSION
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Matrix metalloproteinase-9 (MMP-9) has emerged as a physiological regulator of NMDA receptor (NMDAR)-dependent synaptic plasticity and memory. The pathways by which MMP-9 affects NMDAR signaling remain, however, not well understood. Using single Quantum Dot tracking we demonstrate that MMP-9 enzymatic activity increases NR1-NMDAR surface trafficking but has no influence on AMPA receptor (AMPAR) mobility. Other extracellular protease – Cathepsin G has no effect on both NMDAR and AMPAR lateral diffusion. The mechanism of MMP-9 action on NMDAR is caused neither by change in overall extracellular matrix (ECM) structure, nor by cleavage of NMDAR subunits, but by influence on integrinbeta 1 signaling. These findings describe a new target pathway for MMP-9 action in key physiological and pathological brain processes.

TII.23
INVOLVEMENT OF GROUP I METABOTROPIC GLUTAMATE RECEPTORS IN FORMING HYPOXIC BRAIN TOLERANCE INDUCED IN RATS BY HYPOBARIC PRECONDITIONING
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The assumption that hypoxic tolerance induced by preconditioning with moderate hypobaric hypoxia (MHH) includes modification of intracellular Ca^{2+} (Ca^{2+}_i) transients by modulation of glutamate receptors (GluR) activity was tested. Superfused rat brain slices were prepared 24 hours after three MHH sessions equivalent to 5 000 m above see level and tested by application of selective agonists of different subtypes of GluR. Ca^{2+}_i level in response to the agonists was increased in comparison with the control ones. The most prominent Ca^{2+} level shifts were recorded in response to stimulation of group I metabotropic glutamate receptors (ImGluRs). The modified pattern of the responses showed striking prevalence of signal pathways responsible for Ca^{2+} release from intracellular stores against modulation of Ca^{2+} entry. The selective antagonism of both 1 and 5 subtypes of ImGluRs *in vivo* during MHH did not suppress the preconditioning efficiency, tested in the slices by severe (10 min) anoxia 24 hours after. Moreover by immunocytochemical and Western-blotting methods we found no valid distinctions in a peptide expression of these ImGluRs subtypes between control and MHH-preconditiond rats. Thus it can be supposed that the changed pattern of Ca^{2+} response to the agonist and mechanisms of brain tolerance induced by MHH are determined by a shift in balance of different glutamatergic signal pathways controlling the dynamics of Ca^{2+}_i level rather than by modification of the agonist reception.

TII.24
GLUTAMATE AND NEUROPEPTIDE RELEASED IN THE BRAIN OF MALE GRASSHOPPER *CHORTHIPPUS B.* ALTER THE CONTEXT OF THE PRODUCED SINGING IN A STEP-WISE MANNER
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Injections of neuropeptide proctolin into brain can elicit singing behaviour in male grasshoppers of the species *Ch.biguttulus*. It has been shown earlier that activation of mAChRs lead to prolonged calling song – first level of excitation. Proctolin release might be associated with courtship singing and could probably trigger switches between calling and courtship singing when a con-specific female responds acoustically – second level of excitation. Co-injections of proctolin with glutamate could alter the time course of proctolin-stimulated singing by appearance of precopulatory movements or led to appearance of additional behaviours such as wing flappings – third level of excitation. The singing patterns are produced only by the hind legs stridulatory movements rubbing to the wing's vein and driven by internal neuronal oscillator. The context of the acoustic patterns depends only on the time courses of these hind legs' movements. The arousal of excitation was not linear but in a step-wise manner alternating the described patterns above. These pattern alterations were triggered by co-release of few neurotransmitters – ACh, proctolin and glutamate. There were indications that the intracellular signalling pathways coupled to mAChRs, Proctolin receptors and mGluRs interact each other to achieve the switches between the particular oscillator activity. The latter might alternate the outgoing message context, produced by the male in a given communicative situation.

TII.25
FACILITATION OF TRANSMITTER RELEASE FROM RAT SYMPATHETIC NEURONS *VIA* PRESYNAPTIC $P2Y_1$ RECEPTORS
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P2 receptors for purines and pyrimidines are divided into 7 P2X and 8 P2Y receptors (Abbracchio et al. 2006). Noradrenaline (NA) and ATP are the predominant neurotransmitters in the sympathetic nervous system. In rat superior cervical ganglion (SCG) neurons, endogenous ATP activates presynaptic P2X receptors and thereby mediates a positive feedback regulation of NA release (Boehm 1999). In addition, endogenous ADP mediates a feedback inhibition of NA release, through an inhibition of voltage activated calcium channels (VACCs) most likely *via* $P2Y_{12}$ and pertussis toxin-sensitive G proteins (Lechner et al. 2004). In sympathetic neurons, receptors coupled to Gq type G proteins and PLC, such as B_2 bradykinin and M1 muscarinic receptors, mediate an inhibition of KCNQ channels (Delmas et al. 2005). We found out that presynaptic $P2Y_1$ receptors mediate a facilitation of transmitter release from sympathetic nerve terminals *via* PLC. The present study provides the first evidence for a facilitatory P2Y receptor, in sympathetic neurons. Since $P2Y_1$ antagonists are being developed as antithrombotics, this mechanism can be expected to contribute to their pharmacodynamic profile, in particular with respect to the vascular tone.

TII.26

MONOAMINES EFFECTS ON DIFFERENT TYPES OF HIPPOCAMPAL GAMMA OSCILLATIONS IN ADULT RAT *IN VITRO*

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Monoamines are implicated in cognitive processes in variety of brain regions including the hippocampal formation, where storage and retrieval of information is facilitated by synchronous network activities. We have investigated the effects of norepinephrine, serotonin and dopamine on carbachol-, kainate- and stimulus-induced hippocampal γ-oscillations employing extra and intracellular recordings. Monoamines dose-dependently and reversibly suppressed kainate- and carbachol-induced γ-oscillations. The effect of serotonin was mimicked by fenfluramine, which releases serotonin from presynaptic terminals. Forskolin also suppressed kainate- and carbachol-induced γ-oscillations. This effect was mimicked by 8-Br-cAMP and isoproteronol, an agonist of noradrenergic β-receptor suggesting that the monoamines-mediated suppression of these oscillations could involve intracellular cyclic AMP. By contrast, stimulus-induced γ-oscillations were dose-dependently augmented in power and duration after monoamines application. Intracellular recordings from pyramidal cells revealed that monoamines prolonged the stimulus-induced depolarization and membrane potential oscillations. Stimulus-induced γ-oscillations were also suppressed by isoproteronol, the D1 agonist SKF-38393 forskolin and 8-Br-cAMP. This suggests that the augmentation of stimulus induced γ-oscillations by monoamines involves – at least in part – different classes of cells than in case of carbachol- and kainate-induced γ-oscillations.

TII.27

LEF1/BETA-CATENIN REGULATES $Ca_v3.1$ T-TYPE CALCIUM CHANNEL GENE (CACNA1G) IN THE THALAMUS

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Lef1/Tcf transcription factors together with β-catenin activate genes involved in proliferation and differentiation of neuronal precursor cells. In mature neurons β-catenin participates in dendritogenesis and synaptic function as a component of the cadherin cell-adhesion complex, its transcriptional activity, however, remains elusive. We observed that in the adult brain Lef1 and β-catenin accumulate specifically in the nuclei of thalamic neurons, implying their involvement in regulating gene expression in these cells. The electrophysiological properties of thalamic cells depend on T-type low-voltage-gated Ca^{2+} channels. Since $Ca_v3.1$ is the predominant T-type pore subunit in the thalamus, we hypothesized that the Lef1/β-catenin complex regulates transcription of its gene. The increase of $Ca_v3.1$ gene expression in thalamic cells treated *in vitro* with Wnt3A, an activator of β-catenin, corroborated our presumption. Analysis of the $Ca_v3.1$ gene promoter revealed that its proximal region contains a Lef1 binding site. Indeed, luciferase assays confirm that the promoter is activated by Lef1 and β-catenin. Furthermore, chromatin immunoprecipitation demonstrated that the promoter is occupied by β-catenin in the thalamus. Together, our data indicate that the Lef1/β-catenin complex regulates transcription of $Ca_v3.1$ gene. We propose that β-catenin contributes to neuronal excitability not only by a local action at the synapse, but also by activating gene expression.

TII.28

CYCLICAL EXPRESSION OF THE SODIUM PUMP α-SUBUNIT GENE AND PROTEIN IN THE VISUAL SYSTEM OF *DROSOPHILA MELANOGASTER*

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In the lamina L1 and L2 monopolar cells show circadian rhythms in shape and size changes, however, mechanisms of these rhythms have not been recognized yet. It has been proposed that swelling and shrinking of L1 and L2 interneurons might be due to ionic fluxes, changes in cytoskeleton organization and in protein synthesis. This process also depends on glial cell activity. Since ion channels seem to be involved in morphological plasticity of neurons and glial cells we examined expression of the sodium pump, Na^+,K^+-ATPase, during the day and night. The sodium pump consists of two polypeptide chains: α- and β-subunits encoded by the *Atpα* and *Nrv2* genes, respectively. Using the real-time PCR and immunohistochemistry methods we found the α-subunit shows daily changes in mRNA and protein concentrations while in case the β-subunit such changes were not observed. Since the rhythm in the α-subunit abundance was not observed in the per^0 arrhythmic mutant, this rhythm is controlled by clock genes. It seems to reflect changes in the visual system activity.

TII.29

ROLE OF THE FAST VOLTAGE GATED K^+ CHANNEL FUNCTION IN FIRING BEHAVIOUR OF PURKINJE NEURONES OF ATAXIC RATS

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The role of fast inactivating (IA) voltage gated K^+ channel function in shaping the firing activity of Purkinje cells (PCs) in ataxic rats was investigated. Ataxia was induced in 5 week-old male rats of Sprauge-Dawley by single injection of 3-acethylpyridine (65 mg/kg, i.p.). Four days following induction of ataxia, ether-anesthetized rats were decapitated; brains rapidly removed and 300 μm thick parasagittal slices were prepared from the vermis of the cerebellum. Whole cell patch clamp recording was achieved in the presence of synaptic blockers. To prevent the induction of ataxia, 4-AP (2 mg/kg body weight), IA channel

blocker, was injected prior to 3-AP treatment. Results indicated that in ataxic rats, the latency of the initial spike in PCs was significantly increased ($P<0.001$), while both firing frequency and amplitude of Ca^{2+} spikes were ($P<0.001$) suppressed compared to control. However, pretreatment with 4-AP not only almost completely prevented the neuronal degeneration in PCs layer but also the development of ataxia, which occurred in ataxic rats. PCs in rats co-treated with 4-AP and 3-AP fired predominantly in rhythmic bursts. The amplitude of Ca^{2+} spikes was significantly ($P<0.001$) increased compared to ataxic rats. An increase in the intrinsic excitability of PCs was also observed as a significant decrease in the first spike latency ($P<0.001$). In 4-AP co-treated rats, the duration of action potential was also significantly lengthened ($P<0.001$).

TII.30
THE CONTRIBUTION OF NEURONAL GLUTAMATE TRANSPORTERS IN SHAPING OF eEPSC IN CA1 HIPPOCAMPAL NEURONS

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Glutamate is the major excitatory transmitter in CNS although it causes severe brain damage by pathologic excitotoxicity. Central to efficient neurotransmission is a powerful protection afforded by specific high-affinity glutamate transporters in neurons and glia, clearing synaptic glutamate. In whole-cell patch clamp experiments the influence of neuronal glutamate transporters on spontaneous (sEPSC) and evoked postsynaptic currents (eEPSC) in hippocampal CA1 neurons was examined by manipulating the content of intracellular solution. With Cs+-based internal solution the deficient of presynaptic glutamate transporters affected the occurrence of synaptic event and thus involved in the regulation of transmitter release. eEPSCs were generally suppressed both in amplitude (to 48.73 % vs. control) and in success rate (Rsuc) by TBOA (10 μM) (from 91.1 % in control to 79.57%). In contrast, with K^+-based internal solution (all GluT are intact), amplitude of eEPSC was substantially potentiated by pre-treatment with TBOA (up to 150%), whereas (Rsuc) was reduced to 79.8% in average. The identical reduction of event success rate as well as increased PPF ratio for eEPSC in both cases is indicative for TBOA effect on presynaptic uptake site. In conclusion, presynaptic transporters are suggested to act mainly as negative feedback signal on presynaptic release and/or referred to vesicle refilling processes, when postsynapticaly located transporters are supposed to shape postsynaptic events.

TII.31
RECIPROCAL REGULATION OF KCC2 AND NKCC1 *VIA* SPATIAL DISTRIBUTION IN THE MEMBRANE

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Throughout the nervous system, the Cl^--outward transporter NKCC1 and the Cl^--inward transporter KCC2 play an important role in setting the intracellular chloride concentration. Both transporters are co-expressed in many neurons. This requires tight and reciprocal regulation, due to their opposite direction of Cl^- transport. Here, we investigated the role of cholesterol-rich membrane microdomains, so-called membrane rafts, for NKCC1 and KCC2 function. Membrane rafts were isolated from mature rat brainstems at postnatal days (P) 25–30 *via* solubilization in Brij 58 followed by sucrose-density flotation experiments. This analysis revealed that NKCC1 was codistributed with the membrane raft marker flotillin-1 in lower density fractions. In contrast, two populations were observed for KCC2. One was solubilized, whereas the other population was detergent insoluble. Studies in HEK293 cells demonstrated that disruption of membrane rafts by combined treatment of methyl-b-cyclodextrin and sphingomyelinase impaired NKCC1 mediated $^{86}Rb^+$ uptake, whereas it increased KCC2 dependent $^{86}Rb^+$ uptake. From these data we conclude that NKCC1 requires association with membrane rafts for transport activity, whereas KCC2 is inhibited by membrane rafts. In agreement with this conclusion, transport inactive KCC2 in the neonatal brainstem (P0–2) is exclusively present in membrane rafts. Taken together, our analysis identifies a cellular mechanism that might be involved in reciprocal regulation of NKCC1 and KCC2.

TII.32
GABA TRANSPORTER GAT-1-POSITIVE (GAT-1+) PUNCTA MAY PLAY AN ACTIVE ROLE IN MODULATING GABA ACTION IN THE "TRAINED BARREL" CORTEX

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We have shown previously that classical conditioning training involving stimulation of a row of facial vibrissae (conditioning stimulus, CS) and electrical shock (unconditioned stimulus, UCS) to the tail is associated with increase in the numerical density of GAD67+ boutons located on the cognate barrel hollows. The present study was designed to determine whether the expression of GABA transporter GAT-1 changes after associative learning using the mice classical conditioning training as a model. We found that numerical density of GABA transporter GAT-1+ puncta was increased by about 50% in the neuropil of the "trained barrel" hollow compared with controls: pseudoconditioned, whisker stimulation without UCS, and naive group of mice. The electron microscopic observations confirm that immunoreactivity for GAT-1+ puncta was localized: in single synaptic terminals present on symmetric specialization synapses, on double-synapse spines on symmetric synapse, and on astrocytic processes. The projection of several confocal plans (3D spatial organization) identified GAT-1+ coupled astrocytes. The present evidence supports the hypothesis that GABA transporter GAT-1 localized on GABAergic terminals and the astrocyte networks in the hollow of the "trained barrel" may be involved in learning-dependent changes in layer IV of the barrel cortex.
Supported by MNiSW grant 188 to ES.

TII.33
QUANTAL ATP RELEASE FROM ACUTELY ISOLATED CORTEX CELLS ON SINGLE BOUTON LEVEL
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The ATP postsynaptic currents were measured during local extracellular electrical stimulation of one single bouton of acutely isolated cortex cells. The successful mediator fusion events were visually controlled by stimulus activating staining by SynaptoGreen. The ATP was released from distinct vesicle population as evidence by existence of two separate populations of synaptic currents. As membrane potential was set above the reversal potential for Cl^-, we observed fast inward currents mediated by P2X receptors and slow outward currents mediated by GABA. Furthermore, these two populations of synaptic currents we separated by pharmacological tools. When slow outward postsynaptic currents we inhibited by picrotoxin, the residual fast inward currents were blocked by 30 μM PPADS. The quantal parameters of postsynaptic currents in the cortical cells were obtained and verified using maximum likelihood and autocorrelation techniques. The amplitude histograms for both mediators have a binominal distribution character with quantal size near 6.5 pA for ATP and 10 pA for GABA. In a conclusion, our results demonstrate that release of ATP in cortical inhibitory terminals occurs from separate pool of synaptic vesicles. Upon excitation of postsynaptic site release of ATP and GABA can be synchronized when both transmitters are released in multivesicular package.

TII.34
KISSPEPTIN EFFECTS ON GABA TRANSMISSION TO GONADOTROPIN-RELEASING-HORMONE NEURONS ARE *VIA* BOTH PRE- AND POSTSYNAPTIC MECHANISMS
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Gonadotropin releasing hormone (GnRH) neurons control fertility centrally. The neuromodulator kisspeptin (kiss) strongly activates GnRH neurons *via* its G-protein coupled receptor 54 (GPR54). GnRH neurons express GPR54, implicating direct actions of kiss on these cells; however, GPR54 is broadly expressed in the brain, suggesting indirect actions are possible. Estradiol potentiates the GnRH neuron response to kiss, which recent work showed was due to kiss enhancement of excitatory GABA and glutamate transmission to GnRH neurons. Here we tested if the effect of kiss on GABA transmission to GnRH neurons is due to pre or postsynaptic mechanisms. We performed whole-cell voltage-clamp recordings of GABA miniature postsynaptic currents (mPSC) in GnRH neurons in the presence of tetrodotoxin (TTX), which minimizes presynaptic actions by blocking action potentials. Kiss had no effect on the frequency of GABA mPSCs, but did significantly increase the amplitude of these events. To see if the effect of kiss on GABA PSC amplitude is mediated postsynaptically, we recorded currents generated by local GABA application. Kiss significantly increased GABA-induced current amplitude. These data indicate that the kiss effect on frequency of GABA transmission to GnRH neurons is due to presynaptic actions, but effects on amplitude are postsynaptic. Together with previous findings, these data provide additional insights into the mechanisms of direct and transsynaptic effects of kiss on GnRH neuronal activity.

TII.35
MATRIX METALOPRTEINASE 9 (MMP-9) PLAYS A ROLE IN LONG TERM POTENTIATION (LTP) IN AMYGDALAR BLA TO CE PATHWAY
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Matrix metalloproteinase-9 (MMP-9) is an extracelularly operating protein, capable to cleave several components of extracellular matrix (ECM). It is expressed in neurons in many brain structures. It has been shown to be important for maintenance of LTP in hipoocampal CA3 to CA1 pathway (Nagy et al. 2006) as well as in the prefrontal cortex (Okulski et al. 2007). Amygdaloid body is a small heteromeric structure, important for regulation of memory and autonomic as well as endocrine responses. In the present study, we have examined if LTP is MMP-9-dependent in the pathway from basolateral to central amygdala. The basolateral nucleus of amygdala (BLA) was theta burst-stimulated using a bipolar electrode, and EPSPs were collected from CE. We have found that in slices from MMP-9 knock out mice the late phase of LTP is abolished. The same effect was obtained when inhibitor of MMP-9 was used in rat amygdala slices where LTP lasted for only 30 min after its induction. Finally, we have checked LTP in slices from the transgenic rats with neuron-specific MMP-9 overexpression, driven by Synapsin I promoter. LTP in these rats was lower than in control but stable. The present observation suggests that the proper level of MMP-9 expresion and activity is essential for synaptic plasticity in the BLA-CE pathway, whereas MMP-9 overexpresion may cause destabilization of neuronal environment and decreased activity-dependent strengthening of synaptic transmission.

TII.36
SYNAPTIC CONNECTIONS BETWEEN PYRAMIDAL CELLS AND INTERNEURONS IN SUPERFICIAL LAYERS OF THE NEOCORTEX
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To understand how sensory information is processed in the neocortex it is essential to investigate the neuronal microcircuits and the dynamics of cortical signalling. Layer 1 of the neocortex is a cell-sparse and synaptic dense zone. Layer 1 neurons are exclusively GABAergic. Here, we recorded from layer 1 interneurons in the adult neocortex and we found that layer 1 interneurons displayed highly diverse morphological and electrophysiological properties. Electrophysiologically, L1 interneurons showed accommodating, non-accommodating, accelerating, bursting, irregular and stuttering behaviour upon a sustained current injection. Based on their morphology, various types including neurogliaform cells, basket cells and axo-axonic cells could be identified. Layer 1 interneurons are electrically coupled and provide inhibitory synaptic input to fellow layer 1 interneurons and layer 2/3 pyramidal neurons. However, little is known about the translaminar excitatory innervation of layer 1 interneurons. We performed paired-recordings from layer 1 and layer 2/3 neurons in adult neocortical slices. The properties of unitary EPSPs were found to be dependent on the identity of the postsynaptic neuron. Our results suggest that L2/3 pyramidal neurons efficiently recruit layer 1 interneurons providing a substrate for feedback inhibition, which might contribute to the sparse firing of L2/3 pyramidal neurons observed *in-vivo*.

TII.37
EFFECT OF MATRIX METALLOPROTEASE INHIBITION ON SHORT AND LONG TERM PLASTICITY OF MOSSY FIBER-CA3 SYNAPSES
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Matrix metalloproteinases (MMPs) are a large family of extracellularly-acting endopeptidases. MMPs were shown to play an important role in hippocampal plasticity phenomena such as LTP, learning and memory. In particular, in Shaffer collateral/CA1 (Sch/CA1) synapses, MMPs are crucial for maintenance of long term plasticity requiring NMDA receptor (NMDAR) activation. However, it is not known whether MMPs affect plastic changes in mossy-fiber/CA3 (mf/CA3) synapses, where LTP is basically presynaptic and NMDAR-independent. Field potential (fEPSPs) recordings in CA3 region (mf stimulation) revealed no effect of FN439 (panmetalloprotease inhibitor) on the short-term plasticity but the late phase of LTP was abolished by this agent similar to Sch/CA1 pathway. Since maintenance of LTP in Sch/CA1 was specifically dependent on MMP-9 activity, we have performed immunostaining for MMP-9 in mf/CA3 pathway. Semi-quantitative analysis revealed that induction of LTP resulted in several fold increase in MMP-9 immunoreactivity within hilar and CA3 region relative to control slices. We conclude that MMPs are crucial for long- but not short-term plasticity in mf/CA3 synapses and that LTP induction in this pathway is accompanied by a robust increase in MMP-9 immunoreactivity. It seems thus that consolidation phase of LTP is critically dependent on MMP activity in the two major hippocampal pathways (Sch/CA1 and mf/CA3) where LTP induction mechanisms show profound differences. *Support: Grant No. P-N/030/2006.*

TII.38
LACK OF PRODOMAIN ABOLISHES BDNF CONSTITUTIVE SECRETION BY HEK 293 CELLS
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Brain-derived neurotrophic factor (BDNF) and its proBDNF precursor are released both in constitutive and activity-dependent manner. Prodomain itself proved to be necessary for BDNF targeting to regulated secretory pathway [Egan et al. (2003) Cell, Chen et al. (2005) J Neurosci] but its role in constitutive secretion is elusive. As mature BDNF (mBDNF) conveys trophic and pro-survival signals whereas proBDNF may convey growth inhibiting and death signals, an important issue arises: can we control the type of signal being triggered by BDNF? To verify this we cut off the prodomain and generated plasmid coding only for rat mBDNF. To test the constitutive mBDNF construct secretion we have chosen HEK 293 cell line. Two other plasmids coding either for proBDNF (template for both BDNF forms) or proBDNF protected from prodomain cleavage (source of proBDNF only), served as controls. BDNF secretion was evaluated with WB technique using antibodies detecting (1) both BDNF forms, (2) HA tag (mBDNF construct) and (3) MYC tag (proBDNF constructs). We found all three constructs being stably expressed in HEK cells. However, in contrast to both proBDNF constructs, which were secreted and detected in media fraction, mBDNF construct was revealed only in the cell lysate fraction, not being released to the media. This is the first observation showing that mBDNF can be constitutively released only when accompanied by prodomain. *Support: ASTF 211-00-2007 for EZ, Polish-German grant to MS and SK.*

TIII: Disorders of the Nervous System

TIII.01
INFLUENCE OF AGE UPON THE PHOTOTHROMBOTIC STROKE IN MICE
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Stroke is one of the leading causes of death and disability in humans. Animal models of stroke simulate the human pathology and can help to develop the effective therapies. One of such models is the photothrombosis model in which cerebral ischemia is induced photochemically by injecting the photosensitive dye. The advantages of this model are the small infarct size, minimal surgical manipulation of the animal. Despite the strong connection of stroke with aging process, the majority of experimental studies are conducted in young or adult animals. Here we have used young (3 months old) and aged (12 months old) mice to produce the small (about 2 mm in diameter) infarct neighboring the primary somatosensory barrel cortex area and including the whole depth of cortex. After 7-day reconvalescence we have analyzed the presence and size of the evoked infarct. In both groups stroke has evoked similar changes in cortex cytoarchitecture, however we found that aging has decreased the method effectiveness of about 25%. Moreover, comparing to young animals, in old mice size and extent of infarct evoked with the same parameters and conditions were much less unrepeatable. It can be associated with changes of the nervous system physiology with age that concern the blood flow, diameter of blood vessels, metabolism and physiology of neurons and glial cells. Alternatively, older mice may have adapted functionally to age-dependent damage. *Supported by the Scientific Network: Biovision SNB-504-8.*

TIII.02
ACTIVITY OF METALLOPROTEINASES IN THE BRAIN OF YOUNG AND OLD MICE AFTER FOCAL PHOTOTROMBOTIC STROKE
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Aging is a major risk factor for ischemia. Old experimental animals demonstrate bigger infarct progression and more exten-

sive neuronal degeneration after stroke compared to young animals. Activation and expression of metalloproteinases (MMPs), MMP- 2 and MMP-9 particularly, is strongly up-regulated after stroke and it was proved to have a detrimental role. The present study was designed to compare MMPs activity in old and young brains after ischemia. Cortical photothrombotic stroke was induced in young (3–4 months) and aged (12–13 months) C57/BL mice. MMPs activity on the infarcted area and surrounding tissue was examined at 1, 7 and 14 days after stroke. Although *in situ* zymography revealed that the time-course of gelatinolytic activity and the pattern of distribution of digested products were similar in both groups, old mice exhibited stronger fluorescein signals of MMPs activity in the ischemic and peri-infarct areas, as compared to young animals. Analysis of brain homogenates by SDS-Page zymography showed the up-regulation of MMP-9 activity after stroke in old brains, while in young brains activity of this metalloproteinase was detected at low level. MMP-2 activity remained at stable and comparable level in both groups at all investigated time-points. These results suggest that old brains exhibit stronger proteolytic reaction after stroke, which may contribute to a more severe course of the disease. *Supported by Polish-German Grant P-N/026/2006 and Statutory Funds.*

TIII.03

DISPARITY OF SEASONAL VOLUME CHANGES OF FOREBRAIN STRUCTURES IN THE COMMON SHREW (*SOREX ARANEUS*) SUGGEST ENGAGEMENT OF DIFFERENT MECHANISMS.

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Mechanisms of seasonal changes of brain size in Sorex shrews (Dehnel's effect) remain largely unknown. Hypothesis postulating changes of brain cell numbers has been recently rejected (Bartkowska et al. 2008). Other possible mechanisms are reorganisation of the neuropil and change in the composition of brain tissue (e.g. dehydration). Shrews were captured in the spring, summer and autumn. Some shrews collected in the autumn wintered in captivity. Volumes of selected forebrain structures were measured stereologically on Nissl stained series of sections. Largest oscillations were found in the volume of cortex and basal ganglia. Lack of correlation between the rate of neurogenesis and the volume of hippocampus confirms previous findings. Relative quantity of myelin was determined by densitometry of sections stained with the method of Gallyas. Volumes of neocortex and hippocampus that are myelinised to a similar degree change differently, while volumes of striatum and neocortex, differing in myelin density, reduce their volumes similarly in the winter. Moreover, the overall ratio of forebrain volume to myelin quantity did not change throughout life. This implies that general dehydration may be excluded as a significant reason of brain size alterations and that different mechanisms are engaged in various brain structures. The comparison of analysed structures in terms of connectivity and water permeability by measuring levels of synaptophysin and aquaporin 4 was attempted.

TIII.04

PHONOLOGICAL AND SEMANTIC PROCESSING IN CHILDREN WITH DEVELOPMENTAL DYSLEXIA – AN EVENT-RELATED POTENTIAL STUDY

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Event-related potentials were used to examine if the brain response (N400 component) in dyslexic children is modulated by phonological or semantic priming, similarly to age-matched controls. ERPs were recorded while the children listened to word lists in which the semantic and phonological congruency of the terminal words were manipulated. Dyslexics exhibited a dissociation of priming effects depending on whether semantically or phonologically loaded primes were used. An enhancement of N400 amplitude to semantically incongruent words was observed, although this effect was reduced and delayed compared to that seen in controls. Direct comparison of the performance of the two groups in the semantic priming task revealed that they differed only in their response to semantically incongruent words. In the phonological priming task, rather than an enhancement of the N400 amplitude found in controls, dyslexics displayed a reduction of the N400 to the incongruent condition in comparison to the congruent condition. In this task, the studied groups differed in both the phonologically congruent and incongruent conditions. These results suggest that when faced with phonological priming, dyslexics have problems with both matching for similarities (integration into context) and incongruency detection. In the case of semantic priming, the integration of semantic context seems relatively intact in dyslexics, but they experience difficulties in detecting the shift from one semantic category to another.

TIII.05

HOW NEURONS BECOME GIANT – IDENTIFICATION OF PROTEINS REGULATED BY mTOR KINASE IN ETIOPATHOLOGY OF TUBEROUS SCLEROSIS

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mTOR is a serine-threonine protein kinase for several aspects of neuronal function. Increased mTOR signaling has been implicated in tuberous sclerosis (TS), a multiorgan disease affecting brain. TS is caused by mutations in genes encoding for hamartin and tuberin that lead to increase of the mTOR activity and subsequent abnormal cell growth and proliferation, leading to brain lesions containing giant cells. It is unclear how mTOR contributes to observed changes. Our aim was to identify downstream mTOR effectors important for the disease related abnormal cell growth. To model TS giant cells, rat cortical neurons cultured *in vitro* were transfected with short interfering RNA (siRNA) targeting tuberin that caused a Rapamycin-sensitive increase of neuron soma size. Next, we designed a siRNA library directed against 140 mRNAs encoding potential mTOR targets, selected

based on published data. To select siRNAs decreasing soma size of enlarged cells, cortical neurons were contransfected with tuberin siRNA together with siRNAs from the library. Our screen revealed over 20 genes, whose expression downregulation reversed the giant-cell like phenotype and 6 siRNAs that further increased size of cells with tuberin knockdown. Among proteins contributing to abnormal neuron growth upon mTOR overactivation, we identified those involved in actin cytoskeleton dynamics, vesicular transport and cellular signaling. *This work has been financed by PBZ-MNiI-2/1/2005 and PNRF-96-AI-1/07 grants.*

TIII.06
VACCINE PRESERVATIVE, THIMEROSAL, AFFECTS PAIN SENSITIVITY, BODY TEMPERATURE AND EXTRACELLULAR GLUTAMATE IN THE PREFRONTAL CORTEX IN RATS
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Mercury compounds are known neurotoxins. Thimerosal (THIM), which contains molecularly 49% of mercury, is added as preservative to many child vaccines and is suspected to be a major factor in autism pathogenesis. Data from Adverse Event Reporting of the Center for Disease Control and Prevention (USA) provide epidemiological evidence for a link between mercury exposure from THIM-containing vaccines and autism or other neurodevelopmental disorders. Our earlier studies showed that neonatally administered THIM causes persistent changes in pain reactivity, which are manifested in adult rats. In this study we investigated acute effects of THIM on pain reactivity, body temperature and brain glutamatergic system in mature rats. Single injection of THIM induced marked hypoalgesia, measured in hot plate test. This effect was time- and dose-dependent. It was reversed by administration of naloxone before the test and by two neurosteroids – dehydroepiandrosterone sulfate and androsterone. THIM also caused a dose-dependent hypothermia. Administration of THIM directly to the prefrontal cortex of freely moving rats increased the extracellular concentration of glutamate. The results suggest that acute THIM injection rapidly changes the neurochemical systems related to glutamate, opioid peptides, and probably GABA, which may contribute to developmental and neurotoxic effect of this compound. *Funded by EC grant MEXC-CT-2006-42371 to M. D. Majewska.*

TIII.07
EARLY POSTNATAL THIMEROSAL ADMINISTRATION, IN A VACCINATION-LIKE SCHEME, CAUSES VAST STRUCTURAL DAMAGE IN RAT BRAIN: IMPLICATIONS FOR AUTISM
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Thimerosal (THIM), an organomercury compound added to many child vaccines, is a prime suspect as agent causing autism epidemic. Data analysis from Vaccine Adverse Event Reporting System (CDC,USA) revealed that children immunized with THIM containing vaccines are several times more likely to develop autism and other neurodevelopmental disorders than those, who received THIM-free vaccines. In this study we examined developmental neurotoxic effects of THIM administered to Lewis and Wistar rats i.m. in four equal doses (12 μg Hg/kg to 3 mg Hg/kg) on postnatal days 7–14. Analysis of Hg content in brains of THIM-treated animals showed significant amounts of Hg, which remained there for longer than 30 days. When animals reached maturity their brains were removed and examined for histopathological changes using H&E and immunohistochemistry staining (GFAP, synaptophysin, neurofilaments, dopamine, opiate receptors). Vast structural damage was found in the brains of THIM-treated animals: reduced number of Purkinie cells, ischemic and necrotic changes in the amygdala, ischemic and cell structure abnormalities in the temporal neocortex, dorsal and ventral hippocampus; hippocampal, pontal and cerebellar clasmatodendrosis, loss of synaptic junctions in hippocampus. These neuropathological changes correspond with behavioral alterations observed in THIM-treated rats and seem analogous to structural brain abnormalities found in autistic patients. *Funded by EC grant MEXC-CT-2006-42371 to M.D. Majewska.*

TIII.08
TRANSIENT BRAIN ISCHEMIA AFFECTS BLOOD LEVEL OF BRAIN-DERIVED NEUROTROPHIC FACTOR IN EARLY REPERFUSION
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Brain-derived neurotrophic factor (BDNF) acts on certain neurons of the central and peripheral nervous system, helps to support the survival, encourage the growth and differentiation of new neurons and synapses. In our study we monitored BDNF blood level in course of brain ischemia-reperfusion in rat models of transient focal (FI) and global (GI) ischemia. Blood samples were collected before and during ischemia and at 40 and 90 min of reperfusion. Results showed that in model of FI BDNF concentration in total blood, in plasma and blood cells rapidly decreased during first 40 min of ischemia. In samples of whole blood and blood cells BDNF started to rise at the end of ischemic insult to control level, but in plasma it significantly decreased. In model of GI we didn't observe important changes after insult. During first 90 min of reperfusion in both models BDNF level in total blood and in blood cells continuously decreased. Plasma level of BDNF started to rise at 40 min of reperfusion. At 90 min of recirculation BDNF level in model of FI reached 92%, in GI about 155%. In conclusion we can state that brain ischemia causes reduction of BDNF level during ischemia and subsequent elevation of BDNF concentration in blood cells at the end of ischemic insult followed by decreasing in early period of reperfusion. On the other side, plasma level of this protein reduced during ischemia markedly rises during recirculation. *Supported by VEGA 2/0141/09, VEGA 2/0146/09, APVV LPP-023506, APVV-51-002105.*

TIII.09
THE NEUROPROTECTIVE EFFECTS OF UROCORTIN 2 IN BILATERAL COMMON CAROTID OCCLUSION INDUCED RETINAL DEGENERATION

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Urocortin 2 (Ucn 2) is a CRF paralog that preferentially activates CRF_2 receptors. Little is known regarding potential retinoprotective effects of Ucns despite the known presence of CRF family peptides and their receptors in retina. We investigated the effects of intravitreal Ucn 2 administration on ischemia-induced retinal degeneration (BCCAO). Two-month-old rats were subjected to BCCAO and their retinas were processed histologically after two weeks survival to determine the density of viable cells in the ganglionic cell layer and the thickness of all retinal layers. Immunohistological analysis of PKC-, calretinin-immunoreactivity was also performed. In BCCAO reduced retina thickness by approximately 60% as compared to sham-operated animals. Intraocular Ucn 2 treatment led to a nearly intact appearance of the retinal layers, and the thickness of all layers was significantly increased by 40% compared to ischemic vehicle-treated subjects. Ucn 2 treatment also increased the number of cells by 55% in the ganglionic cell layer as compared to those from carotid-occluded retinas of vehicle-treated subjects. Ucn 2 also reversed the alterations found in the pattern of immunocytochemical markers, such as calretinin and PKC. These findings suggest that intraocular Ucn 2 treatment may protect against ischemia-induced retinal degeneration, results with potential therapeutic implications for ophthalmic diseases. *Support: OTKA: K72592; F67830; 78480; T061766; Richter.*

TIII.10
LOSS AND RESTORATION OF PERINEURONAL NETS AFTER CORTICAL PHOTOTHROMBOTIC STROKE IN RATS

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The distribution and density of cells enwrapped with perineuronal nets (PNs) were examined in the neocortex after unilateral photothrombotic stroke. After different survival times PNs was visualized using *Wisteria floribunda* aglutinin (WFA) staining in the infarct core, peri-infarct and remote cortical areas. Sham operated rats and contralateral hemisphere to the stroke site from experimental rats served as controls. In the infarct core, 4 h after stroke only few cells enwrapped with PNs could be detected and none of numerous dying cells present showed PNs, as revealed by double staining (WFA and FJ-C). No cells with PNs were found 24 h after stroke. In the peri-infarct area, dramatic loss of PNs-enwrapped cells occurred (96%) as compared with the contralateral site, and such cells were almost absent after 24 h. However, 30 days after stroke, a significant number of cells with PNs reappeared. In remote cortical areas, 4h after stroke a reduction in the number of cells with PNs was observed. After 24 h the loss of PNs-enwrapped cells was profound (63%) in comparison to intact hemisphere. However, 7d after stroke complete restoration of the number of cells enwrapped with PNs took place. The density of cells with PNs was similar to that found in the contralateral site and in sham operated rats. These results showed the wide spreading and transient effect of photothrombosis on PNs in remote cortical areas. *Supported by MNiSW Grant N3030300832/0474 and statutory funds.*

TIII.11
RELATIONSHIP BETWEEN ACTIVITY OF MATRIX METALLOPROTEINASE -2 AND -9 (MMP-2, MMP-9) AND ISCHEMIA INDUCED NEUROGENESIS IN ADULT GERBIL HIPPOCAMPUS

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In physiological conditions proteolytic remodeling of extracellular matrix (ECM) participates in the stem cells development. Signal derived from ECM may activate specific intracellular signaling pathways which involve focal adhesion kinase (FAK), a key component responsible for the flow of information to the cell. In our study we checked if there is a spatial and temporal relationship between activity of two matrix metalloproteinases (MMP-2 and MMP-9) and neural stem-cell development in gerbil hippocampus 1, 2 and 4 weeks after short-term global ischemia. Furthermore we also investigate the activation of FAK. Our results show that after the ischemia there is a significant increase in the number of proliferating cells in DG which correlates with the activation of MMPs. Whereas in the damaged CA1 pyramidal layer MMP-2 and -9 activity decreases as a result of delayed death of neurons. In the same region we have also noticed a few immature neurons (NF-200 positive) 2 and 4 weeks after reperfusion, however they did not attain maturity. On the other hand we have noticed higher MMPs activity in the astrocytes surrounding the CA1 region. The diverse localization of MMP-2 and -9 activity in both cell types may be due to their different functions – firstly with the proliferation of newborn cells and secondly with the creation of the environment while the formation of post injury glial scar. *Supported by MSRHE grants: 1266/P01/2006/31*

TIII.12
THE HMGB1 RECEPTOR RAGE MEDIATES ISCHEMIC BRAIN DAMAGE

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In ischemic stroke, the necrotic core is surrounded by a zone of inflammation, in which delayed cell death aggravates the initial insult. Here, we provide evidence that the receptor for advanced glycation end products (RAGE) functions as a sensor of necrotic cell death and contributes to inflammation and ischemic brain damage. The RAGE ligand high mobility group box 1 (HMGB1) was elevated in serum of stroke patients and was released from

ischemic brain tissue in a mouse model of cerebral ischemia. A neutralizing anti-HMGB1 antibody and HMGB1 box A, an antagonist of HMGB1 at the receptor RAGE, ameliorated ischemic brain damage. Interestingly, genetic RAGE deficiency and the decoy receptor soluble RAGE (sRAGE) reduced the infarct size. *In vitro*, expression of RAGE in (micro)glial cells mediated the toxic effect of HMGB1. Addition of macrophages to neural cultures further enhanced the toxic effect of HMGB1. To test whether immigrant macrophages in the ischemic brain mediate the RAGE effect, we generated chimeric mice by transplanting $RAGE^{-/-}$ bone marrow to wild-type mice. RAGE deficiency in bone marrow-derived cells significantly reduced the infarct size. Thus, HMGB1-RAGE signaling links necrosis with macrophage activation and may provide a target for anti-inflammatory therapy in stroke.

TIII.13
NEUROPROTECTION BY AMINOGUANIDINE IN HIPPOCAMPAL CA1 REGION AFTER MIDDLE CEREBRAL ARTERY OCCLUSION (MCAO)
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The present study examined the effects of a selective inducible nitric oxide synthase inhibitor, aminoguanidine (AG) on neuronal cell survival after middle cerebral artery occlusion (MCAO) in hippocampal CA1 region. Transient focal cerebral ischemia was induced in rats by 60 min MCAO, followed by 7 days of reperfusion. Saline as vehicle or AG at the dosage of 150 mg/kg i.p. was administered immediately after occlusion and thereafter twice a day for three days. The evaluation of infarcted volume was made by 2,3,5-triphenyltetrazolium chloride (TTC). For comparison of cellular viability we used Fluoro-Jade B and NeuN staining to examine the evolving phases of infarction induced by MCAO. Treatment with AG significantly reduced total infarct volumes by 55% in comparison with saline group. AG significantly improved the neurological outcome. The number of degenerating neurons was markedly reduced in hippocampal CA1 region compared to groups without AG treatment. These changes were seen in the ipsilateral and contralateral hippocampus. In conclusion, the focal ischemia affects the hippocampus, which responds bilaterally to the injury. Our findings show that AG decreases ischemic brain damage and improves neurological recovery after transient ischemia induced by MCAO. It is suggested that treatment with AG may present a potential therapeutic strategy for the treatment of traumatic brain injury. *This study was supported by VEGA 2/0141/09, VEGA 2/0146/09, VEGA 1/4237/07 and APVV LPP 0235-06.*

TIII.14
IL-1β STIMULATES CYTOKINE SECRETION BY PERIPHERAL LEUKOCYTES FOLLOWING SUBARACHNOID HEMORRHAGE IN RATS
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Much evidence indicates that pathologies observed after subarachnoid hemorrhage (SAH), including vasospasm and neurological defects, are the effects of immuno-inflammatory processes accompanying the hemorrhage. Following SAH, CSF concentration of cytokines increase, what may change the cerebral perfusion and destroy brain–blood barrier. These cytokines are produced by activated leukocytes as well as hypoxic cells in the brain. The aim of this study was to examine the role of IL-1β in stimulation of peripheral leukocytes to increase secretion of cytokines after SAH. SAH was produced by injection of 150 μL of autologous arterial blood into cisterna magna. In 50% of animals, IL-1β activity was inhibited by intracerebroventricular administration of anti-rat IL-1β antibodies (SAH' groups). Control group consisted of sham-operated rats. Ninety minutes or 24 h following surgery, blood samples were collected and subpopulations of mononuclear leukocytes were isolated and cultured for 24 h. Then, the concentration of TNF-α, ET-1 and IL-6 in supernatants was measured by means of ELISA. SAH resulted in significant increase in TNF-α and ET-1, especially after 24 h, and no change in IL-6 concentration. Inhibition of IL1-β decreased the secretion of TNF-α and ET-1 and led to increase in IL-6. We found that peripheral leukocytes are an additional source of cytokines following SAH, and their activation depends on IL1-β produced after hemorrhage.

TIII.15
ALTERATION OF UBIQUITIN IMMUNOREACTIVITY AFTER ISCHEMIC POSTCONDITIONING AND EGb 761 TREATMENT IN CA1 HIPPOCAMPAL EURONS IN RATS
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Ischemic postconditioning used at the right time after initial ischemia is able to protect the most vulnerable CA1 hippocampal neurons. Alteration of ubiquitin immunoreactivity (UIR) was studied after 7 days of reperfusion in 4 experimental groups of rats (1) 10 min of ischemia, (2) 10 min of ischemia followed by ischemic postconditioning (5 min of ischemia done after 2 days of reperfusion). Extract of Ginkgo biloba (EGb 761, 40 mg/kg; p.o.) was administered 5 h after 10 min of ischemia both in ischemic group (3) or ischemia/postconditioning group (4). Quantitative analysis showed significant decrease (45.0 ± 9.7) of ubiquitin positive CA1 neurons in group (1) in comparison to sham control (187.5 ± 15.1) and nonsignificant increase of neuronal UIR (74.8 ± 22.4) in group (3) with EGb 761 administration. Ischemia/postconditioning (2) showed neuroprotection and significant increase of ubiquitin positive CA1 neurons (133.4 ± 14.3). Ischemia/ postconditionig combined with antioxidant EGb 761 (4) revealed suppression of neuroprotective effect of delayed ischemic postconditioning. Moreover, significant decrease of ubiquitin positive neurons (10.8 ± 2.4) in CA1 region was observed. Our results suggest that delayed ischemic postconditioning is able to prevent ischemia induced neurodegeneration while antioxidant used after initial ischemia followed by postconditioning fully abolishes this effect. *This study was supported by VEGA grants: 1/4237/07, 2/0141/09 and APVV LPP 0235-06.*

TIII.16
PRECONDITIONING AND POSTCONDITIONING EXERT STROKE NEUROPROTECTION MODULATING NCX AND ASIC1a EXPRESSION
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Ischemic preconditioning is a neuroprotective mechanism in which a brief non-injurious episode of ischemia protects the brain from a subsequent lethal insult. Recently, it has been reported that modified reperfusion subsequent to a prolonged ischemic episode may also confer neuroprotection, a phenomenon termed postconditioning. Mitogen Activated Kinases (MAPK) play a key role in these neuroprotective mechanisms. Objectives of this study was to evaluate the effect of MAPK activated by preconditioning and postconditioning on the expression of Na^+/Ca^{2+} exchangers(NCXs), and Acid Sensing Ionic Channels (ASICs), two families of proteins that, by modulating intracellular ionic homeostasis, contribute to the progression of the ischemic lesion. NCX and ASIC protein expression was investigated in rats subjected to harmful ischemia, preconditioning or postconditioning, treated with specific MAPK inhibitors or with vehicle alone. Results of this study indicated that NCX1, NCX3 and NCKX2 are up-regulated in those brain regions protected by preconditioning and postconditioning treatment while ASIC1a is downregulated in the same regions. The change observed in the expression of these proteins seems to be due to p-AKT. In fact, p-AKT inhibition reverted the preconditioning and postconditioning neuroprotective effect and prevented NCXs overexpression and ASIC1a downregulation.

