Organiser: Polish Neuroscience Society

Co-organisers: Jagiellonian University

Institute of Pharmacology, Polish Academy of Sciences, Krakow

Committee of Neurobiology of the Polish Academy of Sciences

Polish Chapter of IASP

Committees:

Chairperson: Ryszard Przewłocki
President of the Polish Neuroscience Society

Scientific Program Committee:

Chairperson: Grzegorz Hess
Members: Valerie Askanas, Klaus Peter Hoffmann, Małgorzata Kossut, Marian H. Lewandowski, Jerzy Łazarewicz, Katarzyna Nałęcz, Krystyna Ossowska, Edmund Przegaliński, Ryszard Przewłocki, Elżbieta Pyza, Jolanta Skangiel-Kramska, Krzysztof Turlejski

Local Organising Committee:

Chairperson: Marian H. Lewandowski
Secretary: Tomasz Błasiak, Grzegorz Hess
Treasurer: Krzysztof Turlejski
Members: Anna Błasiak, Bartosz Bobula, Małgorzata Filip, Monika Leśkiewicz, Joanna Mika, Dobromiła Pękala, Magdalena Regulska, Sławomir Siejka, Jadwiga Spyrka, Agata Suder, Hanna Szkudlarek, Katarzyna Werhun

SPONSORS:

IBRO - International Brain Research Organization
Polish Network of Molecular and Cellular Biology UNESCO/PAS
Ministry of Science and Higher Education of Poland
Abcam plc
Alpha Omega
Bank BPH
Biały Kruk
Bilaney Consultants
BIOTREND Chemikalien
Cambridge Electronic Design
Krakow Browary Tyskie
HEKA Elektronik
npi electronic
Olympus Polska
SIGMA-ALDRICH
Wydawnictwo KARPATY

EIGHTH INTERNATIONAL CONGRESS OF THE POLISH NEUROSCIENCE SOCIETY PROGRAMME

Monday, September 24th

2:00 P.M. General Assembly of the Polish Neuroscience Society
4:00 P.M. – 5:00 P.M. Coffee Break
5:00 P.M. – 6:30 P.M. Opening Ceremony
Konorski's Lecture
Multifarious aspects of antidepressant drugs
Jerzy Vetulani (Institute of Pharmacology, Polish Academy of Sciences, Krakow)
6:30 P.M. Get-together Party (Hall of the Auditorium Maximum)

Tuesday, September 25th

9:00 A.M. – 9:45 A.M. **Plenary Lecture**
Properties of glia
Helmut Kettenmann (Max Delbrück Center for Molecular Medicine, Berlin, Germany)
9:50 A.M. – 10:10 A.M. Young Investigator Lecture
10:10 A.M. – 10:30 A.M. Coffee Break
10:30 A.M. – 12:30 P.M. **Symposium I: Neurovision**
Sponsored by the German Neuroscience Society
Organiser and Chair: Klaus-Peter Hoffmann (Ruhr-University, Bochum, Germany)

Thomas Euler (Max Planck Institute for Medical Research, Heidelberg, Germany)
Dendritic processing in the direction-selective circuitry of the retina

Andrzej Wróbel (Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw)
Two streams of attention related beta activity in the visual system of the cat

Alexander Thiele (University of Newcastle upon Tyne, UK)
Mechanisms and neuropharmacology of visual attention in the primate

Frank Bremmer (Philipps-University Marburg, Germany)
Multisensory space and motion encoding

10:30 A.M. – 12:30 P.M. **Symposium II: Microglia in brain pathology**
Organiser and Chair: Bożena Kamińska (Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw) and Helmut Kettenmann (