TIII.17
SYSTEMIC TREATMENT WITH FRESHLY ISOLATED HUCB-MNs CELLS IS THE MOST EFFECTIVE IN RESTORING FUNCTION FOLLOWING BRAIN DAMAGE IN RATS
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The aim of the study was to compare therapeutic effectiveness of intra-arterial infusion of human umbilical cord-blood derived mononuclear (HUCB-MNs) cells at different stages of their neural conversion *in vitro*. Freshly isolated HUCB-MNs (D-0) neuraly directed progenitors (D-3) obtained during 3 days culture of HUCB-MNs and neural stem cells (NSC) line derived from HUCB-MNs were assessed. Focal brain damage of dorsolateral striatum was induced in Wistar rats by stereotactic injection of previously established low dose of ouabain (1 µl or 1,5 µl 5 mmol). Three days later 10^7 HUCB cells were infused into carotid artery. Following surgery rats were housed in large enriched environment cages, in groups of 7–8 animals per cage, for 30 days. Behavioral assessing consisted of: tests for sensorimotor deficits (walking beam task, rotarod), habit learning task, exploratory behavior (open field test) and apomorphine induced rotations. Functional effects of different subsets of HUCB cells were diverse in various behavioral tests and hard to conclude which stage of neural conversion of cord blood cells is the most effective in functional recovery. Additional analysis was applied: scores concerning positive effects of cells treatment visible in all parameters were calculated. The sum of scores revealed that the most effective in functional restoration and reduction of lesion volume were freshly isolated D-0 HUCB cells. *Supported by Medical Research Centre statutory fund.*

TIII.18
PROTEIN KINASE C BETA IN BRAIN MITOCHONDRIA
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PKC is implicated in the regulation of mitochondrial membranes integrity, however its precise function is not fully understood. We have examined association of PKCβ with mitochondria isolated from ischemia-vulnerable (CA1) and ischemia-resistant (CA2-3, DG) hippocampal regions in gerbil model of transient brain ischemia. We show that ischemia-induced translocation of PKCβ to mitochondria in (CA2-3,DG) is significant, rapid and stable and, may bespeak neuroprotection. Furthermore, in an *in vitro* organotypic hippocampal slice culture, the specific PKCβ inhibitor - peptide increases detrimental effect of 100 µM NMDA. Cell death measured with propidium iodide showed 96% ± 25 vs. 63% ± 9 after NMDA alone and vs. 1.5% ± 0.5 dead neurons in unchallenged slices. We have also been looking for PKCβ mtiochondrial partners using "pull-down" method and Mass Spectrometry analysis. It revealed mitochondrial proteins: creatine kinase, ANT, α, β chains of ATP synthase, complex I protein of electron transport chain and thioredoxin, which can bind directly to PKCβ. All of these proteins are connected with energy and superoxide production. Our data suggest that PKCβ translocation to mitochondria might be connected with endogenous neuroprotection in CA2-3, DG hippocampus. PKCβ acting through abovementioned mitochondrial proteins could positively influence the balance in ROS production during reoxygenation after transient ischemia. *Supported by Polish Mitochondrial Network MitoNet.pl.*

TIII.19
THE INFLUENCE OF ISCHEMIA/REPERFUSION ON THE NEURONAL NITRIC OXIDE SYNTHASE IMMUNOREACTIVITY IN LUMBOSACRAL SPINAL CORD AND DORSAL ROOT GANGLIA
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Nitric oxide, a diffusible molecule which is constitutively expressed by neuronal isoform of nitric oxide synthase (nNOS) acts as an important non-conventional neurotransmitter in the nervous system. The distribution of nNOS-immunoreactive (-IR) and nicotinamide adenine dinucleotide phosphate diaphorase (NADPHd) positive neurons was studied by immunohistochemical and histochemical methods in upper lumbar (L1-L3) and lower lumbar and sacral (L4-S4) dorsal root ganglia (DRGs) and in relevant spinal cord segments after 15 min ischemia followed by 7 and 14 days of reperfusion. The intensity of nNOS-IR and NADPHd positivity was evaluated by densitometric analysis, using a software UTHSCSA Image Tool. A quantitative immunohistochemical and histochemical study have shown a strong vulnerability of nitrergic neurons in the spinal cord to transient ischemia and the following reperfusion periods. In addition, our results clearly show the increase of nNOS-IR/NADPHd positivity in spinal ganglia laid outside the spinal cord. Distri-

bution character of nNOS-IR/NADPHd positive fibers coming out from DRGs reflects the increase of nNOS-IR and NADPHd positivity in thin and thick myelinized axons of dorsal roots and dorsal funiculi. These results explain the participation of nitrergic system in nociceptive and proprioceptive circuits in the spinal cord. *Supported by the APVV 0314 -06 and VEGA grants 2/0110/08 and 2/0015/08.*

TIII.20
RESTITUTION OF SENSORY FUNCTION OF VIBRISSAE AFTER STROKE IN THE BARREL FIELD COINCIDES WITH ACTIVITY CHANGES IN SPARED SOMATOSENSORY CORTICAL AREAS

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We described previously a sequence of cortical activity changes after the unilateral stroke in the whiskers somatosensory representation – the barrel field (BF). We aimed to check if this activity remodeling is linked to the restitution of the whiskers sensory function. To characterize poststroke deficits and time related recovery we performed the gap-crossing test for a group of rats 3, 28 and 56 days after the photothrombotic stroke (DPS) in the BF. Rats learned to cross the gap between elevated platforms in darkness, using only sensory information from whiskers. We examined the minimum distances at which rats failed to cross (min) and the maximal distances (max) crossed by the rats in two scenarios at each time point: (1) with all their whiskers and (2) only with unilateral whiskers corresponding to the destroyed BF (contralateral to the stroke). Before the stroke there were no significant differences in min and in max distances when rats used all *versus* unilateral whiskers. At 3DPS the max was lower when rats used whiskers with destroyed sensory representation. The first fall was made by rats at shorter distances when they used just the impaired side whiskers. At 56DPS all the differences between crossing performance disappeared. At this time point, we previously found new activity foci in the spared somatosensory cortex of the injured hemisphere, specific for the whiskers stimulation – probably a new functional representation of the whiskers.

TIII.21
EXPERIMENTAL NEUROPHYSIOLOGICAL MOTOR EVOKED POTENTIAL STUDIES IN THE ISCHEMIC STROKE RAT'S MODEL

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This study was undertaken to ascertain functional changes in areas of ipsi- and contralateral motor cortex involved with the ischemic incidences following experimental closing of flow in cervical aorta uni- (right) or bilaterally when recordings of motor evoked potentials induced with magnetic field (MEPs) were performed.Aims of this study were (1) ascertaining changes in parameters of recordings in motor evoked potentials (MEPs) following experimental ischemic conditions, (2) histological verification of range in pathological changes within motor cortex neurons following ischemia, (3) estimation the correlation between duration of ischemia and changes in activity of cortico-spinal neurons. Studies were performed on 24 Wistar rats. Recordings of MEPs induced in the left motor cortex were performed from right sciatic nerve.Significant fluctuations in MEPs amplitudes from 4.5 mV to 1.5 mV were commonly found within 8 minutes from the experiment onset what might indicate on changes in excitability and transmission of certain number corticospinal neurons. Contrary to the previously found changes in MEPs latencies we observed more often the reversible changes of amplitude parameter. The time of 6 minutes after the significant ischemic incidence is not critical for irreversible and persistent changes in activity of cortico-spinal neurons.

TIII.22
THE EFFECTS OF CPPG ON SPATIAL MEMORY AND THE ACTIVITY OF MMP-2 AND MMP-9 IN THE HIPPOCAMPUS OF RATS WITHOUT OR AFTER HYPOXIA

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CPPG is a highly potent group III metabotropic glutamate receptor ($mGluR_{4/6/7/8}$) antagonist with selective affinity to $mGluR_4$. The aim of the present study was to determine the effects of CPPG on spatial memory in the water maze, and on the activity of MMP-2 and MMP-9 metalloproteases in the hippocampus of rats without and after hypoxia. Short-term hypoxia (2% O_2 and 98% N_2) enhances excitatory synaptic transmission and impairs spatial acquisition in the water maze. We observed that CPPG prolonged only escape latency during the first day of training in water maze in group without hypoxia, while it did not influence escape latency, distance travelled and swim speed during training of rats after hypoxia. It did not change previously acquired escape response during free-swim trial in either group of rats. Hypoxia significantly increased the activity of the proform of MMP-9 in the hippocampus. Gel zymography showed elevations of MMP-2 and MMP-9 activity in the hippocampus of rats without or after hypoxia when CPPG was administered. Concluding, CPPG has no effects on deficits of behavioural processes induced by hypoxia in the water maze. Enhanced activity of MMP-2 and MMP-9 in the hippocampus induced by CPPG does not correspond to the influence on acquisition in the water maze. The reconfiguration of extracellular matrix by MMP-2 and MMP-9 in hippocampus is new central effect of CPPG. *Support: grant No 3-10614L from the State Committee for Scientific Research, Warszawa, Poland.*

TIII.23
IMMUNOSUPPRESSANTS ATTENUATE HMGB1 EXPRESSION AND RELEASE FROM PRIMARY ASTROCYTE CULTURES EXPOSED TO COMBINED OXYGEN-GLUCOSE DEPRIVATION

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Protective potential of immunosuppressants has been reported in many experimental models of ischemia both *in vivo* and *in vitro* suggesting novel therapeutic application of these drugs. On account of fact that high mobility group box 1 (HMGB1) protein has recently

been reported to be involved to ischemic brain injury, the purpose of the present study was to determine whether treatment with immunosuppressants could decrease HMGB1 expression and release in astrocytes exposed to ischemia-simulating conditions (combined oxygen glucose deprivation, OGD). We also studied the influence of these drugs on expression of NFκB, inducible NO synthase (iNOS) and cyclooxygenase-2 (COX-2). In addition, we investigated whether the immunosuppressants could attenuate of necrosis in astrocyte cultures exposed to OGD. Cells were treated with cyclosporine A, FK506 and rapamycin (all drugs at concentrations of 0.1, 1 and 10 mM). Our study has provided evidence that immunosuppressants decrease the expression and release of HMGB1 in ischemic astrocytes. The present results provide further information about the cytoprotective mechanisms of immunosuppressants towards ischemic astrocytes, in relation to the pathophysiology of ischemic brain injury. It appears that the immunosuppressants stimulated protective effects could be mediated in part by suppression of HMGB1 expression and release in astrocytes, what leads to attenuation of ischemia-induced necrosis and neuroinflammation.

TIII.24
MOLECULAR MECHANISMS OF AXON DEGENERATION AND ITS BLOCKADE
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Axon degeneration is a critical feature in peripheral neuropathies and in neurodegenerative diseases. A valuable model for study of axon degeneration is that of Wallerian degeneration (WD) in which the distal portion of a transected axon undergoes a sequential process of degeneration. The discovery of the Wld^s mouse mutant which exhibits strong delay in WD due to expression of nuclear Wld^s protein has completely changed our understanding of WD which is now regarded as an active auto-destruction programme. Dissection of the cellular and molecular mechanisms of this novel death pathway is paramount for the development of new therapies. I will summarise my recent work which shed new light on the mechanisms of WD, chronic axon pathology and the mode of action of Wld^s [e.g. Beirowski et al. (2009) J Neurosci 29: 653–668, Beirowski et al. (2008) Eur J Neurosci 28: 1166–1179].

TIII.25
DYNAMICS OF CHANGES OF EXECUTIVE FUNCTIONS IN PATIENTS WITH TRAUMATIC BRAIN INJURIES
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The purpose of the study was to observe the dynamics of changes of executive functions in patients with light and mild traumatic frontal lobes injuries. Patients, with changes indicated in CT, and the control group accordingly, were presented with a set of neuropsychological methods (i.e. Tower of London (TOL), Trail Making Test (TMT) , Stroop Test etc.), three times during three weeks following the brain trauma and after half a year after the brain trauma. Dynamics of change may differ according to individual factors (Leon-Carrion et al.), however in results obtained an interesting pattern of improvement may be observed. Namely, during the short period after the brain trauma, patients' level of functioning reaches its peak in the first and third week of recovery, whereas decreases rapidly during the second week (e.g. see TOL, $F_{1, 14}$=2.5, P<0.05). Above results may be helpful in the future evaluation of methods in neuropsychological rehabilitation.

TIII.26
THE EFFECT OF NOISE EXPOSURE IN RAT PUPS ON THE ACOUSTIC STARTLE REFLEX IN ADULTHOOD
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Acoustical trauma during the sensitive period of postnatal development may result in the impairment of the auditory system, manifested as deteriorated auditory sensitivity and abnormalities in the processing of auditory signals. The acoustic startle reflex (ASR) and the prepulse inhibition (PPI) of the ASR were examined in 3–6-month-old rats exposed to broad-band noise (125 dB SPL, 8 min) in early postnatal life (day 14). Hearing thresholds in noise-exposed rats, examined by measurements of auditory evoked responses, were found to be normal. The ASR to tones in the range of 2–16 kHz and the PPI of the ASR to a noise burst (110 dB SPL) induced by 2, 4, 8, 12 and 16 kHz tones over a range of 20–80 dB SPL were measured in exposed rats and in age-matched controls. In all exposed rats, a decreased strength of the startle reflex in response to intense stimuli was observed in comparison with controls. The effect of acoustic trauma on the PPI was different from the effect on the ASR: tones of 20–30 dB SPL were able to suppress the ASR in exposed rats significantly more than in control animals, a similar tendency was observed at higher intensities. The results indicate that acoustic trauma in the early developmental period induces abnormalities in the auditory system of rats in the processing of stimulus intensity in addition to abnormalities in the processing of sound frequency [Grécová et al. (2009) Eur J Neurosci, in press]. *Supported by AV0Z50390512, GACR 309/07/1336, LC 554.*

TIII.27
CONSERVED MECHANISMS OF GENE CIS-REGULATION FOLLOWING STROKE AND SEIZURES
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We performed SVD analysis of our data from expression profiling in the rat MCAO model of stroke and of published dataset from the rat hipopocampus following kainate-induced seizures. The contributions (across the genes) of two SVD modes were highly conserved following stroke and seizures, suggesting that they reflect mechanisms of gene regulation conserved following seizures and stroke. Functional annotation revealed that the first conserved SVD mode reflects inflammatory response and apoptosis. The second conserved mode reflects increased expression of genes associated with neuronal synaptic activity. Applying Bayesian networks learning algorithm to the data from Transcription Regulatory regions And Motifs (TRAM) database, we looked for putative cis-regulatory features associated with the conserved modes. We report that two motifs, binding

transcription factor AP1, and the matrix-attachment region binding protein SATB1, had opposing effects on gene expression in the subspace of the mode reflecting inflammation and apoptosis. A number of features; binding transcription factors associated with neuronal plasticity (Egr1-3, Creb, Zfp161), hypoxia/oxidative stress (Nfe2l1-2, Arnt), and neuronal differentiation (Lhx1-3); were associated with the mode reflecting neuronal activity. The results obtained on one dataset were confirmed on the other, which validated the features and – more importantly – revealed mechanisms of gene co-regulation conserved between stroke and seizures.

TIII.28
GELATINOLYTIC ACTIVITY IS PRESENT IN THE INTERCHROMATIN SPACE OF THE NEURONAL CELL NUCLEUS

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Matrix metalloproteinases (MMPs) form an enzyme family which, by mainstream research, is implicated in extracellular matrix processing in physiological and pathophysiological conditions. Some of these proteins termed gelatinases, in particular MMP-2 and MMP-9, cleave gelatin as an artificial substrate. Surprisingly, a number of studies have revealed the presence of gelatinolytic activity in the cell nucleus. Although the phenomenon appears not to be artifactual, neither the identity nor the role of nuclear gelatinases has been established unequivocally. In the nervous system, nuclear gelatinolysis is detectable in normal conditions, yet it is induced by seizures, and stroke. We studied nuclear gelatinolytic activity by high resolution *in situ* zymography (ISZ) in sections of alcohol-fixed, polyester wax-embedded normal rat brain. Ubiquitously distributed among the major brain areas the ISZ signal was present mainly in neurons. At high magnification, our study revealed previously unrecognized mesh-like pattern of nuclear gelatinolytic which, by counterstaing with fluorescent DNA-binding dye, represents an interchromatin space. The ISZ signal colocalized with the ribonucleoprotein compartment enriched in splicing components, identified using an immunoreactivity of spliceosome assembly factor SC-35. This suggesting a function for MMPs in processes of gene-expression and/or RNA-processing and hypothetically involvement in remodeling of chromosome territories.

TIII.29
THE NEUROPROTECTIVE EFFECTS OF PACAP IN SEVERAL MODELS OF NEURODEGENERATION IN THE RAT RETINA

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Pituitary adenylate cyclase activating polypeptide (PACAP) is a neurotrophic and neuroprotective peptide that has been shown to exert protective effects in different neuronal injuries, models of neurodegenerative diseases and cerebral ischemia. PACAP and its receptors are present in the retina. PACAP is neuroprotective in several models of retinal degeneration *in vitro* and *in vivo*. In the present study we summarize the protective effects found with intravitreal PACAP treatment in the following models of retinal degeneration in rats: (1) toxic injury induced by monosodium glutamate (MSG) treatment; (2) ischemic injury (BCCAO) and (3) degeneration induced by different kind of UV-A light. MSG leads to the severe degeneration of the inner retinal layers. BCCAO cause degeneration in all retinal layers. UV-A exposure leads to a severe damage of the outer layers of the retina. In all cases, intravitreal PACAP injection (100 pmol) was administered into one (right) eye, while the other eye (left) received saline treatment, serving as control retinas. Histological and immunocytochemical analysis of the retinas showed that PACAP treatment significantly ameliorated the damaging effects of all treatments. These results provide the basis for future clinical application of PACAP treatment in retinal degeneration. *Support: OTKA: K72592; F67830; 78480; T061766; Gedeon Richter Ltd.*

TIII.30
PROTECTION OF HIPPOCAMPAL NEURONS AGAINST NEUROTOXIC DAMAGE BY ANTI-MYELIN T CELLS MAY INVOLVE DIMINISHING THE ACTIVATION OF MICROGLIA AND NG2+ GLIAL CELLS

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We have previously demonstrated that CD4+ anti-myelin basic protein (MBP) T cells protect hippocampal neurons against trimethyltin-induced damage. In this study, we employ immunocytochemicalmethods to investigate the influence of administration of T cells to the response of microglia and of NG2+ cells to TMT-induced damage. Female Lewis rats were treated with anti-MBP CD4+ T cells (4 million per animal, i.v.) 24 hours after TMT (8 mg/kg, i.p.) intoxication. TMT caused degeneration of CA4 hipppocampal neurons and evoked an abundant reaction of microglial and NG2+ cells in the injured zone. The number of activated cells increased about 4-fold relative to controls as assessed on the 21st day after TMT treatment. A bulk of the cells of ameboid morphology, which expressed NG2 or microglial antigens, appeared in the zone of neurodegeneration. Interestingly, many of the cells of ameboid phenotype shared both antigens. Administration of T cells downregulated the activation of both glial classes and considerably reduced the formation of the ameboid phenotype forms. The latter were decreased, on average, by 60 percent. Our data suggest that the diminished activation of microglia and NG2+ cells, particularly the decrease of the ameboid forms known to release a number of proinflammatory substances, may contribute to the neuroprotective effect of T cells. *Supported by the grant no N401-1293-33 of the Ministry of Scientific Research and Information Technology in Poland.*

TIII.31
ANALYSIS OF PUTATIVE TOXIC INTERACTION OF 1-METHYLNICOTINAMIDE WITH DOPAMINERGIC NEURONS
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It has been proposed that in the Parkinson's disease exo- or endogenous N-methyl-compounds like 1-methylnicotinamide (MNA) could be accumulated by dopaminergic neurons and induces neurotoxic effects, as happens with MPP+. The aim of this study was to test this hypothesis by comparing direct effects of MPP+ or MNA application into the striatum of 7 day old rat pups and to the substantia nigra (SN) of the adult rats. Microinjections of tested substances in doses: MPP+ (20 and 100 μg) and MNA (20, 40, 80 μg), were made unilaterally. Effects of MNA and MPP+ on pups' striatum were evaluated by TTC staining and the lesion volume was calculated using the ImageJ program. In the SN of adult rats alterations in the level of tyrosine hydroxylase (TH) immunostaining were examined at the 5th day after MPP+ or MNA injection, utilizing antibody specific to TH. Moreover the coronal sections (20 μm thick) of the SN were examined to evaluate MNA- or MPP+-induced glial activation with the glia-specific lectin. Using these methods we did not observe any toxic effect of MNA. MPP+ induced statistically significant damages in the striatum of rat pups, whereas there was no lesion after MNA injection. Also in the SN of adult rats MPP+ caused 50% loss of TH-positive neurons and doubled the number of activated glial cells, while MNA injection had no visible effect. Summing up, present data did not demonstrate any toxic effects of MNA on dopaminergic neurons in the rat brain *in vivo*.

TIII.32
THE ROLE OF LOW MOLECULAR WEIGHT THIOLS IN THIMEROSAL TOXICITY – AN *IN VITRO* STUDY
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Thimerosal (TH), an ethylmercury complex of thiosalicylic acid has been used as preservative in vaccines. Inspired by a known high affinity of mercury for thiol groups, we examined whether the presence of L-cysteine (Cys), D,L-homocysteine (Hcy), N-acetyl cysteine (NAC), L-methionine (Met) and glutathione (GSH) in extracellular space could influence the viability, intracellular calcium concentration ($[Ca^{2+}]_i$) and mitochondrial membrane potential in rat cerebellar granule cells. The cells were exposed to 500 nM TH for 48 h or 15 μM TH for 10 min. The loss of cells viability could be prevented partially or wholly, in a dose-dependent manner, by 60, 120 or 600 μM Cys, Hcy, NAC and GSH, but not by Met. The elevation in $[Ca^{2+}]_i$ and mitochondrial potential induced by 25 μM TH were abolished by all compounds studied, except for Met, at 600 μM. The loss of the ethylmercury moiety from TH as a result of interaction with thiols studied was monitored by ^{1}H- and ^{199}Hg-NMR spectroscopy. This extracelullar process may be responsible for the neuroprotection seen in cerebellar cell culture, but also provides a molecular pathway for redistribution of TH derived toxic ethylmercury in the organism.

TIII.33
LENTIVIRAL DELIVERED siRNA AGAINST ICER PROTECTS PRIMARY CORTICAL NEURONS FROM APOPTOSIS CAUSED BY SERUM DEPRIVIATION
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CREB activation and CREB-dependent signaling pathways are crucial for neuronal survival. The term ICER (inducible cAMP early repressor) refers to four protein isoforms that are all endogenous, inducible antagonists of CREB. It was previously shown, that all 4 ICER isoforms are induced upon pro-apoptotic treatment, and also that each of them separately evokes neuronal cell death in cortical culture transfected with these genes. The ICER proteins are believed to be strong repressors of Immediate Early Genes, which are involved in cell response to inter- and/or intra-cellular signals. Herein, we have applied the siRNA approach to silence ICER expression. Because ICERs are members of CREM family of proteins, sharing with them the gene sequence, only the small unique region for ICER was selected to design ICER-directed, specific siRNA. Indeed, we obtained functional siRNA capable of blocking ICERs but not affecting CREM proteins. With this tool, we have investigated if the ICER's silencing protects neurons from apoptosis caused by either serum deprivation or excitotoxicity. Using the lentiviral vector, as a vehicle to deliver siRNA (shRNA) we have found that silencing of ICER mildly, although significantly, protects primary cortical neurons from apoptosis caused by serum deprivation.

TIII.34
IMPAIRMENT OF CHOLINERGIC SEPTAL SN56 CELLS BY THIAMIN DEFICIENCY
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Cholinergic dementias are accompanied by inhibition of pyruvate and ketoglutarate dehydrogenase (PDH, KDH) in affected brains. Therefore, we investigated how amprolium-evoked thiamine pyrophosphate deficits impair acetyl-CoA metabolism as well as function and viability of cholinergic neurons. Two day exposure of differentiated (DC) or nondifferentiated (NC) SN56 cholinergic cells cultured in Minimal Eagles Medium to amprolium caused concentration-dependent inhibition of cell MTT reducing capacity and increased their mortality, that at 5 mM inhibitor concentration reached values 30 and 15% or 43 and 10%, respectively. However, neither PDH nor KDH activities nor cell growth were significantly, inhibited by their culture with amprolium. On the other hand, amprolium decreased acetyl-CoA levels in DC and NC by 39 and 46%, respectively. The inhibitor altered choline acetyltransferase activity neither in NC nor in DC. Acetylcholine content in DC and was suppressed by amprolium for over 40%. On the contrary, in NC it had no effect on the transmitter level. These findings indicate that increased ACh synthesis in

DC made them more vulnerable to acetyl-CoA shortages caused by amprolium-evoked inhibition of thiamine uptake. Low rate of ACh synthesis in NC, allowed them to save more acetyl-CoA to support energy metabolism and thereby facilitated their survival under pathologic conditions. *Supported by MNiSW projects PO5A 11030, NN401 2333 33 and AMG fund W-144.*

TIII.35
ENERGETIC STATUS OF MITOCHONDRIA IN PRIMARY CEREBELLAR GRANULE NEURONAL CULTURES OF RATS PRE- AND POSTNATALLY EXPOSURED TO LEAD

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Lead (Pb^{2+}) is a widespread pollutant and potent central neurotoxin. We have studied its effect on energetic metabolism of cerebellar granule cells (CGC) in pre- and postnatally intoxicated rats. Pregnant Wistar rats received 0.1% lead acetate in water. CGC were prepared from 8-day-old born rats according to a standard procedure. Pb^{2+}concentration was measured in blood and in cerebellum homogenates by AAS. Intracellular Pb^{2+} concentrations in CGC was studied by fluorescent microscopy, in neurons loaded with the Ca^{2+}-sensitive indicator Oregon Green. Intracellular Pb^{2+} was evaluated from the fluorescence intensity and estimate in μM range. Pb-induced decrease in Adenylate Energy Charge (by 10%) and ATP concentration (by 35%) in cerebellum homogenates (HPLC method) was observed at Pb concentrations in whole blood (7.05 ± 2.05 μg/dL).The function of mitochondria of neurons of Pb-treated and control rats were evaluated using: Mitotracker Green FM and JC-1. We observed active mitochondria stained green with Mitotracker in cytoplasm of CGC from control and Pb-treated rats. Incubation of cultured CGC from rats of control groups with the mitochondrial probe JC-1 produced red-orange fluorescence showing mitochondria with high membrane potential ΔΨ. The green fluorescence dependent on mitochondria with low ΔΨ was visible in cytoplasm of cells in Pb-treated rats. Pre- and postnatal low-level exposure to lead caused disruption of mitochondria energetic status in neurons.

TIII.36
SELECTIVE METABOTROPIC GLUTAMATE RECEPTOR 1 ANTAGONIST INHIBITS KAINATE-INDUCED EXCITOTOXICITY *IN VITRO* AND *IN VIVO*

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The aim of the study was to investigate a neuroprotective effect of selective mGluR1 antagonist EMQMCM against kainate-induced excitotoxicity*in vitro* in mouse cortical and hippocampal neurons and *in vivo* in the rat hippocampus. Excitotoxic damage in the cultures was evoked by incubation with 150 μM kainate (KA) for 24 h (hippocampus) or 48 h (cortex). In *in vivo* studies male Wistar rats were unilaterally injected with KA (2.5 nmol/1 μl) into the CA1 hippocampal region. In both models EMQMCM was added 30 min, 1, 3 or 6 h after starting intoxication. *In vitro* KA neurotoxicity was measured by lactate dehydrogenase (LDH) efflux from damaged cells into culture media. The application of EMQMCM (0.1, 1, 10 and 100 μM), 30 min or 1 h after KA, significantly attenuated the KA-induced LDH release both in cortical and hippocampal cultures. Protective effects also appeared when the antagonist was applied 6 h after KA, but only at a concentration 100 μM. Additionally, EMQMCM prevented KA-induced increase in caspase-3 activity, which indicated for its antiapoptotic action. *In vivo* degeneration was examined by counting of surviving neurons in CA pyramidal layer in the dorsal hippocampus, using stereological methods. It was found that EMQMCM (5 or 10 nmol/1 μl) given 30 min after KA, significantly attenuated neuronal damage. The obtained results indicate that EMQMCM can prevent excitotoxic neuronal injury both *in vitro* and *in vivo* studies, soits potential therapeutic role may be suggested.

TIII.37
EFFECT OF LIPOPOLYSACCHARIDE INDUCED INFLAMATION ON NEUROPEPTIDE Y NEURONS IN MOUSE HIPPOCAMPUS

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Neuropeptide Y (NPY), a 36 amino acid neurotransmitter, is involved in the regulation of emotional behavior and its role in the central responses to peripheral immune challenge is postulated. Plasma NPY levels rises in human sepsis and NPY improved survival in the experimental endotoxic shock induced by lipopolysaccharide (LPS) injection. Some of the behavioral effects of LPS, anorexia, depression and anxiety-like behavior are also modulated by NPY. Therefore, in the present study tried to find out if LPS affected NPY neurons in mouse hippocampus, a structure most susceptible to damage and involved in the regulation of emotion. Male C57BL/6 mice were injected with LPS (1 m/kg, i.p.) and their brains were taken after 6 or 24 h. The brains were fixed with paraformaldehyde, cut into frontal sections containing the dorsal hippocampus and processed by immunohistochemistry using an NPY antibody. NPY-immunoreactive neurons were counted stereologically in hippocampal subregions CA1+2, CA3 and DG+hilus, and results were statistically analysed. It was found that after 24 h LPS decreased by ca. 38% the number of NPY-positive neurons in the hippocampal CA regions. The effect was particularly significant in the CA3 area. Moreover, staining intensity was diminished. The obtained results indicate a decrease in NPY expression in the hippocampus, which may be due to the peptide release induced by LPS inflammatory action. *Supported by MS&HE.28/E32/BWSN-0053/2008*

TIII.38
NEUROPROTECTIVE POTENTIAL OF BIPHALIN, MULTIRECEPTOR OPIOID PEPTIDE, AGAINST EXCITOTOXIC INJURY *IN VITRO*

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Biphalin is a dimeric opioid peptide, that exhibits a high affinity for all three opioid receptors types (miu, delta and kappa). When administered intracerebroventricularly it has been shown to be more potent than morphine and ethorphine at eliciting antinociception. Biphalin is currently undergoing intensive preclinical study. It is recognized in recent years that activation of delta-opioid receptor elicits neuroprotection against hypoxia and ischemia, but the underlying mechanisms are not fully understood. In this study we tested the effect of biphalin and the inhibition of opioid receptors by naltrexon (Ntx) on survival of neurons in rat organotypic hippocampal culture challenged with NMDA *in vitro*. Cell survival was estimated with fluorescent cell-death marker propidium iodide. Our main findings are that (1) biphalin in the concentration of 100 μM reduces NMDA-induced neuronal damage; (2) biphalin neuroprotective effect is abolished by opioid receptors antagonist naltrexon; (3) naltrexon by itself does not change NMDA-induced neuronal damage; (4) reduced number of dead cells was clearly shown even when biphalin was applied with a delay after NMDA challenge. These results demonstrate that biphalin, multireceptor opioid peptide, can reduce NMDA-induced neuronal damage. *This work was supported by MSHE Polish Mitochondrial Network grant*.

TIII.39
INFLUENCE OF ISOPROPYL METHYLPHOSPHONOFLUORIDATE INTOXICATION ON CYTOKINE LEVELS IN CHOSEN BRAIN STRUCTURES

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Poisoning with organophosphate compounds induces pronounced neurotransmitter disturbances in central nervous system. There are some data indicating that these poisonings initiate changes in neuro-immune response. Our study was aimed at the evaluation of cytokines levels (interleukin 1β – IL-1β, interleukin 2 – IL-2, tumor necrosis factor α – TNF-α and interferon γ – IFNγ) after isopropyl methylphosphonofluoridate (IMPF) intoxication in chosen brain structures of BALB/c mice. Tests were performed in supernatants of frontal cortex, hippocampus and brain stem homogenates using immunoenzymatic assay after 24 h, 7, 14 and 21 days of IMPF intoxication (1/2 LD_{50}, s.c.). IMPF induced differential changes in cytokines levels (except IL-2) in the examined brain structures. The significant increase of TNF-α and IL-1β levels was shown in frontal cortex after 14 days of poisoning. Raised level of TNF-α in this structure was observed until the end of the experiment (21st day). We also observed pronounced increase of IFNg level after 7 days in hippocampus and after 7 and 14 days in brain stem. Concurrently the level of IL-1β in these structures was significantly decreased, in hippocampus in all measurements, while in the brain stem only after 24 hours following IMPF application.Our results indicate that IMPF intoxication without inducing convulsions can produce marked changes in brain cytokines levels that may lead to immune response disturbances.

TIII.40
CHANGES IN CEREBROSPINAL FLUID CONCENTRATION OF OXIDATIVE STRESS MARKERS DURING CHEMOTHERAPY OF ACUTE LYMPHOBLASTIC LEUKAEMIA IN CHILDREN

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In this study we advised question whether cerebrospinal fluid oxidative stress markers are associated with neurotoxicity of chemotherapy of acute lymphoblastic leukaemia (ALL). Examination of 38 ALL patients revealed a statistically significant increase in 8-isoprostane and decrease in total antioxidative capacity during the treatment. Dynamic analysis revealed a statistically significant increase in isoprostane starting on the 59th day of the treatment when the levels were highest and remained raised during all the treatment course. The mean 8-isoprostane level at the diagnosis was 9.05 ± 5.12 pg/ml, and no correlation with initial leukocytosis, organomegaly and lactate dehydrogenase level was noted. Dynamic data analysis revealed a statistically significant increase in 8-isoprostane on the 59th day of the treatment (24.85 ± 26.28) and at four points during consolidation phase (17.28 ± 8.09; 22.72 ± 21.79; 24.92 ± 22.74; 32.32 ± 26.85) as compared to its level at the diagnosis (P<0.01. The mean total antioxidative capacity level at the diagnosis was (203.98 ± 15.11 μmol/l). Dynamic data analysis revealed a statistically significant decrease in total antioxidative capacity on the 59th day of the treatment (189.76 ± 4.64) and at one point during consolidation phase (188.29 ± 8.46) as compared to its level at the diagnosis (P<0.05). The study suggests that standard ALL treatment may cause neurotoxicity by oxidative stress.

TIII.41
SYSTEMIC INFLAMMATION LEADS TO UP-REGULATION OF PROOXIDATIVE GENES AND TO ACTIVATION OF DEATH SIGNALLING IN HIPPOCAMPUS

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Our recent data have shown that systemic inflammation evoked by lipopolysaccharide (LPS) stimulates NO-mediated signalling pathways in the midbrain. The aim of the present study was to investigate the dynamics of proinflammatory genes expression in mouse hippocampus during 4 days after intraperitoneal injection of LPS (1 mg/kg b.w.). Moreover, the selected biochemical processes involved in cell death pathways were analysed. Real-time PCR analysis indicated the significant time-dependent alteration of several prooxidative genes expression. During first 3 h post-treatment the level of mRNA for COX-2, iNOS and TNFα was increased, then after 6–24 h for cPLA2, TLR4, 5-LOX and 12-LOX, respectively. Immunohistochemical analysis showed an appearance of many activated microglia cells, confirming activation of local inflammatory reaction in hippocampus. The biochemical and molecular studies demonstrated the significant activation of apoptosis inducing factor (AIF)-mediated, caspase-3 -independent

death signalling and cathepsin B-related autophagy. These data were confirmed by electron-microscopic examination of cell ultrastructure. The presented genetic, biochemical and morphologic alterations induced by systemic inflammation could be responsible for impairment of learning and memory. *This study was supported by Scientific Network 28/E-32/BWSN-0053/2008.*

TIII.42
MANIPULATION OF *Ttyh1* GENE EXPRESSION INFLUENCES CELL MORPHOLOGY IN NEUROBLASTOMA AND HIPPOCAMPAL NEURONS *IN VITRO*
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Ttyh1 gene is a member of *tweety* family of putative large conductance maxi-Cl^- channels. According to our previous data, expression of *Ttyh1* mRNA in the brain is localized in neurons and is increased following *status epilepticus* in the animal model of epileptogenesis. The function of *Ttyh1* has not been elucidated. Here we aimed at characterization of *Ttyh1* by overexpressing or silencing it in neuroblastoma cell line or in hippocampal neurons *in vitro.* In hippocampal neurons transfected with plasmid coding Ttyh1-EGFP fused protein, Ttyh1-EGFP was present in dots along the neurites and at the ends of new formed projections. Similar Ttyh1-EGFP localization was observed in neuroblastoma cells. Overexpression of *Ttyh1* in hippocampal neurons *in vitro* induced formation of new, often branched projections as soon as after 24 h. Even more intense branching was observed when cells were transfected with *Ttyh1* 7 or 14DIV. *Ttyh1* silencing in hippocampal neurons *in vitro* using siRNA increased the number and length of primary dendrites. In addition, it affected cell morphology causing abnormal pattern of MAP2 distribution. Since manipulation of *Ttyh1* expression influences cell morphology and distribution of cytoskeleton elements, we propose that *Ttyh1* is involved in cytoskeleton functions. It is tempting to suggest, that by influencing the neurite growth and ramification, *Ttyh1* participates in aberrant network formation and by this – the development of epilepsy. *Supported by MNiSW N N301 162135*

TIII.43
c-Fos MAPPING OF CHANGES IN NEURONAL ACTIVITY DURING THE COURSE OF PTZ KINDLING OF SEIZURES
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In the present study, the c-Fos expression was used to map brain structures recruited during the evolution of seizures after repeated, administration of pentylenetetrazol at the subconvlusive dose (35 mg/kg, i.p.), in rats. It has been found that the earliest expression of c-Fos, at the stage 1,2 of kindling, appeared in the nucleus accumbens-shell, the piriform cortex, the prefrontal cortex and striatum. On the 3rd stage of kindling, the central amygdala nucleus, the entorhinal cortex, and the lateral septal nucleus (LSV), showed an enhanced expression of c-Fos. On the 4th stage of kindling, c-Fos was increased in the basolateral amygdala, and CA1 area of the hippocampus. Finally, c-Fos labelling was enhanced in the dentate gyrus of the hippocampus, only when the stage 5 of kindling, i.e. the clonic-tonic convulsions, appeared. The most potent changes in c-Fos (in a descending order) were shown in the dentate gyrus, piriform cortex, CA1 area, the LSV, basolateral amygdala, central amygdala nuclei, and prefrontal cortex. It appeared, that there are important similarities in the structures recruited at the beginning and at the end of electrically and chemically-induced kindling, i.e. the piriform cortex and the hippocampal dentate gyrus, respectively. On the other hand, the differences gradually disappear at the later stages of kindling, followed by the symmetrical propagation of epileptic activity from the limbic system to the neocortex, during the generalized seizures.

TIII.44
A NOVEL METHOD TO VISUALIZE TISSUE MOLECULAR ARCHITECTURE AT THE LEVEL OF SYNAPSE
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To understand various biological functions of CNS, visualization of the fine details of tissue molecular architectures and synaptic structures is of the major importance. Herein, we use a method for immunfluorescence microscopy called Array Tomography which allows for imaging details of tissue structure. To this end, we cut resin-embedded specimen into serial sections (50–250 nm) by ultramicrotome. Hydrophilic resin permits the antibody penetration into the tissue, thus providing an opportunity for post-embedding immunostaining. The ribbon of serial sections represents tissue volume. Reconstruction of three dimensional distributions of antigens is obtained by imaging and post-processing of consecutive images. The array can be eluted and re-stained, thus allowing for detection of several antigens in the same sample. The above methodology provides visualization at the spatial resolution that is higher than the one obtained using conventional confocal microscopy. Furthermore, we use this method together with the super-resolution (70 nm) stimulated emission depletion (STED) microscopy. Thus, combining of these two methods provides very detailed image of tissue architecture and offers a novel opportunity to reconstruct the molecular architecture with sub-micrometer resolution in three dimensions.

TIII.45
NITRIC OXIDE SYNTHESIS INHIBITOR INCREASES SEVERITY OF HOMOCYSTEINE- INDUCED SEIZURES IN ADULT RATS
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Homocysteine, endogenous sulphur – containing amino acid, displays neuroexcitatory effects and acts as a convulsant agent by still

unclear mechanisms. Reported results on the role of nitric oxide (NO) in epileptogenesis are highly contradictory. NO levels could be decreased by N-nitro-L-arginine methyl ester (L-NAME), an inhibitor of enzyme NO synthase (NOS). The aim of the current study was to examine the effects of L-NAME on seizure susceptibility induced by subconvulsive dose of D,L homocysteine – thiolactone (HCT) in rats. Adult male Wistar albino rats were intraperitoneally (i.p.) treated with HCT 5.5 mmol/kg and observed for seizure behavioral manifestations during next 90 min. Increasing doses of L-NAME (200, 500 and 700 mg/kg, i.p., n=6, 7 and 8, respectively) or saline (n=9) were injected 30 min prior to HCT administration. Seizure episode severity was assessed using descriptive rating scale with four grades. L-NAME in doses of 500 and 700 mg/kg significantly increased severity of homocysteine seizures (P<0.05) when compared with saline –injected rats. The lowest applied dose of L-NAME (200 mg/kg) had no statistically significant effect on severity of seizures induced by HCT. Based on these results it cold be concluded that L-NAME, NOS inhibitor, potentiates convulsive properties of HCT.

TIII.46
PERFUSION SENSITIVE CONTRAST ENHANCED MR IMAGING OF DYSEMBRYOPLASTIC NEUROEPITHELIAL TUMOR (DNT): A NEW NEUROIMAGING FINDING

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Dysembryoplastic neuroepithelial tumors (DNTs) are benign lesions affecting young people and are associated with epilepsy. There have been described more than 300 cases in the literature and the clinical, pathologic and radiological findings are well known. Recent advances in neuroimaging allow the acquisition of cerebral microcirculation parameters by perfusion weighted imaging giving additional diagnostic information improving the diagnostic accuracy. The aim of this study is to show the Perfusion sensitive contrast enhanced MR imaging findings of a case of DNT as an additional neuroradiological finding. Further investigation of microcirculation parameters may be helpful to perform the correct diagnosis of such tumors.

TIII.47
SEIZURES CONTROL IN FREE-RUNNING RATS

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Absence epilepsy is characterized by disturbed consciousness and generalized, synchronous, bilateral, 3–4 Hz (in humans) and 7–11 (in rats) spike-wave discharges (SWD) in EEG. It has been documented that the occurrence of absence seizures shows circadian pattern both in humans and animals. WAG/Rij rats, a well known, validated animal model of human absence epilepsy, show a clear circadian distribution of SWD (maximum in early hours of dark period, minimum after the onset of light). Moreover, a strong correlation between the occurrence of SWD and the level of vigilance exists: SWD are less prone to occur during active wakefulness. SWD rhythm and its relationship with activity in entrained and constant condition were investigated. Chronic EEG and general activity recordings were made in six adult WAG/Rij rats. Animals were kept in 12:12 light–dark cycle. The light regime was changed after 10 days into constant dim light (<6 lux) in which rats were maintained for the following 20 days. The period lengths of both rhythms were estimated by the Cosinor method. Clear 24 h rhythms of activity and SWD were found under entrained conditions, in constant condition both rhythms were free-running. Periods' length of activity was increased while period of SWD rhythm was changed differently for different animals. Observed rhythms' splitting suggests decoupling of these two rhythms in constant condition and that the SWD rhythm is no longer controlled by a master clock.

TIII.48
EEG CLUSTERIZATION PATTERNS IN DIFFERENT EXPERIMENTAL CONDITIONS

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EEG data, obtained in 3 groups of young (18–30 years) subjects during different experimental conditions (background brain activity with open/closed eyes, emotional face recognition and stop-signal task) were analyzed. Event-related desynchronization (ERD) and event-related spectral perturbations (ERSP) were used as functional response measures. Individual cortical distributions of these responses were compared for different modalities. Group 1 (32 women, 16 men) performed stop-signal task, group 2 (21 women, 19 men) – facial expression recognition task, and group 3 (2 women, 13 men) both of these tasks. Background EEG with open and closed eyes was recorded in all three groups. EEGs for groups 1 and 2 were recorded using 32-channel "Neurovisor-24" amplifier, and for group 3- by 132-channel "Neuroscan" amplifier. Event-related reactions were obtained using ERD for group 1 and ERSP for groups 2 and 3. Percentage change was used as measure of reaction on opening eyes. Electrodes were grouped into clusters with similar "behavior" separately for each condition using factor analysis with principal components extraction and varimax rotation. Number of Identified clusters increased and size of clusters decreased with increase of analyzed frequency. Importantly, clusters for each frequency (especially for lower ones) were similar between different conditions, but differed between subjects, supporting hypothesis of stability of brain oscillatory systems' spatial characteristics.

TIII.49
PREDICTION OF PROTEIN SECONDARY STRUCTURE IN BRAIN TISSUES AND MEMBRANES USING NEURAL NETWORKS BASED ON FTIR SPECTROSCOPY

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Membrane and tissue proteins in biological systems are difficult to be isolated and crystallized. In recent years, a low resolution technique, Fourier Transform Infrared (FTIR) spectroscopy, has been improved and proven to be a reliable tool to determine the secondary structure of proteins. Using this technique, it is possible

to see protein bands directly from the spectra of tissues and membranes which allow us to obtain information about protein secondary structure. Generally the information is deduced by monitoring the amide I band, which is located between 1700–1600 cm^{-1}. This band is the result of many overlapping individual peaks arising from different protein conformations, such as α-helix, β-sheet or turns. In the current study, using Neural Network (NN) method based on FTIR spectra [Severcan et al. (2004) Anal Biochem], the radiation, epilepsy and antioxidant-induced variations in the secondary structure of proteins in brain tissues and membranes were determined. The results showed that lipoic acid treatment revealed an unaltered protein secondary structure, while radiation caused a significant increase in random coil structure and epilepsy led to a significant decrease in β-sheet structure in rat brain tissue. This study confirms that NN approach based on FTIR data is a fast and effective method to predict the secondary structure of proteins in different biological systems.

TIII.50
A TEST OF WHETHER N-ACETYL-ASPARTYL-GLUTAMATE AND N-ACETYL-ASPARTATE CAUSE DAMAGE IN LEUKODYSTROPHIES BY ACTIVATING OLIGODENDROCYTE NMDA RECEPTORS
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Elevations of the levels of N-acetyl-aspartyl-glutamate (NAAG) and N-acetyl-aspartate (NAA) are associated with myelin loss in the leukodystophies Canavan's disease and Pelizaeus-Merzbacher-like disease. NAAG and NAA can activate neuronal NMDA receptors, and also act on group II mGluRs. Since NMDA receptors are present on oligodendrocytes [Káradóttir et al. (2005) Nature 438: 1162], we hypothesised that NAA and NAAG may damage oligodendrocytes by activating their NMDA receptors, causing a deleterious Ca^{2+} influx. We show that NAAG, but not NAA, evoked an inward membrane current in cerebellar white matter oligodendrocytes, which was reduced by NMDA receptor block (but not by block of mGluRs). The size of the current evoked by NAAG, relative to that evoked by NMDA, was much smaller in oligodendrocytes than in neurons, and NAAG induced a rise in $[Ca^{2+}]_i$ in neurons but not in oligodendrocytes. In addition, a major part of the response in oligodendrocytes, but not in neurons, was blocked by TTX. We conclude that the NAAG-evoked current in oligodendrocytes is a secondary consequence of activating neuronal NMDA receptors and is unlikely to be a major contributor to white matter damage in the leukodystrophies. *Supported by the Wellcome Trust and Royal Society.*

TIII.51
STREPTOZOTOCIN INDUCED DIABETES CHANGES CONDUCTION VELOCITY DISTRIBUTION OF RAT SCIATIC NERVE IN A TIME DEPENDENT MANNER
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Diabetes is a metabolic disorder based on uncontrolled blood glucose concentration that affects majority of human population. This disorder causes some pathologies in nervous system that called diabetic neuropathies. As a secondary complication of diabetes, diabetic neuropathies causes damage on peripheral nerves depending on time of diabetes. In this study, experimental type 1 diabetes was induced by injection of streptozotocin (50 mg/kg, i.p.) in rats. Diabetic animals were grouped as 2 and 4 weeks diabetic. Control group animals were received only vehicle of STZ (0.1 M Citrate, pH 4.5). Sciatic nerves of experimental animals were dissected and electrophysiological recording experiments were achieved. Recorded compound action potentials were then analyzed to understand the time dependent effects of diabetes on peripheral nerves. Analysis have shown that, current recording techniques that is used in clinics for diagnoses of neuropathologies, carries less information compared to conduction velocity distribution (CVD) histograms obtained by using single fiber action potential (SFAP) models. In parameters obtained by using conventional methods, statistically significant changes were first seen in 4th week of diabetes. But, by means of CVD histograms, diabetes induced significant changes could be seen 2nd week of diabetes in slowly conducting fiber group. So, CVD methods give more information in early diagnosis of neuropathies compared to classical conduction velocity measurements.

TIII.52
AUTOIMMUNE ENCEPHALOMYELITIS IS ACCOMPANIED BY THE INJURY OF HIPPOCAMPAL NEURONS
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Multiple sclerosis (MS) is associated with cognitive deficits, developing independently from motor disorders. These deficits may be associated with brain neuronal damage. In this study, using the experimental model for MS – autoimmune encephalomyelitis (EAE), we investigated whether EAE will result in the damage of hippocampal neurons and selective deficits in learning and memory, and whether there may be a correlation between the two phenomena. Lewis rats 3 months old were injected with 4 millions of anti-MBP CD4+ T cells to evoke EAE. Animals suffered from tail and hind limb paresis and recovered after 10 dpi. T cells infiltrated spinal cord and many brain regions including hippocampus. We demonstrated the decrease of pyramidal neurons in CA4 region by about 20%, as evaluated by stereological measurements, at 21 dpi. This was preceded by prolonged glial activation as well as by a rise of the pro-inflammatory cytokine mRNA expression (IL-1β, IL-6 and TNF α). However, no differences in the water maze test were detected between the EAE and control groups, on 21 dpi and on 90 dpi. In conclusion, anti-MBP CD4+ T cells are capable of injuring hippocampal pyramidal neurons during EAE, probably, through the secretion of pro-inflammatory cytokines. However, in the studied conditions, hippocampal neurodegeneration caused by T cells did not result in memory disturbances. *Supported by the grant no N401-1293-33 of the Ministry of Scientific Research and Information Technology in Poland.*

TIII.53
ASTROGLIAL REACTION IN THE EARLY ASYMPTOMATIC PHASE OF EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS
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Under different pathological conditions activation of astrocytes of neuroprotective or neurotoxic nature is observed. There is a growing number of evidence that many pathological states of brain are characterized by very early active contribution of astrocytes to neurodegenerative axonal damage. Astroglia posses defense mechanisms against glutamate excitotoxicity (transporter systems) but may also contribute to the enhanced release of this potentially toxic amino acid trough exocytosis, P2X7 purinergic receptors, hemichannels or reversing of glutamate transporters. These cells are also a main source of ATP, active signaling molecule, which activates many purinergic receptors in brain, including P2X7R, which participates in development of inflammation and neurodegeneration phenomena. The aim of this study was to investigate the expression of astroglia-specific proteins during the course of EAE using immunochemical and immunohistochemical analysis. We observed early activation of astroglia in the inductive phase of EAE (4 day p.i.) which was connected with overexpression of GFAP and S-100β. Expression of Cx43, protein that forms hemichannels was also enhanced so as the expression of P2X7R. Additionally, the level of GLT-1 glutamate transporter's protein increase significantly. The results suggest that in EAE pathology very early activation of astroglia takes place in the preclinical stage of the disease. The exact nature of this activation will be investigated.

TIII.54
THE 8 kD FORM ENCODED BY THE HTLV-1 ORF-I IS ASSOCIATED WITH LOW PROVIRUS LEVELS AND HEALTHY CARRIER STATE IN A SUBSET OF HTLV-1 INFECTED INDIVIDUALS
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HTLV-1 infection is linked with a neurodegenerative disorder HTLV-1 associated myelopathy/tropical spastic paraparesis (HAM/TSP). Expression of the singly or doubly spliced ORF-I cDNA of HTLV-1 results in the production of the 12 kD and 8 kD protein isoforms. The uncleaved 12 kD form resides in the ER and affects MHC-I and IL-2R. The cleaved 8 kD form traffics to the cell surface, is recruited to the immunological synapse upon T-Cell Receptor (TCR) stimulation decreasing TCR signaling and viral replication. Genetic analysis of ORF-I from *ex vivo* samples of HTLV-1 infected individuals reveals amino acid substitutions that affect its proteolytic cleavage, suggesting that ER or membrane associated functions of ORF-I may contribute to the persistence of HTLV-1 infected T-cells in the host. To investigate a putative relationship between ORF-I forms and provirus level, the best predictor of disease development, we measured the provirus level in the blood and linked it with the presence of ORF-I isoforms. The provirus levels ranged from 0.2 to 165 copies of proviral DNA per 10^6 PBMCs. DNA sequencing and reverse genetics revealed mutations at/in the vicinity of both cleavage sites within ORF-I. A rare mutation found at position 26 in ORF-I resulted mainly in the presence of the 8 kD isoform. Importantly, patients with this mutation had significantly lower virus that those that carried either 12 kD isoform or both forms, and with one exception they belonged to a healthy carrier group.

TIII.55
METABOLOMIC ANALYSIS AND SIGNATURES IN AMYOTROPHIC LATERAL SCLEROSIS: 1H NMR STUDIES OF SERUM
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Amyotrophic Lateral Sclerosis (ALS) is a degenerative motor neuron disease characterized by progressive dropout of motor neurons in the brain, brain stem, and spinal cord. This disease may produce characteristic perturbations of the metabolome, the collection of small-molecules (metabolites). To test this hypothesis, we have investigated metabolite profile in the blood serum of 30 patients with ALS and 25 healthy controls by using 1H NMR. Patients with ALS had significantly higher median concentrations (microM) of creatine/creatinine (43 vs. 30, $P<0.02$), glutamate (92 vs. ND, $P<0.001$), β-hydroxybutyrate (2 vs. ND, $P<0.001$), acetate (15 vs. 8, $P<0.01$), acetone (15 vs. 9, $P<0.05$), and formate (16 vs. ND, $P<0.001$) than healthy controls, and significantly lower concentrations of N-acetyl derivatives ($P<0.001$), glutamine (406 vs. 448, $P<0.02$), histidine (55 vs. 67, $P<0.001$), however, the concentration of alanine, lysine, pyruvate, citrate, glucose, and tyrosine were comparable. Concentration of glutamate, N-acetyl derivatives and histidine correlated with the duration of the disease. These results suggest that metabolomic studies can be used to ascertain metabolic signatures of disease in a single step. Such 1H NMR study of serum provides an insight into aberrant biochemical pathways which may have the potential to serve as a surrogate marker for monitoring ALS disease progression and also could be targets for drug design.

TIV: Sensory and Motor Systems

TIV.01
M100-PEAK AMPLITUDES OF DIFFERENT SUBJECTS, HEMISPHERES, AND STIMULUS CONDITIONS DIFFER BY FACTORS RATHER THAN BY AMOUNTS

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The analysis of MEG data commonly involves arithmetic averaging of the evoked magnetic fields (AEFs) across subjects. A tacit assumption of this practise is that signals from different subjects simply differ by some amount. If true, the standard deviation (SD) of signal strengths across subjects should be independent of the mean signal amplitude. We exploit the dependence of the M100-peak amplitude on the stimulus onset interval (SOI) to scrutinize the variation of SD with mean amplitude. Fifteen subjects were stimulated monaurally with tones of SOIs varying between 250 ms and 12.5 s. For each subject and hemisphere, the M100-peak amplitude was determined, either from the channel with the largest signal or from a cluster of five channels whose signals were averaged arithmetically or geometrically. Irrespective of the measure, we found that the SD of the M100-peak amplitude across subjects scales linearly with the amplitude of the corresponding arithmetic mean, but remains practically constant for the amplitude of the geometric mean. This shows that the M100 amplitudes of different subjects, hemispheres, and stimulus conditions differ by factors rather than by amounts. We also show that the geometric mean of the M100 amplitudes across different conditions provides an excellent measure for normalizing such data. Our results question the common practise of arithmetic averaging across subjects, or subtraction procedures between conditions, and thus are likely to have widespread implications.

TIV.02
THE EFFECT OF POSTNATAL BRIEF NOISE EXPOSURE ON SOUND LEVEL PROCESSING IN INFERIOR COLLICULUS NEURONS OF ADULT RATS

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The development of the central auditory system is an activity-dependent process. Hence, any manipulation of the hearing organ that temporarily alters the input to the central auditory nuclei can have a serious and mostly permanent impact on their structure and function. Our previous study showed that adult rats exposed to intense brief noise as juvenile had significantly deteriorated frequency selectivity of the inferior colliculus neurons. In this study, we focused on sound level processing in inferior colliculus neurons in rats exposed for 8 min to intense broad-band noise on postnatal day 14. At the age of 3–6 months, the neuronal activity in the central nucleus of the inferior colliculus was recorded under ketamine-xylazine anaesthesia, and the parameters of the rate-level functions were compared between the neurons of noise-treated rats (n=116) and age-matched controls (n=94). While the neuronal thresholds in the two groups did not differ significantly, the neurons of the exposed animals exhibited a significantly shorter dynamic range and a steeper slope of the rate-level function compared with controls. The results indicate that a short-lasting exposure to intense sound during the sensitive period of postnatal development disrupts the normal coding of sound intensity in the inferior colliculus neurons. *Supported by grants AV0Z50390512, GACR 309/07/1336, GACR 309/08/H079, IGA NR 8113-4 and LC 554.*

TIV.03
ALTERNATIVE SPLICING OF L7/pcp2 GENE RESULTS IN TRUNCATED PROTEIN

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The L7/pcp2 have been described for the first time in 1988 by two independent groups. Transcripted RNA is source of 99-amino-acid peptide of 16kD with some homology to NH2-terminal part of PDGF. pcp2 protein is known for its very specific expression in cerebellar Purkinje cells and bipolar retinal neurons. Initial experiments showed, that isolated Purkinje cell specific mRNA did not hybridize to cerebellar mRNA from mice with Purkinje cell degeneration mutation. It was a strong indication, that pcp2 protein could play an important role in Purkinje cell physiology and development. Mice null mutant for that gene however, do not display any morphological or physiological abnormalities. Up to now there are only few experimental data suggesting the function of pcp2 protein. Experiments on G protein interactions revealed pcp2 as possible GDP exchange factor for Go subunit. This function of pcp2 depends on the presence of G-protein regulatory motifs (GPR) known also as GoLoco motifs. Our experiments regarding expression pattern of pcp2, revealed a new splice variant of its mRNA – pcp2B. Additional ~350bp long band was always prominent in PCR products amplified on cDNA from mouse eye preparations. Incorporation of the new exon 3B results in truncated protein, because of STOP codon present in its structure. Missing region consists of some putative phosphorylation sites with possible biological function important the L7/pcp2 protein.

TIV.04
SIGNAL FREQUENCY TRANSFORMATION BY HODGKIN-HUXLEY NEURONS

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The response of a Hodgkin-Huxley neuron strongly depends on the form of input current. We study the model with a periodic post-synaptic current, where each of the current pulses has the form $I(t) \sim g_{syn} \sum (t/\tau) \exp(-t/\tau) \Theta(t) (V_a - V_{syn})$, where g_{syn} is the synapse conductivity, τ is the time constant associated with the synapse conduction, V_a is the maximum membrane potential and V_{syn} is the reversal potential of the synapse. The other parameters are typical for the Hodgkin-Huxley model. There are three resonant frequencies at 57 Hz, 28.5 Hz, and 19 Hz, where the 57 Hz feature is the main resonance of the neuron. In the resonant regime the system has the tendency to mode locking with high values of k, where $k=T_o/T_i$ is the ratio of the output ISI to the input ISI. Cha-

otic states are present in many areas of the resonant regime. The mode-locked states within the resonance may have large values of *k*. The incoming signal frequency may be substantially reduced when passing through such neuron. A chain of two or more neurons may decrease the signal frequency by more than an order of magnitude. Noise in the input signal lowers the response threshold and improves the signal-to-noise ratio. Network effects are also discussed. *Acknowledgements: Part of the numerical computation was performed in the Computer Center of the Tri-city Academic Computer Network in Gdansk, Poland.*

TIV.05
MELANOPSIN-BASED PHOTORECEPTION: CONTRIBUTION TO THE MECHANISM OF GENERATION OF INFRASLOW RHYTHM IN THE RAT OLIVARY PRETECTAL NUCLEUS
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In mammals, environmental light signals are captured by the eye's photoreceptors: rods, cones and intrinsically photosensitive melanopsin retinal ganglion cells (ipRGCs). During the last few years, ipRGCs were extensively studied and their membrane properties, projections and physiological role in regulation of circadian rhythms and pupillary light reflex were documented. However, these studies do not explain how activity of ipRGCs affects physiology of target cells in suprachiasmatic nuclei (SCN), intergeniculate leaflet (IGL) or olivary pretectal nucleus (OPN). Some of the neurons constituting above mentioned structures, express slow oscillatory activities that are modulated by light and depend on functional input from the retina. This implicates, that mechanism of expression of slow oscillation may include synaptic drive from ipRGCs. Recently, 2-aminoethoxydiphenylborane (2-APB) was described as an acute inhibitor of ipRGCs activity. This study combines intravitreous injections of 2-APB with extracellular recordings from oscillatory OPN neurons in urethane anesthetized Wistar rat. The experiments showed that inactivation of ipRGCs activity abolish oscillatory pattern and reduce firing rate of OPN neurons. Injection of comparable volume of physiological saline in control experiments had no effect on oscillatory activity. To our knowledge, this is the first study that directly links extraretinal neuronal firing with activity of ipRGCs.

TIV.06
CORTICAL FEEDBACK MODULATES TEMPORAL PATTERN OF SPONTANEOUS NEURONAL ACTIVITY IN THE CAT'S DORSAL LATERAL GENICULATE AND PERIGENICULATE NUCLEI
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In cats with pretrigeminal brainstem transections, reversible inactivation of the primary visual cortex by cooling results in changes of the magnitude of response and spatio-temporal structure of receptive fields of neurons from lateral geniculate and perigeniculate nuclei (LGNd and PGN; Waleszczyk et al. 2005). Since cortical inactivation changes also the spontaneous activity of these thalamic neurons, we hypothesized that cortical feedback affects their membrane potential. Both types of investigated cells displayed two modes of activity: a tonic mode, during depolarization; and a burst mode, when the cell is hyperpolarized. In this report we investigated the effect of elimination of cortical feedback by cooling on the temporal pattern of the spontaneous "bursty" activity of single neurons in LGNd and PGN. During such reversible inactivation of areas 17 and 18, in both LGNd and PGN cells, spontaneously occurring bursts exhibited longer interspike intervals (ISIs) and lower number of spikes, while the average burst duration remained unchanged. Longer ISIs suggest that cortical feedback influences not only visual responsiveness of thalamic neurons, but also temporal pattern of their spontaneous firing, in line with the hypothesis of a tonic cortical modulation of their membrane potential. *Supported by the Ministry of Science and Higher Education grant COST/127/2007.*

TIV.07
A NEW METHOD FOR IDENTIFICATION OF MODULATION IN NEURAL RESPONSES TO DRIFTING GRATING STIMULATION
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Temporal modulation of responses to drifting grating stimulation is observed in visual neurons in different brain structures. The common measure of intensity of such modulation is the modulation index (MI; Movshon et al. 1978), defined as the ratio of the amplitude of the response component at the stimulus temporal frequency (f_1) and the net response of the cell. However MI works correctly in a limited range of net responses. If stimulation causes only a weak change in mean firing rate, MI can take any value independently of actual modulation in the response. Here we present a new, simple method of determining the strength of modulation, based on the detection of a peak in an amplitude spectrum at the frequency of stimulation. We define modulation strength (MS) as the ratio of f_1 value above the mean value of amplitude spectrum and standard deviation (SD) of amplitude values along all frequencies in the spectrum. We assume the response to be modulated if f_1 value exceeds the mean amplitude by at least one SD (MS>1). We test the method on data recorded from a number of structures of the extrageniculate visual pathway and compare it to the classical MI and the modulation depth (measure used in the amplitude modulation radio transmission – here the change of the spectrum component at the stimulation frequency between the spontaneous activity and the response). *Supported by MNiSW grants: N303 070234, COST/127/2007, N303 046 31/1483, OTKA/NKTH Hungary grant 68594, OTKA grant PD75156.*

TIV.08
MOTOR IMAGERY MEASURED BY FUNCTIONAL NEAR-INFRARED SPECTROSCOPY (fNIRS)
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Functional near-infrared spectroscopy (fNIRS) is a non-invasive technique that utilizes light in the near-infrared (NIR) range to determine the oxygenation of localized regions of the brain. The proposed study aims to establish fNIRS as a valid tool for use in brain-comput-

er interfaces (BCI). Specifically we will use multi-channel fNIRS to provide feedback of neural activity in a virtual reality rehabilitation system. BCIs are feedback systems that allow human brain signals to control devices in real time. fNIRS instruments are non-invasive and easily adapted to wireless or portable applications, offering decisive advantages for the development of BCIs compared to fMRI or EEG. Our project focuses on the development of novel BCI feedback systems based on NIR technology that, in the longer term, will be used in conjunction with a training systems for the rehabilitation of motor function. We present results of mental performance of motor imagery (MI) in response to no, positive and negative feedback conditions indicating that there exist distinct activation pattern concerning occurrence, amplitude and timing. Although, limitations of the current study require further evaluation, these parameters could be utilized as a basis to develop pattern classifiers for fNIRS controlled BCIs.

TIV.09
EFFECTS OF PRISM ADAPTION ON VOLUNTARY AND REFLEXIVE ORIENTING OF ATTENTION
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Hemispatial neglect is a failure of attending to the left half-space resulting from lesions of the right hemisphere. It is believed to be an attentional dysfunction, involving deficits in disengagement of attention from the objects in right visual field, as well as in inhibition of return to the right visual field. Rosetti and Rode (1998) proposed a method of ameliorating symptoms of neglect by means of prismatic lenses, which shift vision (e.g. 15° to the right or left) and thereby trigger changes in visuo-spatial integration. However, it is not clear, which particular cognitive mechanisms are affected by the method. Furthermore, leftward prism adaption in healthy subjects causes an effect similar to hemispatial neglect. Similarly, cognitive and neural mechanisms underlying this effect remain yet to be identified. Two experiments were conducted, which aimed at indicating attentional processes modified by prism adaptation in case of "neglect-like" effect in healthy individuals. Posner's location-cuing task paradigm was used in order to differentiate processes of endogenous and exogenous orienting of attention, as well as to measure the effect of inhibition of return. Participants performed the task before and after prism adaptation. Results are discussed in light of recent theories of neglect.

TIV.10
HYPERSENSITIVITY AND NOCICEPTIVE BEHAVIOUR OF da-DREAM TRANSGENIC MICE
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DREAM is a Ca^{2}-dependent transcriptional repressor that regulates the expression of multiple genes, some of which are implicated in pain mechanisms. To further investigate the role of DREAM in pain we have developed transgenic lines of mice overexpressing dominant active mutants of DREAM (daDREAM). They were tested for thermal and mechanical sensitivity in basal conditions and following CFA-induced inflammation or CCI-induced neuropathic pain. Naïve transgenic (TG) mice showed significantly higher responses to noxious heat as compared to wild type (WT). In a visceral pain model,TG animals were markedly hyperalgesic in comparison to WT. In models of chronic inflammation (CFA), TG animals show a delay in the development of thermal hypersensitivity as compared to the WT animals. In a model of neuropathic pain (CCI), within 5 days post op, TG animals were as hyperalgesic as WT animals. However, considering that baseline sensitivity was lower in TG animals, the degree of hyperalgesia was less than that of WT animals when compared to pre-operative values. Thus, daDREAM is implicated in various pain states as well as baseline noxious sensitivity.The behavioural data will be supplemented with histochemical data characterising sensory neurone populations in both groups of animals.

TIV.11
MODULATION OF $P2X_{2/3}$ RECEPTORS BY OPIOIDS IN RAT NODOSE NEURONES
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$P2X_{2/3}$ receptors, localized on peripheral and central terminals of primary sensory neurons, are involved in persistent nociceptive signalling. Opioid peptides are known to produce analgesia through central as well as *via* peripheral mechanisms. We have shown that in rat nodose neurons $P2X_{2/3}$-mediated responses were modulated by opioids in a biphasic manner: an initial short phase of potentiation (300–400 s) was followed by long-lasting inhibition of the response (for about 50% at the steady-state level). Addition of GTP-gamma-S, GDP to the intracellular solution and preincubation with pertussis toxin indicated that $P2X_{2/3}$ receptors were affected by opioids *via* G-protein dependent pathways. We have also shown that sensitivity of $P2X_{2/3}$ receptors to endomorphin-1 is altered after co-culturing of nodose neurons with fibrosarcoma cells (NCTC 2472). In co-cultured neurons ATP-activated currents with "slower" desensitization kinetics were less inhibited by the opioid. "Ultra-slow" responses were completely insensitive to endomorphin-1. The occurrence of these responses increased with the duration of co-culturing. Thus, co-culturing decreases sensitivity of ATP responses to endomorphin-1, which can account for low sensitivity of cancer pain to opioids.

TIV.12
THE EXTRASYNAPTIC AMPA RECEPTORS FUNCTIONING IS ALTERED UNDER INFLAMMATORY PAIN
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We have recently shown that synaptic Ca^{2+}-impearmable AMPA receptors (AMPARs) internalization in dorsal horn neurons underlies the maintenance of nociceptive hypersensitivity in inflammatory pain. Here we have analyzed if trafficking of extrasynaptic AMPARs is also changed during development and maintenance of persistent pain. We report that Complete Freund's Adjuvant (CFA)-induced inflammation causes an increase in functional expression of extrasynaptic AMPARs in rat substantia gelatinosa (SG) neurons during

the maintenance rather than development of persistent pain. This increase, revealed as a significant enhancement of AMPA-induced membrane currents and $[Ca^{2+}]_i$ transients, was observed only in neurons characterized by an intrinsic tonic firing properties whereas no changes were observed in neurons exhibiting a strong adaptation. The increase was also accompanied by an enhancement of surface GluR1 expression and of the total amount of cobalt-positive neurons indicating an increase in a pool of GluR2-lacking AMPARs in extrasynaptic plasma membrane. These results suggest that functional changes in extrasynaptic AMPARs of tonic SG neurons that are associated with the maintenance of nociceptive hypersensitivity may also contribute to inflammatory pain. We also suppose that there is a different contribution of tonic and transient neurons to the detection of peripheral painful stimuli and to maintenance of nociceptive hypersensitivity.

TIV.13
ASSOCIATIVE LEARNING MODIFIES REPRESENTATION OF VIBRISSAE IN SII CORTEX –A 2DG STUDY
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Secondary somatosenory cortex (SII) in rodents receives input from vibrissae trough reciprocal cortico-cortical connections from the barrel cortex and directly from thalamic sensory nuclei. In humans, SII is activated bilaterally during attentional tasks and it is consider to play a role in tactile memory and sensimotor integration. We examined it behavioral paradigms that modifies SI evokes also plasticity in SII. We measured area of SII activation by stimulation of a row of vibrissae previously activated in classical conditioning paradigm, in which stimulation of a row of vibrissae was paired with a tail shock. The training consisted of 3 daily 10 min sessions, during which 40 pairings were delivered. Activation was mapped with [14C]-2-deoxyglucose (2DG) autoradiography one day after the end of conditioning. The autoradiograms were analyzed with computerized image analysis system, which aligned the 2DG uptake pattern with Nissl stain. We reported previously that conditioning results in enlargement of cortical representation of the "trained" row of vibrissae in SI. Here we found that in SII the representation of the "trained" row is increased bilaterally, by 37% on the average. The increase was observed in cortical layers II/III and IV. Clearly, plasticity in SII is not simply a reflection of changes in SI. It may be supposed that in response to activation of a pathway involved in conditioning, structures involved in attention respond more strongly to sensory stimuli.

TIV.14
EXPLORATION OF NEW ENVIRONMENT REVEALS EXTENSIVE DEPRIVATION-INDUCED PLASTICITY
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Sensory deprivation elicits alterations in the functional organization of the primary somatosensory cortex. It was shown that plucking out all but one row of whiskers in adult mice evokes broadening of the functional representation of the spared row, as measured with radioactive 2-deoxyglucose (2-DG) uptake during passive stimulation of whiskers. We would like to establish whether changes in metabolic activity caused by sensory deprivation are paralleled by changes at genomic levels visualized by immediate early genes immunohistochemistry. Exploration of enriched environment is a powerful trigger to induce immediate early genes in the barrel cortex. In this study we show that plasticity of the functional representation of the spared row of whiskers can be estimated by radioactive 2-DG method in animals actively using their whiskers while exploring new environment. Expansion of the spared row representation in the deprived hemisphere reaches 140% of the control (non-deprived) hemisphere after 1 week of deprivation and spreads to whole barrel field after four weeks of deprivation. We also show that induction of some early immediate genes during exploration of new environment is limited to the non-deprived barrels after one day of deprivation. In further course of studies we will perform immunohistochemical reactions for proteins encoded by early immediate genes in brains from animals deprived for 1 or 4 weeks.

TIV.15
ESTIMATING FUNCTIONAL BRAIN CONNECTIVITY THROUGH ANALYSIS OF MULTI-SITE EVOKED POTENTIALS RECORDINGS
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Local field potential (LFP) – the result of summed postsynaptic potentials from cell populations – reveal the most characteristic neural activity at the recording site in the brain. Thus LFPs are well suited for study of neural networks, specifically those involved in processing of sensory information. We propose a method of assessing functional brain connectivity through LFP analysis. The method is applied to multi-site signals representing potentials evoked by a repeated, stereotyped stimulus. In spite of a stereotyped stimulus, all responses are different due to, *inter alia*, ongoing background activity of the brain and this trial-to-trial variability is utilized in our analysis. The method is based on calculation of correlations between trial-to-trial LFP variations at every post-stimulus latency at every recoding site. The results show how neuronal activities at different sites and latencies correspond to activation at other sites with a given time delays. We used this method to analyze the functional connectivity in thalamocortical network involved in processing of somatosensory (vibrissal) information in non-anaesthetised rat. One result is that the cortical activation at 25–50 ms post-stimulus correlates with thalamic LFP measured at 50–150 ms post stimulus, thus implying this late latency thalamic activity depends on a corticothalamic feedback. Apart from raw LFP, the method is applicable to results of various decomposition methods of brain signals (PCA, ICA, etc.).

TIV.16
INTEGRATION OF MULTIPLE-WHISKER STIMULATION IN RAT BARREL CORTEX
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The vibrissal system of rodents has become one of the dominant models for investigating the mechanisms of sensory information processing. However, the mechanisms underlying integration of multiple whisker input is not well understood. To address this question, we recorded local field potentials (LFP) from the barrel cortex in of anaesthetised rat. Recording points were distributed on the 4 × 22 grid covering two neighbouring cortical columns and septa between them. Potentials evoked (EP) by deflection of single whiskers and sets of three whiskers (within arc and/or row) were analy-

sed by two dimensional current source density method (2D CSD). The multiple whisker response was compared to linear predictor, defined as a sum of corresponding single whiskers responses. CSD performed on the data revealed significant differences between linear prediction and multiple whisker deflection. Multiple input responses had lower amplitudes as compared to linear prediction condition. The earliest differences were observed in infra- and supragranular layer approximately 8 ms after stimulation. Differences in granular layer appeared 10 ms after stimulation. Our data suggest that supra- and infra granular layers are involved in initial phase of the integration of multiple whisker inputs

TIV.17
SPINAL CORD SORTILIN DISTRIBUTION AND LEVELS CHANGE IN REGION-SPECIFIC MODE AFTER SPINALIZATION: p75 COMPLEXING IN MOTONEURONS?

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Membrane receptor sortilin is involved in sorting and processing of proteins. In complex with p75 receptor binds proneurotrophins what can lead to apoptosis and remodeling of neuronal network. To study sortilin involvement in spinal cord (SC) remodeling after injury, we characterized its distribution patterns, levels and relation to p75 expression in L3/L4 segments, 6 weeks after SC transection at low thoracic level. In intact rats sortilin immunoreactivity (IR) was widely distributed in white and grey matter. The strongest IR was observed in glial cells of lateral and ventral funiculi. In the grey matter sortilin IR appeared in number of neurons and glial cells, except for the lamina 2, where it rarely occurred. In contrast, p75 was limited to the bundle of strongly IR fibers in dorso-lateral lamina 2. Sortilin and p75 did not colocalise there and overlapped in isolated large neurons of lamina 9. Spinalization caused a 5% decrease of sortilin IR in the white matter not accompanied by p75 IR changes. In the grey matter sortilin IR level was not changed but frequency of p75/sortilin IR overlapping increased in lamina 9 neurons. This result indicates postlesion increase of p75/sortilin complexing in motoneurons which may reflect activation of proneurotrophin-mediated dysfunction. Our data show lack of such interaction in primary sensory afferents. Sortilin IR in numerous cells devoid of p75 labeling confirms that it plays also other roles. *Supported by MSE P-N/029/2006 grant.*

TIV.18
DIFFERENCES IN MORPHOMETRIC PROPERTIES OF MUSCLE FIBERS AND INNERVATION RATIO OF MALE AND FEMALE MOTOR UNITS IN THE RAT MEDIAL GASTROCNEMIUS MUSCLE

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The study aimed at demonstration of sexual differences in morphology and innervation of the medial gastrocnemius muscle (MG) in male and female Wistar rats. The experimental project involved three stages: (1) the functional isolation and analysis of contractile properties of as many motor units (MUs) as possible, (2) recording the contraction force of the whole muscle evoked by stimulation of the sciatic nerve and, (3) the morphological examinations of MG. The experiments revealed that MG of males contained 46% of fast fatigable (FF) MUs, 40% of fast resistant (FR) MUs and 14% of slow (S) MUs, whereas in females 40% of FF, 37% of FR and 23% of S type MUs were found. The force of MUs in male muscle was higher, and the twitch time parameters were longer in males. Moreover, the muscle mass and force in males was significantly higher. The relation of the muscle force to the mean value of MUs force revealed that male MG contained approximately 10% more MUs than in females. The male muscles which had approximately 1.5 times bigger mass than in females were composed of over 11 800 muscle fibers, whereas in females 8 000 fibers. These results strongly suggested that the average number of muscle fibers in individual MUs of males was higher about 47% comparing to females. In addition, the mean diameter of muscle fibers was 29% bigger in males. Finally, the cross-section area of muscle fibers was also 15% larger in males.

TIV.19
SUMMATION OF MOTOR UNIT FORCES IN THE RAT MEDIAL GASTROCNEMIUS MUSCLE

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The purpose of the study was to examine the summation of forces of two and four individual motor units (MU), and four groups of units in the medial gastrocnemius muscle during parallel stimulation of axons innervating these units. Experiments were performed on Wistar rats under pentobarbital anesthesia. Single motor axons were prepared by splitting the L5 and L4 ventral roots until electrical stimulation of these filaments evoked "all-or-none" type motor unit action potentials and twitch forces in the studied muscle. The examination of groups of units followed division of the L5 ventral root into four bundles of axons. After the isolation, a number of activities were recorded: single twitches, unfused and maximal tetani. The effects of summation of motor unit contractions were evaluated as differences between the recorded force during parallel stimulation and the algebraic sum of individual forces. As more units were activated simultaneously it was found that the recorded force became progressively lower than the estimated algebraic sum of forces. Furthermore, in all cases, the force of unfused tetani summated more effectively than the force of twitches or of fused tetani. Finally, a decrease in the amplitude of force oscillation in the unfused tetanus was observed in relation to values expected based on algebraic summation of oscillations for individual MUs. The main finding of this study is that forces produced by MUs during simultaneous stimulation summate nonlinearly.

TIV.20
INFLUENCE OF 5-WEEK WHOLE BODY VIBRATION TRAINING ON MOTOR UNIT CONTRACTILE PROPERTIES IN THE RAT MEDIAL GASTROCNEMIUS MUSCLE

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The aim of the study was to determine the effect of the whole body vibration training on motor unit contractile properties in rat medial gastrocnemius.Two groups of Wistar rats trained 30 s (V30, n=4) or 4 × 30 s (V120, n=4) daily, 5 days weekly, for 5 weeks on vibratory plat-

form (Power Plate®, USA, 2 mm vibration at 50 Hz) were compared to the control group (C, *n*=10). The employed vibration excited motor units through the stretch reflex loop. Functional isolation of units was achieved by electrical stimulation of thin filaments of the ventral roots. A total of 267 motor units were studied (56 in V30, 69 in V120, and 142 in C). They were classified into: fast fatigable (FF), fast resistant to fatigue (FR) and slow (S) types.In the V30 and V120 group, no changes in the distribution of motor unit types were noted. For both groups the specific and the only change was a significant increase in the twitch and maximal tetanus force in FF motor units. Additionally, for V120 group an increase in maximal tetanus force of S units was noted. For V120 group the contraction and relaxation times shortened in fast motor units and FF motor units became also less resistant to fatigue. It is shown that only a little increased daily physical activity induced by vibration considerably influences motor unit properties and FF motor units seem to be the most sensitive to vibratory stimulus.

TIV.21
AGEING AFFECTS THE RATE OF FORCE DEVELOPMENT OF MOTOR UNITS IN RAT FAST MUSCLE

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The rate of force development (RFD) of skeletal muscle decreases with ageing. The purpose of this study was to determine whether this phenomenon may be connected with the age-related changes in motor unit (MU) properties. One group of young (5–10 months old, *n*=9) and two groups of old (24–25 months old, *n*=6 and 28–30 months old, *n*=8) male Wistar rats were studied. Contractile activity of isolated MUs in the rat medial gastrocnemius muscle was evoked by electrical stimulation of ventral root filaments. MUs were classified into fast fatigable (FF), fast resistant (FR), and slow (S) according to susceptibility to fatigue and twitch contraction time. The RFD, force and force-time integral (FTI) of twitch, contraction following doublet pulse stimulation and maximal tetanus were measured and compared. Absolute and relative RFD of studied MUs evidently increased with ageing. We observed gradual augmentation of absolute force and FTI of evoked doublet contractions during ageing in S MUs while in FF units this increase was transient (only for 24–25 months old animals). However, the relative values of force and FTI after doublet stimulus were lower in S and FR MUs and unchanged in FF units in old animals as compared to the young. The increase in MU RFD is opposite to decrease in muscle RFD with ageing. The decline of the muscle performance capabilities can not be attributed to deterioration of mechanical parameters of survived MUs but rather is due to overall loss and change in proportion of MUs.

TIV.22
VARIABILITY OF CONTRACTILE PROPERTIES AND ACTION POTENTIALS OF MOTOR UNIT IN FLEXOR DIGITORUM BREVIS AND MEDIAL GASTROCNEMIUS MUSCLES IN THE RAT

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The differences between motor unit (MU) properties in foot (flexor digitorum brevis, FDB) and sural (medial gastrocnemius, MG) muscles in anaesthetized Wistar rats were studied. In experiments functional isolation of single MUs was achieved by stimulation of thin filaments from the L5–L6 and L4–L5 ventral roots for FDB and MG muscles, respectively. The mean muscle mass amounted to 76 mg for FDB and 895 mg for MG. The force and the motor unit action potential (MUAP) properties were analysed. The three types of MUs (FF, FR and S) in the MG muscle were found. Sag phenomenon was visible in all fast units at 40 Hz stimulation. In FDB, the slow MUs were not noted. Sag was visible for only a part of fast MUs at lower frequencies of stimulation. In the MG muscle the fatigue index had bimodal distribution, whereas in FDB the distribution was continuous. The twitch and maximum forces were approximately 8 times lower in FBD then in MG, whereas the contraction time was significantly longer. The force-frequency curves of all motor units in FDB were shifted towards lower frequencies and comparison to the same type of MUs in MG. The MUAP durations for FF and FR MUs were longer whereas the MUAP amplitudes were higher for FF units in MG in relation to FDB. In conclusion, the variability of MU properties in distal and proximal muscle concerns all studied contractile parameters and likely reflects different motor control strategy.

TIV.23
DOUBLET OF ACTION POTENTIALS EVOKED BY INTRACELLULAR INJECTION OF CURRENT INTO RAT MOTONEURONES

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Experiments were based on electrophysiological method of direct stimulation and recording from motoneurones located in L4–L5 segments of the rats spinal cord. Records from spontaneously firing motoneurones were collected after intracellular application of a current of gradually increasing intensity. Current threshold for generation of the first action potential was determined. Additionally, intervals between six first potentials were analyzed. It was demonstrated that the gradually increasing current intensity caused the generation of doublets in 68% of motoneurones studied. Doublets appeared when stimulating current increased to 120–500% of the threshold value. Moreover, the appearance of the doublet resulted in a significant prolongation of time interval to the subsequent, third action potential. These results suggest that doublets in motoneuronal firing appear as a result of their strong synaptic excitation. The prolonged interpulse interval after the doublet firing can be due to an increase of duration and amplitude of afterhyperpolarization (AHP), previously observed in antidromic doublet stimulation.

TIV.24
CHANGES IN THE FORCE-FREQUENCY RELATIONSHIP AFTER THE TREADMILL AND WHOLE BODY VIBRATION TRAINING OF MOTOR UNITS IN RAT MEDIAL GASTROCNEMIUS MUSCLE

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The aim of the study was to compare changes in the force-frequency relationship of motor units (MUs) in rat medial gastrocnemius muscle, caused by treadmill training and whole body vibration training. 24 Wistar rats were studied. Rats were divided into 3 groups: tread-

mill training group (*n*=7, locomotion speed 27 cm/s, 1 km daily, 5 days weekly, for 4 weeks), whole body vibration training group (*n*=6, Power Plate® vibration platform, 50 Hz vibration of 2 mm amplitude, 30 s daily, 5 days weekly, for 4 weeks) and a control group *n*=11. Functional isolation of single MUs was achieved by electrical stimulation of ventral roots filaments. A total of 424 MUs were studied (148 in treadmill training, 142 in vibration training and 134 in control). MUs were classified as fast fatigable – FF, fast resistant – FR or S – slow. The frequency causing 60% of maximum force of MUs has decreased in FF units of both trained groups and increased in FR units. In S units the increase of the parameter was noted only in treadmill trained group. Additionally, the slope of the force-frequency curve has increased in all MUs types in the vibration training group.

TIV.25
CHANGES IN MOTOR UNITS PROPERTIES IN A RAT MODEL OF AMYOTROPHIC LATERAL SCLEROSIS (ALS)
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ALS is a fatal neurodegenerative disorder characterized by developing muscle weakness and paralysis which is an effect of selective and progressive death of motoneurons. The experiments were carried out on motor units (MUs) of the medial gastrocnemius muscle in transgenic rats with hSOD1$^{(G93A)}$ mutation. The animals were divided into three groups: 3 months old, 4 months old and approximately 200 days old in nearly terminal state, when nerve stimulation revealed on average 6 MUs in the studied muscle. Because of considerable disturbances in contraction time (CT) and disappearance of sag effect, usually applied for MUs classification as fast and slow, the division of MUs was based on 20 Hz tetanus index. Meanwhile, to distinguish fast fatigable (FF) from the fast resistant (FR) MUs the fatigue index (FatI) was applied. Results showed that MUs proportion changed radically in the course of the disease and in terminal state an increase of S and a decrease of fast MUs was observed. Moreover, CT increased gradually in all MU types. FatI of FF MUs increased but, at the same time, for FR and S gradually decreased. Twitch force as well as tetanus force in terminal group increased especially for FR and S MUs what indicates plastic changes in examined muscle (sprouting of surviving axons and collateral reinnervation), leading also to increased amplitude of MUs action potentials. EMG analysis additionally showed slowing of axonal conduction velocity or/and motor plate transduction.

TIV.26
SUMMATION OF MMG SIGNALS DURING CO-ACTIVITY OF TWO ISOLATED MOTOR UNITS IN MEDIAL GASTROCNEMIUS MUSCLE OF THE RAT
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The MMG signal generated in contracting pennate muscle is due to a transversal displacement of the its surface. It was shown on the base of *in vivo* experiments and a computer model that the MMG signal recorded during an isolated motor unit (MU) contraction is dependent on the stimulation frequency, the position of the laser distance sensor (LDS) and MU architecture. The three different profiles of the MMG can be observed. The first type denoted as N (negative) is observed for the LDS located over the proximal connection of the MU (the distance from the muscle surface decreases with contraction force increase). In class P (positive) the relationship is opposite to N and the LDS is located over the area between distal connection of the MU and tendon of insertion. Finally, for the third class denoted as M (mixed) the MMG is initially positive, and when the contraction force exceed a certain level it starts to decrease and becomes negative. The process of the MMG summation during two MUs contraction was also investigated. It was observed that for the MUs with MMG-N or MMG-M profile the MMG summation was quasi linear. In case of twitch and unfused contractions it was equal the algebraic sum of the individual MMGs. In case of the fused contraction the resulted MMG was slightly lesser than the algebraic sum. In opposition, the contraction of two MUs MMG-P induced the MMG signal lesser than the algebraic sum and the resulted signal was nearly equal the MMG presented by stronger MU.

TIV.27
CONTRACTILE PROPERTIES AND DISTRIBUTION OF TYPES OF MOTOR UNITS INNERVATED BY AXONS FROM L4 AND L5 VENTRAL ROOTS IN THE RAT MEDIAL GASTROCNEMIUS
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There is a limited data concerning the rostro-caudal distribution of motoneurons within the motor nucleus. In present experiments single motor units in rat medial gastrocnemius were studied and their contractile properties as well as percentage participation of different types of motor units belonging to subpopulations innervated by axons in L4 and L5 ventral roots were analyzed. Experiments were based on functional isolation and electrical stimulation of as many as possible axons from L4 and L5 ventral roots. 35 motor units innervated by L4 and 90 units innervated by L5 ventral root were studied. We found that the composition of the three physiological types of motor units (fast fatigable, fast resistant and slow) in the two subpopulations of motor units was similar. However, the twitch time parameters (the contraction time and half-relaxation time) were slightly longer in L4 in comparison with L5 subpopulation (the difference was significant for fast resistant units) although the difference has not been reflected in expected shift of a steep part of force-frequency of stimulation curve towards lower frequencies in L4 subpopulation of motor units. Force parameters were similar for motor units belonging to two subpopulations. The small differences between L4 and L5 subpopulations of motor units can be due to possible differences in a spatial distribution of their muscle fibres in the semipennate medial gastrocnemius.

TIV.28
THE TETANIC DEPRESSION IN FAST MOTOR UNITS OF MAMMALIAN SKELETAL MUSCLE CAN BE EVOKED EVEN BY LENGTHENING OF ONE INITIAL INTERPULSE INTERVAL
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The tetanic depression is phenomenon observed when higher frequency of stimulation of fast motor units immediately follows the lower one. However, it is not known does only one prolonged first interpulse interval can evoke this effect. This study was conducted on 27 fast

motor units of cat and 47 units of rat medial gastrocnemius muscles. In experiments performed on the two species the following stimulation protocol was applied in the main part of experiment: (1) the 6-pulses train of stimuli at 20 Hz (cat) or 40 Hz (rat), i.e. at 50 and 25 ms interpulse intervals, (2) the 6-pulses train of stimuli with the first interpulse interval prolonged from 50 ms to 100 ms (cat) or from 25 to 50 ms (rat) followed by 5 stimuli at 20 Hz (cat) or 40 Hz (rat), (3) the 6-pulses train of stimuli at 20 Hz (cat) or 40 Hz (rat). Effects of two-time prolongation of the first interpulse interval were observed as a decrease of the force following the sixth stimulus in a train. The tetanic depression in rat motor unit tetani for fast fatigable (FF) units amounted to 5.39 ± 4.94% and 9.24 ± 4.13% for fast resistant (FR) units whereas in cats the mean values were 15.00 ± 10.15% and 27.00 ± 13.95% for FF and FR motor units, respectively. This results prove that the studied phenomenon influence the force development when the motoneuron begins the activity even with only one, first prolonged interpulse interval.

TIV.29
THE LINEAR SUMMATION OF THE MOTOR UNITS ACTION POTENTIALS RECORDED WITH WIRE ELECTRODE IN THE RAT MEDIAL GASTROCNEMIUS MUSCLE

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The summation of the motor units action potentials (MUAPs) in the rat medial gastrocnemius muscle was studied. Experiments were performed on anesthetized animals and single motor units (MUs) were functionally isolated by electrical stimulation of thin filaments of ventral roots. MUAPs were recorded with two silver wires inserted into the muscle. Algebraic sum of the MUAPs recorded from two to four individual MUs were compared to the action potentials recorded during their simultaneous stimulation. The peak-to-peak amplitude, the longitudinal center of the signal square, the number of phases and turns, correlation coefficient and mean-square error were measured. In all studied cases of summation the number of phases and turns were the same in the two compared signals. When summation effects of two MUs were compared, there were no significant differences in the peak-to-peak amplitude. When 3 and 4 MUs were co-activated significant differences between a sum of individual MUAPs and the recorded action potential were noted in some cases. The most variable parameter was the longitudinal center of the signal square which was the major source of differences. It was also shown that in a case of contractions of low force MUs the studied parameters of the algebraic sum of their MUAPs was similar to parameters of action potentials recorded during simultaneous stimulation.

TIV.30
PROJECTIONS FROM THE BASILIAR PONTINE NUCLEI TO THE CEREBELLAR CORTEX: FLUORESCENT TRACING STUDY IN THE RABBIT

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Pontine nuclei (PN) are large center transferring cortical afferents to the cerebellum. The study addresses to distribution of PN neurons in the pontocerebellar link. Two different neuroplasmatic and nuclear fluorescent tracers were injected in separate experiments into: (1) paramedian lobule (PML) and pyramis, and (2) pyramis and uvula. Following both combination of injections, retrogradely labeled neurons were found bilaterally in all PN apart from the ventral nucleus. These neurons, parent for the pontocerebellar projection, clustered in two groups. First larger group occupied the dorsolateral nucleus and the dorsolateral region of lateral and peduncular nuclei. Second smaller group involved the dorsal region of paramedian nucleus and the dorsomedial region of peduncular nucleus. Projection to the uvula arises from nearly entire rostrocaudal extent of PN, and that to the pyramis and PML originates from medial and caudal extent of PN. In spite of substantial overlap of projection area in PN, no neurons were found to project by way of axonal collaterals. The strongest PN projections arise from the dorsolateral nucleus and these from the paramedian, lateral and peduncular nuclei are about two, three and five times weaker, respectively. PML and the uvula receive the most numerous projections (mean 4 744 and 4 283 neurons) whereas projection to the pyramis (mean 1 172 neurons) is apparently smaller. The differences in the projection seem to be cause of different function of these lobules.

TIV.31
PROJECTION FROM THE DORSAL COLUMN NUCLEI TO THE CEREBELLAR CAUDAL VERMIS: COMPARISON OF COLLATERAL AND NON-COLLATERAL CONNECTIONS

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The study was performed to determine projection from the dorsal column nuclei (DCN), both independent and by way of axonal collaterals, to the pyramis and uvula. Two different fluorescent tracers were unilaterally injected into the two vermal targets. Single and double retrogradely labeled neurons, parent for non-collateral and collateral projections, were found in defined regions of DCN. Non-collateral bilateral projections directed to the pyramis and uvula are stronger to the latter, and originate from neurons in the lateral cuneate nucleus (CuL; n= 5 040 vs. 6 637), the complex of gracile and medial cuneate nucleus (Gr+CuM; n=820 vs. 2 190) and CuM (n=32 vs. 92). Weak projection from the dorsal Gr (n=57) is destined for the pyramis. Neurons in the rostral and middle CuL, projecting to the pyramis and uvula occupy ventrolateral and ventromedial positions, respectively, and a narrow region between them is common. More caudally, small areas in CuL, Gr+CuM and CuM supply the uvula, but in majority, areas of projection from these subnuclei to both lobules overlap. Weaker ipsilateral projections by way of axonal collaterals (n=175) arise from neurons in common projection areas in CuL and Gr+CuM. To sum up, neurons for the DCN-pyramis and DCN-uvula projections vary in number and distribution, and there is a small population of neurons participating in divergent projection to both lobules. Different extent of DCN influences may be due to disparate function of the pyramis and uvula.

TIV.32
DISTRIBUTION OF THE PARVALBUMIN, CALBINDIN-D28K AND CALRETININ IMMUNOREACTIVITY IN THE STRIATUM OF THE BRAZILIAN SHORT-TAILED OPOSSUM
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To understand the organization of inhibitory circuitries in the striatum of the opossum, the distribution of parvalbumin (PV), calretinin (CR) and calbindin (CB) was investigated within the nucleus accumbens (Acc), caudate nucleus (Cd) and putamen (Pu). Brains from six adult opossum (both sexes) were stained for PV, CR and CB and analyzed using fluorescent or confocal microscopy. Within neurons immunostained for each calcium-binding protein four major types of neurons were distinguished. Type 1 – small ovoid or roundish somata with three to five thin dendrites of approximately equal thickness; type 2 – medium-to-large-sized multipolar neurons with dendrites of variable thickness; type 3 – fusiform neurons of variable size emanating dendrites from the opposite poles of the somata; type 4 – pyramidal neurons. Moderate-to-high density of CB immunoreactive (-ir) neurons was observed in the Cd and Pu, but staining in the Acc was lighter. Light density of PV-ir neurons was present in the Cd, Pu and Acc, but PV-immunoreactivity of neuropil was high. Only single CR-ir neurons were scattered through the all studied structures, but the immunostaning of neuropil was much higher. Our data provide baseline information for comparisons of distribution of calcium binding proteins in different species, including rat, monkey and human.

TIV.33
CHANGES IN EXPRESSION OF nNOS AND PARVALBUMIN IN DORSAL HORN OF THE LUMBAR SPINAL CORD AFTER SPINAL CORD INJURY
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The aim of the present study was to examine changes in the level of neuronal nitric oxide synthase (nNOS) and parvalbumin (PV) in dorsal horn of the rat lumbar spinal cord segments (L2–L6) 7 and 14 days after thoracic spinal cord transection. Our data show that staining of control transverse spinal cord sections with nNOS antibody provided an intense labeling of small round or bipolar nNOS- immunoreactive (IR) cells bodies, fibers and terminal like structures which were distributed throughout lamina II and formed a dense IR band. The large multipolar nNOS-IR cells with stellate or elongated cell bodies were occasionally seen in the deeper layers (laminae III–IV). A decreased density of fiber-like and the loss of somatic nNOS staining was found in dorsal horn layers after transection at both time points. A quantitative assessment of nNOS IR disclosed a strong decrease in the number of small neurons in superficial laminae (laminae I–III). In addition, an intense PV-IR band, consisting of terminal like structures in lamina II under normal conditions, was significantly reduced due to spinal cord transection. The present study indicates a considerable decrease in the number of nNOS-IR neurons in superficial dorsal horn, where NO modulates the activity of inhibitory GABA-ergic interneurons. The changes in expression of both the calcium binding proteins in dorsal horn may modulate nociceptive transmission. *Support: APVV grant 0314-06 and VEGA Grant 2/0015/08 from the SAS.*

POSTER SESSION II

TV: Neural Excitability, Synapses and Glia: Synaptic Plasticity and Networks

TV.01
CENTRAL RETINAL LESIONS INDUCE TIME-DEPENDENT PROTEIN EXPRESSION CHANGES THROUGHOUT AREA 17
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Central retinal lesions lead to loss of visual input in the central part of area 17 of the adult cat. Eventually this results in topographic map reorganization within the sensory-deprived cortical lesion projection zone (LPZ) as measured by electrophysiology. This recovery coincides with changes in gene/protein expression. We therefore investigated these alterations as a function of post-lesion survival time in three regions of primary visual area 17: the center of the LPZ, the border of the LPZ and far peripheral area 17. We analysed the differential protein expression patterns between control and experimental animals with 2-dimensional differential gel electrophoresis. We have traced 74 differential spots for the three regions in area 17. Central retinal lesions clearly induced region-specific and time-dependent protein expression changes. Even the protein expression in far peripheral area 17 spared from a direct impact of the central retinal lesions differed from the corresponding region in normal subjects. We succeeded in identifying 93% of the differential spots by means of mass spectrometry and software processing helped functional grouping within the list of proteins. This analysis allowed us to select two plasticity-related proteins for further validation experiments.

TV.02
A SINGLE EXPOSURE TO AN ENRICHED ENVIRONMENT STIMULATES THE ACTIVATION OF DISCRETE NEURONAL POPULATIONS IN THE BRAIN OF THE fos-tau-lacZ MOUSE
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Storage of experience, including learning and memory, is thought to involve plasticity within pre-existing brain circuits. One model for looking at experience-dependent changes is environmental enrichment (EE), which involves exposing animals to a complex novel environment. Animals exposed to EE have previously been shown to exhibit a variety of behavioural and structural alterations in the brain, including decreased stress, improved

learning and memory, altered levels of immediate early genes and synaptic change in the visual cortex. We were interested in understanding what regions of the brain are activated during the initial stages of EE. We used the fos-tau-lacZ (FTL) transgenic mouse to examine changes in functional activation throughout the brain after a single exposure to EE. Female C57BL6 FTL mice (n=19) were divided into three groups; enriched, home cage and handled control. We found that early in the process of EE, there was a high level of FTL expression in a series of brain regions in the enriched group compared to the home cage group and the handled control group, indicating that multiple circuits were activated. These regions include the claustrum, infralimbic cortex, hippocampus, amygdala and the hypothalamus. We believe that EE stimulates an initial strong increase in activation of multiple functional circuits. These circuits are presumably involved in the initial response of the animal to the enriched environment.

TV.03
DOPAMINERGIC D1/D5-RECEPTOR BLOCKADE WITHIN THE NUCLEUS ACCUMBENS AND ITS EFFECT ON HIPPOCAMPAL LONG-TERM POTENTIATION IN THE DENTATE GYRUS OF FREELY MOOVING ANIMALS

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Hippocampal long-term potentiation (LTP) is considered to be a cellular model of learning and memory. It has been shown that stimulation of modulatory brain regions can influence hippocampal LTP. Quite little is known of the role played by the nucleus accumbens (NAc) on hippocampal LTP. The NAc is the central component of the basal ganglia positioned to integrate signals arising from corticolimbic areas and to translate them into motor behavior. Electrical stimulation of the NAc core and shell region has been shown to modulate LTP in the dentate gyrus. We were now interested to study if the dopaminergic system within the NAc affects LTP in the dentate gyrus in freely moving animals. Thus, we have applied a specific dopaminergic D1/D5-receptor blocker, SCH23390, into the NAc and studied its possible effect on control potentials as well as on LTP in the dentate gyrus in freely moving rats. Interstingly, treated animals show a depression in baseline responses recorded in the dentate gyrus, just by D1/D5-blockade within the NAc. Furthermore, these animals were characterized by a reduced LTP after weak tetanic stimulation of the perforant path when compared with controls. As there is no direct dopaminergic connection between NAc and dentate gyrus, the effects must involve indirect mechanisms, such as glutamatergic and/or GABAergic pathways.

TV.04
DIFFERENT REGIONS OF THE APICAL BRANCH OF CA1 DENDRITES EXPRESS SPECIFIC FORMS OF LTD IN HIPPOCAMPAL SLICES *IN VITRO*

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Long-term potentiation (LTP) and long-term depression (LTD) of excitatory synaptic transmission are widespread phenomena expressed at many excitatory synapses in the mammalian brain. Because of its long duration, input specificity and associative properties, LTP and LTD have been used as cellular models for memory. We wanted to investigate whether different locations on the apical dendritic branch could influence the induction of LTD and its related properties. Late-LTD could be induced in the apical CA1 dendrites by strong low-frequency stimulation (SLFS) pattern if the synapses were located distally, whereas, proximally located synapses were not able to maintain late-LTD. However, SLFS in both locations was able to trigger the synthesis of plasticity-related proteins, which could be evidenced by cross tagging experiments. In addition, we have investigated if hippocampal CA1-LTP prevents/occludes the establishment of LTD in the same synaptic input at specific time points after LTP-induction. We show induction of LTP occludes longer-lasting but not short-term LTD about 1 h after LTP-induction. However, after 4 h, i.e. after transformation of early- into late-LTP, also later forms of LTD can again be induced in the same synaptic input. Our results demonstrate that hippocampal neurons do not lose their capacity for the induction of longer forms of LTP or LTD after the establishment of late-LTP in the apical dendrites of hippocampal CA1-neurons.

TV.05
THE EFFECTS OF PROTEIN SYNTHESIS INHIBITOR ON THE EXPRESSION AND RE-CONSOLIDATION OF PENTYLENETETRAZOL KINDLED SEIZURES

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In the present study the effects of a protein synthesis inhibitor, cycloheximide (125 μg, i.c.v.), on the expression and reconsolidation of pentylenetetrazol-induced kindled seizures, were studied in rats. Cycloheximide given repeatedly (every second day) to fully kindled rats, immediately after 4 consecutive sessions of PTZ-seizures, did not modify the strength of subsequent fits of convulsions. On the other hand, the protein synthesis inhibitor significantly attenuated the strength of convulsions when the drug was administered 1 h before the PTZ injection, every second day for 5 consecutive experimental sessions. However, when cycloheximide was omitted in a consecutive session, PTZ induced a fully developed fit of tonic-clonic convulsions, indicating that cycloheximide-induced changes in seizure intensity were transitory, not related to a stable modification in the function of neuronal circuits responsible for kindling seizures. The present findings suggest that the mechanisms underlying epileptogenesis are very resistant to modification, and as such, are not the subject to permanent changes even under the influence of protein synthesis inhibition. One possible reason may be the depth and multiplicity of changes induced by seizures (i.e. alterations in enzymes, receptors, structural proteins, growth factors, etc.), that may cause permanent biochemical and morphological alterations in the brain that give rise to the kindled seizures.

TV.06
REINFORCEMENT OF AN EARLY-LTP IN THE HIPPOCAMPAL CA1 BY STIMULATION OF THE VENTRAL TEGMENTAL AREA IN FREELY MOVING RATS
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It has been shown that the prolonged maintenance of hippocampal long-term potentiation (LTP) requires heterosynaptic events during its induction. The ventral tegmental area (VTA), a modulatory input to the CA1 region is a heterogeneous group of dopaminergic cells and a major component of the mesolimbic dopamine system. We used a method which allowed us to simultaneously record both, the field-EPSP and the population spike (POP) in the hippocampal CA1 in freely moving rats by stimulation of the contralateral CA3. We could show that an early-LTP in the CA1 region can be reinforced into a longer-lasting form by high-frequency stimulation of the VTA 15 min after its induction. This reinforcement of an early-LTP in CA1 was dependent on dopaminergic receptor activation and was also dependent on protein synthesis. We have now extended our studies and could show that high-frequency stimulation of the VTA alone, i.e. without LTP-induction in CA1, caused a delayed-onset potentiation for the recorded field-EPSP and POP in the CA1 region in response to test stimulation of the contralateral CA3. This delayed-onset potentiation was dependent on the synergistic activation of both the glutamatergic and the dopaminergic receptor activation, because paused glutamatergic test stimulation abolished this potentiation.

TV.07
PROTEOMIC ANALYSIS OF SYNAPTIC PROTEINS FROM MICE EXPRESSING LOW LEVELS OF p25
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Cdk5 is a neuronal kinase involved in synaptic plasticity and memory formation. When overactive, Cdk5 can induce cell cycle arrest, tau hyperphosphorylation and apoptosis. This dichotomy in function is correlated with the degree of Cdk5 activation by small regulators. P25 is the most potent activator of Cdk5, absent in neurons under physiological conditions but induced during neuronal insults. In mouse models, high levels of p25 lead to neurodegeneration. Consistently, aged mice with life long exposure to low p25 levels exhibit tau hyperphosphorylation. However, expression of low p25 levels can improve learning in a sex-specific manner in young adult mice. The underlying molecular mechanisms of this dose effect are still poorly understood. Therefore, in the project presented we have undertaken comparative proteomics on hippocampal synaptosomes from wildtype and p25 mutant mice of both sexes. Four different approaches of tandem mass tag labeling were employed. From more than 500 quantifiable proteins, we identified sex-specific changes in the p25 transgenics compared to wildtype mice. Selected proteins will be introduced and implications for the role of Cdk5-p25 in memory and neurodegeneration will be discussed. *This work was supported by an MRC PhD studentship.*

TV.08
INDUCTION OF LONG-TERM POTENTIATION AND DEPOTENTIATION BY THETA-FREQUENCY STIMULATION IN THE CA1 FIELD OF RAT HIPPOCAMPUS *IN VITRO*
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One of the most important features of hippocampal long-term potentiation (LTP) is that it is elicited by naturally occurring patterns of neuronal activity. Long term depression of synaptic transmission in hippocampus is induced by low-frequency stimulation protocols that completely different from those required for LTP induction. Depotentiation is another form of synaptic plasticity that has been hypothesized as a candidate for LTP counterpart in learning processes. Depotentiation can be induced by theta-frequency stimulation patterns similar to those required for the LTP induction. We showed that short stimulating sequences applied at theta frequency to Schaffer collaterals can produce complex population spikes in the CA1 field of rat hippocampal slices if they were preincubated in modified artificial cerebrospinal fluid. Such theta-stimulation induces LTP if the complex spikes emerge. In these conditions, associative LTP is also produced if weak and strong pathways are stimulated synchronously. We found that associative LTP induced by synchronous theta stimulation is reversed after application of asynchronous theta stimulation. Thus, opposite changes of the efficacy of synaptic transmission can be induced by similar stimulation protocols. Our results confirm the hypothesis that in some conditions depotentiation may underlie learning rules in the CA1 field of hippocampus.

TV.09
MATRIX METALLOPROTEINASE-9 AT THE NEUROMUSCULAR JUNCTION
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Matrix metalloproteinases (MMPs) are considered to play a pivotal role in remodeling of the extracellular matrix in health and disease. Recent studies suggest that MMP-9 plays an important role in synaptic plasticity, learning and memory. It was shown that MMP-9 occurs in postsynaptic part of hippocampal synapses, where the amount and activity of MMP-9 were increased by stimulation leading plasticity changes. At the neuromuscular junction, a role, related to the turnover of agrin, has been demonstrated for MMP-3. Although MMP-9 has been localized to NMJ, neither its role nor precise distribution in relation to synaptic elements is established unequivocally. Here, we have carried out the investigation of the gelatinolytic activity of NMJ in rats, in order to establish whether high-intensity exercise can promote expression of MMP-9 at the NMJ. Our finding of the present work is that training increases the gelatinolytic activity of the NMJ in the extensor digitorium longus and soleus muscles. The 4-week endurance training program elicited alterations at the presynaptic side of the NMJ, where the increasing gelatinolytic activity was localized in the Schwann cells. These data shows that endurance training influences on the gelatinolytic activity of NMJ, possibly through synaptic transmission.

TV.10

CD44-ErbB RECEPTOR INTERACTION STUDIED BY FLUORESCENCE RESONANCE ENERGY TRANSFER ANALYSIS USING FLUORESCENCE LIFETIME IMAGING MICROSCOPY

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CD44 is a multifunctional cell surface glycoprotein which regulates cell-cell and cell-matrix interactions in a variety of tissues. CD44 was implicated in the development of peripheral nerves, functioning as a coreceptor for ErbB class of growth factor receptors. However, it is not known whether CD44-ErbB interaction may occur at the adult peripheral synapses. Here we studied, using Fluorescence Lifetime Imaging Microscopy, the proximity between CD44 and ErbB3 at the rat neuromuscular junction (NMJ). This was performed in muscle sections co-immunostained for CD44 and ErbB3, using secondary antibodies coupled to Alexa488 and Alexa647 respectively. Neuromuscular junctions were visualized using Alexa555-bound α-BT. The FRET between Alexa488 (donor) and Alexa647 (acceptor) was judged by measuring an accompanying changes in the donor fluorescence lifetime. We found that the mean fluorescence lifetime of the donor fluorophore labeling CD44 protein was considerably shorter over the NMJ than in non-synaptic sites. Then we compared normal rat muscle to the muscle affected by denervation in the transgenic model of amyotrophic lateral sclerosis (ALS). Importantly, ALS-like neurodegeneration resulted in significant increase in molecular proximity of CD44 and ErbB3 at the NMJ. The specific complex formation between the two proteins was confirmed using immunoprecipitation analysis. Our study provides novel data on the molecular architecture of the neuromuscular synapse in both health and disease.

TV.11

DYNAMICS OF CORTICAL REMODELING DURING REMOTE MEMORY STORAGE

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There is evidence that reorganization of neuronal circuits in the anterior cingulate cortex (aCC) occurs during remote memory storage. Here we describe how morphological changes progressively develop on aCC neurons during formation and stabilization of an aversive memory trace. Using a contextual fear conditioning protocol, we measured memory performance and spine density on aCC pyramidal neurons at several time points (1, 5, 7, 14, 21 days) following the training episode. Results show that aversive memory, estimated by the percentage of time spent freezing, was robust already 1 day posttraining and remained noteworthy stable until day 21. Morphological measurements in the trained animlas then revealed no evidence of cortical remodeling on day 5 posttraining, but a significant increase in spines on day 7 which persisted until day 21. Interestingly, despite an equivalent number of spines was counted in aCC on days 7, 14, and 21, the number of single neurons showing increased spine density strongly varied across time-points. These findings suggest that long term storage of the memory trace requires a relatively stable increase in spines provided by a variable number of recruited neurons.

TV.12

EVIDENCE FOR CROSS-MODAL PLASTICITY IN ADULT MOUSE VISUAL SYSTEM

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Since the pioneering work of Hubel and Wiesel in the ’60, research in cat, monkey and humans has led to a central dogma that beyond the critical period the brain only retains a reduced capacity for reorganization. Current work in rodents however challenges this view since specific treatments are capable of reinstating cortical plasticity in the adult. The goal of our work was to study this adult cortical reorganization in detail. A combination of deprivation of one eye and stimulation of the remaining eye previously led to the identification of input-specific subdivisions (Van Brussel et al. 2009). Using this information as a reference map, we established to what extent each of these functional subdivisions take part in cortical reorganization upon enucleation. Briefly, there seemed to be two waves of recovery, the first characterized by the expansion of the supragranular binocular zone of V1 and V2L and the second affecting the infragranular layers, initiated at the outer border of the visual cortex with neighboring non-visual cortex and accompanied by hyper-activity of this adjacent cortex. To test a possible non-visual nature of this recovery, we combined monocular enucleation with the inactivation of the remaining eye or a second sensory modality. Both lack of complete visual cortex deactivation upon enucleation of the remaining eye, and strong effects of auditory and somatosensory deprivation on infragranular visual cortex suggest cross-modal plasticity in adult mice.

TV.13

NEUROPLASTICITY IN THE HIPPOCAMPUS AND THE STRIATUM OF THE MOUSE DURING VISUO-MOTOR LEARNING: ANALYSIS OF IMMEDIATE EARLY GENE EXPRESSION PATTERNS

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Visuo-motor learning, a dynamic process in which visual stimuli are associated with certain motor responses, leads to goal-directed, complex behaviours. Literature states that the striatum and the hippocampus are two important brain areas for this type of learning since neural mechanisms and changing activation patterns within these areas are involved in the processing of visual stimuli and the subsequent motor act. The neural activity of mice trained in the Morris water maze was molecularly char-

acterized by *in situ* hybridization for the immediate early gene activity markers *arc* and *homer1a* and this in two timeframes, namely the early learning and the late learning (overtrained/automatisation) phase. First results show that in the early learning phase *arc* mRNA expression appears to be upregulated in the ventrolateral striatum, while in the overtrained phase a higher expression is noticeable in the dorsal striatum. *Arc* and *homer1a* mRNA expression levels in the hippocampus indicate that this structure is more active in the early learning phase compared to the overtrained phase. Subregional analysis of hippocampal *homer1a* mRNA expression patterns implies an opposite dynamic of CA1/DG and CA3 in response to learning. While CA1/DG activity decreases when mice become overtrained, CA3 activity seems to slightly increase. Further analysis will reveal how differential molecular activity in the subregions of hippocampus and striatum relates to both phases of the visuo-motor learning process.

TV.14
PROTEOMIC DISCOVERY OF MATRIX METALLOPROTEINASE-9 SUBSTRATES

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Elucidation of protease substrates ("proteodegradomes") is essential for understanding the proteolytic pathways and their networks and thus their role in the regulation of cell function. Matrix metalloproteinase-9 (MMP-9) is expressed by the adult brain and released in response to enhanced neuronal activity. It is well established that MMP-9 is involved in neuronal plasticity, including long-term potentiation, learning and memory. Under pathological conditions, during excitotoxicity, stroke and traumatic brain injury, MMP-9 is detrimental to the brain tissue, probably because of its enhanced activity. MMP-9 is locally inhibited by endogenous tissue inhibitors of metalloproteinase 1 (TIMP-1). In the current studies we optimized the isolation of synaptoneurosomal fractions from the murine hippocampus. In order to induce MMP-9 activity the synaptoneurosomal fractions were treated with 50 μM of glutamate for 20 min. To identify MMP-9 substrates, we compared the synaptic fractions isolated from wild type and MMP-9 knockout mice by two-dimensional electrophoresis (2-DE). We have found the differences in the 2-D gel patterns. Further studies will be complemented by in-gel digestion of the protein spots of interest, mass spectrometry of the resultant peptides, and peptide mass fingerprinting to identify each protein.

TV.15
HIGHER- ORDER NUCLEAR STRUCTURE IN EPILEPTOGENESIS

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Although the molecular mechanisms of gene-expression in neurons are comprehensively described in the literature, little is known about the relationship between these processes and the architecture of the neuronal cell nucleus. For example, it has never been examined whether bursts of transcriptional activity associated with seizures involve any regulation at the level of higher-order nuclear structure. Accordingly, we have performed studies on the structure of neuronal nucleus in epileptic animals. Using electron microscopy, we found the striking appearance of large interchromatin granule clusters (IGCs) in epileptic nuclei. We confirmed this observation by immunofluorescence-confocal analysis of IGC marker, a spliceosome assembly factor SC-35. Interestingly, there was also aggregation of spots immunoreactive for phophorylated and acetylated Histone H3, a marker of transcriptionally active chromatin. The findings are consistent with the results of bioinformatic analysis of transcription profiling in the rat kainate-induced status epilepticus (public gene expression data), showing tendency for coordinated expression of positional gene-clusters along chromosomes. Taken together, our results suggest that upon epileptogenesis there is prominent reorganization of neuronal nucleus, putatively involving formation of molecular factories, where transcription, splicing, and export of pre-mRNA are orchestrated.

TV.16
LOCOMOTOR EXERCISE OF SPINAL RATS CAUSES AN INCREASE IN SIZE OF CHOLINERGIC TERMINALS IN EXTENSOR MOTOR NUCLEI

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Spinal cord transection causes dramatic, sustained decrease of vesicular acetylcholine transporter VAChT in terminals contacting motoneurons, as reported by Kitzman (2006, Exp Neurol 197). Cholinergic projection is known to regulate excitability of motoneurons during locomotion. The question arises if locomotor exercise of spinal, paraplegic animals might restore the role of this projection. Three groups of adult rats were tested: intact control (n=6), spinal (n=7) and spinal trained subjected to 5 weeks of treadmill locomotor training (n=8). Animals were spinalized at low thoracic segments. Gastrocnemius/soleus and anterior tibial motoneurons were prelabeled with fluorescent dyes (FG, DY), injected to the muscles. VAChT immunoreactivity (IR) was detected using polyclonal Sigma antibody. We have found that the spinal cord transection caused a decrease of VAChT IR in boutons synapsing on cell bodies and proximal dendrites of motoneurons in L3 and L4 segments compared with that of intact rats. Surprisingly, training caused its further decay. Mean number of VAChT IR boutons did not differ consistently between groups. However, in the extensor motoneuron pools of trained animals the number of bigger VAChT IR boutons was clearly higher than in spinal non-trained and intact animals. The latter effect is in line with functional improvement of spinal trained animals, which is the most prominent in the support phase of locomotion. *Supported by MSE P-N/029/2006 and N N401 0480 33 grants.*

TV.17
MONOCULAR ENUCLEATION INDUCES PROMINENT THYMOSIN BETA 4 EXPRESSION IN MICROGLIA AND NEURONS OF THE SUPERIOR COLLICULUS OF ADULT MICE
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Thymosin beta 4 (Tβ4) is a peptide of 43 amino acids, mainly recognized as a regulator of actin polymerisation by sequestering G-actin. The peptide has been implicated in apoptosis, angiogenesis and lesion-induced neuroplasticity. Tβ4 is widely distributed in the brain, in neurons as well as in microglia. In this study we examined possible changes in Tβ4 expression in the visual system of the mouse after surgical removal of one eye. Adult mice were deprived of vision and sacrificed 1, 3 and 5 days and 1, 2 and 7 weeks after enucleation of the right eye. After 3 days of monocular deprivation, a substantial up-regulation of Tβ4-positive microglia was visible in the left superior colliculus, which faded with post-lesion survival time. The Tβ4-positive microglia appeared activated for clearance of debris of degenerating axons and myelin. Apart from this microglial activation, a remarkable neuronal response was observed. In the deeper layers of the left and right superior colliculus Tβ4-positive neurons with very long Tβ4-positive neurites became apparent. These cells were visible within 1 week but became more obvious 7 weeks post-lesion. Tβ4 may therefore participate in growth or remodeling of neuronal processes in deep layers of the superior colliculus possibly to induce some form of recovery of the visual system upon sensory deprivation.

TV.18
THE INVOLVEMENT OF GLUCOCORTICOID AND MINERALOCORTICOID RECEPTORS IN THE EFFECTS OF BRIEF RESTRAINT STRESS ON LTP AND LTD IN THE DENTATE GYRUS OF MICE
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Long-term potentiation (LTP) and long-term depression (LTD) are two cellular models of synaptic plasticity. These phenomena can be modulated by stress. Stress is correlated with an increased release of glucocorticoids (GCs) from the adrenal cortex. GCs act *via* two receptor types, glucocorticoid receptors (GRs) and mineralocorticosteroid receptors (MRs) which are expressed in a high density in the dentate gyrus (DG). Here we studied whether single brief restraint stress affects LTP and LTD in the DG and if these effects are mediated by GR and MR receptors. C57BL/6 male mice were subjected to the restraint, lasting 10 minutes. 1 hour before immobilisation animals were subcutaneously injected with glucocorticoid receptor antagonist RU38486 or mineralocorticoid receptor antagonist spironolactone. Hippocampal slices were prepared immediately after the end of restraint. Field excitatory postsynaptic potentials (fEPSP) were evoked in the molecular layer of the DG by the stimulation of the lateral perforant path. LTP was induced by 4 trains of high-frequency stimulation (100 Hz, 1 s, repeated every 25 s). LTD was evoked by low-frequency stimulation (1 Hz, 15 min). In slices from stressed mice LTP was enhanced, but LTD was impaired. The data indicate that facilitatory effects of restraint on LTP are diminished by RU38486 and spironolactone indicating the involment of both GRs and MRs. Preliminary results suggest that the inhibitory effects of restraint on LTD are decreased by RU38486.

TV.19
THE INVOLVEMENT OF MINERALOCORTICOID RECEPTORS IN THE EFFECTS OF REPEATED BRIEF RESTRAINT STRESS ON LTP IN THE DENTATE GYRUS OF MICE
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Increased level of corticoid hormons during stress results in activation of hippocampal mineralocorticosteroid receptors (MRs) and glucocorticoid receptors (GRs). Our earlier studies showed impairment of LTP in the dentate gyrus (DG) of mice after 3 daily sessions of brief (10 min) restraint stress and LTP augmentation after 14 and 21 daily sessions of immobilization. In contrast, 1 and 7 daily sessions of immobilization did not affect LTP level. We demonstrated that the GR antagonist RU38486 selectively blocks the effect of 3 sessions of restraint. In the present study we investigated whether the effects of repeated brief restraint stress are mediated by MRs. C57BL/6 male mice were exposed to the restraint, lasting 10 minutes, for 3 and 14 days. 1 hour before immobilization the animals were subcutaneously injected with the MR receptor antagonist spironolactone. Mice were sacrificed 24 h after the last exposure to restraint and hippocampal slices were prepared. Field excitatory postsynaptic potentials were evoked by the stimulation of the lateral perforant path and recorded from the molecular layer of the DG. LTP induction was attempted by applying 4 trains of high-frequency stimulation (HFS, 100 Hz, 1 s, repeated every 25 s). In both experimental groups immediately after HFS recorded responses were potentiated but 2 h later they were diminished relative to control. Thus, the present results show that spironolactone blocks LTP induction after 3 and 14 days of daily restraint.

TV.20
IDENTIFICATION OF CELLS UNDERGOING PLASTIC CHANGES IN THE BARREL CORTEX AFTER SIMPLE ASSOCIATIVE LEARNING USING TRANSGENIC fosGFP MICE
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Learning is assumed to be connected with neuronal c-*fos* expression. In this study we investigate whether associative learning involving tactile stimulation of one row of whiskers induced changes in c-*fos* expression level in neurons within the cortical representation of the stimulated vibrissae. We use transgenic mice in which the expression of green fluorescent protein (GFP) is controlled by c-*fos* promoter [Barth et al. (2004) J Neurosci]. This construct allows us to identify individual neurons undergoing plastic changes

in acute (living) brain slices using standard fluorescence imaging. In the trained mice prior to experiments a 3-day sensory stimulation (20 min/day) of row B of whiskers (conditioned stimulus) paired with an electrical shock (unconditioned stimulus) was performed. The second group comprised of untrained animals. Acute slices from the somatosensory cortex (cut orthogonally to the rows of barrels) were prepared after the end of training and investigated using upright fluorescent microscope. Our preliminary data indicate high variability of fosGFP expression throughout cortical layers. Biggest amount of GFP-labeled cells are observed in layer 2/3 and much smaller in layers VI and V. Sensory training alter amount of fosGFP+ cells in layer 2/3 of barrel B as compared to other barrels with little or no effect in layers IV and V. *Supported by the Ministry of Science and Education grant: N30308131/2682.*

TV.21
ALTERATIONS IN SYNAPTIC PLASTICITY CAUSED BY ST. JOHN'S WORT MAY MITIGATE NEGATIVE EFFECTS OF STRESS ON SPATIAL WORKING MEMORY
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St. John's wort (*Hypericum perforatum*) is one of the popular herbal drugs in Europe and USA. We have recently described beneficial effects of this herb in the treatment of stress-evoked memory impairments. The aim of the present study was to test a hypothesis that St. John's wort alleviates stress- and corticosterone-related memory impairments by restoring levels of synaptic plasticity proteins: neuromoduline (GAP-43) and synaptophysin (SYP) in hippocampus and prefrontal cortex. Stressed and corticosterone treated rats presented a significantly delayed acquisition of spatial working memory ($P<0.001$) in the Barnes maze (BM). Chronic administration of H. *perforatum* (350 mg kg^{-1} for 21 days) potently and significantly improved processing of spatial information in the stressed and corticosterone treated rats ($P<0.001$). Also the herb increased levels of GAP-43 and SYP, in both St. John's wort treated rat's hippocampus ($P<0.05$ and $P>0.05$, respectively) and prefrontal cortex ($P<0.05$ and $P<0.05$) as measured by western immunoblotting. We found that *H. perforatum* prevented the deleterious effects of both chronic restraint stress and prolonged corticosterone treatment on working memory measured in BM test. These findings indicate that at least part of the beneficial effect of *H. perforatum* on memory may be mediated by GAP-43 and SYP proteins.

TV.22
MMP-9 ACTIVATION AFTER NEURONAL STIMULATION IS A POLYADENYLATION DEPENDENT PROCESS
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Recent studies indicate that MMP-9 (gelatinase B that regulates pericellular environment through the cleavage of protein components of the extracellular matrix) plays a role in synaptic plasticity. Szklarczyk et al. (2002), Konopacki et al. (2008) and Wilczynski et al. (2008) have demonstrated the presence of mRNA and protein for MMP-9 at postsynaptic sides of rat hippocampal neurons. It was also shown by Michaluk et al. (2007) that gelatinolytic activity of MMP9 increases after stimulation of rat neuronal cultures with either glutamate or bicuculine. We observed the presence of MMP-9 protein and mRNA in synaptoneurosomes, the synaptic fraction isolated from hippocampus. To detect the activity of MMP-9 we measured the cleavage of its substrate, β-dystroglycan. By the use of this readout we showed that MMP-9 is activated 5 to 10 mintes after neuronal stimulation. We postulate here that MMP-9 is translated from dendritically-localized mRNA and the protein is produced in response to synaptic stimulation. Its rapid and local translation and secretion is a polyadenylation- and local translation-dependent process.

TV.23
EPIGENETIC REGULATION OF MATRIX METALLOPROTEINASE-9 TRANSCRIPTION DURING EPILEPTOGENESIS
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Matrix Metalloproteinase 9 (MMP-9) plays an important role in a physiological and aberrant synaptic plasticity, like that occurring during epileptogenesis. Consequently, enhanced synaptic plasticity is accompanied by a prolonged upregulation of MMP-9 expression due to an activation of its transcription. Epigenetic mechanisms are often responsible for a long lasting changes in a gene expression. Accordingly, our recent data suggested that MMP-9 gene transcription in brain neurons can be regulated by these molecular phenomena. Here, we have studied epigenetic changes occurring in a *Mmp-9* promoter in the rat hippocampus during PTZ-induced kindling, which is a model of an epileptogenesis. Using the methylation-specific polymerase chain reaction, we have evaluated pattern of alterations of the methylation status in the 5'CpG sites of the *Mmp*-9 promoter which arise during different stages of the kindling. Then, we have confirmed these data with the bisulfite sequencing.

TV.24
SPATIO-TEMPORAL CHARACTERIZATION OF mTOR KINASE ACTIVITY INDUCED BY KAINIC ACID EVOKED SEIZURES
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The common effects of epileptic seizures are aberrant plastic changes in synaptic connectivity and morphological rearrangements in hippocampal mossy fibers. This phenomenon is believed to be a basis of facilitation of further epileptic episodes, however its molecular mechanism remains elusive. mTOR kinase is crucial for rearrangements of neuronal morphology and for long term synaptic plasticity. Overactivation of mTOR was reported in brain pathologies associated with seizures (tuberous sclerosis, cortical dysplasia). Experimental evidence supports causative role of mTOR in these forms of epilepsy. So far, however, links of mTOR signaling pathway to other types of epilepsy are unknown. To address this issue we investigated activation of mTOR and its downstream effectors in rat brain after kainic acid (KA) induced epilepsy. Animals were sacrificed 2, 6, 12, 24 and 72 hours after KA administration and levels of phosphorylated mTOR and S6 has been examined. Both, biochemical methods and immunohistochemistry proved S6 activation in hippocampus and cerebral cortex at all time points after KA. Immunohistochemical analysis additionally revealed that

2 h after KA, activities of mTOR and S6 are upregulated mainly in neurons while after 6 h there was also enhancement of glial S6 immunoreactivity. Our data suggest that KA induced seizures activate mTOR signaling in various cell types with specific spatio-temporal pattern. *This work has been financed by PNRF-96-AI-1/07 grant.*

TV.25
STIMULATION OF THE VENTRAL TEGMENTAL AREA INDUCES HIPPOCAMPAL THETA RHYTHM AND *c-fos* EXPRESSION IN THE RAT BRAIN
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Our previous study indicated that microinjection of procaine or electrolytic lesion of the ventral tegmental area (VTA) suppressed hippocampal theta rhythm in urethane-anaesthetized rats. The aim of this study was to verify the hypothesis that electrical stimulation of the VTA induces hippocampal theta rhythm and to find brain structures particularly active during this phenomenon and probably involved in its mechanism. The study was performed on urethane anaestethized male Wistar rats with an electrode implanted unilaterally in the VTA or zona incerta (ZI – control group). Stimulation was applied as 0.1-ms rectangular impulses of 50 Hz frequency and duration of 30 s at 10-min intervals. VTA stimulation within the current intensity range of 100–240 mA evoked hippocampal theta rhythm, manifested as synchronization of the EEG signal and an increase in the power at 3–6 Hz band. ZI stimulation did not elicit such effects. After VTA stimulation we also found induction of *c-fos* expression in brain regions connected to the VTA: nucleus accumbens, lateral septum, or engaged in the regulation of hippocampal theta rhythm: medial septum, midline thalamic nuclei, hypothalamic nuclei, pedunculopontine, laterodorsal and cuneiform tegmental nuclei. The results indicate that the VTA may be a part of the brainstem theta synchronizing system and may influence the hippocampal EEG through indirect pathway *via* hypothalamus and the medial septum, simultaneously increasing thalamic activity.

TV.26
EFFECT OF EXTRA HIGH POWER PULSES AND HYDROGEN PEROXIDE ON FUNCTIONAL ACTIVITY OF NEURONS
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It was recently shown that the exposure of aqueous solutions to high-peak-power microwave pulses leads to the formation of hydrogen peroxide (H_20_2). The aim of this study was to evaluate the effects of extra high power pulses (EHPP,9.3 GHz, 101.2 kW/kg) millimeter waves and H_20_2 on action potentials, acetylcholine chemosensitivity and cell volume of giant neurons from the parietal ganglion of *Helix Pomatia* by means of standard voltage-clamp method. H_20_2 containing and EHPP treated physiological solutions increased the interburst period of neuronal pacemaker activity. In K-free physiological solution (when Na^+/K^+ pump is inactive) the interburst period was higher than in normal one. Application of H_20_2 containing and EHPP treated physiological solutions changed the number of active receptors on neuromembrane surface which caused depression of its chemosensitivity and decreased as a consequence of cell volume (cell shrinking). These comparative studies allowed us to suggest that EHPP induced formation of H_2O_2 is one of the main messenger through which the non-thermal biological effect of EHPP on functional activity of neurons is realized.

TV.27
SINGLE AND ENSEMBLE NEURAL VARIABILITY WITH SERIAL CORRELATION
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The activity of spiking neurons is frequently described by renewal point process models. However, the assumption of independent intervals must be questioned for many different types of neurons. We review experimental studies that reported the feature of a negative serial correlation of neighboring intervals, commonly observed in neurons in the sensory periphery as well as in central neurons, notably in the mammalian cortex. In our experiments we observed the same short-lived negative serial dependence of intervals in the spontaneous activity of mushroom body extrinsic neurons in the honeybee. To model serial interval correlations of arbitrary lags, we suggest a family of autoregressive point processes. Its marginal interval distribution is described by the generalized gamma model, which includes as special cases the log-normal and gamma distributions, which have been widely used to characterize regular spiking neurons. In numeric simulations we investigated how serial correlation affects the variance of the neural spike count. We show that the experimentally confirmed negative correlation reduces single-neuron variability, as quantified by the Fano factor, by up to 50%, which favors the transmission of a rate code. We argue that the feature of a negative serial correlation is likely to be common to the class of spike-frequency-adapting neurons and that it might have been largely overlooked in extracellular single-unit recordings due to spike sorting errors.

TV.28
MODIFICATION OF EXCITABILITY OF CORTICAL NEURONS INDUCED BY ASSOCIATIVE LEARNING – *EX VIVO* EXPERIMENTS
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In adult mice, whiskers stimulation paired with an electrical shock to the tail induces expansion of the cortical representation of stimulated vibrissae and enhances inhibitory interactions within the "trained" barrels. In present experiments we investigated whether such simple associative learning paradigm induced also changes in the neuronal excitability. We prepared brain slices through the barrel cortex and performed whole-cell recordings from visually identified layer IV neurons. Our results showed that excitatory cells located in layer IV of the cortical representation of the "trained" row B of vibrissae had higher frequency of spikes recorded at threshold potential, as compared to both neurons from "untrained" barrel D and to cells from control animals. Additionally, cells within the "trained" barrels displayed bigger gain in the input-output function and decrease in the activation of BK channels responsible for

the fast afterhyperpolarisation, which suggests the source for their enhanced intrinsic excitability. The increased excitability of cells within the "trained" barrels may express their homeostatic plasticity which parallels enhanced inhibitory interactions found previously within layer IV of the cortical representation of the "trained" vibrissae. This may be the way to increase the cortical selectivity of response to sensory input from "trained" whiskers. *Supported by the MNiSW grants: N30308131/2682, N40114631/3239, COST/127/2007 and PBZ/MNiSW/07/2006.*

TV.29
ELUCIDATING THE FUNCTION OF CPEB PROTEINS IN MICROGLIA
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Cytoplasmic Polyadenylation Element Binding proteins (CPEBs) modulate local protein synthesis in neurons crucial for LTP maintenance, memory, and learning. Our recent results indicate they are involved in the progression of epilepsy in a mouse model of temporal lobe epilepsy (TLE). Neuronal proteins regulated by CPEBs include tissue plasminogen activator (tPA), but the primary source of tPA in brain is microglia. CPEB function in glial cells has hardly been investigated. Since microglial tPA is a crucial neurodegenerative factor in TLE, we explore the attractive hypothesis of microglial CPEB involvement in tPA release and, thereby, neurodegeneration following seizures. By single cell RT-PCR we observed CPEB1-4 mRNAs in mouse microglia. This corroborates RT-PCR and immunoblot results, showing abundant CPEB transcript and protein levels in the BV-2 microglial cell line and ES-cells-derived microglia. We use CX3CR1 EGFP/+ knock-in transgenic mice to isolate microglial cells by FACS. In such primary microglia cultures, we will compare CPEB and tPA expression, polyadenylation state of tPA mRNA, and phosphorylation status of CPEBs before and after microglial activation. Finally, we will look at CPEB-mediated control of tPA expression with a luciferase reporter assay. We hope the abovementioned studies to shed a new light on the function of microglial CPEBs and their potential role in neurodegeneration associated with TLE.

TV.30
FACILITATED EXTRACELLULAR DIFFUSION IN THE CORPUS CALLOSUM OF BRAL1-DEFICIENT MICE
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The extracellular matrix molecule complex stabilized by Bral1 at the nodes of Ranvier might play a role in neuronal conduction as this complex represents a reservoir and/or diffusion barrier for Na^+ ions. We measured the apparent diffusion coefficient of water (ADC_w) by diffusion-weighted MRI and the extracellular space (ECS) diffusion parameters volume fraction α (α = ECS volume/total tissue volume) and tortuosity λ (λ^2 = free/apparent diffusion coefficient) by the real-time iontophoretic method. Measurements were done in the somatosensory cortex and along the mediolateral (x), rostrocaudal (y) and ventrodorsal (z) axes in the corpus callosum (CC) of Bral1 +/+ and -/- mice. In the cortex, we did not find any significant differences in the ECS diffusion parameters or ADC_w values between Bral1 +/+ and -/- mice. In the CC, λ in Bral1 +/+ mice was significantly higher along all three axes (λ_x = 1.41 ± 0.03; λ_y = 1.70 ± 0.02; λ_z = 1.72 ± 0.01; mean ± SEM) compared to -/- mice (λ_x = 1.32 ± 0.02; λ_y = 1.56 ± 0.01; λ_z = 1.58 ± 0.02); there were no significant differences in α. These data were confirmed by a lower ADC_w in the CC of Bral1 +/+ than of -/- mice, along the x (1 158 ± 55 $\mu m^2 s^{-1}$ and 1 340 ± 25 $\mu m^2 s^{-1}$, respectively) and y axes (442 ± 19 $\mu m^2 s^{-1}$ and 521 ± 24 $\mu m^2 s^{-1}$, respectively). We conclude that the deletion of Bral1 results in a reduction of diffusion barriers at the nodes of Ranvier, which in turn facilitates diffusion in all directions in the myelinated white matter.

TV.31
LACK OF NEUROPROTECTIVE EFFECT OF ASTROGLIA-DERIVED BDNF ON HIPPOCAMPAL CELL CULTURES EXPOSED TO TRIMETHYLTIN
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It is well known that in response to neuronal injury activated glial cells produce pro-inflammatory cytokines and neurotrophic factors. These immunoregulatory molecules may play either neurotoxic or neuroprotective roles. In previous studies on mixed neuronal-glial cultures of rat hippocampal dentate gyrus we demonstrated that trimethyltin (TMT) induced neuronal cell death accompanied by an enhanced production of tumor necrosis factor alpha (TNF-α) in microglial cells and a strong increase in TNF-α receptor 1 (TNFR1) expression on astrocytes. Since evidence has been provided that TNFR1 could mediate the production of neurotrophic factors, in the current studies we examined whether the brain-derived neurotrophic factor (BDNF) is up-regulated after TMT exposure and whether it may exert neuroprotective effect on dentate granule cells. Using western blot analysis and immunocytochemical staining we have shown a dose-dependent increase in BDNF production, mainly in astrocytes. Quantitative fluorescence analysis revealed that addition of anti-TNFR1 antibody to TMT-treated cultures suppresses the astroglial synthesis of BDNF. Nevertheless, immunocytochemical studies of active caspase-3 demonstrated the high level of its expression in cultures exposed to TMT, as well as in cultures pre-treated with BDNF. Our data suggest the involvement of TNFR1 pathway in BDNF production in astrocytes and indicate that this neurotrophic factor does not protect granule neurons against TMT injury.

TV.32
INFLUENCE OF ANTIDEPRESSANTS ON CYTOKINES (TNF-α, IL-1β, IL-10) IN LIPOPOLYSACCHARIDE-STIMULATED PRIMARY RAT MIXED GLIAL CELL CULTURES
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It is suggested that glial activation play an important role in the pathogenesis of psychiatric and neurodegenerative diseases. Ac-

tivated glial cells secrete various cytokines. Anti-inflammatory effect of imipramine, moclobemide, fluoxetine was investigated using 13–14 day primary rat mixed glial cultures prepared from cerebral hemispheres of one-day old newborn Wistar rats. LPS-stimulated levels of TNF-α, IL-1β, IL-10 were measured with ELISA kits in culture medium. Antidepressants were used at concentrations from 10^8 to 100 μM. mRNA for cytokines was evaluated by RT-QPCR. Moclobemide, fluoxetine (10^8 to 10 μM) and imipramine (10^6 to 100 μM) reduced TNF-α release. IL-1β concentration was diminished by moclobemide, imipramine (10^6 to 100 μM) and fluoxetine (10-6 to 10 μM) but level of IL-10 was not changed significantly after drug administration. The levels of TNF-α or IL-1β mRNA were reduced by the studied drugs (10 μM), whereas IL-10 mRNA level was only attenuated. Our results support observation that antidepressants have anti-inflammatory effects in CNS because they affected the balance between pro- and antiinflammatory cytokines (TNF-α, IL-1β/IL-10) in mixed glial culture. *This work was supported by Grant N 401 130 31/2871 from the Ministry of Science and Higher Education.*

TV.33
CELL-TYPE SPECIFIC REGULATION OF MORPHINE- AND GLUCOCORTICOID INDUCED GENES
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Single morphine administration to mice leads to changes in the expression of numerous genes in mouse striatum. Among them are genes regulated by the glucocorticoid receptor activation, like Sgk1, Gilz and Plzf [Korostynski et al. (2007) Genome Biol]. Analysis of their expression patterns with the use of *in situ* hybridization revealed their different regional distribution throughout the brain. To verify the cellular specificity of expression, the double immunohistochemical stainings were performed with antibodies directed against investigated proteins and cellular markers (NeuN, GFAP, CC1, OX42). The results show that certain morphine- and GR-induced proteins are expressed in neurons, whereas others in glial cells. Hence, their regulation depends on the cell-type specific context.

TVI: Disorders of the Nervous System II

TVI.01
DISASSEMBLY OF SHANK AND HOMER SYNAPTIC CLUSTERS IS DRIVEN BY SOLUBLE β-AMYLOID THROUGH DIVERGENT NMDAR-DEPENDENT SIGNALLING PATHWAYS
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Disruption of the postsynaptic density (PSD) is thought to be responsible for synaptic dysfunction and loss in early-stage Alzheimer's disease. We show that Aβ disrupts two other scaffold proteins, Homer1b and Shank1. Treatment of cortical neurons with soluble Ab results in rapid thinning of the PSD, decreased synaptic levels of Homer1b and Shank1, and reduced synaptic mGluR1 levels; the latter changes require NMDAR and voltage-dependent calcium channel (VDCC) activity. We also show that de novo protein synthesis is required for the declustering effects of Aβ on Homer1b (but not Shank1) and that, in contrast to PSD-95, Aβ-induced Homer1b and Shank1cluster disassembly does not depend on proteasome activity. The regulation of Homer1b and Shank1 by Aβ diverges in two other respects: (1) whereas the activity of both NMDAR and VDCC is required for Aβ-induced declustering of Homer1b, Aβ-induced declustering of Shank1 only requires NMDAR activity; (2) whereas the effects of Aβ on Homer1b involve engagement of the PI-3K pathway and PP2B activity, those on Shank1 involve activation of ERK/RSK. Thus, Aβ recruits discrete signaling pathways to rapidly reduce the synaptic localization of major components of the PSD and to regulate the availability of mGluR1.

TVI.02
INVOLVEMENT OF THE CacyBP/SIP PROTEIN IN TAUOPATHY
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The CacyBP/SIP protein is highly expressed in brain neurons, where it localizes in somata and in neuronal processes. The role of CacyBP/SIP is not clear at present but recently it has been suggested that the interaction between CacyBP/SIP and tubulin might be important for stability of microtubules in neuronal cells. Particularly, it was found that CacyBP/SIP changes its localization in an age-dependent way. Moreover, these changes in localization are similar to those observed for tau, a well known tubulin binding protein. Since it is known that changes in localization of tau during aging are similar to those observed in tauopaties (such as Alzheimer's disease), in this work we examined the localization and possible role of CacyBP/SIP in mouse model of tauopathy (mice overexpressing the tau protein). We found that in young tauopathic mice CacyBP/SIP and tau are mainly present in neuronal somata whereas in wild type animals both proteins are localized in somata and in neuronal processes. Moreover, the tubulin staining pattern in transgenic mice was different than in wild animals. This might suggest that translocation of tau together with CacyBP/SIP affects stability of microtubules in neuronal cells. At present we examine whether posttranslational modification(s) of CacyBP/SIP, such as phosphorylation, might be responsible for the observed translocation of CacyBP/SIP during tauopathy. *This work was supported by statutory funds from the Nencki Institute of Experimental Biology.*

TVI.03
THE TRIPEPTIDE RER AND ITS POTENTIAL ROLE IN THE AMELIORATION OF SYMPTOMS IN ALZHEIMER'S DISEASE ANE OTHER DEMENTIAS
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Alzheimer's disease (AD) is a neurodegenerative syndrome with high prevalence in the elderly population and whose early symp-

toms include impairment in spatial memory. Inherited forms of AD can be caused by dominant, single missence mutations in the Amyloid Precursor Protein (APP) gene, and this has been used to generate several animal models for the disease by overexpression of a mutated version of the protein. We have established at our institute a breeding colony of transgenic mice expressing the London mutation of APP as a transgene (APP[V717I]), and tested them at three and six months of age on the Morris Water Maze to confirm a previous report on their impairment in the acquisition of this particular task. Moreover, APP[V717I] mice were used as a model to determine whether treatment with the tripeptide RER, a compound that has previously been shown to ameliorate amyloid-induced amnesia in young chicks, is capable of modifying their performance in the maze in a way that compensates the deficits in spatial memory derived from the expression of the transgene. *This work was supported by Grant #88 provided by the Alzheimer's Society.*

TVI.04
CELL TYPE SPECIFIC UPTAKE AND CLEARANCE OF β-AMYLOID PEPTIDES
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Aggregation of β-amyloid peptides (Aβs) into toxic oligomers, amyloid fibrils, and its deposition in senile plaques initiate the neurodegenerative cascade of Alzheimer`s disease. Intraneuronal accumulation of Aβs starts prior to the appearance of senile plaques, and contributes to neurodegeneration by affecting protein trafficking, mitochondrial metabolism, tau phosphorylation, and synaptic plasticity. The aim of this study was to compare the uptake and clearance of Aβs by various cell lines. SK-N-SH neuroblastoma cells rapidly metabolized Aβ clearing 40 μM of Aβ40 from the medium within 48 h. Pulse-uptake experiment demonstrated that Aβ40 was totally metabolized inside SK-N-SH cells within 6 h. Presence of apolipoprotein E4 accelerated the Aβ40 uptake. In comparison, primary hippocampal neurons internalized only small fraction of Aβ40 present in the medium but showed notable intraneuronal Aβ40 accumulation. In pulse-uptake experiment, intraneuronal presence of Aβ40 monomers and oligomers could be demonstrated after 24 h. Accumulation of Aβ40 by hippocampal neurons affected cell viability and membrane integrity as determined by MTT and LDH release assays, respectively. Our results demonstrate that neuroblastoma cells internalize and metabolize Aβ40 more efficiently than primary hippocampal neurons. Therefore, under conditions of elevated Aβ level, hippocampal neurons are susceptible to Aβ accumulation and intraneuronal oligomerization what leads to downstream toxic effects.

TVI.05
INCREASED VULNERABILITY TO OXIDATIVE STRESS IN LYMPHOCYTES FROM SAD AND FAD PATIENTS
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Alzheimer's disease (AD) is the most common neurodegenerative disorder in which certain molecular changes are observed not only in neurons but also in peripheral cells. Growing evidence suggests that AD post-mitotic neurons exhibit increased apoptotic response to oxidative stress, mitochondria dysfunction and calcium dyshomeostasis. We hypothesized that some of these alterations could be observed in peripheral lymphocytes and studied for potential diagnostic purposes. We analyzed apoptotic response to the redox stress evoked by 2-deoxy-D-ribose in immortalized lymphocytes from 18 patients with sporadic AD (SAD), from 2 familial AD (FAD) patients with novel mutations in presenilin 1: P117R and I213F, and from 20 age-matched healthy individuals. Using two independent flow cytometry methods for quantification of apoptosis, we found that SAD and FAD lymphocytes show enhanced apoptotic response to the redox stress. This apoptotic response was accompanied by decline in mitochondrial membrane potential measured with JC-1 as well as by increased activities of caspase 9 and caspase 3. However, no changes in the expression of two calcium-binding proteins: calmyrin 1 and calreticulin were observed. This study emphasizes that increased susceptibility to redox stress and associated upregulation of mitochondrial apoptotic pathway is characteristic not only for AD neurons, but also for AD lymphocytes. Thus, human lymphocytes could be used in further studies on AD pathogenesis.

TVI.06
RESVERATROL AND NITRIC OXIDE CHOLINERGIC NEUROTOXICITY
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Several scavengers of free radicals like resveratrol (RV) are claimed to be useful in complementary therapy of Alzheimer's dementia (AD) and other cholinergic encephalopathies, through the protection of mitochondrial energy metabolism. NO excess is one of recognized pathogens in AD brains. Therefore, we investigated whether RV might overcome cytotoxic effects of NO excess on septal cholinergic neuroblastoma SN56 cells. Chronic, 16 h exposure of nondifferentiated SN56 cells (NC) to 0.2 mM sodium nitroprusside (SNP) caused 30 and 27% suppression of pyruvate dehydrogenase (PDH) activity and acetyl-CoA level and the increase of nonviable cells fraction to 30%. In cAMP/retinoic acid-differentiated cells (DC), similar 30% inhibition of PDH by SNP caused 50% suppression of acetyl-CoA content and 47% loss of cell viability. RV (0.005 mM) itself affected none of parameters, neither in NC nor in DC. It did not overcome SNP-evoked inhibition of PDH and suppression of acetyl-CoA content in NC and DC. Despite of that RV partially prevented SNP-induced increase of DC and NC mortality to 31 and 14%, respectively. These data demonstrate that greater cytotoxic effects of SNP in DC than in NC correlated with evoked acetyl-CoA deficits that might be linked with higher level of acetylcholine metabolism in the former. Protective effects of RV in NO-challenged cholinergic neurons were not connected with improvement of their acetyl-CoA metabolism. *Supported by MNiSW projects NN401 2333 33, P05A 11030.*

TVI.07
TRANSMEMBRANE INTRANEURONAL DELIVERY OF TAU PROTEIN IN RAT MAY SERVE AS AN ANIMAL MODEL OF HUMAN TAUOPATHY
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Filamentous inclusions of tau protein are hallmarks of tauopathies including Alzheimer's disease. Here a rat model for tauopathies was developed using pore-forming halitoxin Poly-APS. Tau protein was delivered through the membrane into the neurons where it is metabolized. Rats were injected with recombinant human Tau441 only (controls), Tau+Poly-APS (double) and Tau+Poly-APS+Okadaic Acid (triple). Cognitive and neurodegenerative changes were examined with Morrris water maze behavioral test and immunohistochemistry, respectively. Acquisition of the spatial reference memory was unaffected by administration of Tau or Tau+Poly-APS. However, enhanced phosphorylation of exogenously delivered tau with the triple infusion impaired learning. Hippocampal tau was visualized using antibodies against Tau441 and phosphorylated Tau-S404. The higher intensity of Tau441 immunostaining in double and triple groups suggests that exogenous tau is sequestered from the infusion solution into the cells. With respect to phospho-tau izoform we observed different cellular compartmentalization of P-Tau-S404 in double and triple treated rats. In double treated rats P-TauS401 was distributed both in the cell bodies and neuronal fibers, while in triple group presence of OA caused redistribution of phospho-tau staining from neuronal processes to the perikaryon. This is similar to age-dependent tau redistribution between cellular compartments and could be a result of hyperphosphorylation.

TVI.08
PAIRED ASSOCIATES LEARNING IN ALZHEIMER'S DISEASE, MILD COGNITIVE IMPAIRMENT AND HEALTHY INDIVIDUALS: A PRELIMINARY STUDY
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CANTAB Paired Associates Learning test (PAL) involves the ability to associate the stimulus (what) with its spatial location (where).There is some evidence that structures of the medial temporal lobe plays a crucial role in formation of these associations. Evidences from both lesion (in rats, monkeys, humans) and neuroimaging studies suggest that medial temporal lobes structures such as hippocampus, parahippocampal gyrus and enthorinal cortex are affected in early stages of Alzheimer's disease (AD). These stages can be detected using PAL which is a vulnerable tool for early preclinical detection and assessment of AD. We compared the performance of PAL in 5 patient with AD, 20 Mild Cognitive Impairment patients (MCI) and 19 normal healthy controls (NC). The performance in AD group was significantly lower than those from MCI and NC group. The main difference was in committed errors at more difficult, 6-pattern stage, where spatial location of 6 patterns had to be remembered. Moreover, in this situation the learning of pattern location in AD group was slowed down; more trials were needed to complete 6- patterns stage. In summary, these results confirmed that PAL may be the sensitive tool in clinical diagnosis of AD. *Supported by MIND no. WKP_1/1.4.3.1 /2004/11/11/52/2005/U.*

TVI.09
MITOCHONDRIAL AND NUCLEAR TARGETS OF AMYLOID β-EVOKED OXIDATIVE STRESS
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Amyloid β (Aβ) is responsible for mitochondrial failure and biochemical alterations linked to Alzheimer`s disease (AD). To better understand mechanisms of Aβ toxicity we investigated its mitochondrial and nuclear targets, apoptosis-inducing factor (AIF) and Poly(ADP-ribose) polymerase-1 (PARP-1) in PC12 cells transfected with wild type (APPwt) or double Swedish-mutated human Amyloid Precursor Protein gene (APPsw) characterized by different Aβ concentrations. We found close relationship between Aβ level and cyclooxygenase (COX)- and lipoxygenase (LOX)-related free radical formation leading to p65/NF-κB nuclear translocation. COX and LOX inhibitors protected APPsw cells against p65 translocation. Aβ-evoked oxidative stress enhanced mitochondrial AIF level and inhibited PARP-1 in APPsw cells. Nitrosative stress evoked by 0.5 mM sodium nitroprusside (SNP) had no further effect on Aβ-altered PARP-1 activity and mitochondrial AIF level in APPsw cells. However, SNP evoked death of 70–80% of all cell types after 24 h. COX and LOX inhibitors had ameliorating effect in these conditions. Our data indicated that double Swedish mutation in APP significantly increased cell vulnerability to oxidative stress. Enhanced mitochondrial AIF level and PARP-1 inhibition might be responsible for cell survival under oxidative stress evoked by accumulating Aβ in APPsw cells. COX and LOX inhibitors protected the cells against death caused by simultaneous Aβ toxicity and nitrosative stress.

TVI.10
PRIMING OF ROTATIONAL BEHAVIOR BY A DOPAMINE RECEPTOR AGONIST IN HEMIPARKINSONIAN RATS: MOVEMENT-DEPENDENT INDUCTION
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Repetitive stimulation of basal ganglia dopamine receptors leads to abnormal motor responses in dopamine-denervated rats. To study whether such responses were influenced by the previous execution of movement, we evaluated how "priming", a phenomenon mimicking an abnormal motor response, depends on movement performance. To this end, unilaterally 6-hydroxydopamine-lesioned rats received apomorphine (0.2 mg/kg s.c.), being either allowed to move or immobilized (1 h) before, concomitantly to, or after its administration. Three days later, the dopamine D1 receptor agonist

SKF 38393 (3 mg/kg s.c.) was administered. Rats that had rotated following apomorphine showed contraversive rotational behavior following SKF 38393, whilst rats that had been immobilized concomitantly to apomorphine, but neither before nor after, did not. To clarify whether immobiliztion-related stress influenced the results, additional rats received apomorphine plus immobilization and the corticosterone-synthesis inhibitor metyrapone (100 mg/kg i.p.), or apomorphine plus a tail stressor, being not immobilized. Metyrapone did not affect the prevention of priming by immobilization, and tail stressor did not affect priming magnitude, suggesting that stress has minimal effect on the results observed. This study demonstrates how movement performance following initial dopaminergic stimulation governs the occurrence of an abnormal motor response to a subsequent dopaminergic challenge in dopamine-denervated rats.

TVI.11
STUDY OF THE ROLE OF ALPHA-SYNUCLEIN PHOSPHORYLATION AT Ser 129 IN DOPAMINERGIC NEURODEGENERATION IN PARKINSON'S DISEASE

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Studies during the last decade have established a central role in Parkinson's disease (PD) neurodegeneration for the protein alpha-synuclein (α-syn). Among the different post-translational modifications that human a-syn can undergo, phosphorylation has been related to the disease. α-syn phosphorylation at Ser 129 seems to be related to the disease progress in PD, since S129P-α-syn is found in low levels in healthy humans and it is related to increased inclusion formation. In order to elucidate the role of phosphorylation of α-syn at Ser 129 in the disease process we used a rat PD model based on the local injection of recombinant adeno-associated viral vectors (rAAV). We created mutations in α-syn at Ser129, replacing the serine either with alanine (S129A) to block phosphorylation or with aspartate (S129D) to mimic phosphorylation. We overexpressed wt, S129A and S129D α-syn in rat midbrain and thereafter have compared the effect of the overexpression on dopaminergic neurons at different time points post-injection. Our results show that pseudophosphorylated α-syn S129D induced faster motor deficit compared to S129A. On our hands, the phosphorylation of α-syn at Ser129 seems to have a role in the progression of the disease but is not necessary for pathology, motor deficits or cell death to occur.

TVI.12
CHRONIC, INTRAVENTRICULAR INFUSION OF MPP+ WITH ALZET OSMOTIC MINIPUMPS IN RATS AS A MODEL OF PARKINSON'S DISEASE

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An underlying mechanism of degeneration in Parkinson's disease (PD) is unknown. The animal models of PD, developed so far have certain disadvantages; hence a search for a new model of PD seems necessary. Chronic, unilateral, intraventricular delivery of MPP^+ (0.284 and 0.428 mg/kg/day for 28 days) using an ALZET osmotic minipump, implanted s.c., produced a marked, dose-dependent loss of DA and its metabolites DOPAC and HVA (50–90%) in the striatum, ipsilateral to the infusion site. DA concentration was normal in the non-infused, right striatum. Also no changes in the 5-HT level were observed. The stereological counting of the number of dopaminergic neurons in the substantia nigra pars compacta (SNc), stained with the antibody against tyrosine hydroxylase, showed their 30–50% loss on the lesioned side. Those changes were accompanied with a diminished expression of mRNA for the dopamine transporter in the SNc (by ca. 30%). Additionally, *in situ* hybridization studies indicated an enhanced expression of mRNA for both adenosine A_{2A} and dopamine D_2 receptors in the striatum and diminished expression of mRNA for BDNF in the hippocampus. The obtained results showed that this chronic model of continuous, intracerebral infusion of MPP^+, produced a selective nigrostriatal DA cell loss and number of other neurochemical changes resembling PD.
Study supported by the grant No. NN401 1137 33 (MS&HE) and by a statutory fund from the Institute of Pharmacology, PAS, Poland.

TVI.13
A_{2A} ADENOSINE RECEPTOR ANTAGONISTS EFFECT ON HYDROXYL RADICAL GENERATION IN DA DEPLETED STRIATUM WITH RESERPINE

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Although the cause of DA neurons neurodegeneration is still unknown, oxidative stress is paramount in the pathogenesis of Parkinson's disease. An accumulation of cytosolic DA has been shown to be neurotoxic through the generation of free radicals (FR). Searching for FR scavengers, we studied the effect of selective A_{2A} adenosine receptor antagonists, shown to have neuroprotective properties, on hydroxyl radical (HR) production in rat striatum with reserpine impaired DA storage. We found an increase in extracellular glutamate and HR levels in DA-depleted striatum. CSC (1 mg/kg), ZM 241385 (3 mg/kg) and L-DOPA (25 mg/kg) normalized glutamate release and combination of A_{2A} antagonists and L-DOPA showed similar effect. CSC increased DA and HR levels but ZM 241385 given alone did not affect DA nor HR levels. L-DOPA enhanced DA extracellular level but did not change the production of HR. Combination of L-DOPA and CSC further elevated DA extracellular level and markedly increased HR production while combination of L-DOPA and ZM 241385 attenuated, enhanced by L-DOPA DA level and had no effect on HR production. This data suggests that disrupted balance between DA and glutamate in DA depleted nigrostriatal neurons results in generation of neurotoxic HR. Both A_{2A} antagonists, like L-DOPA, redress the DA/glutamate balance. However, A_{2A} antagonists in combination with L-DOPA show different pharmacological profile in their effect on DA release and subsequent generation of HR.

TVI.14
HIGH FREQUENCY STIMULATION OF THE SUBTHALAMIC NUCLEUS INCREASES BLOOD NK CELL NUMBER IN HEMIPARKINSONIAN RATS DIFFERING IN RESPONSE TO NOVELTY
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High frequency stimulation (HFS) of the subthalamic nucleus (STN) is a surgical therapy for improving of motor symptoms in Parkinson's disease. In this study, we assessed the HFS effects on cytometric analysis of the peripheral blood lymphocytes (T, B, NK, T helper, T cytotoxic) in freely moving hemiparkinsonian rats. Before unilateral lesion of the right substantia nigra pars compacta (6-hydroxydopamine), all rats were divided into two behavioral groups: high-HR and low-LR responders in novelty test. As compared to the sham controls, HFS of STN significantly increases NK cell percentage number (25.86 ± 9.68% vs. 18.79 ± 5.63%, $P<0.05$). In contrast, significantly ($P<0.05$) lower B lymphocytes level in stimulated (18.86 ± 3.91%) then sham group (22.80 ± 4.63%) was observed. These general changes in NK and B lymphocyte numbers were reflected in HR stimulated animals (31.08 ± 7.19% vs. 20.44 ± 5.65% for NK cells number and 17.67 ± 2.57% vs. 23.23 ± 3.37% for B lymphocyte, stimulated vs controls, respectively, $P<0.01$). On the other hand, significantly lower T lymphocyte level in LR stimulated animals in comparison to LR controls was found (38.32 ± 4.18% vs. 45.91 ± 5.68%). Moreover, significant differences between stimulated HR_S and LR_S in the NK and T cytotoxic percentages were found (31.08 ± 7.19% vs. 17.63 ± 6.74%, $P<0.05$; 8.46 ± 1.83% vs. 12.29 ± 1.76%, $P<0.01$). The results emphasize the importance of individual differences in reactivity to novelty on immune response to HFS of STN.

TVI.15
AN INVOLVEMENT OF MAPK/ERK1/2 PATHWAY IN MECHANISM OF LACTACYSTIN-INDUCED CELL DAMAGE IN PRIMARY CORTICAL NEURONS
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Proteasome dysfunction is involved in pathomechanism of several neurodegenerative diseases where an accumulation of aberrant proteins occurs (e.g. Parkinson's disease, Alzheimer's disease). Lactacystin (LC) has been used for induction of proteasome inhibition-dependent neuronal cell death for several years but mechanism of its toxic action on neurons is still poorly understood. In the present study we showed time- and concentration-dependent toxic action of lactacystin (0.25–50 μM) in mouse cortical neurons. Although, lactacystin induced caspase-3 activation, its toxic action was not attenuated by caspase-3 inhibitor AcDEVD-CHO. We demonstrated that inhibitors of MAPK/ERK1/2 cellular signaling (U0126 and PD98052) were protective against LC-evoked cell death as confirmed by LDH and MTT reduction assays. Moreover, these data were verified by Western Blot analysis, where we observed the increase in ERK1/2 activity after LC treatment and this effect was inhibited by U0126. The obtained data point to engagement of activation of MAPK/ERK1/2 in toxic action of lactacystin and give a rationale for using agents which inhibit this intracellular pathway in treatment of neurodegenerative diseases connected with proteasome dysfunction. *Supported by Polish MNSW Scientific Network Fund no 26/E-40/BWSN-0023/2008.*

TVI.16
PARKINSON'S DISEASE LEADS TO DISTORTED INTERACTION OF EPISODIC AND WORKING MEMORY SYSTEMS
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Patients with Parkinson's disease (PD) exhibit impairments in executive function and working memory. As opposed to motor symptoms of the disease the physiological origins of cognitive deficits are not well understood. The aim of the present study was to delineate mechanisms of cognitive impairment in PD using blood oxygen level-dependent (BOLD) functional magnetic resonance imaging (fMRI). Twenty-two patient and 10 matched control subjects accomplished Wisconsin Card Sorting Test (WCST) – standard clinical measure of executive function and then underwent fMRI during performance of the N-Back working memory task.The correlation analysis comparing BOLD signal intensity changes with number of categories achieved in WCST revealed a significant positive correlation within supramarginal gyrus, inferior parietal gyrus, middle and inferior frontal gyrus and caudate nucleus. Significant negative correlations with executive impairment were observed within fusiform and parahippocampal gyrus, as well as enthorhinal and perirhinal cortex. These results suggest that cognitive impairment in PD may origin in consequence of distorted interaction between episodic and working memory systems.

TVI.17
INHIBITORY EFFECTS OF 17β-ESTRADIOL ON PRODUCTION OF BOTH MOLECULAR AND CELLULAR FACTORS IN NEUROINFLAMMATORY REACTION IN MODEL OF PARKINSON'S DISEASE
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The neuroprotective action of 17β-estradiol (E2) against 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) has been shown in both female and male mice, however the exact mechanisms of that phenomenon remain obscured. We studied the chronic effects of E2 (0.25 mg per pellet, 21-days release) administered 7 days prior (Experiment 1) to or 3 days after (Experiment 2) MPTP intoxication (40 mg/kg, i.p.) in C57BL aged male mice on neurodegenerative and inflammatory processes in nigrostriatal pathway. We estimated striatal: tyrosine hydroxylase (TH), glial fibrillary acidic protein (GFAP) content (Western blotting); cytokines (TNFα, $TGFβ_1$, IFNγ), trophic factor (GDNF) gene expression (RT-PCR);

CD4+ and CD8+ cells influx (immunohistochemistry) at 1, 7, 21 (Exp. 1) and 7, 21 (Exp. 2) days post intoxication. E2 exerted a neuroprotective effect upon nigrostriatal system when administered prior but also when administered after intoxication (E2 attenuated the MPTP-induced loss of TH). E2 also decreased the GFAP content. MPTP caused a rapid increase of $TGF\beta_1$, TNFα and IFNγ. Pre-treatment with E2 decreased the early expression of $TGF\beta_1$ and IFNγ but failed to suppress the MPTP-induced increase of TNFα. E2 pre-treatment also induced an increase of the GDNF and CD4+ cells influx to the injured brain areas but decreased the CD8+ cells infiltration. The neuroprotective effects of E2 indicated in MPTP model might mediate through a modulation of neuroinflammatory reaction in lesioned nigrostriatal system.

TVI.18

THE MOLECULAR AND BEHAVIORAL CHANGES IN THE LONG-TERM OBSERVATIONS IN MICE MODEL OF PARKINSON'S DISEASE

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The pathogenesis of non-motor symptoms in Parkinson's disease (PD) is complex and not fully understood, but is believed to be related to the widespread nature of dopaminergic dysfunction involving structures beyond the substantia nigra. Therefore we've also focused in our experiments on specific brain regions (e.g. hippocampus and prefrontal cortex) critically involved in spatial learning and memory processes. One year old C57/BL male mice received 1-methyl-4-phenyl-1,2,3,6-tetrahydropyrindine (MPTP) 40 mg/kg, and control group received 0.9 % NaCl. To evaluate spatial learning and memory abilities, the Morris water maze (WM) behavioral test was provided after 6 months from the intoxication. The brain concentration of monoamines: dopamine (DA), noradrenaline (NA), 5 – hydroxytryptamine (5-HT) and amino acid: glutamate (Glu) were determined by using high – performance liquid chromatography (HPLC). Statistical significances differences in monoamines levels between groups were noticed in hippocampus (NA, DA content) and prefrontal cortex (NA content). We didn't notice significant differences in the WM test parameters between MPTP and the control animals. Correlation between the results of the behavioral testing in the probe trial and the level of monoamines were calculated. Swim distance to reach the hidden platform was negatively correlated with NA level in the hippocampus (r_p=−0.62, P<0.05). There is need for a further investigation to confirm the role for NA in spatial memories.

TVI.19

NEUROPROTECTIVE EFFECTS OF AAV2-hIL-10 IN MOUSE MODEL OF PARKINSON`S DISEASE

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Parkinson's disease (PD) is a neurodegenerative disorder which is characterized by abnormal loss of nigrostriatal dopamine (DA) neurons, accompanied by DA deficiency in the striatum. The pathomechanism by which DA neurons degenerate is still unknown, however, there is increasing evidence that is possible immunological mechanisms involvement in the etiopathogenesis of PD. The aim of the present study was to examine the effect of an adeno-associated viral type-2 (AAV2) vector containing human interleukin-10 (hIL-10) gene on dopaminergic system restoration in the murine model of PD induced by by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). Male C57BL/6 mice 12 months-old were used in this study. MPTP was injected in four intraperitoneal injections at 1-h intervals. AAV2-hIL-10 vector was bilaterally administered into striatum at 14, 21 or 28 days prior to MPTP intoxication. Animals were sacrificed at 7 days following MPTP injection. Striatal DA, DOPAC, HVA concentrations were quantified by HPLC method; tyrosine hydroxylase (TH) mRNA expression was measured by RT-PCR method. MPTP treatment dramatically decreased DA concentration, significantly decreased TH mRNA gene expression. AAV2-hIL-10 exerted a neuroprotective effect upon dopaminergic system (lower decrease in DA concentration). Additionally, viral vector administration prevented depletion of TH mRNA expression induced by MPTP. Our data suggest that AAV2-hIL-10 may play a neuroprotective role in MPTP mouse model of PD.

TVI.20

EFFECTS OF AAV2-hIL-10 ON IMMUNE RESPONSE FOLLOWING TOXIC DEGENERATION CAUSED BY 1-METHYL-4-PHENYL-1,2,3,6-TETRAHYDROPYRIDINE IN MICE

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Interleukine 10 (IL-10) – an antiinflammatory cytokine produced by lymphocytes and mononuclear phagocytes including microglia. IL-10 modulates the biological activities of immune cells resulting in a decreased production of pro-inflammatory mediators including cytokines, chemokines and adhesion molecules. The aim of the present study was to examine the effect of an adeno-associated viral type-2 (AAV2) vector containing human interleukin-10 (hIL-10) gene to evaluation of immune response to the AAV2-hIL-10 (measured as IFN-γ, GFAP and TGFβ mRNA expression) in the murine model of Parkinson's disease (PD) induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). Male C57BL/6 mice 12 months-old were used in this study. MPTP (40 mg/kg) was injected in four intraperitoneal injections at 1-h intervals. AAV2-hIL-10 vector was bilaterally administered into striatum at 14, 21 or 28 days prior to MPTP intoxication. Animals were sacrificed by spinal cords dislocation at 7 days following MPTP injection. TGFβ, IFN-γ and GFAP mRNA expression was examined by RT-PCR method. MPTP treatment significantly increased IFN-γ mRNA expression. AAV2-hIL-10 administration strongly increased IFN-γ as well as TGFβ and GFAP (21 and 28 day) gene expression compared to control and MPTP group. Our results point to the necessity of the reinterpretation of the role of the inflammatory reaction in nerodegenerative processes

TVI.21
THE SLEEP PROFILE IN PARKINSON'S DISAEASE
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Sleep disorders are reported by up to 90% of the Parkinson's disease (PD) patients. The main causes of poor sleep quality are the altered dopaminergic transmission in the subcortical areas, playing role in the sleep wake regulation, the influence of dopaminergic medication and the negative effect of rigidity, painfull dystonias, and other motor symptoms of PD. The aim of this study was the objective polysomnographic assessment of sleep quality in PD with respect to the possible gender differences. We evaluated: seven female and 10 male PD patients (mean age 66.3 ± 9.2 vs. 63.9 ± 10.2 years; mean Hoehn Yahr stage 2.2 ± 0.5 vs. 2.3 ± 0.5 and mean UPDRS score 28.0 ± 9.3 vs. 33.9 ± 15.9) respectively, with no significant differences between both sexes. The sleep was examined with nocturnal polysomnography and with two questionnaires – the Pitsburger sleep quality index (PSQI) and the Parkinson disease sleep scale (PDSS). Both genders showed worsened sleep quality. In particular the decreased sleep efficiency (61.1 ± 17.5% women, 45.5 ± 23.9% men) and prolonged sleep onset latency (95.2 ± 101.6 min – women, 52.6 ± 77.7 min – men) showed marked differences with respect to estabilished norms for the Polish population. The statistical comparison of the polysomnographic parameters revealed no significant differences between both genders. The poor sleep quality seen in our polysomnographic findings stays in accordance with frequent complainings of disordered sleep among PD patients.

TVI.22
INHIBITION OF INFILTRATION AND ACTIVATION OF BRAIN MACROPHAGES UNDERLIES ANTITUMOR ACTIVITY OF SYSTEMICALLY ADMINISTERED CYCLOSPORIN A
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Malignant glioblastomas are characterized by infiltration of tumour tissue with brain macrophages that contribute to tumour progression through release a variety of growth factors, cytokines/chemokines supporting tumor growth, invasion and the immune system evasion. Thus targeting of cytokine production, infiltration and activation of macrophages may be a novel antitumor strategy. In the present study, we investigated an effectiveness and molecular mechanisms mediating antitumor effects of CsA in the murine glioma model. EGFP-GL261 glioma cells were injected into the striatum of C57BL/6 mice and tumor-bearing mice received CsA (2 or 10 mg/kg/i.p.) every 2 days from 2nd or 8th day after implantation. CsA-treated mice had significantly smaller tumors than control mice. When the treatment was postponed to 8th day, only the higher dose of CsA was effective. CsA-treated mice showed a diminished number of tumor-infiltrating, amoeboid brain macrophages. TUNEL staining revealed a DNA fragmentation mostly within infiltrating macrophages after CsA treatment. At concentrations <10 μM CsA did not affect proliferation or viability of cultured glioma cells. Elevated IL-10 and GM-CSF levels were found in tumor-bearing hemisphere in comparison to naive controls and CsA treatment reduced significantly cytokine levels. Our findings demonstrate that CsA-induced decrease of cytokine production, infiltration and activation of macrophages results in reduction of glioma growth and progression.

TVI.23
EVALUATION OF MIGRATION OF BONE MARROW-DERIVED MACROPHAGES AND LYMPHOCYTES TOWARDS GLIOMA CELLS AND AND ITS MODULATION BY CYCLOSPORIN A
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Malignant glioblastomas are characterized by infiltration of tumour tissue with brain macrophages that may consist up to 30% of tumour mass and contribute to tumour progression. A relative contribution of resident microglia and peripheral monocyte/macrophages in gliomas is poorly defined. We generated chimeric mice with the immune system reconstituted after irradiation with hematopoietic GFP-bone marrow cells. The dsRed-GL261 glioma cells were implanted to the brains of 16-weeks old C57BL/6 chimeric mice Two weeks after implantation, tumour bearing hemispheres were isolated and the number of $CD11b^{+}CD45^{low}$ microglial cells or $CD11b^{+}CD45^{high}$ macrophages was determined by flow cytometry. We found that peripheral GFP^{+} macrophages comprise above 60% of GFP^{+} cells in the tumor. A co-localization of Iba-1^{+} cells (macrophages/microglia) with GFP^{+} cells has been detected by confocal microscopy. Tumor associated peripheral macrophages can facilitate glioma invasion and promote angiogenesis. We have previously demonstrated that cyclosporin A (CsA) blocks activation of microglia and its promoting effects on glioma invasion *in vitro* and *in vivo* (Sliwa et al. 2007). In chimeric mice treated CsA, a percentage of GFP^{+} macrophages in gliomas was reduced suggesting an inhibitory effects of CsA on immune cell migration. Our studies demonstrate that blood-borne macrophages migrate to the tumour and consist a significant population of tumour-associated macrophages.

TVI.24
CELL SIZE AND PROLIFERATION OF SUBEPENDYMAL GIANT CELL ASTROCYTOMAS ARE REGULATED BY mTOR AND Ras-ERK SIGNALING PATHWAYS
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Tuberous sclerosis complex (TSC) is characterized by cortical developmental malformations that are associated with epilepsy and appearance of benign brain tumors – subependymal giant cell astrocytomas (SEGAs) which are composed of distinct cell types, including giant cells and dysplastic neurons. TSC results from

mutations in *TSC1* and *TSC2*, which lead to the mTOR pathway activation, and p70S6 kinase and ribosomal S6 protein phosphorylation. ERK pathway is also aberrantly activated in SEGAs. Clinical trials with the mTOR inhibitor – rapamycin have demonstrated reduction in size of SEGAs, but the molecular mechanisms are unknown. In the present study, we evaluated the effects of rapamycin and the ERK pathway inhibitor – UO126 on cell size, proliferation and viability of cell cultures derived from SEGA. Rapamycin or UO126 alone did not affect viability and proliferation of SEGA cells. However, treatment with both drugs reduced proliferation of SEGA cells. Staining of F-actin revealed decrease of cell size in SEGA cultures exposed to rapamycin alone or in combination with UO126, but treatment with UO126 alone did not influence cell size nor morphology. Our studies demonstrate that simultaneous inhibition of both mTOR and ERK signaling pathways reduces proliferation of SEGA cells. Moreover, inhibition of mTOR signaling with rapamycin diminishes a volume of giant SEGA cells. It implicates that inhibitors of mTOR and ERK pathways should be considered for clinical trials of SEGAs

TVI.25
DIFFERENTIAL MODULATION OF BASAL AND IFN-INDUCED STAT TARGET GENES EXPRESSION BY SPECIFIC STAT DIRECTED OLIGODEOXYNUCLEOTIDE DECOYS IN GLIOMA C6 CELLS
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Constitutive activation of STAT3, leading to up-regulation of some proliferation-related and anti-apoptotic genes, was described in a number of tumors, including glioblastomas and is considered as an important drug target for cancer therapy. The binding sites for STAT3 are highly similar to that of STAT1, and the two factors may have opposite effects on expression of the same target genes. We took advantage of differences in binding sequences, relatively specific for some but not all STAT proteins, to elucidate the interplay between different STATs in the regulation of gene expression in the rat glioma C6 cells. A number of double-stranded oligodeoxynucleotide (ODN) decoys, each carrying a particular STAT binding sequence, were employed as competitive inhibitors of binding sites in the genome. We compared the effects of the decoy containing ISRE/GAS (binding complexes of STAT1, STAT2, IRFs) or STAT1 site (specific for STAT1 homodimer) on the expression of endogenous genes. We report that the decoy against STAT1 reduced the constitutive expression of endogenous STAT3 target genes under basal conditions. The decoy ISRE/GAS blocked IFNg-induced increase in expression of STAT target genes. Decoys with mutated and/or scrambled STAT-binding motifs were used as controls for specificity. Our results demonstrate a limited usefulness of some ODN decoys in manipulation of endogenous gene expression, probably due to limited sequence specificity of STAT binding sites.

TVI.26
CRITICAL PERIOD FOR MOTION PERCEPTION IN CATS
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Visual input guides the development of visual system in which distinct critical periods are connected with separate functions. Monocular deprivation during critical period leads to acuity deficits, while binocular deprivation impairs more specific visual functions. Children with later onset of binocular cataract have normal global motion perception, while patients with congenital cataract show a deficit (Lewis and Maurer 2005). Similarly global motion detection is impaired in cats binocularly deprived for 6 months (Burnat et al. 2002). The exact time-frame for global motion perception critical period still needs to be established. Two experimental groups of cats: congenital (2BD) binocularly deprived for first 2 months of life and developmental (2N2BD) with 2 months of normal vision followed by 2 months of deprivation were used. Controls had normal visual input. Animals were trained in two-choice apparatus for a food reward. Visual discrimination tasks with random dot patterns differing in velocity and, or direction were used. In 2N2BD group we found significant impairment as compared to 2BD group in velocity discrimination tasks. Moreover, we found in 2N2BD group that the detection of global motion signal at low contrast is impaired by the addition of dark noise. Surprisingly, visual experience for first 2 months limited to the moving shadows may facilitate global motion perception as the 2BD group learned motion tasks faster than control group. *Supported by MNiSW grant N40132/1002.*

TVI.27
A MIFEPRISTONE-REGULATED ADENO-ASSOCIATED VIRAL VECTOR SYSTEM FOR REGULATED NEUROTROPHIC FACTOR EXPRESSION IN THE CENTRAL NERVOUS SYSTEM
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Neurotrophic factors (NF) are potent molecules with great promise for treatment of devastating neurodegenerative disorders like Alzheimer´s and Parkinson´s disease. Targeted delivery of NF like NGF or GDNF by means of viral vectors to distinct populations of brain cells may significantly enhance bio-availability and safety of such treatment approaches. However, gene therapy in its current understanding means to introduce e.g. a cDNA coding GDNF into neurons or astrocytes and expressing the factor from that time on continuously, without an option to tune expression levels according to individual patient's demands or to shut down expression in case of unforeseen side effects. In order to increase efficacy and safety of gene therapy vectors for treatments of human patients, we here describe the development of a tightly regulatable vector system based on the approved human drug, mifepristone, for applications in the CNS. A two-vector based layout allows modulating levels of the transactivator in order to achieve a very tight off-state while maintaining sufficient potency for activation of transgene expression. We describe characterization of the vector system in cultured primary brain cells and in the rat striatum and cortex in terms of dependence of vector titres, inducing drug levels, and repeated responsiveness. We believe that such vector system will significantly promote gene therapeutic applications in patients but will also have great impact on basic research applications.

TVI.28
PRESYNAPTIC AND POSTSYNAPTIC EFFECTS OF NORADRENERGIC FUNCTION IN AN ATTENTIONAL SET-SHIFTING TEST
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Chronic stress is a major risk factor for many psychiatric disorders, which are also associated with prefrontal cortical dysfunction. We found that elevating norepinephrine (NE) in rat medial prefrontal cortex (mPFC) with atipamezole enhanced extra-dimensional (ED) cognitive set-shifting on the attentional set-shifting test (AST). By contrast, a 2-week exposure to chronic unpredictable stress (CUS) induced an antidepressant (DMI)-reversible deficit on ED. To assess whether CUS-induced cognitive deficits represent a loss of NE modulation in the mPFC, we tested if elevating NE activity in the mPFC is still capable of enhancing cognition after CUS. Again, CUS impaired ED cognitive set-shifting in saline treated rats, but this deficit was attenuated in CUS rats given atipamezole prior to testing. Extracellular NE was not different between saline-treated CUS and control rats. However, atipamezole enhanced NE release in both control and CUS rats throughout the duration of the test. In the second experiment, CUS produced a cognitive deficit, which was prevented by chronic DMI. Acute blockade of α_1-adrenergic receptors in mPFC of CUS/DMI-treated rats prior to ED caused a cognitive deficit similar to that of CUS/saline rats. These results suggest the beneficial effects of chronic antidepressant treatment on cognitive performance are mediated through α_1-adrenergic receptors in the mPFC. Furthermore, NE facilitation of cognitive flexibility in the mPFC remains intact after CUS.

TVI.29
ELEVATED EXPRESSION OF *α*1-ADRENOCEPTOR SUBTYPES IN THE HIPPOCAMPUS OF RATS NON-RESPONDING TO IMIPRAMINE TREATMENT IN THE CHRONIC MILD STRESS
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Cerebral α_1-adrenoceptors (α_1-AR) are known to be changed by stress conditions and involved inthe mechanism of antidepressant action. The chronic mild stress (CMS) procedure that induces depression-like symptoms in animals is a useful tool to study the mechanisms of action of antidepressant drugs in animals. We aimed to investigate the expression of α_{1A}-, α_{1B}-, and α_{1D}-AR mRNAs in the hippocampus of rats subjected to the standard CMS procedure and then treated with an antidepressant drug, imipramine (IMI). Five groups of male Wistar rats were considered in the study: sham-saline; stress-saline; sham-IMI; stress-IMI and IMI-non-responders (i.e., stressed rats, which did not respond to IMI treatment). The expression of α_1-ARs was measured at the level of mRNA (by quantitative real-time PCR) and their total density analyzed by [3H]prazosin autoradiography. We found that both CMS procedure and IMI-treatment did not affect the expression of all α_1-AR mRNAs. However, in rats non-responding to IMI treatment in behavioral test, the expression of α_{1A}-, α_{1B}- and α_{1D}-AR mRNAs was significantly increased (respectively, by 81%, $P<0.01$; 78%, $P<0.01$; 46%, $P<0.5$) compared to sham-saline and stress-IMI groups. Similar direction of change was seen in α_1-AR total density. Our results suggest the involvement of all subtypes of α_1-adrenoceptor in the phenomenon of resistance of depressive animal to IMI treatment. *Supported by statutory funds of the Institute of Pharmacology, PAS.*

TVI.30
EXAMINATION OF SCHIZOPHRENIA ENDOPHENOTYPES IN THE HETEROZYGOUS NEUREGULIN-1 KO MOUSE: A PSYCHOPHARMACOLOGICAL AND MAGNETIC RESONANCE IMAGING STUDY
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Meta-analysis has confirmed the association between the neuregulin-1 (NRG1) gene risk to develop schizophrenia. Neuregulin-1, alongside several other risk genes, is known to play a role in glutamatergic neurotransmission. There is a substantive body of evidence for hypoglutamatergic function in schizophrenia; the psychotomimetic activity of non-competitive NMDA antagonists MK-801 and phencyclidine (PCP) is complemented by NMDA deficits in *post-mortem* brain in schizophrenia. In the present study, differential sensitivity to the behavioural effects of MK-801 and PCP was examined in mice containing partial deletion of the NRG1 gene in order to elucidate the relationship between NRG1 genotype and the psychotomimetic effects of NMDA receptor antagonism. Changes in social behaviour and exploratory activity were assessed in NRG1 mutants vs. wildtypes following acute or subchronic administration of MK-801 or PCP. Neuregulin-1 mutants and wildtype controls mice were also examined by magnetic resonance (MR) volumetry using high-resolution T2-weighted imaging at 7 T in order to examine effects of NRG1 genotype on presence of schizophrenia-relevant structural brain endophenotypes. The results indicate that NRG1 knockout mice display (a) altered responsivity to NMDA receptor antagonists compared to controls (b) volumetric changes in brain areas implicated in schizophrenia. *This work was funded by Science Foundation Ireland & Health Research Board of Ireland*.

TVI.31
LOCAL BLOCADE OF NMDA RECEPTORS IN THE RODENT PREFRONTAL CORTEX MODIFIES OSCILLATORY ACTIVITY AND c-Fos EXPRESSION IN THE NUCLEUS ACCUMBENS
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In humans subanesthetic doses of noncompetitive NMDA receptor (NMDAR) antagonists can produce symptoms similar to those observed in schizophrenia. In rodents, systemic application of NMDAR antagonists produce hyperactivity, stereotypies and abnormal social interactions used to model certain features of schizophrenia. It was also shown that NMDAR antagonists induce the high frequency oscillations (HFO, 140–180 Hz) in the nucleus accumbens (NAc). Several neurophysiological models of schizophrenia emphasize the role of interactions between frontal cortical areas,

especially medial prefrontal cortex (mPFC) and NAc. The aim of this study was to examine the effects of local infusion of MK-801 (a selective NMDAR antagonist) in the mPFC on oscillatory activity recorded in the NAc. Experiments were performed on freely moving rats, bilaterally implanted with guides in the mPFC and an electrode in the right NAc. Bilateral administration of MK-801 to the mPFC produced a substantial increase in the power of HFO, which was associated with NMDAR antagonist-induced changes in locomotor activity and behavior. In another group of animals we examined the effects of local infusion of MK-801 into mPFC on c-Fos expression in NAc. Prominent c-Fos response was observed in the NAc in relation to saline and control groups. These results suggest that local administration of MK-801 to the mPFC modifies oscillatory activity and c-Fos expression in the NAc.

TVI.32
CANNABINOID CB1 RECEPTOR ANTAGONIST RIMONABANT ATTENUATES REINSTATEMENT OF NICOTINE PLACE PREFERENCE PROVOKED BY NICOTINE OR MORPHINE IN RATS
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Reinstatement of drug-seeking behaviour in animals is relevant to relapse to drug taking in humans. In the present study, we used the conditioned place preference paradigm to investigate the establishment, extinction, reinstatement and cross-reinstatement of nicotine-induced place conditioning in rats. We have revealed that nicotine produced a place preference to the compartment paired with its injections during conditioning. Once established, nicotine place preference was extinguished by repeated testing. Following this extinction phase, nicotine-experienced rats were challenged with nicotine (0.5 mg/kg) or morphine (10 mg/kg). These priming injections of both drugs renewed a marked preference for the compartment previously paired with nicotine. In the second step, we examined the influence of CB1 cannabinoid receptor antagonist rimonabant (0.5, 1 and 2 mg/kg) on the reinstatement of nicotine place conditioning. It was shown that rimonabant, dose-dependently, attenuated the reinstatement of nicotine-conditioned response. Our findings may further indicate similar endocannabinoid-dependent mechanisms involved in the development of reinstatement of nicotine-conditioned place preference provoked by priming injections of nicotine and morphine. As reinstatement of drug-seeking is a factor for the development of drug dependence, CB1 receptor antagonists may be useful in the relapse-prevention phase of addiction treatment, including nicotinism and polydrug abuse.

TVI.33
ADENOSINE RECEPTOR AGONISTS ATTENUATE THE EXPRESSION OF SENSITIZATION TO MORPHINE-INDUCED PLACE PREFERENCE IN RATS
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Behavioural sensitization is an enhancement of certain drug-induced effects which develops following repeated, intermittent exposure to opioids and psychoactive drugs. The sensitization can be measured in various animal models, e.g. conditioned place preference paradigm. In the present studies we undertook to investigate an involvement of adenosine receptor agonists in expression of sensitization to morphine-induced rewarding effects in rats. Three, intraperitoneal (i.p.) injections of morphine (5.0 mg/kg) induced conditioned place preference. Five days later, in animals with prior history of morphine administration, ineffective dose of morphine (0.75 mg/kg i.p.) was administered. That dose was able to induce a significant intensification of morphine response in conditioned place preference paradigm. It showed that morphine-induced sensitization to rewarding effects had been developed. To determine the effects of adenosine receptor agonists on expression of sensitization, selective and non-selective drugs were administered 15 min before the last injection of morphine. We showed that all adenosine drugs were able to attenuate the expression of morphine sensitization and the strongest effects were produced by selective (A_1) and non-selective (A_1 and A_{2A}) adenosine receptor agonists. We conclude that adenosine agonists may play an important role in drug-seeking behaviour underlying the development of addiction.

TVI.34
CHARACTERISTIC OF ALCOHOL-PREFERRING WHP AND NON-PREFERRING WLP LINES OF RATS
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The selected lines of rats WHP (Warsaw High Preferring) and WLP (Warsaw Low Preferring) determine the animal model to investigate neurobiological mechanism of ethanol action. WHP rats drink excessive amounts of alcohol whereas WLP rats drink only small amounts. Patterns of ethanol consumption in WHP and WLP lines are constant. Due to cessation of alcohol intake the WHP rats have developed features of withdrawing signs. Level of dopamine and serotonine is lower in some brain regions of the WHP rats. WHP rats show an increased responsiveness to the stimulatory effects of low dose of ethanol.It has been suggested that acquisition of acute tolerance to ethanol may promote increased ethanol consumption. The effects of acute administration of ethanol (dose – 2.3 g/kg i.p.) were established by assessing ethanol-induced motor impairment, hypothermia and blood ethanol level in WHP and WLP rats. The rotarod model was used to measure motor impairment. Results have been revealed that WHP rats recovered motor activity and normal temperature when blood ethanol was at the highest level, indicating the development of acute tolerance to ethanol. The WLP rats showed similar recovery to WHP rats but at the lower blood ethanol level.Tolerance to ethanol may have a great value as a predictor of susceptibility to alcoholism therefore may significant means in the relation to alcohol consumption and dependence. Generally tolerance is thought as the neuroadaptive processes to intoxicate effect of ethanol.

TVI.35
EFFECT OF NICOTINE ON LOCOMOTION OF *CAENORHABDITIS ELEGANS*
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The locomotion of the soil nematode *Caenorhabditis elegans* is a simple behavior that has been widely used to dissect genetic components of behavior, synaptic transmission, and muscle function. Numerous

studies have reported that exogenous nicotine caused contractions and spastic paralysis of worms at a concentration of 30 mM. The aim of this study was to estimate nicotine-induced behavioral response of *C. elegans* wild-type (N2) L1 larvae. The L1 larvae were transferred to NGM plates containing 300 μM (–)-nicotine. The Parallel Worm Tracker was used to measure average locomotion speed and nicotine-induced paralysis in *C. elegans* [Ramot et al. (2008) PLoS ONE 3: e2208). The tracker utilizes a modified web camera (attached to zoom stereo microscopes), which records the position of tens of worms in sequential video frames. In each experiment, approximately 40 worms were tracked for 30 s every 10 min. A rapid increase in the mean speed of worm locomotion (up to 2-fold) was observed after 10 min of exposure of L1 larvae to nicotine. At the same time, the lowest number of paralyzed and immobile worms was noticed. The lowest mean locomotion speed and the maximum of paralyzed individuals was observed after 40 min of nicotine treatment. It was followed by a recovery period within 60 min after exposure, during which the worms acquired tolerance. Further research will be undertaken to analyze the nicotine dose-dependent locomotion response of all developmental stages of *C. elegans*.

TVI.36
METABOTROPIC GROUP I GLUTAMATE RECEPTORS (mGluR) ANTAGONISTS ATTENUATE COCAINE-INDUCED TOXICITY AND COCAINE-INDUCED BEHAVIORAL SENSITIZATION IN MICE
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Cocaine abuse and dependence is a worldwide health problem. However, there are no currently approved medications to reduce cocaine abuse/relapse and toxicity. Published data showed that group I mGluR antagonists (mGluR1 and mGluR5) possess an anti-addictive potential in various animal models of cocaine abuse. In the present study we assessed the impact of mGluR1 antagonist – EMQMCM and mGluR5 antagonist – MTEP on the cocaine-induced toxicity (lethality) in mice. Moreover, we evaluated the influence of these antagonists on motivational effect of cocaine measured in the sensitization test to its hyperlocomotor effect in mice. Our study indicated that EMQMCM and MTEP, at the doses of 2.5; 5, and 10 mg/kg, dose-dependently decreased cocaine-induced lethality produced by 75 mg/kg of cocaine. The effect of EMQMCM was stronger than MTEP, and EMQMCM at the dose of 10 mg/kg completely reduced the lethality induced by cocaine. Furthermore, EMQMCM but not MTEP, significantly reduced the expression of cocaine-induced (10 mg/kg) behavioral sensitization. Our results suggest that mGluR1 receptors are implicated in motivational effect of cocaine but both receptors (particularly mGluR1) play a role in cocaine-induced toxicity.

TVI.37
ARE DELTA OPIOID RECEPTORS INVOLVED IN THE REWARDING ACTION OF COCAINE IN RATS?
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Delta opioid receptors are involved in modulation of nociception, thermoregulation, locomotor activity and rewarding properties of drugs of abuse. The new analog of deltorphin – DK-4 is a peptide with high affinity to delta opioid receptors in mouse vas deference bioassay. The aim of our study was to indicate whether delta opioid receptors are involved in the rewarding effects of cocaine. Therefore, we tested influence of (1) opioid receptor antagonists (μ, κ, δ) on the expression of cocaine-induced conditioned place preference (CPP); and (2) influence of DK-4 on the cocaine reinstatement of CPP (Sleipness et al. 2007). Rats were conditioned daily with cocaine (5 mg/kg, i.p.), tested on the 6th day (the expression of CPP), and then retested for 9 days to monitor the extinction of the CPP. On the day 10 animals were treated with a single injection of cocaine (5 mg/kg) or DK-4 (5, 10 nmol, i.c.v.) to reinstate the CPP. Our results indicated that naltrindole – a delta opioid receptor antagonist decreased cocaine rewarding effect (the expression of CPP). DK-4 increased the reinstatement of cocaine-induced CPP. These studies suggest that delta opioid receptors are involved in the cocaine-induced rewarding effects.

TVI.38
THE ROLE OF THE SYMPATHETIC NERVOUS SYSTEM IN AMPHETAMINE-INDUCED MODULATION OF NATURAL KILLER CYTOTOXICITY IN RATS
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It is well known that the main site of the action of amphetamine (AMPH) are the catecholaminergic neurons located both in central nervous system and in the sympathetic nervous system (SNS). To analyse the potential role of the SNS in the mechanism of AMPH-induced changes in natural killer cells cytotoxicity (NKCC) rats were sympathectomized by 6-hydroxydopamine (6-OHDA, 3 × 50 mg/kg, i.p.) prior to AMPH (1 mg/kg, i.p.) administration. In a separate experiment, rats were pretreated with a α-adrenergic receptor antagonist phentolamine (5 mg/kg, i.p.), β-adrenergic receptor antagonist propranolol (5 mg/kg, i.p.) or both. NKCC (^{51}Cr-release assay) and the number of LGL (NK cells) were evaluated in the peripheral blood and spleen. In the peripheral blood AMPH-induced stimulation of NKCC was completely blocked by 6-OHDA. The increase in LGL number in the peripheral blood evoked by AMPH was partially inhibited by sympathectomy. In the spleen both effects of AMPH i.e. reduction of NKCC and decrease in LGL number were completely reversed by 6-OHDA. β-antagonist attenuated the AMPH-induced changes in NKCC and LGL number in the peripheral blood and spleen. In contrast, changes in NKCC and LGL number were not affected by α-blockade. These data clearly show that both AMPH-induced stimulation of the peripheral blood NKCC and suppression of spleen NKCC are mediated by the SNS. Furthermore, catecholamines elevated by AMPH modulate the NKCC *via* β-adrenergic, but not α-adrenergic mechanism.

TVI.39
EFFECTS OF BINGE-LIKE ETHANOL ADMINISTRATION ON MACROMOLECULE PROFILES IN HIPPOCAMPUS OF YOUNG ADULT WISTAR RATS AS ASSESSED BY FT-IR SPECTROSCOPY
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Despite of widespread abuse and the presence of vast data about ethanol's adverse effects on behavior, brain morphology and physi-

ology, molecular mechanism underlying deteriorating effects of chronic ethanol intoxication on the nervous system still remain elusive. The aim of the present study is revisiting the potential effects of adult alcohol insult and/or withdrawal on the protein, nucleic acids, and lipid profiles in the rat hippocampus by using Fourier Transform Infrared (FTIR) spectroscopy. It is a rapid, sensitive and non-disturbing technique which gives information about different functional groups belonging to biomolecules, simultaneously [Garip et al. (2007) Appl Spectrosc]. In the current study the rats were intragastrically administered ethanol at a dose increasing from 4.5 g to12 g/kg for 15 days and then the mean blood alcohol concentration was measured as 605.67 ± 36 mg/dl. FTIR results revealed an increase in the total content of proteins, lipids, as well as amino, fatty, and nucleic acids. Moreover, an increase in lipid disordering and a decrease in membrane fluidity were obtained. Chronic ethanol intoxication was also found to cause an increase in random coil and aggregated β sheet content indicating structural changes in proteins, such as denaturation. Significant variations in DNA and RNA conformation were observed. These molecular changes are discussed in terms of their potential effects on the neural function.

TVI.40
TETRAHYDROCANNABINOL MAY ENHANCE NATURAL KILLER CELL CYTOTOXICITY IN RATS
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It is well known that Δ^9- tetrahydrocannabinol (THC), the main active component of marijuana, exerts mainly suppressive influence on the immune system. Here we present data indicating that THC induce immune enhancing effects. In this study we examined the effects of small doses of THC on the natural killer cell cytotoxicity (NKCC) (^{51}Cr – release assay) and also on the number of NK cells (identified as large granular lymphocytes, LGL) (Timonen method) in the peripheral blood in rats. Animals received acute or chronic (10 days) i.p. injections of THC at the doses of 0.2 and 0.5 mg/kg. It was found that acute injection of THC significantly increased NKCC after the dose of 0.2 mg/kg (+ 131 Δ%; $P<0.001$ vs. control and + 52 Δ%; $P<0.05$ vs. 0.5 mg/kg dose). After chronic exposure to THC, a significant NKCC increase was observed after the dose of 0.5 mg/kg (+ 209 Δ%; $P<0.001$ vs. control and + 97 Δ%; $P<0.01$ vs. 0.2 mg/kg dose). The number of LGL remained unchanged after both acute and chronic treatment with THC. It has been reported repeatedly that the doses of over a dozen milligrams of THC suppress NKCC. These results negate the common opinion about unidirectional suppressive effects of cannabinoids on the immune system. It seems that the pharmacodynamics rule of hormesis may also concern the influence of THC on the immune system.

TVI.41
EFFECTS OF CONTINGENT AND NON-CONTINGENT ADMINISTRATION OF MORPHINE ON THE DOPAMINE RECEPTORS AND OPIOID PEPTIDES GENE EXPRESSION IN THE MOUSE BRAIN
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Changes in the gene expression produced by drugs of abuse differ depending on whether the drug is self-administered by the subject (contingent administration) or injected to the animals by the experimenter (non-contingent administration). To verify whether expression of the opioid peptide precursors and dopamine receptors depend on the direct pharmacological effect of the drug or reflect the cognitive processes associated with self-administration of morphine we employed "yoked" self-administration procedure. The experiment was performed on C57BL/6J mice that were trained to self-administer morphine. We used the technique of *in situ* hybridization to measure the dopamine receptors (D1R and D2R) and opioid propeptide (proenkephalin and prodynorphin) mRNA levels in several brain regions implicated in addiction. Differences in the D1R and D2R gene expression were found in the dorsal striatum and nucleus accumbens, where an increase of mRNA levels upon active, but not passive morphine administration was observed. No changes in the expression of both opioid propeptide genes were detected in all investigated brain regions of mice receiving contingent or non-contingent morphine injections. The observed increase in the D1R and D2R genes expression suggests that changes in the dopaminergic system may be specifically associated with the motivational and cognitive processes underlying self-administration of morphine. *Supported by EU grants: LSHM-CT-2007-037669 and LSHM-CT-2004-005166.*

TVI.42
EFFECT OF DAIDZIN AND PUERARIN ADMINISTRATION ON BEHAVIORAL AND COGNITIVE FUNCTIONS IN CHRONICALLY ETHANOL DRINKING OUTBRED RATS
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Administration of isoflavones present in *Pueraria radix,* daidzin (DAI) or puerarin (PUE), reduces ethanol intake in rodents. The paradigm showed by these compounds is probably mediated centrally, therefore an assessment of influence of DAI and PUE on association between alcohol consumption and changes in memory and motor functions seems to be of scientific importance. Ethanol "preferring" (PR) and "non-preferring" (NP) rats were treated with DAI (40 mg/kg, p.o.) and PUE (150 mg/kg, p.o.) for 28 consecutive days and their motor activity, motor coordination, anxiety-related reactions, short- and long-term memory were assessed. It was found that DAI (23%) and PUE (52%) lowered alcohol intake in PR rats. PUE produced a facilitation effect on long- (significantly) and short-term (tendency) memory in PR rats. The effects seemed to be specific since PUE did not affect motor activity and anxiety-related reactions and led to improvement motor coordination in PR rats. On the contrary, DAI did not change cognitive and behavioral functions in PR animals, whereas positive effect especially on short-term memory in NP rats was found. Unfortunately, DAI lowered motor activity and coordination in NP animals. In conclusion, since PUE attenuated the deficits of inhibitory avoidance performance induced dizocilpine (Hsieh et al. 2002), therefore its positive effect on long-term memory *via* NMDA receptor activation in PR rats can be considered. *The study has been supported by grant N405024 32/1684.*

TVI.43
EFFECTS OF CANNABIDIOL ADMINISTRATION ON BODY WEIGHT GAINS IN RATS
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Cannabidiol (CBD), the major non-psychoactive compound of Cannabis has been reported to display neuroprotective, anti-inflammatory, and anti-tumor actions. Despite growing interest in pharmacological effects of CBD, there is no data concerned with the effects of CBD on body weight, food intake or metabolism. The present study examined whether CBD administration affects body weight, during 13 days of systemic CBD treatment. Adult male Wistar rats (n=54), weighing approximately 250 ± 20 g at the beginning of the experiment received intraperitoneal injections of CBD at dose of 2.5 or 5 mg/kg/day, or the vehicle, for 13 consecutive days. The animals were weighed each day 2 h before each injection. Body weight on the first day of treatment served as baseline (100%), then in following 13 days percentage body weight gains were calculated. Repeated CBD administration significantly slowed body weight gains. Two-way ANOVA revealed significant difference between weight gains of rats receiving CBD and the control group (P<0.0001).Rats receiving CBD at dose of 5 mg/kg gained, on average, 8.0 ± 0.9 g (SEM) less weight than control animals, which is 19.6% of total weight gain of controls during that period. Mean body weight gain of rats receiving 2.5 mg/kg of CBD was 6.8 ± 0.9 g lower than controls (16.6% of total weight gain of controls).The results indicate that CBD administered in low doses lowers body weight gain. The data suggest that CBD may affect food intake or metabolism.

TVI.44
WARSAW HIGH ALCOHOL-PREFERRING (WHP) AND LOW ALCOHOL-PREFERRING (WLP) RATS DIFFER IN BEHAVIORAL AND MIDBRAIN DOPAMINE SYSTEMS' RESPONSES TO MORPHINE
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Rats were given 14 "daily" (6 doses/week) s.c. doses of 0.9% NaCl (Sal), morphine (Mor), or methadone (Met), then left untreated for 14 days, and finally challenged with Mor, except that half each of the Sal groups were given Sal instead. All the rats were then tested for open field behavior, and were sacrificed 2 h post-challenge. Striatal (CPu), accumbal (Acb), sensorimotor cortex (S1) and prefrontal cortex contents of dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) and 3-methoxytyramine (3 MT, assessed only in the CPu and Acb) were measured by HPLC. Analysis of total distance covered by the rats showed Mor and Met-induced sensitization to Mor in the WHP rats only, whereas the distance covered in the central part of the test arena was significantly affected (increased) only by the Mor pretreatment and only in the WHP rats. There was no significant between-line or treatment-related difference in DA content, and no significant between-line difference in baseline DA metabolite contents, except that S1 HVA content was significantly higher in drug-naive WLP rats than in drug-naive WHP rats. HVA and – to a lesser extent – DOPAC contents were, in general, higher in the Mor-challenged rats than in the respective Sal-treated controls, but the differences were more pronounced in the WLP rats than in their WHP counterparts, whereas the opioid -pretreated WHP rats showed higher CPu and Acb 3 MT contents than their WLP counterparts.

TVI.45
CHANGES OF ERK1 AND ERK2 PHOSPHORYLATION IN HIPPOCAMPUS DURING MORPHINE-INDUCED CONDITIONED PLACE PREFERENCE IN MOUSE BRAIN
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Extracellular signal-regulated kinases (ERK1 and ERK2) may play an important role in the molecular mechanisms of opiates addiction. Recently, the role of hippocampus in the process of addiction has attracted an attention as addictive drugs like morphine may affect the normal function of this region leading to a formation of aberrant learning. Our previous results demonstrated that acute and prolonged morphine treatment leads to changes of ERK1 and ERK2 phosphorylation. However, the specific pattern of alterations of ERK1 and ERK2 phosphorylation (which is required for ERKs activity) in the hippocampus, during the development of morphine reward has not been studied yet. We discovered that first morphine conditioning increased ERK1 and ERK2 phosphorylation in CPP paradigm, but after third morphine session during CPP, we observed the decrease of ERK1 and ERK2 phosphorylation. Moreover, an increase of pERK2/pERK1 ratio correlated linearly with the expression of place preference following morphine administration. The changes of phosphorylation were observed in mossy fibers, the structure, which is involved in spatial learning and memory. In hippocampus, the observed changes of ERK phosphorylation favoring mainly ERK2 activity together with the putative role of ERK2 in learning and long-term memory suggest that morphine-induced interactions within ERK pathway participate in reward-controlled learning. *Research supported by Ministry of Science and Education Grant N401 066 31/168.*

TVI.46
INTRAVENOUS COCAINE ALTERS LYMPHOCYTE SUBSETS DISTRIBUTION IN PERIPHERAL BLOOD OF RATS
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Cocaine use has been associated with the increased risk for HIV and HCV infection in humans. The addicts have faster progression of HIV infection and increased incidence of AIDS suggesting that cocaine use results in a specific functional impairment of the immune system. Present study aimed to evaluate lymphocyte subsets number and distribution in peripheral blood after acute series of intravenous cocaine infusions. Adult male Wistar rats received 3 injections of cocaine hydrochloride (5 mg/kg/ml) in 30 min intervals. Blood samples were collected 30 min after the last infusion. Total leukocyte number, percentage numbers of leukocyte subpopulations and T, B, NK, T helper and T cytotoxic lymphocyte subsets were assessed. Intravenous cocaine caused significant decrease in total leukocyte and lymphocyte number and sig-

nificant fall in total numbers of T, B, NK and both T helper and T cytotoxic lymphocyte subsets. Furthermore, intravenous cocaine altered proportions between lymphocyte subsets by increasing T, mainly T helper and decreasing B, T cytotoxic and NK lymphocyte percentage portion. Immunosuppressive effect of intravenous cocaine infusions may thus be connected with alterations in lymphocyte subsets proportions. Such destabilization of lymphocyte subsets balance, especially T helper lymphocyte, could constitute basis of increased susceptibility for HIV and HCV infections.

TVI.47
GENE PROFILING THE REWARDING EFFECT OF MORPHINE IN STRIATUM AND HIPPOCAMPUS
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Persistent changes that take place during the development of opioid addiction are thought to be due to adaptive changes in gene expression in the brain. In the present study we used DNA microarrays (Illumina) to analyze the expression of about 45 000 transcripts in the selected brain regions (striatum and hippocampus) of C57Bl/6J mice during the morphine-induced conditioned place preference (CPP) test. Repetitive morphine treatment during morphine conditioning led to an altered expression of 257 genes. Many of these genes coded for heat shock proteins, neuronal transport and kinases including mitogen-activated protein kinases. The observed alterations in the gene transcription resulted from the morphine-induced conditioning rather than CPP test alone. The genes meeting criteria for statistical significance revealed strong correlation coefficients with the expression of place preference (measured as increased time spent on the morphine-paired side). These findings suggest that morphine-conditioning induces the persistent changes in gene transcription that correlate with the rewarding behavior. *Research supported by Ministry of Science and Education Grant N401 066 31/168.*

TVI.48
EVALUATION OF SPONTANEOUS PARTIAL WİTHDRAWAL AND EFFECT OF CLONIDINE IN INTERMITTENT MORPHINE ADMINISTRATION IN RATS
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Using novelty-induced grooming as a marker of stress response and clonidine as an anti-stress drug, present study tested the hypothesis that intermittent injection of morphine may induce stress and allow some withdrawal during each interval (Houshyar et al. 2003). Male Wistar rats were injected s.c. with saline or morphine twice daily (08:30 and 18:30) for 4 days with increasing doses (10, 20, 30, 40 mg/kg/injection/day). On days 1–4, animals were given tap water with or without 5 μg/ml clonidine (18:30–8:30). On day 5, at the time of morning injections, animals were observed for 5 minutes for grooming and spontaneous withdrawal [14-h withdrawal assessed with global withdrawal score (GWS)]. Then, animals were injected s.c. with morphine (40 mg/kg) or saline and 2 hours later with naloxone (1 mg/kg) and observed for 15 minutes for withdrawal symptoms. Data were analyzed using ANOVA and Bonferroni test. Morphine and morphine+clonidine groups spent less time in grooming and produced marked GWS and naloxone-precipitated withdrawal signs. Present study could not demonstrate an increase in grooming reflecting stress before the next dose of intermittent use of morphine, but provides evidence that the injection regimen allows manifestations of partial morphine withdrawal. Clonidine, possibly due to insufficient water intake, could not prevent withdrawal signs. Further experimentation may provide more data to clarify spontaneous withdrawal associated with the treatment regimen.

TVI.49
EXTRACELLULAR CORTICAL SEROTONIN AND DEPRESSION-RELATED BEHAVIOUR IN THE FORCED SWIM TEST IS INFLUENCED BY INTERLEUKIN-2
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It is assumed that cytokines (like interleukins) can influence depression and anxiety. Interleukin-2 (IL-2) is suggested to be one factor, which may mediate behavioural and neurochemical (e.g., serotonergic) features of depression in the brain. Previously, we have shown in rats that IL-2 mRNA in the striatum and prefrontal cortex is correlated with anxiety-like avoidance behaviour in an elevated plus-maze. Additionally, striatal IL-2 microinjections affected anxiety-like behaviour in a biphasic way. In the present study, we investigated the impact of systemically (i.p.) injected IL-2 (2.5 μg/kg) on serotonegic (5-HT) and dopaminergic neurotransmission in various cortical areas by *in-vivo* microdialysis in anaesthetised rats (Exp. 1). Furthermore, based on the serotonergic time profile, we conducted two experiments to test for delayed (test 2 h post injection, Exp. 2) and acute (Exp. 3) behavioural effects of systemic IL-2 (0–5 μg/kg) on depression-related behaviour in a forced swim test (FST). The neurochemical results revealed that systemic IL-2 lastingly reduced extracellular 5-HT levels in the medial prefrontal (−75%), occipital (−70%), and temporal cortices (−45%). In contrast, dopamine was moderately reduced only in the medial prefrontal cortex. The functional relevance of these specific neurochemical changes were supported by the subsequent behavioural evaluation showing IL-2 dose-dependent effects on depression-related behaviour in the FST after delayed testing.

TVI.50
THE INVOLVEMENT OF NITRIC OXIDE (NO) IN THE AMNESIC EFFECTS OF DIAZEPAM IN MICE
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Literature data show the relationship between L-arginine:NO:cGMP pathway and g-aminobutyric acid (GABA)-mediated transmission in the central nervous system. Benzodiazepines are known to enhance the GABA-ergic neurotransmission and well established as inhibitory modulators of memory processing. More-

over, the role of NO in learning and memory processes has been proposed. The present studies were designed to evaluate the role of L-arginine:NO:cGMP pathway in the amnesic effects of diazepam (DZ) in the modified elevated plus-maze (mEPM) task in mice. Our experiments indicated that DZ (1 mg/kg, s.c.) impaired elevated plus-maze memory performance in mice. Pretreatment with L-arginine, the NO precursor, (500 mg/kg, i.p.) prevented the amnesic properties of DZ. While, 7-nitroindazole (7-NI), the neuronal NO synthase inhibitor (nNOS), (40 mg/kg, i.p.) and methylene blue (MB), the soluble guanylate cyclase (sGC) inhibitor, (5 mg/kg, i.p.) enhanced the DZ-induced memory deficits. Moreover, the effect of both 7-NI and MB were reversed by L-arginine (250 mg/kg, i.p.). It is important to note that presented data are not due to either 7-NI-, MB-induced impairment of memory or changes in locomotor activity, because 7-NI and MB given alone, had no impact on the mEPM behaviour and locomotor activity of mice. Taken together, these results suggest that an inhibition of the L-arginine:NO:cGMP pathway may be involved in the amnesic effects of benzodiazepines.

TVI.51
INTERSTRAIN DIFFERENCES IN 5-HT$_3$ RECEPTOR CENTRAL-INDUCED HYPOTHERMIA AND COMPARISON WITH HYPOTHERMIA MEDIATED *VIA* 5-HT$_{1A}$ RECEPTOR

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The selective agonist of 5-HT$_3$ receptors 1-(3-Chlorophenyl)biguanide hydrochloride (m-CPBG) administered intraventricularly (40, 80 or 160 nmol) produced distinct hypothermic response in AKR/2J mice. At the same time, intraperitoneal administration of m-CPBG in wide dose ranges (0.5, 1.0, 5.0 or 10.0 mg/kg) caused no effect on the body temperature. These findings suggest: (1) the implication of central, rather than peripheral 5-HT$_3$ receptor in thermoregulation; (2) the inability of m-CPBG to permeate through blood-brain barrier in mice. The comparison of brain 5-HT$_3$-induced hypothermic reaction between six inbred mouse strains (DBA/2J, CBA/Lac, C57BL/6, BALB/c, ICR, AKR/J) was performed and two highly sensitive to m-CPBG mouse strains (CBA/Lac and C57BL/6) were found.In aforenamed six inbred mouse strains the functional activity of 5-HT$_{1A}$ receptors was studied. Considerable interstrain differences were found in the intensity of hypothermic response produced by intraperitoneal administration of selective agonist of 5-HT$_{1A}$ receptors – 8-OH-DPAT. The comparison of hypothermic reaction induced by central administration of m-CPBG and hypothermia produced by 8-OH-DPAT revealed significant correlation between 5-HT$_3$ and 5-HT$_{1A}$-induced hypothermia in five out of six investigated inbred mouse strains. The data suggest the association of the brain 5-HT$_3$ and 5-HT$_{1A}$ receptors in the mechanisms of serotonin-conditioned hypothermia.

TVI.52
EVIDENCE FOR REGIONALLY SPECIFIC CHANGES IN HIGH-FREQUENCY OSCILLATIONS AFTER INJECTION OF KETAMINE IN FREELY MOVING RATS

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We have shown previously that the NMDAR antagonists ketamine and MK-801 enhance high-frequency oscillations (140–180 Hz, HFO) in the rat nucleus accumbens (NAc). However, it is not known whether NMDAR antagonists can modify HFO recorded in structures outside the NAc. Thus we have examined the effect of a single subanesthetic dose of ketamine (25 mg/kg) on oscillatory activity in local field potentials recorded in the neuroanatomically related dorsal striatum and in the hippocampus, where spontaneous high-frequency oscillations (ripples) have been well described. We used both monopolar and bipolar recordings to evaluate oscillatory activity recorded at baseline and after injection of ketamine. In monopolar recordings ketamine-induced increases in the power of HFO were present in all structures, although the power was always substantially larger in the NAc. Bipolar recordings, known to remove common-mode input, were used in an attempt to more precisely localise the source of HFO. In all cases ketamine-induced HFO were still present in the signals recorded from the NAc, but not from the dorsal striatum or hippocampus. Notably, spontaneous sharp-wave ripples also remained in the bipolar signal from the hippocampus. In a separate study of the depth-profile analysis of oscillatory activity we found the power of HFO was substantially larger in areas closest to the NAc. These findings suggest that ketamine may produce some regionally specific changes in HFO.

TVI.53
STATE-DEPENDENT MODIFICATION OF HIGH-FREQUENCY OSCILLATIONS IN THE RODENT NUCLEUS ACCUMBENS

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Spontaneous high frequency oscillations (HFO) in the local field potential recorded in the nucleus accumbens (NAc) are typically represented by a small peak in the power spectra in the range of 140–180 Hz. These HFO are known to occur in the awake state, but their distribution over the sleep-wake cycle has not been investigated. To address this issue we firstly examined the power of HFO during periods of quiet waking, slow-wave sleep (SWS) and REM sleep. Since the electrophysiological activity during general anesthesia resembles certain features of naturally occurring SWS we went on to examine the effect of pentobarbital, isoflurane or urethane anesthesia on spontaneous and ketamine-induced increases in HFO. We found that the power of spontaneous HFO decreased significantly during periods of SWS with respect to both quiet waking and REM sleep. General anesthetics also reduced the power of spontaneous HFO recorded in the NAc and completely blocked the ketamine-induced increase. These findings suggest that behavioural states where the generation of mental activity is most intense are associated with the presence of HFO in the NAc. In line with this, states which lead to decreased mentation, such as naturally occurring SWS and general anesthesia are associated with reductions in the power of HFO. Together these findings suggest that the presence of HFO may have behaviourally meaningful consequences.

TVI.54
EFFECT OF CO-TREATMENT WITH FLUOXETINE AND AMANTADINE ON THE BEHAVIORAL AND IMMUNOLOGICAL PARAMETERS OF RATS SUBJECTED TO THE FORCED SWIMMING TEST
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Considerable attention has been paid to a possible role of immunological dysregulation in the pathogenesis of depression. It has been reported that combined administration of antidepressant drugs and the non-competitive NMDA receptor antagonist amantadine (AMA) reduces immobility time in the forced swimming test. Moreover, preliminary clinical data show that such a combination of drugs has beneficial effects in treatment-resistant depressed patients. Since immune activation and a pro-inflammatory response are clearly evident in treatment-resistant depression, the aim of the present study was to examine the effect of an antidepressant combination of FLU and AMA on immune parameters in rats subjected to the forced swimming test. The obtained results revealed synergistic antidepressant effects of combined administration of FLU (10 mg/kg) and AMA (10 mg/kg) – drugs otherwise ineffective when given separately in such doses. The antidepressant activity was accompanied with a reversal of the stress-induced increase in the proliferation of splenocytes in response to concanavalin A, and a significant enhancement in the production of the negative immunoregulator interleukine-10. Moreover, the relative spleen weight in these rats was also reduced after joint administration of FLU with AMA in comparison with rats treated with the vehicle. In summary, the antidepressive efficacy of a combination of FLU and AMA given in suboptimal doses may be related to their negative immune effects.

TVI.55
THE EFFECT OF ANTIDEPRESSANT DRUGS ON DELAYED-TYPE HYPERSENSITIVITY REACTION IN BETA2M-/-, TCRDELTA-/- AND CD1D-/- MICE
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Delayed-type hypersensitivity (DTH) is a T cell-mediated immune reaction that plays a major role in the pathogenesis of various inflammatory disorders. One of the most characteristic DTH phenomena is contact hypersensitivity (CS) used to assess cell-mediated immunity against tumor cells and microbes that survive within macrophages. Recently it was suggested that activation of immune system plays a role in etiology of depression and that antidepressive agents have negative immunoregulatory effects. Our present studies showed that chronic fluoxetine and desipramine administration significantly inhibited CS reaction to picryl chloride (PCL) in B10.PLwild type mice (by 58% and 48%, respectively, when compared to positive control) and their inhibitory effect was even stronger in TCRd knockout mice. On the other hand in b_2m-/- and CD1d-/- mice the prolonged antidepressant administration did not affect CS reaction. PCL sensitization significantly increased spleen weight and proliferative activity of splenocytes in B10PL, TCRd-/- and b_2m-/- mice. Prolonged antidepressant treatment attenuated this effect but only in B10PL mice. Presented data might suggest that inhibitory effect of desipramine and fluoxetine on CS reaction could be caused by their influence on $CD8^+$ and NKT regulatory cells that attenuate T cell-mediated immune response.

TVI.56
EFFECT OF BACTERIAL LIPOPOLYSACCHARIDE AND INTERLEUKINE 6 ON CATALEPSY AND SPONTANEOUS LOCOMOTION IN MICE
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Catalepsy is a state of pronounced motor inhibition and is found in all vertebrates. The exaggerated form of catalepsy is a syndrome of some grave mental disorders in human. The selective breeding for high predisposition to catalepsy showed that the major gene of catalepsy is located on the distal fragment of chromosome 13 and seems to be associatied with the *Il6st* gene coding the gp130 protein, which is the shared protein in the signal transduction from interleukins, participating in the cell differentiation, immune and endocrine regulation. Experiments were carried out on adult C57BL/6J catalepsy-resistant mouse males. Bacterial lipopolysaccharide (LPS; 100, 200 μg/kg, i.p.) significantly depressed all activity in the open-field and it induced cataleptic immobility (more than 20 s three times) in 50% of mice in each group. Interleukine 6 (IL-6; 200ng/mouse, i.p.) did not effect on behavior in open-field, but it induced immobility in 70% of mice. Administration of LPS or IL-6 increases the level of IL-6 which binds to the IL-6 receptor, this reaction results to activation of gp130 protein. So, caleptogenic effect of LPS and IL-6 is in good correlation with association of predisposition to catalepsy with *Il6st* gene. Since depressive-like behavior and immunity disturbances were found in mice selected for catalepsy and LPS treatment could model depression, gp130 protein seems to be involved in regulation of both depression and catalepsy.

TVI.57
STUDY ON THE POTENTIAL EFFECTS OF FETAL EXPOSURE TO DICLOFENAC SODIUM ON BRAIN MORPHOLOGY AND BEHAVIOR IN YOUNG WISTAR RATS
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Diclofenac sodium (DS) acts as a potent cyclooxygenase inhibitor, reducing arachidonic acid release and prostaglandins formation. It is commonly used as a non-steroid anti-inflammatory drug and the potential adverse effects of its administration during pregnancy are of medical and public concern. In the present study, the

DS in a dose of 1 mg/kg/day was subcutaneously injected to the pregnant Wistar rats throughout 5–20 gestational days. One group of 4-week old pups was sacrificed and the counts of principal neurons in cerebellum and hippocampus were done using stereological methods. Another group of pups was subjected to a battery of behavioral tasks. A significant decrease in the total number of Purkinje cells but not CA pyramidal neurons was observed in the drug-treated juvenile pups. No substantial between-group differences were found in most of the applied behavioral tasks including plus maze anxiety test, and learning/memory tasks such as 12-arm radial maze and the Morris water maze, the latter run both under allo- and idiothetic stimulus conditions. Only in the Open Field test, fetal DS male but not female pups showed slower rate of habituation compared to controls. The obtained results indicated that the moderate doses of DS administered between 5–20 gestation days in rats equivalent of the second half of the 1st and the whole 2nd trimester in human) resulted in some neuronal losses which, however, had no adverse behavioral impact.

TVI.58
(+)-UH 232, A PARTIAL AGONIST OF THE D_3 DOPAMINE RECEPTORS, ATTENUATES COGNITIVE EFFECTS OF ANGIOTENSIN IV AND des-Phe^6-ANGIOTENSIN IV IN RATS
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We have recently found that the postsynaptic D_3 dopamine (DA) receptors appear not to participate in the memory enhancing effects of the angiotensin AT_4 receptor agonists angiotensin IV (Ang IV) and des-Phe^6-Ang IV. In this study we evaluated role of the presynaptic DA D_3 receptors in these effects. For that purpose effects of (+)-UH 232, a selective D_3 DA receptors partial agonist preferring presynaptic sites, on the pro-cognitive action of intracerebroventricularly (icv) injected Ang IV and des-Phe^6-Ang IV were examined. Male Wistar rats weighing 180–200 g were used. Both peptides given at the dose of 1 nmol facilitated recall of a passive avoidance (PA) behaviour, improved object recognition (OR), and increased apomorphine-induced stereotype behaviour. In the auxiliary tests performed to control for the unspecific influence of motor (open field, OF) and emotional ("plus" maze, PM) effects of our treatments on the results of memory tests they had either no (OF) or negligible (PM) effects. Intraperitoneal pre-treatment of the animals with an ineffective on its own dose (1 mg/kg) of (+)-UH 232 abolished or markedly diminished effects of both peptides on PA and OR but did not influence enhancement of stereotypy caused by the peptides. These results indicate that the functional presynaptic DA D_3 receptors are necessary for the pro-cognitive effects of Ang IV to occur.

TVI.59
THE GENETIC BACKGROUND IS RESPONSIBLE FOR RESPONSE TO NATURAL REWARD
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The increased inclination to natural rewards like food and sensation seeking have been associated with elevated drug intake in humans. We aimed to compare four inbred mouse strains with known different susceptibility to drug addiction in respect to response to natural rewards. In this study, we investigated four inbred strains C57Bl/6J, DBA/2J, SWR/J and 129P3/J in the self-administration of food (SAF) and operant sensation seeking (OSS) models in three paradigms: (1) the fixed ratio operant task (FR), (2) the progressive ratio operant task and (3) the concurrent choice operant task. No difference in response to reward in SAF FR paradigm was found among the studied strains. In contrast C57Bl/6J mice performed better then DBA/2J and 129P3/J strains in OSS paradigm while SWR mice did not react at all. In turn, the highest score of correct choice in SAF was observed in 129P3/J mice. The systemic administration of dopamine D1 receptor antagonist SCH 23390 or opioid receptor antagonists (naloxone or naltrexone) reduced the response to reward in the C57Bl/6J and DBA/2J mice. Our data suggest that genotype background is responsible for reward-seeking in SAF and OSS models with C57Bl/6J mice being the most responsive to the reward. We observed also that food as a reward has different motivational value than sensation seeking. Also our study indicates that opioid and dopaminergic systems may be involved in the response to natural rewards.

TVII: Homeostatic and Neuroendocrine Systems

TVII.01
BRAIN RESPONSE TO PERIPHERAL TUMORS IN RATS – THE STUDY OF NEUROBIOLOGY OF CANCER
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A significant amount of data has emerged supporting the hypothesis that the central nervous system might monitor and modulate tumor growth. The aim of our study was to investigate whether intraperitoneal tumor growth may induce detectable changes in brain structures that are involved in the response to immune challenges. Using Fos immunohistochemistry we investigated the effect of a tumor induced by a single intraperitoneal injection of BP6-TU2 fibrosarcoma cells to male Wistar rats on the activity of selected brain structures. Twenty eight days after the tumor cells administration we found an increased Fos expression in the nucleus tractus solitarii/A2, A1 noradrenergic cells, parabrachial nucleus as well as in the hypothalamic paraventricular, supraoptic and accessory neurons. These structures are involved in the processing of signals related to immune challenges and consequent elaboration of neuro-endocrine responses. Obtained data supports the view that the signals related to peripheral tumor development might be transmitted to the brain. Further studies are necessary to reveal whether our findings can be attributed to a specific effect of cancer or whether observed changes in the activity of neuronal structures reflex processes that only accompany the cancer progression.

TVII.02
HYPOTHALAMIC CRH AND VASOPRESSIN EXPRESSION IN ANIMAL MODELS OF DEPRESSION AND ANXIETY
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Hypersecretion of the hypothalamic corticotropin-releasing hormone (CRH), the main neuroendocrine hypothalamic-pituitary-adrenal (HPA) axis stimulator, is supposed to be the reason of HPA axis abnormalities observed in depression and anxiety. It was also suggested that vasopressin as the major CRH secretagogue might be involved in HPA axis hyperactivity described in depression. But neuroendocrine mechanisms of depression and anxiety are still not revealed. The aim of the present study was to elucidate the role of hypothalamic CRH- and vasopressin-producing centres in the development of depressive- and anxiety-like states in rats. Animal models have been applied, including the "learned helplessness" as a model of depression and Time-dependent sensitization paradigm as a model of anxiety disorder (posttraumatic stress disorder, PTSD, in particular). The depressive- and anxiety-like states in rats are characterized by significant increase of CRH-immunoreactivity in the parvocellular division of the paraventricular nucleus (PVN). Overexpression of CRH in the magnocellular PVN is involved in the development of anxiety- but not depressive-like state. Anxiety-like state in rats is accompanied by increase of vasopressin expression in the magnocellular part of the PVN, suggesting its implication in the pathogenesis of PTSD. Our data give evidence of common and specific mechanisms of depression and anxiety and might be useful in working out new approaches in treatment of these disorders.

TVII.03
STRESS-INDUCED CHANGES IN GENE EXPRESSION OF CATECHOLAMINE ENZYMES AND ADRENOCEPTORS IN HEART OF SHAM-OPERATED AND PVN-DEAFFERENTATED RATS
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The paraventricular nucleus of the hypothalamus (PVN) regulates neuroendocrine, autonomic and cardiovascular responses to stress. Stressors activate catacholaminergic systems which consequently modulates activity of organism *via* adrenergic receptors (ARs). Our work was focused on investigation of changes in gene expression of epinephrine-synthesizing enzyme phenylethanolamine-N-methyltransferase (PNMT), neuropeptide Y (NPY) and adrenoceptors in the heart of sham-operated and PVN-deafferentated rats exposed to immobilization stress (IMO). PNMT and NPY mRNA levels in the heart of sham-operated rats were not significantly increased after a single IMO. However, posterolateral deafferentation of the PVN reduced PNMT mRNA, whereas levels of NPY mRNA were elevated. These data suggest a stimulatory role of PVN on PNMT and an inhibitory role on NPY gene expression. Moreover, β1-AR mRNA levels were significantly reduced in heart of PVN-deafferentated animals comparing to sham-operated rats. On other side, after single IMO β1-AR mRNA levels were significantly increased in PVN-deafferentated rats. Gene expression of other ARs (β2, β3, α1B) in hearts of stressed PVN-deafferentated animals were decreased. The found down-regulation of gene expression of ARs might suggest a rise in sympathetic activity induced by PVN deafferentation. Our data suggest that PVN plays an important role in stress-induced activation of cardiac sympathetic system.

TVII.04
PROPHYLACTIC USE OF DESIPRAMINE IN WISTAR RATS WITH HIGH STRESS-SENSITIVITY INCREASES RESISTANCE TO EXPERIMENTAL TUMOR METASTASIS
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The effect of antidepressant drugs on tumour progress in animals models of stress is very poorly recognized. Further more a role of susceptibility to stress in modulatory effect of antidepressant drug on tumour growth have not been studied. The aim of present study was to establish the effect of individual reactivity to stress and prophylactic two weeks desipramine administration on metastatic colonization of MADB 106 cells in lungs of Wistar rats. Rats were subjected by three weeks to chronic mild stress (CMS) model of depression and high-reactive and non-reactive rats were selected. Three months after CMS termination high-reactive and non-reactive animals were further subdivided: for two additional weeks the rats received daily injection of desipramine or saline. Tumor cells were injected two hours after last desipramine or saline administration. In stress-reactive, vehicle treated rats increase of number of lung metastasis in comparison to stress non-reactive, vehicle treated rats were observed. On the other hand chronic desipramine pretreatment significantly increased survival rate and diminished number of lung metastasis in stress-sensitive animals although did not show such effect in stress-resistant animals. Increase in rate of animal survival and decrease in lung metastasis can be connected with stimulatory effect of chronic desipramine pretreatment on NK cell activity. *This study was supported by grant N40109732/2074 from Poland's MS and HE.*

TVII.05
STRESS INDUCES CHANGES OF CATECHOLAMINERGIC SYSTEM IN THE RAT SPLEEN
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There is evidence that stress-induced activation of sympathoadrenal system (SAS) affects the function of immune system. The aim of our work was to investigate catecholamine (CA) levels, gene expression of CA biosynthetic enzymes and adrenoceptors (ARs) in the spleen of rats exposed to single and repeated (14×) immobilization stress (IMO). Acute IMO did not affect adrenaline or noradrenaline (NA) levels in the spleen. Repeated IMO induced a rise in both CAs suggesting increased CA biosynthesis. This stimulus was associated with the increase in tyrosine hydroxylase (TH) protein, but with un-

detectable TH mRNA levels. That indicates predominantly neuronal origin of this enzyme. Detection of dopamine-β-hydroxylase and phenylethanolamine-N-methyltransferase gene expression points to their local production and responsiveness to IMO. Stress differently affected individual types of splenic ARs. Exposure to acute and chronic IMO increased β_2-AR mRNA, which is the major immunomodulatory AR. Gene expression of α_{2A}- and α_{2C}-AR, the main NA autoregulatory ARs, was reduced. As a consequence, NA level increased. Thus, exposure to stress produces the rise of CAs in the spleen. The elevation of β_2-AR and a drop in α_2-ARs mRNA, indicate their predominant function in immune system regulation, most probably cytokine production *via* β_2-AR and CA turnover *via* α_2-AR uptake as well as *via* CA biosynthetic enzymes activity. However, the physiological role of this observation needs further investigation.

TVII.06

THE INFLUENCE OF AGING ON THE POPULATION OF NGF CONTAINING NEURONS OF LIMBIC SYSTEM IN THE RAT FOLLOWING OPEN FIELD STIMULATION

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NGF (nerve growth factor) is involved not only in growth and survival of neurons but also promotes their age-dependent morphological changes (repair and remodeling) in normal life and during stress. This study aimed to investigate an influence of ages, on the changes of NGF immunoreactive (-ir) cells in the: amygdala, hippocampus and hypothalamus caused by acute (one-time for 15 min) or repeated (21 days for 15 min daily) exposition to open field (OF) test. Each group of age consisted of experimental and control (non-stressed) Wistar male rats. To detected NGF-ir cells single immunofluorescence staining was applied. Each control groups revealed many of NGF-ir neurons in the studied structures. Following OF acute stimulation, the number of NGF-ir cells in all the studied structures was higher in the three months old rats than that of control ones; the level of NGF-ir cells in the one year old rats was higher only in paraventricular nucleus of hypothalamus and in central nucleus of amygdala. In two years old rats no changes was observed in comparison with control animals. After OF repeated exposition, the level of NGF-ir cells was similar to that observed under acute one. These data demonstrated that the aging affected the level of NGF-ir neurons caused by acute and repeated OF stimulation in the structures of limbic system. Stress duration did not influence the level of NGF-ir neurons.

TVII.07

DEHYDROEPIANDROSTERONE SULPHATE (DHEAS) IS REWARDING AND ALTERS THE REWARDING EFFECT OF COCAINE IN THE CONDITIONED PLACE PREFERENCE PROCEDURE (CPP)

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DHEAS is endogenous allosteric antagonists of the GABA(A) receptor and has other direct and indirect actions in CNS. It is metabolized to other neuroactive steroids. Prior studies revealed an ambiguous role of DHEA(S) in cocaine dependence, suggesting interactions with cocaine effects in the brain. This preclinical study was undertaken to evaluate direct influence of DHEAS on the rewarding effects of cocaine, using the CPP test in rats. Male Wistar rats (250–300 g) were used. The conditioned place preference experiment consisted of preconditioning conditioning and post-conditioning phases. Drug injections were done i.p. only during the conditioning phase. Cocaine had a biphasic dose-dependent rewarding effect (inverted U type). The doses 5 and 10 mg/kg were clearly rewarding, as they increased rats' preference for cocaine injection site, while at the dose 20 mg/kg, cocaine rewarding effect was lost. DHEAS alone had a similar biphasic rewarding effect (the effect of 40 mg/kg was maximal and statistically significant). Pretreatment of rats with 40 mg/kg of DHEAS before injection of cocaine noticeably changed its rewarding effect, shifting cocaine dose response curve to the left. DHEAS, *per se* has rewarding properties and it enhances the rewarding effects of low doses of cocaine, but decreases the rewarding effect of higher doses of cocaine. *Funded by EC grant MEXC-CT-2006-42371 to M.D. Majewska.*

TVII.08

ANDROSTERONE HAS REWARDING AND AVERSIVE PROSPERITIES AND ALTERS THE REWARDING EFFECT OF COCAINE IN THE CONDITIONED PLACE PREFERENCE PROCEDURE (CPP)

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Our previous study showed that the neurosteroid dehydroepiandrosterone sulfate (DHEAS) alters the rewarding effects of cocaine. Because DHEAS is metabolized to androsterone, which has opposite synaptic activity, we wondered about contribution of androsterone to the above phenomenon. Here we examined androsterone's effect on the rewarding properties of cocaine in CPP test. Male Wistar rats (250–300 g) were used. The CPP procedure consisted of pre-conditioning, conditioning and post-conditioning phases. Drug injections were done i.p. Androsterone alone was rewarding at doses 1 mg/kg and 10 mg/kg, while the dose of 40mg/kg was visibly aversive. Cocaine had a biphasic dose-dependent rewarding effect (inverted U type): the doses 5 and 10 mg/kg were rewarding, while at dose 20 mg/kg, cocaine rewarding effect was lost. Pretreatment with 1mg/kg of androsterone increased the rewarding effect of all cocaine doses, especially the dose of 20 mg/kg, but the rewarding dose of 10 mg/kg of androsterone had no effect on the rewarding prosperities of cocaine. All doses of cocaine decreased the aversive effect of 40 mg/kg of androsterone. Androsterone *per se* has a biphasic rewarding-aversive effects in the CPP test and influences the rewarding properties of cocaine, which might be explained by two mechanisms: potentiation of reward at low doses and reduction of the aversive effect of the high doses. *Funded by EC grant MEXC-CT-2006-42371 to M.D. Majewska.*

TVII.09
INTERACTIONS OF NEUROSTEROIDS – DEHYDROEPIANDROSTERONE SULPHATE (DHEAS) AND ANDROSTERONE - WITH COCAINE IN BEHAVIOURAL DESPAIR TEST (PORSOLT) IN RATS
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Previous studies showed that high plasma levels of endogenous DHEAS correlated with resiliency and ability of cocaine addicts to maintain prolonged abstinence after the therapy (Wilkins et al. 2005), while addicts treated with high dose of exogenous DHEA used more cocaine that those administered placebo (Shoptaw et al. 2004). Such ambiguous outcomes could be due to mixed actions of DHEAS and its metabolite, androsterone, which have opposite neuronal activities. Here we examined acute effects of both neurosteroids in Porsolt test and their interactions with 5 mg/kg of cocaine – the dose reported to have antidepressive action, but which does not influence locomotor activity in the open field. Wistar rats (250-300 g) were used. All substances were injected i.p.; steroids 30 min and cocaine 5 minutes before the test, which measures locomotor activity of rats in narrow water containers. Cocaine at dose 5 mg/kg significantly increased rats' locomotor activity in the Porsolt test. Neither DHEAS (1, 5, 10, 40, 80 mg/kg) nor androsterone (0,1, 1, 10, 40 mg/kg) *per se* had any effect on activity in this test. DHEAS dose-dependently potentiated the effect of cocaine, but androsterone had no influence on this cocaine action. Acutely administered DHEAS appears to increase antidepressive effects of cocaine, but androsterone has no such influence. *Funded by EC grant MEXC-CT-2006-42371 to M.D. Majewska*.

TVII.10
ROLE OF THE AFFERENT VAGAL PATHWAY IN MODELING OF CARDIO-RESPIRATORY RESPONSE TO ARVANIL IN ANAESTHETIZED RATS
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Arvanil is metabolically stable hybrid between anandamide and capsaicin and an agonist of cannabinoid CB1 and vanilloid VR1 receptors. Arvanil is able to alleviate hyperkinesia typical in rat model of Huntington's disease, spasticity, pain, tremor and other signs of disease in rat model of multiple sclerosis. The drug reveals anti-tumour and anti-inflammatory action. The present study was designed to test the role of the vagal pathway in post-arvanil cardio-respiratory response. Cardio-respiratory effects of an intravenous injection of arvanil were investigated in 21 urethane-chloralose anaesthetised and spontaneously breathing rats. Bolus injection of 0.8 mg kg^{-1} of arvanil into the right femoral vein induced in all neurally intact rats a significant increase of tidal volume (V_T) and diaphragm activity as well as hypertension coupled with fall in respiratory rate (f). Bilateral midcervical vagotomy precluded the alteration of respiratory parameters without any changes in cardiovascular effects. Arvanil-induced increase in mean arterial blood pressure (MAP) still persisted even after supranodose vagotomy. Results indicated that the respiratory effects evoked by arvanil administered *via* the peripheral circulation require intact midcervical vagi. Supranodose vagotomy failed to eliminate the hypertension evoked by arvanil.

TVII.11
ROLE OF CHEMOSENSITIVE RTN NEURONS IN MEDITATING RESPIRATORY ACTIVATION AFTER HYPOTHALAMIC STIMULATION
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Stimulation of the dorsomedial nucleus (DMH) or the perifornical area (PeF) of the hypothalamus activates breathing. The descending neuronal pathways that mediate this response are unknown. In the present experiments we test the possibility that this pathway includes a relay in the retrotrapezoid nucleus (RTN). The RTN resides at the rostral end of the medulla oblongata and consists of intrinsically pH-sensitive glutamatergic neurons that are proposed to contribute to CO2 stabilization by regulating the activity of the Central Respiratory Pattern Generator (CPG). The experiments were done in adult isoflurane-anesthetized rats. Stimulation of DMH/PeF with the selective $GABA_A$ receptor antagonist gabazine increased phrenic nerve activity and the discharge rate of simultaneously recorded RTN units. This upregulation was observed at all levels of end-expiratory CO2. After silencing the CPG with intravenous morphine (10–15 mg/kg), hypothalamic stimulation was still capable of enhancing RTN activity. In addition, both DMH/PeF stimulation or hypercapnia greatly increased the number of RTN neurons that expressed Fos when compared to rats maintained under control hypocapnic conditions. We conclude that the DMH/PeF region of the hypothalamus controls respiration at least in part by regulating the activity of the retrotrapezoid nucleus. *Support: HL74011, HL 28785.*

TVII.12
BINGE ALCOHOL EXPOSURE DURING PUBERTY INCREASES THE EXPRESSION OF GENES INVOLVED IN STRESS RESPONSES
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Teenage alcohol abuse is a fundamental health concern and it can have permanent effects on brain function. We identified the effects of adolescent binge exposure on regulators of stress and anxiety responses: corticotrophin-releasing hormone (CRH) and arginine vasopressin (AVP). We hypothesized that binge ethanol exposure during puberty increases the expression of these genes in the paraventricular nucleus (PVN) of the hypothalamus. Animals wew handled daily for 7 days and then divided into 4 groups (n=6/8): (1) untreated, (2) intraperitoneal (i.p.) saline injection (for 8 days), (3) acute ethanol exposure (saline for 7 days and ethanol (3 g/kg) on the last day) and (4) binge ethanol exposure (3 days of ip ethanol injection, 2 days of saline and 3 days of ethanol). Treatments started at PND37 and animals were sacrificed at PND44 1 hour after the last injection; trunk blood was collected, brains removed, and rapidly frozen. Blood alcohol level reached 153 ± 12 mg/dl in males and 187 ± 23 mg/dl in females. Both acute and binge alcohol treatments increased CORT levels. Brains were sectioned at 200 µm on a freezing microtome and the PVN was microdissected. Total RNA

was isolated from the PVN and quantitative real time RT-PCR was performed. In the PVN binge ethanol exposure increased CRH and AVP gene expression ($P<0.05$) suggesting that it might have profound effects on the development of the hypothalamo-pituitary-adrenal axis and could increase the risk of adult anxiety disorders.

TVII.13
MATERNAL SEPARATION AFFECTS TYROSINE HYDROXYLASE AND NCAM EXPRESSION IN DOPAMINERGIC STRUCTURES OF JUVENILE, ADOLESCENT AND ADULT RAT BRAIN
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Stressful experiences in early life can influence brain development and increase the risk for mental disorders. However the specific impact of early life stress on maturation of dopaminergic (DA) system is still unknown. We applied maternal separation (MS) paradigm in rats, on postnatal (P) days 1–14, to investigate its impact on expression of tyrosine hydroxylase (TH) and NCAM proteins in DA structures of juvenile (P15), adolescent (P35) and adult (P70) rat brain. On P15 MS decreased the number of TH+ neurons in the substantia nigra (SN) pars compacta of females as well as in the SN pars reticulata (SNr) and the ventral tegmental area (VTA) of both females and males. The effect persisted until P35 only in the VTA of MS females. Oppositely on P70, an increase in the number of TH+ neurons was observed in the VTA of females. Additionally on P35 a transient rise in TH+ cells was detected in the SNr of MS females. MS did not change the expression of TH in the striatum, nucleus accumbens and medial prefrontal cortex (mPFC). On P15 a decrease in the level of 140 kDa NCAM was observed in the SN and striatum of MS females and a decrease in 180 kDa NCAM in the VTA of MS males. On P35 MS diminished the expression of 180 kDa NCAM in the SN and mPFC of females. Finally, on P70 a decrease in 180 kDa NCAM was observed in the mPFC of MS males. The results of the present study strongly indicate that early life stress can affect maturation and plasticity of DA system at different life stages.

TVII.14
COMBINED ACTION OF VIBRATION AND LOW-INTENSITY ELECTROMAGNETIC RADIATION ON SPIKE ACTIVITY OF SUPRAOPTIC CELLS
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Spontaneous spike activity of supraoptic cells of hypothalamus after 30-day-long exposure of low-intensity extremely-high frequency radiation (42.2 GHz, 0.19 mW/cm^2, daily for an hour) on vibration-stimulated rats (60 Hz, 0.4 mm, 2 hours daily) was analyzed. The distributions of registered cells by the degree of regularity of interspike intervals (ISI), dynamics of succession of ISI and discrete frequency ranges, as well as the mean spike frequency and the coefficient of variation of ISIs in naive control and after treatment were evaluated. 30-day-long exposure was followed by significant ($P<0.05$/$P<0.01$) shifts in distribution by degree of regularity and dynamics of succession of ISI. These changes were significant in comparison with isolated influence of vibration. The significant decrease of mean spike frequency up to 13.5 ± 1.7 imp/s ($P<0.01$) was revealed. It was accompanied by reliable ($P<0.01$) abrupt increase of quantity of low-frequency cells as well as decrease of numbers of high- and medium-frequency units. These changes were statistically insignificant in comparison with data from isolated action of vibration. The coefficient of variation of ISIs after treatment was increased up to 105.3 ± 4.3% ($P<0.05$). Thus, we suppose that the effect of low-intensity extremely-high frequency radiation depends on initial state of organism.

TVII.15
TIME-DEPENDENT EFFECTS OF SWIM STRESS ON EARLY-LTP IN THE HIPPOCAMPAL CA1 IN FREELY MOVING RATS
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Stress has been well-documented to affect hippocampal long-term potentiation (LTP), which is widely believed to underlie learning and memory at the cellular level. However, only few reports addressed the issue regarding the timing of presenting a stress episode in relation to LTP induction. Previously we could show that in the dentate gyrus a protein synthesis-independent early-LTP can be reinforced by swim stress into a protein synthesis-dependent late-LTP when the stress episode was applied within a time window of about 30 min around tetanization (Korz and Frey 2003). Considering the important role that the hippocampal CA1 plays in the processing of spatial and temporal information, we were now interested in studying the influence of swim stress on functional plasticity events in the CA1 region. Field potentials were recorded in freely moving rats subjected to a brief episode of swim 15 min before or after early-LTP induction in the CA1 by stimulating the contralateral CA3. Our results revealed that a swim episode alone exerted a transient depressing effect on baseline values of recorded field potentials. Swim stress presented shortly after the induction of early-LTP resulted in its depotentiation. Furthermore, if the same stress protocol preceded early-LTP induction, it prevented the potentiation and induced a long-lasting depression of field potentials. These data suggest that swim stress differentially alters synaptic plasticity in the CA1 when compared with the dentate gyrus.

TVII.16
c-Fos, 5HT AND CRF IMMUNOSTAINING OF THE PREFRONTAL CORTEX AREAS THE IN THE RATS DIFFERING IN THE STRENGTH OF A FEAR RESPONSE
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The aim of the study was to examine the neurochemical background of differences in the individual responses to conditioned aversive stimuli, using the strength of a rat conditioned freezing response (the contextual fear test), as a discriminating variable. It was shown that high responder (HR), i.e. rats with duration of a freezing response one standard error, or more, above the mean value, had a higher c-Fos activity in the FrA and PrL prefrontal cortical areas, and stronger 5HT immunostaining in the FrA. However, these animals had lower CRF immunostaining in the

same cortical areas in comparison with low responder (LR), i.e. rats with the duration of a freezing response one standard error, or more, below the mean value. The LR group vocalized more during test session in the aversive band, and had higher serum levels of corticosterone, examined 10 min after test session. It was shown that different natural patterns of responding to conditioned aversive stimuli are associated with different expression of CRF and serotonergic- innervation of prefrontal cortical areas.

TVII.17
THE INFLUENCE OF CRF AND α-HELICAL $CRF_{(9-41)}$ ON RAT FEAR RESPONSES AND AMINO ACIDS CONCENTRATION IN THE CENTRAL NUCLEUS OF AMYGDALA

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In the previous study we showed that exogenous CRF (1 μg/rat, icv) enhanced rat-freezing responses and increased expression of CRF-related immunoreactive complexes in the amygdala. On the other hand, a non-selective CRF receptor antagonist, α-helical $CRF_{(9-41)}$ (10 μg/rat, i.c.v.) decreased rat fear response and increased the concentration of GABA in the amygdala (*in vitro*). The aim of the present study was to examine the effects of CRF and α-helical $CRF_{(9-41)}$, on the freezing reaction in the conditioned fear test and the release of amino acids in the central nucleus of amygdala (CeA), using microdialysis technique. It was found that CRF increased the concentration of aspartate, glutamate and Glu/GABA ratio in the CeA, an effect that preceded an increased expression of anxiety-like responses, which appeared 15 min after drug administration. α-helical $CRF_{(9-41)}$, decreased rat freezing responses and increased the local concentration of GABA, during the first 30 min of observation. The present data show an important role of CRF in the central nucleus of the amygdala in the integration of anxiety-related, biochemical and behavioural responses, and suggest an involvement of amino acids innervation of the CeA, in the effects of this neurohormone.

TVII.18
THE POWER OF FREQUENCY BANDS IN ELECTRICAL ACTIVITY OF LIMBIC STRUCTURES OF THE ROMAN HIGH AND LOW AVOIDANCE RATS DIFFERS IN STRESS-DEPENDENT MANNER

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The psychogenetically selected Roman High (RHA/Verh) and Roman Low (RLA/Verh) Avoidance rats constitute a widely accepted model of diverse emotional reactivity. They show divergent stress-related behavioral and neuronal responses when confronted with a novel and/or stressogenic environment (Meyza et al. 2009). Here, we have explored the possibility that the difference might be also reflected in the electrical activity of prefrontal cortex (mPFC), amygdala (Amy) and hippocampus (CA1) – structures involved in generation of emotions. Spectral analysis of telemetrically recorded local field potentials (LFPs) from mPFC, Amy and CA1 in freely moving rats performing behavioral tests differing with aversiveness (Open Field, Elevated Plus Maze, Hole Board test and Acute Restraint) showed that power of 4 distinct frequency bands (delta: 0–3 Hz, theta: 4–12 Hz, beta: 13–30 Hz and gamma: 31–90 Hz) is higher in the LFPs of RLA/Verh than of RHA/Verh rats, especially in the CA1 and mPFC. This difference seems to be stress-dependent (among tests measuring spontaneous behavior). Acute restraint elicited differences only in the delta and gamma bands in the mPFC. Strikingly, the difference is most clear while the animal is performing a decisive/risk assesment behavior (e.g. leaning towards aversive part of the arena). Moreover, individual differences in the power of bands can be observed at that time, thus they may be considered a neuronal correlate of individual differences in emotional reactivity.

TVII.19
SEROTONIN AND CORTICOSTERONE – NEUROCHEMICAL INDICATORS OF STRESS

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The anxiety tests are widely used to study behaviour of rats in the stress-inducing situation. The study presents a comparative analysis of the influence of different stressogenic stimuli on the serotonergic activity in the emotional brain regions and on plasma corticosterone concentration in rats. The animals were subject to two various behavioral tests, and subsequently the concentration of serotonin (5-HT), its metabolite 5 – hydroxyindoleacetic acid (5-HIAA) and of plasma corticosterone concentration were determined using high-performance liquid chromatography with electrochemical detection (HPLC-ED). Stressogenic stimuli in the tests performed were: light of high volume (light dark transition test – LDT) or open field (open field test – OF). HPLC-ED analysis showed a significant increased of 5-HT level in the amygdala (AM) after using both tests. HPLC-ED analysis showed also an increased of 5-HIAA concentration in the frontal cortex (CTX), in the hippocampus (HI), in the AM after using the LDT-test and an increased of 5-HIAA concentration in the CTX and in the AM after using OF-test. Simultaneously, the analysis showed elevation of corticosterone concentration in rats exposed to LDT and OF. These results confirm that serotonin and coricosterone participate in the regulation of emotional response and they are important neurochemical indicators of stress.

TVII.20
THE ROLE OF CLONIDINE IN BEHAVIORAL AND CORTICOSTERONE RESPONSES TO SOCIAL COMPETITION

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It is postulated that the noradrenergic brain neuronal activity is closely related to the control of the hypothalamo-pituitary-adrenocortical (HPA) system. Our previous observations showed that the brain noradrenergic system is implicated in the control of social position studied in social competition tests. In order to obtain some further evidence speaking for a specific role of noradrenaline system activity in the control of social position we studied the effects of administration of the α2-adrenoceptor agonist clonidine on social behavior analyzed in the social competition test. Simultaneously, the concentration of corticosterone (RIA) was measured in the plasma of rats after social competition test and tested individually. We used an acute social competition situation that consisted

of simultaneous placing of two experimental rats into the familiar cage supplied with feeder of glucose pellets. The obtained data revealed that social competitive behavior was affected by peripherally administered clonidine. The animals got better position for competition and received higher social rank in pairs after stimulation of α2-drenoceptor. Plasma corticosterone concentration was elevated in rats injected peripherally with clonidine in comparison with individually tested rats. The obtained results suggest that stimulation of α2-adrenoceptor affects HPA system activity and may facilitate appearance of tendency to social confrontations in rats.

TVII.21
THE EFFECT OF THE AUTONOMIC NERVOUS SYSTEM ON THE SURVIVAL OF RATS IMPLANTED WITH YOSHIDA ASCITES TUMOR CELLS

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Even if it is suggested that stress does not directly induce cancer there is plenty of evidence that shows that stress represents an important factor facilitating cancer progression, however the exact mechanisms and pathways are not known in details. Because the autonomic nervous system plays an important role in the elaboration of stress response, we investigated the effect of the disruption of the sympathetic or parasympathetic system on the survival of tumor bearing rats. We used male Wistar rats in which we performed either chemical sympathectomy induced by intraperitoneal application of 6-OH dopamine or subdiaphragmatic vagotomy by surgical dissection of the vagus nerve. After a regeneration period we administered intraperitoneally to sympathectomized, vagotomized and sham operated rats Yoshida ascites cells. We have found that whereas chemical sympathectomy significantly reduced the survival of tumor bearing rats, subdiaphragmatic vagotomy had only a slight effect on reducing the survival of rats implanted by Yoshida cells. Our findings suggest that the autonomic nervous system, especially its sympathetic division, plays an important role in the regulation of the development of Yoshida ascites tumor cells in rats. We suggest that whereas long lasting sympathetic activation as a consequence of exposure to chronic stress might have a promoting effect on cancer growth, the sympathetic system might have, during basal conditions, a modulatory effect on tumor progression.

TVII.22
RELAXIN-3 INNERVATION OF THE INTERGENICULATE LEAFLET IN THE RAT

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The intergeniculate leaflet of the thalamus (IGL), a subdivision of the lateral geniculate body, is a component of the biological clock, responsible for, amongst other things, the synchronizing effect of timed caloric restriction on locomotor activity in rodents. The IGL has a distinct functional and anatomical profile amongst thalamic nuclei, including strong peptidergic inputs (e.g. orexin and PACAP). Recently a dense network of terminals immunoreactive for relaxin-3 (RLX3) was identified in IGL. RLX3 is a newly discovered neuropeptide expressed in high levels in brain. The physiological actions of RLX3 are largely unknown, but recent studies have suggested influences on feeding, stress responses and spatial memory. The majority of RLX3 neurons are located in the nucleus incertus (NI), but prior anatomical studies have excluded the NI as a source of the RLX3 in the IGL. In this study, we used neural tract-tracing and immunohistochemistry to demonstrate that the major source of RLX3 fibers within IGL originates from RLX3 neuron populations in the periventricular- and periaqueductal gray. Furthermore, some of these IGL-projecting RLX3 neurons express corticotropin-releasing factor receptors, indicating that the RLX3 innervation may transmit stress-related responses to the IGL. Since stress is one of the non-photic factors capable of synchronizing behavioural rhythmicity, our data suggest that RLX3 might be another key element in the complex mammalian circadian system.

TVII.23
ANALYSIS OF THE INTENSITY OF ANXIETY RESPONSE MEASURED WITH ELEVATED PLUS-MAZE TEST

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Anxiety plays undoubtedly important role in the life of the individual units and leads to the search for better mechanisms for adaptation engaged in mobilizing for action. An important role in the regulation mechanisms for the escape and providing increased activity of the organisms plays catecholamines. The neurochemical background of anxiety is increase of concentration of noradrenaline and dopamine in the emotional structures of the brain. In the present work we used Elevated Plus-Maze test (EPM test). The EPM test is widely used to study behavior of rodents for exploration and emotionality. It was made an analysis of changes in concentration of noradreneline (NA), dopamine (DA) and their metabolites in selected brain regions in rats after the EPM. HPLC analysis showed elevation of MHPG level in the hypothalamus (HPT) and DOPAC in the frontal cortex (CTX) of rats. These results indicate stimulation of the dopaminergic system activity in the CTX with simultaneous increase of the noradrenergic system activity in the HPT.

TVII.24
EFFECTS OF MATERNAL DEPRIVATION ON BEHAVIOR AND BRAIN DEVELOPMENT IN ADULT LIFE OF MICE

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Maternal deprivation model was used for studying the influence of perinatal stress on the behavior of adult mice. Swiss mice pups were separated from their mothers for two hours daily from postnatal day 1 (P1) to P7. One group of pups has been treated with buspirone immediately before each period of maternal separation (P1–P7). At the age of 1–3 months mice were subjected to a battery of behavioral tests. Then they were sacrificed, their brains were removed and used for immunohistochemistry staining or immunoblotting (Western blot) with antibodies directed against glucocorticoid or mineralocorticoid

receptors. We found that in the open field test and the plus maze test the stressed mice showed greater anxiety than non-stressed animals. Anxiety in stressed mice treated with buspirone was lower than in stressed only mice. Next, mice were tested either in a water maze test or an object recognition test where their memory and learning performance was assessed. We did not find any significant differences between groups in these two tests. These results demonstrate that stressing early life events, like maternal separation, permanently disturb emotionality of the adult mice but do not affect their learning or memory functions. Deleterious effects of stress may be prevented by pharmacological treatment. *Supported by the Polish Ministry of Science and Higher Education grant No 3757/B/P01/2007/33.*

TVII.25
ELECTRICAL STIMULATION OF THE MEDIAL SEPTAL NUCLEUS INCREASES CYTOTOXIC ACTIVITY OF NK CELLS IN THE SPLEEN AND PERIPHERAL BLOOD IN RATS
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The medial septal nucleus (MS) is a forebrain limbic structure involved in learning and memory mechanisms. In previous study we found that electrolytic lesion of the medial septal nucleus caused depression of the peripheral blood natural killer cell cytotoxicity (NKCC) and the leukocyte number. In the sham operated group mere insertion of electrodes into the MS evoked transient NKCC enhancement, probably resulting from mechanical MS stimulation. To check this effect in the present study, we evaluated both spleen and blood NKCC (^{51}Cr-release assay) and large granular lymphocytes (LGL) number (a morphological method) after chronic electrical stimulation (constant current 0.1 ms duration cathodal pulses delivered at a frequency of 50 Hz during 30-min daily session for 14 consecutive days) of MS in conscious, freely moving rats (n=12). Additionally, peripheral blood leukocyte, lymphocyte and neutrophile number was mesaured. Chronic MS stimulation caused significant blood NKCC augmentation and LGL number (25.86 ± 9.31% vs. 15.75 ± 4.75%, P<0.01) in comparison to the sham group (n=13). No significant effect was found in the spleen (27.14 ± 9.99% vs. 28.58 ± 8.04%). A week after termination of the stimulation procedure all measured parameters returned to the baseline. The results obtained indicate that such limbic structure as medial septum enhances antitumor and antiviral function and number of NK cells.

TVII.26
UNILATERAL DAMAGE TO THE NUCLEUS ACCUMBENS DECREASES PERIPHERAL BLOOD NK CELL NUMBER IN RATS
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The nucleus accumbens (Acb), which is major terminal fields for the dopamine fibers originating in the ventral tegmental area (VTA) plays a pivotal role in the integration of signals arising from the limbic and cortical areas that mediate the motivational, homeostatic and motor aspects of behaviour. In the present work, we assessed the effects of unilateral electrolytic lesion of the Acb on peripheral blood lymphocyte number in freely moving rats. Before unilateral Acb lesion, all rats (n=10) were exposed to chronic (14 consecutive days) VTA electrical stimulation to obtain feeding or exploration response and the baseline level of lymphocyte numbers. On the 2nd day after electrolytic lesion of Acb (2 mA, 15 s) the percentage of blood T, B and NK lymphocytes, TCD3$^+$CD4$^+$ and TCD3$^+$CD8 subsets were determined by flow cytometry. As compared to the baseline, the significant decrease in NK cell percentage (51.10 ± 14.05% vs. 20.3 ± 5.84%, P<0.01) following Acb lesion was observed. In contrast, there were no significant differences in the percentage number of B (17.7 ± 2.38% vs. 22.25 ± 5.28%) and T (39.31 ± 9.75% vs. 42.10 ± 7.21%) cells between the baseline and post-lesion values. Accordingly, post-lesion values of T helper and T cytotoxic subset were as follow: 30.35 ± 5.31%, 8.02 ± 1.11%. These findings suggest that Acb, which is partially responsible for motivational states, plays a crucial role in innate antitumor and antiviral response.

TVII.27
CHANGES OF BRAIN-DERIVED NEUROTROPHIC FACTOR (BDNF) IMMUNOREACTIVE CELLS NUMBER IN HYPOTHALAMIC NUCLEI AFTER CHRONIC STRESS IN YOUNG AND AGED RATS
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It is postulated, that brain-derived neurotrophic factor (BDNF) have been implicated in the neurobiological mechanisms underlying brain plasticity after chronic stress. The objective of this study was to evaluate influence of chronic stress on brain plasticity measured by BDNF immunoreactivity in brain structures of young (P28) and adult (P360) rats. 26 male Wistar rats were exposed to 15 min daily open field (OF) or forced swim test (FS) during three weeks. Fluorescent immunohistochemistry was used to localize BDNF positive cells in hypothalamic areas connected with stress response: both parvo- and magnocellular divisions of the paraventricular nucleus (PVp and PVm) and the supraoptic nucleus (SO). In animals aged P28 chronic OF i FS stress caused a statistically significant (P<0.001) decline in the number of BDNF-ir cells in both parts of the PV and SO. In contrast, in rats P360 was not observed any change in the number of BDNF-ir cells after chronic OF stimulation compared to control in PVp and SO. In summary: age of rats subjected to chronic stimulation OF FS or stress had an impact on changes in the number of BDNF-ir cells in the tested hypothalamic nuclei.

TVII.28
GAP JUNCTION IN THE HIPPOCAMPAL FORMATION NETWORK: INTERACTION BETWEEN GAP JUNCTION BLOCKER AND OPENER
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Gap junction (GJ) channels provide an aqueous pathway for the passage ions current and small molecules between cell cytoplasms. It has been demonstrated that various physiological stimuli, experimental treatments and chemical agents can modulate activity of those structures. In our previous studies we showed that intrahippocampal injection of carbenoxolone (GJ blocker) abolished local recorded theta rhythm in anesthetized rats. However, intrahippocam-

pal formation (HPC) administration of trimethylamine (GJ opener) evoked an opposite effect i.e. well synchronized theta activity. The similar findings were also obtained in many experiments carried out on awake animals and in studies conducted in *in vitro* conditions. In the present studies, performed on anesthetized rats, the interaction between GJ blocker and opener, separately injected into HPC on locally registered theta rhythm, were established. When diminishing effect of HPC injection of carbenoxolone (100 μg/1 μl) on theta rhythm amplitude and power was observed, trimethylamine (30 μg/1 μl) was locally administered. Trimethylamine was found to alleviate blocking effect on spontaneous HPC theta rhythm. In the next experiment, all rats pretreated with trimethylamine were injected with carbenoxolone. GJ blocker was found to prevent facilitating effect of trimethylamine on theta rhythm. The mechanism underlying GJ involvement in theta rhythm generation *in vivo* is discussed. *These studies were supported by grant MNiSzW NN 401 2811 33.*

TVII.29
THE EFFECT OF CARBACHOL ON HIPPOCAMPAL THETA IN CARBENOXOLONE PRETREATED RATS
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Among the number of the mechanisms of signal transmission evolved in biological systems, a distinctive feature of gap-junction coupling is ability of direct high-speed information spreading over the space of coupled cells. It is performed on cytoplasmic tracks provided by the channel structure of gap junction assemblies (electrical synapses). In the nervous system this high-speed pathway is considered as a promoter of neuronal synchrony, and so – an underground for oscillatory events. On the other hand, it is well known that to produce oscillations, the brain tissue needs qualitatively and quantitatively balanced neurotransmitter input feeding the oscillation-generating networks. The question arise what is the real contribution of the two signalling systems: electrical and chemical to oscillatory activity. Hippocampal formation (HPC) theta rhythm is one of the best synchronized brain oscillations. Our earlier experiments demonstrted that the cessation of gap junction coupling had a spectacular effect on HPC spontaneous theta rhythm. HPC administrations of carbenoxolone (CBX), a gap junction blocker, produced progressive impairment of theta oscillations up to their abolition. In order to estimate if high-level enhancement of chemical transmission is capable of counteracting the gap junction blockage, in the present work carbachol, a cholinergic agonist, was injected into CBX pretreated hippocampus. Under CBX-blockage no effect of carbachol was noticed. *Grant MNiSzW NN 401 2811 33.*

TVII.30
THETA GENERATORS IN COMBINED TRANSECTED HIPPOCAMPAL FORMATION SLICES
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Rhythmic slow activity (RSA, theta) is commonly associated with the hippocampal formation (HPC). It is generally agreed that HPC theta rhythm is dependent on an intact septal area that act as a pacemaker for the RSA. However, hippocampal neurons cannot be considered as passive followers of rhythmic septal inputs. The local hippocampal networks may also contribute to the RSA – application of the cholinergic agonist, carbachol produce theta rhythm in HPC slices *in vitro*. The evaluation of theta amplitude and phase profile indicates the existence of two theta generators located in CA1 and DG areas. Moreover, model of slice preparation demonstrated that in the CA3c HPC region the third RSA generator is localized. In our previous experiments we hypothesized that completely isolated DG area is not capable of theta rhythm generation. In the present study we attempted to verify this hypothesis, using model of combined transected HPC slices. We analyzed theta activity in two different transected slices: DG slices containing CA1 area and DG slices containing CA3c region. We demonstrated that both CA1 and CA3c regions are capable of independent generation high amplitude RSA, whereas theta recorded in DG was characterized by significantly lower theta amplitude. Our *in vitro* obtained findings are consistent with earlier reports suggesting that HPC theta generators are localized in CA1 and CA3c but not in DG region. *Supported by grant MNiSzW NN 303 091 834.*

TVII.31
DISCHARGE PATTERNS OF THETA-RELATED CELLS IN TRANSECTED HIPPOCAMPAL FORMATION SLICES
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Hippocampal formation (HPC) theta rhythm is approximately sinusoidal, most prominent and well-documented EEG pattern recorded from the mammalian brain. Following the discovery of cholinergically induced theta rhythm recorded in slice preparations of HPC, central mechanisms underlying theta generation have been successfully studied in the *in vitro* conditions. In our previous work we have attempted to record an EEG activity of three types of transected hippocampal slices (CA1 trans-slice, CA3c trans-slice, and DG trans-slice) examining activity of separated intrahippocampal theta generators. Data obtained in those studies revealed that dentate gyrus isolated from generators located in CA1 and CA3c fields respectively, is not able to generate theta rhythm in the *in vitro* conditions. The purpose of the present study was to extend our previous *in vitro* studies concerning the activity patterns of theta related cells. This time theta cells were recorded in transacted hippocampal slices i.e. CA1 trans-slice, CA3c trans-slice, and DG trans-slice. Sixty cells were isolated and recorded from transected slices, including thirty cells from CA3c (20 theta-on cells, 6 theta-off cells, and 4 gating cells), and thirty cells from CA1 (15 theta-on cells, 13 theta-off cells, and 2 gating cells). None of DG transected slices tested generated any synchronous field potentials and accompanying cell activity. *Supported by MNiSW grant No. N N 303 091 834.*

TVII.32
EFFECT OF (R)-(+)-8-OH-DPAT ON THE PHASE SHIFT OF LOCOMOTOR ACTIVITY RHYTHM INDUCED BY DARK PULSES IN CONSTANT LIGHT IN MICE
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The evidence from a variety of studies suggests the involvement of serotonin (5-HT) in both the response of the suprachiasmatic nuclei neurons to light and the phase-resetting properties of non-photic stimuli. Although it is well known that 5-HT agonist causes a phase shift of locomotor activity rhythm, there are few reports of experiments on the influence of serotonin upon the locomotor ac-

tivity rhythm of nocturnal rodents kept under constant light (LL) conditions. In our previous experiments we characterized the phase response curve (PRC) for (R)-(+)-8-OH-DPAT in LL in mice and we discovered that the shape of this PRC is similar to the PRC for dark pulses under LL. It suggests that mechanism response for phase shifts of locomotor activity rhythm induced by 5-HT_{1A} agonist and by dark pulses may be the same. Therefore, we have examined the effect of (R)-(+)-8-OH-DPAT on the phase shifts of locomotor activity rhythm induced by dark pulses. Eight week old C57BL/10 male mice were kept in a soundproof room in individual cages with free access to a running wheel. The wheel-running activity rhythm was recorded under 12:12 LD and under LL. In the LL (R)-(+)-8-OH-DPAT was i.p injected every two hours during twenty-four hours. Injections were given at 30 minutes before 4 hours dark pulses. The analysis of the phase shifts of the locomotor activity rhythm indicated that at some time points (R)-(+)-8-OH-DPAT increases dark pulses phase-shifts whereas in other points decreases were observed.

TVII.33
CHANGES OF THE FIRING PATTERNS OF SEROTONERGIC NEURONS IN THE RAT MEDIAN RAPHE NUCLEUS DURING LONG LASTING RECORDINGS

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The median raphe nucleus (MRN) is one of the main sources of serotonergic ascending projections. Previous electrophysiological studies have shown that serotonergic neurons spontaneously discharge single broad action potentials in a slow clocklike firing pattern. Subsequent studies have revealed another subpopulation of serotonin (5-HT) containing cells, which possess the same electrophysiological attributes of classical 5-HT neurons, simultaneously displaying bursting pattern of activity. More recently it has been shown additional class of 5-HT neurons, with relatively fast firing activity (more than 8 Hz), implying larger diversity of 5-HT cells populations. The present data confirm formerly proposed supposition that individual 5-HT cells are able to switch between different activity pattern, earlier described as distinctive subpopulation of these cells. In the present study *in vivo* extracellular single unit recordings were made from the presumptively identified 5-HT neurons within the MRN of the urethane anesthetized rat. The activity patterns were assessed by constructing interspike interval histograms. In the course of long lasting (up to 10 hours) recordings we have found, that in some cases individual 5-HT neurons have changed the pattern of activity from regular to less regular, bursting or nearly silent. This heterogeneity of firing modes is more suitable to an explanation of the role that serotonergic system plays in diverse physiological and pathological processes.

TVII.34
LOCALIZATION OF CRYPTOCHROME-IMMUNOPOSITIVE NEURONS IN THE VISUAL SYSTEM OF *DROSOPHILA MELANOGASTER*

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Cryptochrome (CRY) is a blue light absorbing protein involved in the photic entrainment of circadian clock in the fruit fly. It has also been suggested that CRY may play role in molecular circadian clock that generates circadian rhythms in the visual system. The aim of our study was to examine localization of CRY-immunopositive neurons in the visual system of *Drosophila* and changes in abundance of CRY protein in these neurons. We used *Drosophila* transgenic lines which express green fluorescent protein (GFP) under control of the *cry* promotor. Analyses of GFP fluorescence showed that CRY is present in the dorsal neurons DN_3, and in the dorsal (LN_d) and ventral lateral neurons (LN_v) and its density decreases during the day and increases during the night. In the LN_v and in the processes of DN_3, the CRY abundance was the highest at ZT1 (1 h after lights-on) and the lowest at ZT13 (1 h after lights-off) in both males and females. In addition we found that the LN_vs project to the second visual neuropil (medulla) and form CRY-immunopositive network of many varicose processes in the medulla. We also detected numerous CRY-immunopositive processes in the first optic neuropil (lamina) which origin from a single fibre extending from the LN_vs. In the lamina it divides into many thin branches next to the retina. Our results showed for the first time that the lamina is invaded directly by processes of clock neurons which seem to control circadian plasticity of neurons and synapses in the lamina.

TVII.35
THE INFLUENCE OF OREXIN B ON THE ACTIVITY OF THE INTERGENICULATE LEAFLET NEURONS

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The most known function of the intergeniculate leaflet (IGL) is regulation of circadian rhythms by integration of photic and non-photic cues and conveying this signal to the site of main oscillator – suprachiasmatic nuclei. Information about non-photic cues enter the IGL from various brain nuclei, including those involved in regulation of sleep/wake states. Hypothalamic peptides – orexins has been implicated in numerous physiological functions including maintenance of arousal and wakefulness. Since it has been shown that fibers containing orexins are distributed in the area of the IGL, the orexinergic system may constitute one of the possible candidate to transmit non-photic/arousal related cues to the IGL. Using the immunohistochemical and electrophysiological recording techniques we evaluated the innervation of the two major types of the IGL neurons by fibers containing orexin B (OXB) and its influence on the activity of the IGL cells. Experiments were performed on brain slices, obtained from adult male Wistar rats, using extracellular single unit recordings. For each individual neuron, after reaching the stable baseline activity, the OXB (1 μM) was applied by local pressure injection. Administered drugs evoke an increase in the firing rate of the IGL neurons. Obtained data are in agreement with the results of our immunohistochemical staining revealing the presence of orexin fibers in the area of the IGL.

TVIII: Cognition and Behavior

TVIII.01
THE COGNITIVE NEUROSCIENCE OF AUDITORY DISTRACTION

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Background sound can disrupt cognitive performance, even when the person performing that task tries to ignore that sound and even when the sound is unrelated to the task being performed. There are several forms of auditory distraction, each of which may implicate multiple cognitive processes that, in turn, could be related to multiple brain processes. One form of auditory distraction can disrupt the performance upon a memory task that involves holding lists of to-be-remembered digits in memory briefly, before attempting to report back those to-be-remembered items in the original order. This auditory distraction effect occurs during a retention interval when those items are held in memory and when the sounds are presented. This disruption of immediate memory by auditory distraction could be related to the generation of brain processes in response to the presented sounds, as may be indexed by an auditory ERP protocol. Support for the N1 hypothesis that distraction can involve factors related to the generation of the N1 component of the auditory ERP is offered by the finding that increases in token set size produce a disruption of performance alongside an increase in N1. This increase in the disruption of immediate memory by auditory distraction is not associated with MMN, but rather is associated with a spatiotemporally and functionally-distinct increase in N1, which has been shown to occur without the concomitant elicitation of P3a.

TVIII.02
DOES THE SPATIAL-NUMERICAL ASSOCIATION INTERFERE WITH ORIENTING AND EXECUTIVE ATTENTIONAL PROCESSING?

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Brain representations of numbers are spatially organized on the so-called mental number line (MNL). We investigated the relationship between this spatial-numerical association and attention processes. In the first task subjects responded to four-digit numbers (targets) preceded by a centrally presented digit. They had to indicate the side of occurrence of this previous digit in the target (left/right). The digit location in the target could be congruent with its position on MNL (e.g. "8" on the right) or incongruent (e.g. "9" on the left). In the second task subjects assessed the parity of the central digit in the five-digit numbers (right key = even; left key = odd). The condition was defined as congruent when the reaction side corresponded to the digit position on MNL and incongruent when there was no such correspondence. The results showed more accurate and faster reactions in congruent than incongruent conditions. We conclude that: (1) the digit magnitude representation shifts attention to the direction of its representation on MNL, what confirms the interaction between orienting attention processes and digit positions on MNL; (2) the incongruence between the correct response side (left/right) and the digit spatial location on MNL induce the conflict, which proves the involving of the executive attention system.

TVIII.03
IS PICTURE RECOGNITION MEMORY ENHANCED THROUGH SENSORY OR COGNITIVE FACILITATION?

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Different features of pictures can be used as recognition clues, and colour is one of them. However, it is unclear whether enhanced recognition memory by colours is due to the distinctiveness of features highlighted by colours (sensory facilitation), or it is due to the colour representation in memory (cognitive facilitation). One way to define mechanisms through which colour enhances memory is to investigate the role of colour diagnosticity in picture recognition memory which can be defined as the degree to which an object was associated with a specific colour. Colour diagnosticity was manipulated by using pictures in two colours modes: naturally (high colour diagnosticity) and unnaturally (low colour diagnosticity) coloured pictures. Unnatural coloured mode was complementary to the natural. Since there were two different versions of picture in the encoding phase and two in the recognition phase, there were four possible combinations of encoding and recognizing pictures. 60 participants were exposed to different combinations of encoding and recognizing pictures. Accuracy and recognition time were measured. Results showed strong encoding-specificity effect, with no differences in correct recognitions of naturally and unnaturally coloured pictures. In other words, colour diagnosticity effect was not found. Furthermore, it was shown that colour diagnosticity takes part only during recognition time, but does not affect the accuracy itself.

TVIII.04
PSYCHOPHYSIOLOGICAL MECHANISMS OF THE TEENAGE AGGRESSION'S AND ITS CORRECTION

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Current research is aimed at reveal psychophysiological basis of teenage aggression and finding ways of reducing aggressive behavior that is most essential and topical problems of modern society. To understand the investigated phenomenon it was applied a complex approach. The Bass-Darky and Eysenk qiestionnaires, drawing a non existing animal and Wagner's Hand tests were used for determining the level of aggression. Then, the influence of playing aggressive computer games was investigated. The brain visual evoked potentials were recorded in frontal, orbito-frontal, temporal and anterior inferotemporal area before and after playing the game. The obtained results have shown that the activity of frontal cortex of highly aggressive subjects of both genders increased as a result of playing the game, but in the anterior inferotemporal area it decreased which correlated with decreased level of situational agression. The reversed changes were observed at examinees with a low level of aggression of both genders. Therefore, the direction of changes depended on the initial level of aggression, but intensity of these changes depended on genderss. The correction methods such as "Danish boxing", "push", "internal shout", "pressing" and "arttherapy" were used for psychocorrection residual aggression. The above-mentioned correction methods led to decrease of situational aggression at high-aggressive boys.

TVIII.05
GENDER-DEPENDED ROLE OF FRONTAL AND ORBITO-FRONTAL CORTEX AREAS IN REGULATION OF TEENAGE CONFLICTNESS
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The most of conflictogenic factors should be concerned as open or latent aggression. Thus, "aggressive" computer games can be used as an experimental model for study brain mechanisms of conflictness. The aim of this study was investigation of gender-depended role of anterior cortex areas in regulation of teenage conflictness. According to Utkin's conflictness questionnaire test results subjects were divided into 4 groups: boys and girls with a high or low level of potential conflictness. The influence of aggressive computer gameplay was investigated. The brain visual evoked potentials were recorded from frontal and orbito-frontal cortex before and after playing the computer game. The analysis of obtained data has shown that, as a result of gameplay, the boys' brain anterior cortex activity increased, which correlated with decreasing level of conflictness. In the group of girls the activity of anterior cortex areas was decreased. However, there was a difference between frontal and orbito-frontal areas activity depended on the initial conflictness level. Initially high level of orbito-frontal activity was recorded at girls with high conflictness. The aggressive computer gameplay reduced orbito-frontal activity that caused decrease of conflictness level. Non-conflicting girls had initially high frontal activity which decreased through aggressive gameplay. It is connected with weakening of cortex control and corresponding increase of conflictness level.

TVIII.06
IMAGINED AND PERCEIVED ITEMS: AN EVENT-RELATED POTENTIAL STUDY OF SOURCE-MEMORY
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Mental processes that are initiated without any input from outside world may differ from those related to the direct external stimulation. Specifically, a question arises whether memory traces for previously imagined items may differ from memory traces for perceived items. The aim of our study was to test this issue, using the event-related potentials (ERP). It is well documented that remembered old items elicit more positive-going ERPs than correctly judged new items. This so-called "old/new effect" indexes neural activity associated with correct retrieval of information about a prior event. In this study the old/new effect was investigated separately for previously imagined and perceived items. In the first part of the study, word labels of common objects were presented. Half of them were followed by a colour picture of the corresponding object. The other half was followed by a black screen, signalling to the participants to mentally visualize the object. In the second part, the participants discriminated between new words, words corresponding to previously perceived or imagined pictures. Correctly identified old items were associated with more activity than correctly judged new items over the left parietal areas. Importantly, this statistically significant old/new effect, representing the recollection index, was greater for imagined than for perceived items. Thus brain representations of imagined items might be more detailed and precise than those of real objects.

TVIII.07
CAN WE PREDICT THE CREATION OF FALSE RECOGNITIONS FROM EVENT-RELATED BRAIN POTENTIALS RECORDED DURING ENCODING?
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The poster depicts the project of research on event-related brain potentials (ERPs) recorded during the creation of false recognitions of words. The magnitude of literature on this field focuses on the stage of recognition when subjects decide whether or not given words were presented to them previously. Only two recent studies analyzed ERPs recorded during encoding when subjects mere try to remember visually presented words which subsequently lead to falsely recognize (or correctly reject) the corresponding associates. However, the results of these studies are inconsistent. Urbach et al. (2005) reported higher amplitude of LPC component (500–800 ms poststimulus) for words not eliciting false recognitions while Geng et al. (2007) reported higher amplitude of LPC component (500–640 ms poststimulus) for words eliciting false recognitions. The authors claim that these effects reflect item-specific information encoding or associative encoding, respectively. However, it seems highly improbable that the same ERP component reflects different cognitive processes. Therefore, the current project proposes framework for understanding that discrepancy, namely the Activation Monitoring Theory developed by Roediger et al. (2001). Moreover, the project points two factors that can influence the creation of false recognitions and their electrophysiological correlates during encoding: the associative strength of words and their presentation duration.

TVIII.08
DYNAMICAL PROPERTIES OF "INTERNAL CLOCK" AS A NEURAL BASIS OF INDIVIDUAL DIFFERENCES IN THE LEVEL OF INTELLIGENCE
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Neuropsychological evidence has suggested that both intelligence and temporal resolution in information processing may be determined by similar neural mechanisms, related to internal timing mechanism. Personal tapping tempo (PTT) can be considered as a promising measure of such "internal clock" properties, as it reflects spontaneous tempo in human motor activity. As the existing studies do not provide detailed description of temporal characteristics of PTT, conclusions on temporal control underling timing and intelligence are still not clear. The present study investigated in 55 highly intelligent and 50 average intelligent students dynamical properties of temporal control of repetitive finger movements in PTT. Nonlinear elements for reconstruction of dynamical properties of PTT were performed. We found fractal properties of tapping performance. Additionally, advanced mathematical analyzes showed grouping in frequencies of PTT, which constituted characteristic optima of subjects' performance. In these optima intelligence-related differences were revealed. These findings supported the notion that 'internal clock system' in the brain could be fractal in its nature which has a different structure in highly and average intelligent individuals. *The study was supported by Grant N N106 109636 from Ministry of National Education and Science.*

TVIII.09
NEUROANATOMICAL CORRELATES OF FAST FORWORD TRAINING: AN fMRI STUDY
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Using fMRI we tested brain activations involved in temporal processing following Fast ForWord (FFW) training in 11 young volunteers. They performed the Temporal Order Judgment task in two sessions: before and after the training. The task was to report the order of two sounds presented in rapid succession. Each pair consisted of a short (10-ms) and a long (50-ms) sound separated by Inter-Stimulus-Intervals of 160 ms (easy condition), 60 ms (medium) and 10 ms (difficult). FFW training was applied for ca. 8 weeks with four 1-hour sessions per week. The brain activations during particular conditions ("before vs. after" and "after vs. before") the training were compared using paired sample *t*-tests from SPM5. 'Before vs. after' comparisons resulted in the following activations: (1) easy condition – left cerebellum, (2) medium condition – cerebellum and insula bilaterally, left thalamus and putamen and moreover, bilaterally selected areas of the temporal, frontal and parietal lobes, (3) difficult condition – left insula and the left temporal, frontal and parietal gyri. "After vs. before" comparisons showed no significant differences for each condition, thus, indicating no increase of activation "after" than "before" the training. These results support the involvement of above structures in timing and suggest decreased brain activation in timing tasks following FFW training. *We thank Scientific Learning for the opportunity to use FFW grant MSHE no.: 1082/P01/2006/31.*

TVIII.10
SEX DIFFERENCES IN IMPLICIT MEMORY: AN fMRI STUDY USING THE WEATHER PREDICTION PROBABILISTIC CLASSIFICATION TASK
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Several recent studies have shown sex-related differences in the neural organization of memory processes. However, as the majority of investigations involved explicit memory tasks, little is known about potential differences between men and women in the organization of implicit memory processes. Our study addressed this issue. We used functional magnetic resonance imaging (fMRI) to compare the patterns of brain activity in men and women performing the weather prediction probabilistic classification task which is a tool for assessing implicit learning. In this task, subjects learn to predict the weather using associations that are formed gradually across many trials, because of the probabilistic nature of the cue-outcome relationships. Although both men and women exhibited similar level of behavioral performance, between-sex group analysis of the BOLD response demonstrated greater activity in men than in women in dorsolateral prefrontal (BA 9, 46), parietal (BA 7, 40), and occipital (18, 19) cortices. In contrast, women activated insular (BA 13), temporal (BA 21, 22) and ventrolateral prefrontal (BA 44) cortices. Differences between men and women in the patterns of brain activity might reflect distinct strategies used in the weather prediction probabilistic classification task. Our results indicate that neural activity underlying implicit memory differs between sexes, despite of lack of differences in behavioral efficiency.

TVIII.11
INTERHEMISPHERIC RELATIONS IN ATTENTION EVIDENCED BY THE LATERALIZED ATTENTION NETWORK TEST (LANT)
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Attention has been convincingly demonstrated to involve anatomically and functionally distinct networks that subserve conflict resolution, spatial orienting and alerting. On the one hand, partially independent symmetric attention networks can be postulated within the two brain hemispheres (Zaidel 1995). On the other hand, large body of data, mostly clinical, suggests asymmetric control of attention (Heilman and Van Den Abell 1980). Not much is known at present on the nature of interhemispheric relations in attention. To address this issue, the Lateralized Attention Network Test (LANT) was designed. The introductory study of Greene et al. (2007) has suggested some hemispheric independence and similarity but no clear asymmetry. To gain better insight we conducted three consequent experiments recruiting 170 participants. We supplemented the original LANT procedure with modifications that allowed comparisons between goal-directed and stimulus-driven control of attention. The results show that interhemispheric relations within the attentional networks do depend on whether the "endogenous" or the "exogenous" control dominates in the task.

TVIII.12
ANXIETY-RELATED BEHAVIOR IN RATS WITH CONSTITUTIONALLY ALTERED SEROTONERGIC ACTIVITY
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Serotonin (5HT, 5-hydroxytryptamine) is involved in regulation of both central and peripheral physiological functions, and disturbances in serotonergic neurotransmission are common in neuropsychiatric disorders. Using selective breeding, we have developed Wistar-Zagreb 5HT rats that include two sublines: one with high (high-5HT) and other with low (low-5HT) levels of peripheral (platelet) serotonin and activity of 5HT transporter, representing a rodent model with constitutionally altered serotonin homeostasis. We hypothesized that in these animals, beside peripheral, brain serotonergic activity is also altered which should have effect on their behavioral response. In the present study, we have tested the anxiety-related behavior of 5HT sublines (3 months old males, n=20–21 per subline) in elevated plus maze and open field paradigms. Animals from high-5HT subline had lower score in time spent in open arms and number of entries to the open arms of elevated plus maze in comparison to low-5HT subline (P<0.05). Also, the score of head dip time in open arms was lower in high-

5HT subline (P<0.005). In open field test, high-5HT subline had lower score in total rearing time (P<0.005). Altogether, these results confirm results of previous behavioral tests (hole-board and zero maze) showing differences in anxiety-related behavior in Wistar-Zagreb 5HT rats and indicating higher level of anxiety-related behavior in high-5HT subline.

TVIII.13
COLOCALISATION OF GLUCOCORTICOID AND 5-HT$_{1A}$ RECEPTOR IMMUNOREACTIVITY-EXPRESSING CELLS IN THE BRAIN STRUCTURES OF LOW AND HIGH ANXIETY RATS

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In recent years we have elaborated an animal model to examine the neurochemical background of differences in the individual responses to conditioned aversive stimuli, using the strength of a rat contextual fear test, as a discriminating variable: low responders (LR), i.e. rats with duration of a freezing response one standard error, or more, below the mean value and high responders (HR), i.e. rats with duration of a freezing response one standard error, or more, above the mean value. It was found that 1.5 h after a testing session of contextual fear test, the LR animals showed a higher density of 5-HT$_{1A}$ and glucocorticoid immunoreactivity-expressing cells (GRs-ir) in the cortical M2 area and hippocampal dentate gyrus as well as an increased number co-expressing 5-HT$_{1A}$/GRs-ir in the same areas. The HR rats had a significantly higher concentration of 5-HT$_{1A}$ and GRs-ir in the basolateral amygdala. The present data add more arguments for the neurobiological background of differences in individual responses to aversive conditioned stimuli.

TVIII.14
SOCIAL MODULATION OF AVERSIVE LEARNING IN RATS

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It is well known that emotions participate in regulation of social behaviors and that the emotional states displayed by a conspecific can seriously affect the behavior of other animals. In its simplest forms empathy can be characterized as the capacity to be affected by and/or share the emotional state of another. However, to date, relatively little is known about the mechanisms by which the animals that are not in a direct danger can share emotions. In the present study we used the model of between-subject transfer of fear to characterize the social interaction during which fear is transmitted, as well as the effects of socially transmitted fear on behavior of its recipients. We found that: (1) during social interaction with a recently fear conditioned partner, observers and demonstrators exhibit social exploratory behaviors rather than aggressive behaviors; (2) learning in a shock-motivated shuttle avoidance task is facilitated in rats that underwent the social interaction with a partner that had been either fear conditioned or trained in two-way avoidance; (3) a brief social interaction with a recently fear conditioned partner immediately before fear conditioning improves conditioned freezing measured on the next day; this effect can be also seen in rats that are unfamiliar to each other. Collectively, the obtained data suggest that a brief social interaction with a cage-mate that had undergone an aversive learning experience promotes aversive learning in an otherwise naïve animal.

TVIII.15
DIFFERENTIAL CONTRIBUTION OF DORSAL HIPPOCAMPUS IN THE REORGANIZATION OF HOMOGENOUS ALLOTHETIC OR IDIOTHETIC SPATIAL REPRESENTATION IN RATS

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The hippocampus is engaged in the processing of visual (allothetic) and self-motion (idiothetic) information for spatial memory function. Beyond memory, it is involved in cognitive coordination processes (Wesierska at al. 2005). We hypothesised that the hippocampus is also critical for the reorganization of spatial representations. To study this we used a place avoidance (PA) method to create homogenous allothetic (Room: R+) or idiothetic (Arena: A+) spatial representations. We then rearranged these representations according to the A+ or the R+. In the PA task hippocampal lesioned (HL) and intact (I) rats learn to avoid a place on the arena where shocks are administered. In the R+ variant this place was in a fixed position according to the room frame (lit room, rotating arena, water-dissolved proximal cues); the A+ variant was represented by a fixed position according to the arena information (dark, rotating arena). The HL rats were impaired during acquisition of avoidance in the R+ ($F_{1, 14}$=13.97; P<0.002) but not in the A+ variant. When spatial conditions were rearranged all lesioned rats showed impaired learning (from R+ to A+: $F_{1, 13}$=12.04; P<0.004; from A+ to R+: $F_{1, 14}$=10.59; P<0.005). Our results suggest that acquisition of allothetic representation, compared to idiothetic representation is more highly hippocampal-dependent. Conversely, an unimpaired hippocampus seems to be necessary for the reorganisation of spatial representation. *Supported by grant 3120/B/P01/2007/33.*

TVIII.16
RECIPROCAL PATTERNS OF c-Fos EXPRESSION IN THE MEDIAL PREFRONTAL CORTEX AND AMYGDALA AFTER EXTINCTION AND RENEWAL OF CONDITIONED FEAR IN RATS

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Substantial evidence indicates that extinguished fear can be recovered after a change in experimental context (i.e., the renewal effect). The aim of this study was to characterize the neural circuitry underlying the retrieval of extinguished fear

memories using c-Fos immunohistochemistry. Firstly, rats received auditory fear conditioning. Subsequently, they were extinguished by presenting CS-alone trials in either the same context as conditioning or in a second context. Then, all rats were tested for their fear of the auditory CS in the second context and sacrificed 90 min after testing. The presentation of the extinguished CS outside of the extinction context resulted in renewal of the freezing response relative to animals tested to the CS in the extinction context. In addition, the renewal of fear was associated with c-Fos expression in the prelimbic division of the medial prefrontal cortex, the lateral and basolateral nuclei of the amygdala, and the medial division of the central nucleus of the amygdala. In contrast, the presentation of the CS in the extinction context induced c-Fos expression in the infralimbic cortex, the intercalated nuclei of the amygdala and the dentate gyrus. Hippocampal areas CA1 and CA3 exhibited c-Fos expression when the CS was presented in either context. These data suggest that the context-specificity of extinction may be mediated by prefrontal modulation of amygdala activity, and that the hippocampus may have a fundamental role in contextual memory retrieval.

TVIII.17
LOCAL TRANSLATION – THE MECHANISM OF LONG-TERM MEMORY FORMATION IN THE ABSENCE OF ALPHA CaMKII AUTOPHOSPHORYLATION

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Alpha calcium and calmoduline-dependent kinase II (CaMKII) is the major protein in glutamateric neurons in the forebrain. Its activity is regulated by autophosphorylation on the threonine 286. CaMKII-T286A mutant mice have severe deficits in context memory formation. They form fear memory of the context in fear conditioning task only after very intensive training with 5 shocks. Here we present the data showing that foreground fear conditioning training does not induce expression of any of the analysed immediate early genes (c-Fos, Zif268, Nur77 or JunB) in CaMKII-T286A mutant mice. Furthermore, long-term fear memory in the mutants cannot be blocked by administration of mRNA or protein synthesis inhibitors (actinomycin D or anisomycin) into dorsal hippocampus. Moreover, as revealed by Illumina microarrays, fear memory training does not induce context-shock association specific expression in the hippocampus. On the other hand, the training of the CaMKII-T286A mutant mice induces in the hippocampus expression of locally-translated proteins, Arc and PSD-95. In addition, both training-induced PSD-95 expression and long-term fear memory can be blocked by intrahippocampal administration of rapamycin (inhibitor of mammalian target of rapamycin kinase, the main regulator of local translation). Thus, our data strengthen the notion that local translation of PSD-95 in the dorsal hippocampus is the mechanism of long-term memory formation in the absence of alpha CaMKII autophosphorylation.

TVIII.18
IMMINENT VS. REMOTE DANGER: EMOTIONAL REACTIONS AND PATTERNS OF c-Fos EXPRESSION IN THE RAT BRAIN

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It has been shown that emotional states displayed by animals are able to influence their conspecifics' behavior and that such transferred emotions help animals to adapt to specific requirements of changing environment. Animals that are not in direct danger can share fear by either observation of a conspecific in danger or being informed by a conspecific about danger. To date, relatively little is known about mechanisms by which the emotional transfer occurs. Aiming to gain more insights into the neural substrates of two types of socially transferred fear, we designed behavioral models of indirect emotional stimulation. To allow observation of a conspecific in danger, we placed a rat designated as an observer in a safe compartment of two-compartment conditioning box and exposed the animal to a demonstrator being fear-conditioned in the other compartment. To model a situation in which an observer cannot witness a demonstrator in direct danger, we used a rat that had previously undergone fear conditioning as stimulus source for the other individual. Witnessing conspecific's distress evoked an increase in defensive responses and a decrease in exploratory behaviors. On the other hand, interaction with a stressed partner increased behaviors directed towards gaining information (sniffing and allogrooming). Activation of various brain structures following observation of a conspecific in danger and interaction with a conspecific that underwent a stressful stimulation were analyzed.

TVIII.19
IL-6 DEFICIENCY ALTERS COGNITIVE PROCESSES IN MICE

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Interleukin-6 (IL-6) in addition to its role in the immune system has the potential to modulate several brain functions including learning and memory processes. In the present study we investigated the role of IL-6 in CNS function in male mice not expressing IL-6 (C57BL/6J IL6-/-$^{tm\ 1\ Kopf}$) and wild type mice (WT) used as controls. The animals were kept in standard conditions with water and food available ad libitum except during experiments. All testing took place between 8.30 AM and 12.30 PM. Each group consisted of 13 animals. In order to evaluate a role of endogenous IL-6 in cognitive functions "object recognition test" for the evaluation of recognition memory was used. In an attempt to evaluate whether observed effect was memory specific, the level of anxiety and psychomotor activity of mice was evaluated in an "elevated plus maze" test and in an open field, respectively. Recognition memory, measured by the difference in exploration of the new object and a duplicate of the familiar one, presented 1 h earlier, was impaired in IL-6 deficient mice. Moreover, lack of IL-6 significantly attenuated locomotor and exploratory activity

measured in an open field test and enhanced anxiety evaluated in an "elevated plus maze" test. Results of this study indicate that IL-6 deficiency impairs recognition memory, attenuates locomotor and exploratory activity and enhances anxiety in mice. *The study was supported by the Polish Ministry of Science grant Nr 2P05B01826.*

TVIII.20
NEW HIPPOCAMPAL NEURONS ARE NOT OBLIGATORY FOR MEMORY FORMATION; MICE WITH NO ADULT BRAIN NEUROGENESIS SHOW LEARNING

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New neurons are produced in the brains of adult animals, including humans, throughout the lifespan. Since one of the site of adult brain neurogenesis is the hippocampal formation, a brain structure involved in learning and memory, new neurons were expected to be involved in these phenomena. However, the very evidence supporting this hypothesis remains limited, inconsistent and in most cases indirect. Also, the experiments in which adult neurogenesis is blocked use irradiation and drugs known for their side effects. We used a novel approach, cyclin D2 knock-out mice (D2 KO mice), specifically lacking adult brain neurogenesis (which was verified using DCX and NeuN staining) to verify its importance in learning and memory. D2 KO mice and their wild type siblings were tested in several behavioral paradigms including those in which the role of adult neurogenesis has been postulated. D2 KO mice showed no impairment in sensorimotor tests with only sensory impairment in an olfaction-dependent task. However, D2 KO mice showed proper procedural learning as well as learning in context (including remote memory), cue and trace fear-conditioning, Morris water maze, novel object recognition test, and in a multifunctional behavioral system – Intellicages. Our results suggest that adult brain neurogenesis is not obligatory in learning, including the kinds of learning where the role of adult neurogenesis has previously been strongly suggested. *Support – MNiSW grant no N303 051 31/1624.*

TVIII.21
PHENOTYPING OF TRANSGENIC MICE: COMPARISON OF INTELLICAGE–SYSTEM TO AUTOMATIC MONITORING OF BEHAVIOR IN SOCIAL CONTEXT WITH STANDARD TESTS

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There is a growing need for new strategies allowing for screening, monitoring and phenotyping of genetically modified animals. One of the instruments is IntelliCage system, an automated testing device allowing to investigate behavior in social groups of mice in repetitive conditions, without excessive contact with experimenter. We compared results of 4 strains of genetically modified mice tested individually in standard battery of tests with the animal performance in social context in IntelliCage. The overall behavior of tested strains, namely D2 knock-out mice lacked adult neurogenesis, GLT1 knock-out mice deprived of glial glutamate transporter, APP.V717I transgenic mice expressing the mutant human amyloid precursor protein and Dicer mice with forebrain specific and inducible inactivation of Dicer gene, were at first analyzed in sensorimotor tests, open field and elevated plus maze. Activity, exploration and anxiety level were then investigated in IntelliCage system and we found that all the tests were able to detect differences in phenotype. Furthermore, learning and memory tests were conducted in IntelliCage, e.g. place preference and confronted with traditional learning tasks, such as Morris water maze. Again, IntelliCage as well as standard tests differentiated mice with mutation from their wild-type siblings. We also found on example of APP.V717I transgenic mice prospects for investigation the effect of the social interaction on learning in IntelliCage system.

TVIII.22
OLD TRANSGENIC RATS WITH OVEREXPRESSION OF MMP-9 ARE MORE ACTIVE THAN CONTROLS AND REMEMBER BETTER IN BEHAVIORAL TESTS

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Matrix metalloproteinases are a major group of enzymes regulating cell-matrix composition, which are essential for many biological processes. Matrix Metalloproteinase-9 (MMP-9) has recently emerged as an important molecule in control of extracellular proteolysis in the synaptic plasticity. Using conventional transgenesis, we have created rats with overexpression of MMP-9 limited to the neurons of the hippocampus, cerebral cortex and cerebellum (MMP-9-gene is under control of neuronal, synapsin-1 promoter). A battery of behavioral tests, including Open Field and Elevated Plus Maze tests, revealed no significant difference between young transgenic and control rats. However, age-related changes in expression level of MMP-9 in wild type rats led to some dysfunctions in behavior, as we noticed differences in general activity between the transgenic and wild type rats increasing with age. The old transgenic rats demonstrated higher activity and better motor functions and coordination than controls. Moreover, the level of anxiety was decreased in the old transgenic rats in comparison with their wild type siblings. Also, the old transgenic rats showed better taste memory than wild type. These results strongly suggest that MMP-9 may have an important role in control of the behavior of animals.

TVIII.23
INSIGHTS INTO SPATIAL MEMORY FORMATION IN RETROSPLENIAL CORTEX
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The development of two-photon imaging techniques has created new ways to study the function of the mouse brain. We are using this method to monitor *in vivo* the activation of a FosGFP reporter in the retrosplenial cortex (RSC) in mice with chronically implanted optical windows. This study was designed to explore the mechanisms of RSC involvement in spatial memory encoding. RSC receives projections from the anterior thalamus and the CA1 region of hippocampus; it is also reciprocally connected with the subiculum, anterior cingulate and parietal cortices. Therefore this structure has been proposed to have a critical role in the circuitry responsible for spatial learning and memory. For the imaging experiment mice were trained for 10 days alternatively in a "spatial" or in a "cued" version of the Morris water maze task (MWM). In the "spatial" version (days 1, 3, 5, 7 and 9 of training) mice could use extra-maze cues in the experimental room. In the "cued" version (days 2, 4, 6, 8 and 10), the extra-maze cues were blocked with a curtain placed around the pool and the platform position was marked with metal rod. On days 7–10, 90 minutes after the 4th and 5th sessions, specific regions of the RSC were imaged in lightly anesthetized FosGFP mice. Analysis revealed that approximately 30% of cells imaged showed reliable changes in activity when alternating between the spatial and cued task. Among those cells, the majority showed higher activity after the spatial task than after the cued task.

TVIII.24
EMG ACTIVITY IN RESPONSE TO DYNAMIC AND STATIC FACIAL EXPRESSIONS
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Facial expressions are complex signals caused by rapid changes in facial muscular activity that are brief and last only a few seconds. The suggestion that dynamic facial expressions of emotion induce more evident facial mimicry than static ones remains controversial. We investigated this issue by recording EMG from the three muscles: corrugator supercilii, zygomatic major and orbicularis oculi. Dynamic (video movie) and static (pictures of faces with equivalent static emotion) facial expressions of four emotions: anger, fear, surprise and happiness were presented. The analysis of data showed that subjects reacted spontaneously and rapidly to happy faces with increased zygomatic and orbicularis oculi EMG activity and with decreased corrugator EMG activity. In both zygomatic and orbicularis oculi muscles alteration of activity was greater in response to dynamic than to static stimuli. Moreover angry faces evoked alteration of corrugator and orbicularis oculi EMG activity, although dynamic expressions evoked stronger reaction in orbicularis oculi only. Unexpectedly static angry faces evoked greater alterations in corrugator EMG activity than dynamic faces. Two other expressions of emotion: fear and surprise did not evoke significant changes in EMG activity of none of three muscles. The obtained results only partially confirmed the hypothesis that the dynamic stimuli evoked stronger facial muscle reactions.

TVIII.25
THE KNOWLEDGE OF THE NEUROSCIENTIST MISSES THE HAND BUT HITS THE MIND OF NEUROREHABILITATION PROFESSIONAL
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The professional of neurorehabilitation, evaluates the neuromuscular dysfunctions of man based mostly on the result of the coded test than the instrumental ones. Such professional, in the Evidence-Based Medicine era, needs the suitable education (Kinalski 2008). The experimental and clinical neurophysiologists should be more often the partners of the interdisciplinary research. At present in Poland take place two international congresses, in which clinicians, who are interested to utilize in clinical practice the results of the preclinical research participate actively (http://ptreh.home.pl, http://www.pns2009.pl). The aim of the report is to show: (1) how often the clinicians participate actively in the International Congresses of the Polish Neuroscience Society organized since 1991; (2) what type of research results were presented by the clinicians on the symposia and poster sessions; (3) which of the domestic and the foreign establishments were affiliated by the clinicians.

POSTER SESSION III

H: History of Neuroscience in Poland

H.01
HOW DID THE INSTITUTE OF EXPERIMENTAL BIOLOGY COME TO BE NAMED AFTER MARCELI NENCKI (1847–1901)?
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Marceli Nencki was a great Polish scientist of the XIX century. He began his research at the University of Berne, Switzerland, in 1872. In 1876 he was appointed Associate Professor there, and one year later he became a full Professor and Director of the Institute of Medical Chemistry. In 1891 Marceli Nencki accepted an invitation to organize, together with Ivan P. Pavlov, the Institute of Experimental Medicine in St. Petersburg, where he spent the last decade of his life. In research he concentrated on topics related to urea synthesis, the chemistry of purines, and biological oxidation of aromatic compounds. He also examined the structure of proteins, enzymatic processes in the intestine, and bacterial biochemistry. Among Nencki's greatest successes was showing, together with Leon Marchlewski, a close chemical relationship between hemo-

globin and chlorophyll. The idea to establish the research institute named after Marceli Nencki was born shortly after his death in 1901. Among his friends who pushed forward this idea, the most effective was Nadine Sieber-Shumova, his close co-worker from Berne and St. Petersburg. However, many years were to pass until finally in 1918/19 the Nencki Institute was founded. Today, the Nencki Institute, where the neurobiology, neurophysiology, biochemistry and cell biology are widely represented, is the only research centre in Poland where the investigations are performed from the molecular to the whole animal and human subject level.

H.02
NAPOLEON NIKODEM CYBULSKI (1854–1919) – THE FOUNDER OF ELECTROPHYSIOLOGICAL SCHOOL IN CRACOW
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Napoleon Cybulski was one of the most prominent Polish physiologists. He defended his PhD thesis in 1885 and became the Chair of the Department of Physiology at the Faculty of Medicine of Jagiellonian University, till 1919. He is known as a founder of the physiology school in Cracow. One of his greatest achievements was the construction of a device for precise measurements of blood movement in the vessels – the photohemotachometer. It provided a better insight into the physiology and pathophysiology of the circulatory system. In the field of endocrinology Cybulski, together with Szymonowicz, found out that adrenal extracts contain biologically active substances that elevate blood pressure. He was also a constructor of an extremely sophisticated microcalorimeter to measure the quantity of heat produced during isolated muscle contraction. He applied, for the first time, condensator discharges to stimulate nerves and analyzed changes in the excitability of the muscles. Cybulski proved that the cause of the electrical excitability of tissue depends on the electrical energy and the time of its duration. Together with Adolf Beck, he performed the experiments on the sensory centers in the central nervous system by means of the observations of the electrical evoked potentials. They discovered the continuous electrical oscillations in the brain and recorded the negative electrical potentials in brain areas. This invention had the great contribution to the development of physiology.

H.03
IMPACT OF ORGANIC SEMIOLOGY ON DEVELOPMENT OF NEUROLOGY IN THE WORK OF JOSEPH JULES FRANCOIS FELIX BABIŃSKI (1857–1932)
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Joseph Jules François Félix Babiński was a son of emigrants from Poland. He was born in Paris where he also went to school and studied medicine. He graduated with honours in 1879. He made his training as an apprentice of Cornil, Vulpian and Buequoy. When he was assistant to Jean-Martin Charcot, he chose his specialization in internal medicine and neurology, soon achieving mastery in these subjects. In 1890 he was appointed head of the Pitié Hospital and worked there till the end of his life. He wrote over 300 papers on physiology of the nervous system and neuropathology. Babiński is the author of organic semiology of hemiplegia and paraplegia, which helps to differentiate them from functional and hysterical paralysis. He discovered most of the pyramidal symptoms. In his studies on defense reflex he defined its relationship with injuries to the pyramidal tract, named the "Babiński Sign", a test known also as Babiński reflex, introduced to neurology and widely used to assess upper motor neuron disease. Symptomatology of cerebellar diseases helped to differentiate cerebellar disorders from atrial disorders, enabling to determine such symptoms as: hypermetria, asynergy, adiadochokinesis, tremor, catalepsy. Babiński is to be remembered as a pioneer of neurosurgery in France. He was an extremely modest person of great intuition and of analytical and synthetic mind. He was an extraordinary researcher and clinician. He died on 29.10.1932 and was buried in Montmorency cemetery near Paris.

H.04
ADOLF BECK (1863–1942) AND HIS SHARE IN DEVELOPMENT OF ELECTROPHYSIOLOGY
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Adolf Beck lived in years 1863–1942. He graduated from the Jagiellonian University and was an outstanding physiologist, pupil and the closest collaborator of Prof. Napoleon Cybulski. In 1890 he became a PhD in medical sciences. His research was devoted to the physiology of the central nervous system and opened the fundamentals of electrophysiology and in consequence, to electroencephalography. Cybulski's and Beck's studies had a pioneering character and they were the first to discover electric activity of the brain and changes of this activity in response to various stimuli. The first Beck paper was published in German (1890) and produced response from English researchers who claimed the primacy of Richard Caton (1875). However, the Caton studies differed from Beck's experiments and were not known to him before. Beck's didactic and academic work has to be underlined when presenting his profile. At 32, as Associate Professor, he became chair of Physiology Department at Medical Faculty of the Jan Kazimierz University in Lviv and three years later he was promoted to Full Professor. He worked as dean of Medical Faculty and later as chancellor. He was the author and co-author of many manuals and original scientific papers. His book entitled "Physiology of the Central Nervous System", published in 1913, meets all the requirements of a modern manual for neurophysiology. He published a manual entitled "Human Physiology", first in 1915 together with Napoleon Cybulski and then, in 1922, by himself.

H.05
EDWARD FLATAU (1868–1932) – THE CLASSIC OF POLISH NEUROLOGY, CO-FOUNDER OF THE NENCKI INSTITUTE
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Edward Flatau established neurobiological and neuropathological sciences in Poland and at the same time was an outstanding doctor. He was born in Płock, spent several years studying and working abroad, in Moscow and Berlin. In 1894 he wrote a key work "Atlas of the Human Brain and the Course of the Nerve-Fibres" which was published in many languages. He formulated the statement, known

as Flatau`s Law, that "greater the length of the fibres in the spinal cord the closer they are situated to the periphery". For this work he received Ph.D. in medical sciences. By 1899 he returned to Poland, as a scientist with a world known name. For many years he shared his responsibilities as experimentalist and neurologist between the laboratory and the hospital (he was the head of neurology in the Jewish Hospital in Warsaw). He had a large private practice. He is the author of more than 100 publications in many languages. His most referenced book is classical book about Migraine (1912). Another fundamental paper was on progressive torsion spasm in children. He established the first neurobiological laboratory in 1911 in Warsaw which he headed until 1923. From the initiative of the directors of Warsaw Scientific Society` laboratories: Edward Flatau (Neurobiological Laboratory), Kazimierz Białaszewicz (Physiological Laboratory) and Romuald Minkiewicz (General Biology), an autonomous organization was formed in 1918 under the name of Marceli Nencki Institute of Experimental Biology.

H.06
JAN PILZ (1870–1930) – THE PIONEER OF POLISH NEUROLOGY
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The presentation aims to describe the early Polish neurology and its pioneer, Professor Jan Pilz. During his medical study in Zurich and Bern, Jan Pilz was so impressed by Swiss achievements in psychiatry that he decided to work in psychiatric clinics in Zurich and Geneva. He took his doctorate in neuropathology under a well-known Russian physician, Bekhterew. Pilz visited neurological and psychiatric clinics in France, Germany, The Netherlands, and Russia. From 1905, he took the first Polish Department of Neurology and Psychiatry in Cracow. Professor Pilz was engaged in study of the topography of cortical pupilary motor centers in animals. He took into consideration only isolated pupilary movements, which earlier investigators as Bekhterew, Schiff, Brown-Séquard had not considered. Pilz proved that there were not only isolated pupilary centers in the cortex but that it was also possible to point out in the cortex of various species of animals an analogy in the topography of the contraction and expansion centers of the pupils. He also was interested in hereditariness of mental diseases and homosexuality. Pilz was the President of Eugenics Society in Cracow, a founder of the Neurological and Psychiatric Society, the Society for Caring Mental Patients. His co-workers were first researchers on neurological and psychiatric diseases in Poland. References: Archive of Jagiellonian University in Cracow, Jan Pilz's records.

H.07
ROMUALD MINKIEWICZ (1878–1944) – FOUNDER OF THE WARSAW SCHOOL OF ETHOLOGY
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Romuald Minkiewicz was an outstanding Polish biologist and ethologist, as well as a devoted socialist activist. After biological studies in St. Petersburg (Russia), he acted as assistant at the University of Kazan, where he received (1904) his PhD degree. Initially, he was interested in hydrobiology and biological oceanology, and studied in various marine biological stations. After the WW I, he was active in the fields of physiology of perception, memory, acquired and innate behaviour of animals, and worked as professor at the Free Polish University and in the Nencki Institute of Experimental Biology in Warsaw (Head of the Department of General Biology, 1918–1939). In 1926–1931 he was Chairman of that Institute. Minkiewicz was interested in taxes (in that time called "tropisms"), in particular in photic reactions of various animals to colour lights. He was among the first ones to study modifications of body colouring adapting animals to their environment, including the so called "disguising behaviour" of the crab *Maia squinado* and behavioural patterns employed by animals to find sites matching their own colouration. He also studied ethology of ants and of flying aculeate *Hymenoptera*. He devoted an extensive study to nest architecture and prey of digger wasps (*Sphegidae*), today still cited in relevant papers. He wrote poems and dramas. He died of injury in 1944 during Warsaw uprising.

H.08
STEFAN BOROWIECKI (1881–1937) – THE FOUNDER OF THE DEPARTMENT OF NEUROLOGY AT THE POZNAN UNIVERSITY
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The presentation aims to describe the life and works of Professor Stefan Borowiecki (1881–1937), a typical representative of the first generation of Polish neurologists, well educated in both neurology and psychiatry at Swiss, German, Russian and French universities. As a young doctor, he took a job at the hospital for mental patients in Kochanówka near Łódź. Next, he rounded out his medical education at the outpatient's clinic run by Professor Jan Pilz in Kraków, the anatomical laboratory run by Prof. K. Monakow, the hospital wards run by Dr. J. Babiński in Paris, and some hospitals in Rheinau, Berlin, and Zurich. For some years he run a ward for nervous and mental diseases at the St. Lazarus's Hospital in Kraków. From 1921 to his premature death, he had taken the Chair of Neurology and Psychiatry at the Poznan University. He carried out research on brain anatomy, congenital brain defects, psychoanalysis, and mechanism of persecution. References: Public Record Office in Warsaw and Archive of the University of Medical Sciences in Poznań, S. Borowiecki's records.

H.09
MAKSYMILIAN ROSE (1883–1937) – THE WORLD FAMOUS POLISH NEUROANATOMIST
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Maksymilian Rose was born in 1883. In 1908 he graduated from faculty of medicine at Jagiellonian University in Cracow. Thereafter he worked in Berlin with T. Ziehen and H. Oppenheim specializing in neurology. Working with K. Brodmann in clinic of R. Gaupp in Tubingen he became interested in anatomy of the brain and especially in cytoarchitectonic of the cerebral cortex. Anatomy was still in the centre of his attention while he was working in Anatomy Institute under direction of K. Kostanecki. Between 1925–1928 he was appointed as director of the Department of Neurology in the Kaiser Wilhelm Institute in Berlin. Between 1928–1931 he worked as assistant professor (docent) at the Warsaw

University. At the same time, in Warsaw he organized one of the first Institutes for Brain Research in Europe. He moved to Wilno, where he was appointed in the University in 1931 as head of the psychiatric clinic, and since 1934 as head of the neurological clinic as well. In the same time he moved the Warsaw Brain Research Institute to Wilno and became his Director. The cytoarchitectonic of the cerebral cortex was his main topic of research. He founded the theory of the division of the cortex based on its development. He was the author of several cytoarchitectonic atlases and many scientific publications from neuroanatomy, neurology and psychiatry field. Professor Rose died in 1937 in Wilno.

H.10
JAN DEMBOWSKI (1889–1963), ANIMAL PSYCHOLOGIST AND FOUNDER OF BEHAVIOURAL PROTISTOLOGY IN POLAND

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Jan Dembowski was an outstanding Polish biologist, protistologist and animal psychologist. After biological studies in St. Petersburg (Russia), he received his PhD degree at the Warsaw University. He was then professor at the Free Polish University, and worked at the Nencki Institute of Experimental Biology in Warsaw, and then at the Stephen Báthory University in Vilnius. After WW II he worked at the Łódź University, and took part in the restoration of the Nencki Institute, where he became Head of the Department of Biology and Director (1947–1960). He was the first President of the new Polish Academy of Sciences (1952–1956). Dembowski wrote numerous books and scientific papers, both theoretical and experimental. His most important studies dealt with the behaviour of a protozoan *Paramecium caudatum*, larvae of the caddisfly *Molanna angustata* and various crabs. In *Paramecium* he analysed, among others, active choice of food on the basis of its physico-chemical features, patterns of swimming and geotactic behaviour. He also demonstrated the inability of *Paramecium* to show avoidance learning in response to light or shadow coupled with the electric shock. His research on behaviour of arthropods was focused mainly on the question of behavioural plasticity. In his book "Animal Psychology" (1950) he put forward a precursory thesis of scientific revolutions, broadly accepted only in 1962 when it was proposed again by Th. S. Kuhn. Dembowski played the piano and was a passionate hunter.

H.11
STEFAN KAZIMIERZ PIEŃKOWSKI (1885–1940) – THE FORGOTTEN POLISH NEUROLOGIST AND PSYCHIATRIST

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This presentation shows a profile of Professor Stefan Kazimierz Pieńkowski, the eminent neurologist and psychiatrist, the successor of Professor Jan Pilz at the Department of Neurology and Psychiatry at the Jagiellonian University in Cracow. He studied medicine in Warsaw and Kiev, next, medicine, philosophy and natural sciences in Cracow. He specialized in embryology, bacteriology, neurology and psychiatry in Cracow and some universities in France. His main scientific interests were focused on hereditariness of mental diseases, encephalitis, brain injuries, and influence of blood circulation disorder on nervous system. He published papers on encephalitis in French and Polish. He was the Editor-in-Chief of the journal "Neurologia Polska" and a correspondent for the French journal "Revue Neurologique", the Vice-President of the Psychiatric and Neurological Society, the Commission for Research on the Brain of Marshall Józef Piłsudski, the Eugenic Commission in the Polish Psychiatric Society. He was murdered by Soviets in Katyń. References: Public Record Office in Warsaw and Archive of Jagiellonian University in Cracow, S. K. Pieńkowski's records.

H. 12
JERZY CHORÓBSKI (1902–1986) – THE FOUNDER OF POLISH NEUROSURGERY

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Jerzy Choróbski, a distinguished neurosurgeon, who established Polish modern neurosurgery, graduated in medicine from the Jagiellonian University in 1926 and developed his career in European and American clinics. In 1920s he studied in Paris and mastered his talent among the elite European neurologists, neurosurgeons and psychiatrists of those days (Bidziński 2008). In 1930s, as a grantee of M. Ottman and Rockefeller Foundations, Choróbski specialized in neurosurgery under supervision of Prof. Penfield in Montreal. Many of his scientific relations outlasted the time of World War II and resulted in visits of leading neurosurgeons in Poland. Under difficult facility circumstances, on 6 November 1935 Choróbski opened a 15-bed Neurosurgery Ward at the Department of Neurological Diseases of the Warsaw University. He considered that date as the beginning of Polish modern neurosurgery. Choróbski broaden the spectrum of neurosurgeries being performed so far and many were conducted in Poland for the first time. In addition to brain, cerebellar or spinal cord tumors, he developed surgical treatment of sympathetic system diseases, which couldn't be cured pharmacologically at that time. Together with progress of diagnostic techniques, Choróbski introduced surgical treatment of epilepsy based on Penfield school approach and developed therapy of brain vascular diseases. This talented neurosurgeon established also surgical treatment of involuntary movements (Choróbski 1961, 1962).

H.13
JERZY KONORSKI (1903–1973) – THE PIONEER OF INVESTIGATIONS ON VOLUNTARY MOTOR BEHAVIOR

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Jerzy Konorski was one of the most eminent world leaders in research on brain physiology, the author of instrumental (Type II) conditioned reflex theory and the founder of the Polish school of neurophysiology. Already as a student of medicine, Konorski discerned experimentally a new type of conditioned reflexes, which he called motor (Type II) as opposed to classic (Type I) salivary reflexes, studied by I.P. Pavlov. In 1928 he described, for the first time, the specificity of Type II reflexes and the methods of their training. In 1931–1933 he stayed in Pavlov's famous laboratory in Petersburg. Since 1933 he was associated with the Nencki Institute of Experimental Biology, where after World War II, he created and became the Head of the

Department of Neurophysiology, the research of which was devoted to the analysis of mechanisms controlling voluntary motor behavior. Konorski published more than 180 papers, among them three monographs, concerning principles of brain functioning. His passion for research, creativity and profound knowledge attracted many scientists from around the world who visited him and his Department. In recognition of his scientific achievements Konorski's name was put on a strict list of candidates for Nobel Price. His death in 1973 was a great loss for both the Polish and world scientific community. His ideas are still appreciated and continue to inspire further research with the use of modern techniques.

H.14
LILIANA LUBIŃSKA (1904–1990) – THE PIONEER OF INVESTIGATIONS ON AXONAL TRANSPORT AND NERVE REGENERATION

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Professor Liliana Lubińska has been working in the Nencki Institute since 1933. In 1946 she has founded together with Professor Jerzy Konorski the Department of Neurophysiology in the Institute. Liliana Lubińska devoted practically her whole life to studies on peripheral nervous system with the main aim to elucidate the mechanism of functioning of the neuron with its small cell body and long axon. She has studied thus the events taking place during regeneration and Wallerian degeneration as well as the mechanisms of axonal transport. She has used simple methods elaborated and tested in every detail. In the late fifties Professor Liliana Lubińska encouraged Professor Stella Niemierko – the biochemist, to begin joint studies on transport of some compounds along axons. The joint work on this project as well as concomitantly carried on studies with Professor Jirina Zelena, brought to a formulation of basic, largely citated and referred to, hypothesis of bidirectional movement of axoplasm. Liliana Lubińska was an outstanding scientist with strong personality and with very brought knowledge of neurobiological phenomena and a unique intellectual capacity for posing and solving scientific problems. She has been the author of several original papers and reviews of fundamental significance, still frequently quoted in world literature. They had a great impact on neurobiology, influenced the research of many scientists and triggered a new line of experiments.

H.15
STELLA NIEMIERKO (1906–2006) – THE FOUNDER OF FUNCTIONAL NEUROCHEMISTRY IN POLAND

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All the long and most fruitful scientific activity of Professor Stella Niemierko has been inseparably connected with the Nencki Institute of Experimental Biology which she entered in 1926 as a third-year student. For many years she was involved in the studies of various aspects of phosphorus metabolism. In 1959 Stella Niemierko established the first national team to introduce a neurochemical approach to systems based physiology. Four years later she officially founded the Laboratory of Neurochemistry which she guided until her retirement in 1977. She actively participated in its further development until the end of her life. Collaborative work between Professor Stella Niemierko and Professor. Liliana Lubińska led to significant advances in the field of axonal transport. The major findings which brought to a formulation of basic hypothesis of bidirectional movement of axoplasm were published in the leading scientific journals Nature and Science. Encouraged by Prof. Jerzy Konorski, she undertook the studies on neurochemical basis of learning and memory. She also initiated investigations concerning biochemical changes occurring in the brain after first visual stimulation, which were awarded a national prize. Professor Niemierko has contributed to the development of neurochemical research projects in several centers in Poland gaining the respect and admiration of the whole Polish biochemical and neurobiological Community. She was honored member of the Polish Neuroscience Society.

H.16
EWA OSETOWSKA (1919–1978) – DISTINGUISHED POLISH NEUROPATHOLOGIST

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Professor Ewa Osetowska was one of the most distinguished Polish neuropathologists. She was born in 1919. In 1958 she underwent training in Bunge Institute, Antwerp, working under the supervision of Prof. Ludo van Bogaert. This experience together with the inspiration of her Polish mentor, Prof. Adam Opalski, proved decisive for her following scientific career. In 1963 she became the head of the Neuropathology Department of the Polish Academy of Sciences. She put her effort into organizing work in this new and completely unknown in Poland field of science. She assembled a group of people interested in the field of neuropathology and inspired them to the scientific work and to the organization of their own laboratories in different towns of the country. Professor E. Osetowska promoted 20 PhD's and 5 postdoctoral degrees. Among her students we can find many of later prominent scientists, such as: Professor Mirosław J. Mossakowski–President of the Polish Academy of Sciences, Professor Henryk Wiśniewski – Director of the Institute for Basic Research in New York, Professor Maria Dambska, a renowned specialist in the field of the development of the nervous system. At the age of 49, after long and severe illness, Professor. Ewa Osetowska passes away, leaving behind a stunning scientific legacy – altogether over 150 scientific publications in which clinical and neuropathological diagnosis was always a starting point for further search of the pathogenesis of the diseases.

H.17
MIROSŁAW J. MOSSAKOWSKI (1929–2001) AND HIS CONTRIBUTION TO NEUROPATHOLOGICAL RESEARCH IN POLAND

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Professor Mirosław Mossakowski, was a leading figure in neuropathology and neuroscience in Poland. For more than three decades he remained active in the study of various neurological disorders. Foremost was his brilliant early work on the morphological changes of neural cells exposed to ammonia, copper and sera from patients with hepatic encephalopathy, highlighting the critical role of astrocytes in the mechanism of this disorder. The biology and pathology of glial cells studied from the morphological and biochemical points

of view, the observation of primary fibrillary gliosis, were particularly innovative. His studies of neurodegenerative diseases brought the opportunity to observe and published first the description of the coexistence of lipid storage diseases and leucodystrophies. His work on cerebral ischemia brought about one of the first demonstrations of imbalance between glycogen synthesis and metabolism in the CNS. In addition to his considerable scientific contributions, he extended his inexhaustible energy and untiring devotion to the promotion of neuroscience and neuropathology. Professor Mossakowski served for two terms as a vice-president of the International Society of Neuropathology and on the editorial boards of many journals. He received many prestigious awards and nominations. Professor Mossakowski was a kind and generous man, a superb teacher, and role model. He will be sorely missed by all who were fortunate to experience his matchless wit, knowledge and wisdom.

H.18
KAZIMIERZ JAN ZIELINSKI (1929–2004) AND HIS STUDIES ON DEFENSIVE CONDITIONING
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Professor Kazimierz Jan Zielinski had his over-forty-year-long career path very closely connected with the development of the Nencki Institute. The general scope of his research was behavioral neuroscience. Much of his scientific work was devoted to systematic study of the defensive Pavlovian and instrumental conditioning, and to functions of the prefrontal cortex and the amygdala in aversive learning. Topics, to which Professor Zielinski had made important contributions, ranged from stimulus intensity dynamism to conditioned inhibition and processes of inhibition of delay. The director of the Nencki Institute for many terms, he also organized the Laboratory of Defensive Conditioned Reflexes within the Department of Neurophysiology. The scope of the Laboratory reflects one of Professor Zielinski's biggest scientific passions – the quest for understanding the mechanisms of avoidance learning, as well as internal complexity of this instrumental defensive response. Numerous studies at the Laboratory, many designed, performed or supervised by himself, were devoted to study parameters affecting the performance and differentiation of the avoidance response, and to examine the neuronal processes that mediate the states of fear and safety. Professor Zielinski hypothesized that different physiological mechanisms are responsible for performance of short- and long-latency avoidance responses, and that these two classes of avoidance response evoke diverse emotional states.

H.19
BOGUSŁAW ŻERNICKI (1931–2002) – INVESTIGATIONS ON SLEEP, LEARNING AND PLASTICITY
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Professor Żernicki described himself as a "physician by education, physiologist by profession, but admirer of psychology and philosophy". His most known and cited research concerned the pretrigeminal preparation. He proved that brain, isolated from the majority of sensory stimuli, preserves its basic behavioral functions and continues to learn. He studied also the perceptive and associative mechanisms of learning after deprivation of pattern vision at early developmental stages, elaborating the role of subcortical visual pathways in visual development. Professor Żernicki conducted research at the University of Pisa, University of Paris, Chilean University in Santiago, University of Rochester, and the University of Nice. He supervised NIMH grants for the Nencki Institute, hosted many foreign scientists, and organized several international conferences and meetings. Professor Żernicki was an author of more than 100 papers on central mechanisms of conditioning, isolated brain, physiology of the visual system and developmental neurophysiology. He also wrote four books in the Recent Discoveries of Science series, many chapters, textbooks and articles on policy in science. As a mentor in neuroscience he supervised 11 PhD theses. Most of his pupils continue research work in tenured positions in and outside the Institute. Several generations of neurophysiologists from the Nencki Institute were inspired by his scientific passion, talent for research and organization.

H.20
STANISŁAW WOLFARTH (1933–2007) AND HIS WORK RELATED TO PARKINSON'S DISEASE
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Stanisław Wolfarth graduated at the Medical Academy in Kraków in 1963. He worked for over 40 years at the Institute of Pharmacology PAS, where he was a head of the Department of Neuropharmacology (1971–2003). Studies on the role of the basal ganglia in development of Parkinson's disease became a passion of his life. One of his first achievements was the finding that clinical relationship termed dopaminergic-cholinergic balance resulted from interactions of complex neuronal systems including the substantia nigra, striatum and other structures. He demonstrated that GABAergic pathways connecting the substantia nigra, thalamus and zona incerta - lateral hypothalamus controlled the muscle tone, and played a crucial role in initiation of movements. He was one of the authors of a prototypic device for objective measurement of the muscle tone of rat's hind paw which is now the only apparatus of this type worldwide. Within the last 15 years he found antiparkinsonian properties of ligands of NMDA and metabotropic glutamate receptors in animal models, and discovered that muscle stiffness in the elderly had completely different pathophysiological basis than parkinsonian muscle rigidity. He was deeply involved in several international co-operations, was an author of over 90 scientific papers, and for his pioneer discoveries was highly prized by many scientific organizations. His works are characterized by amazing coherence of research objectives, consistency and up-to-dateness.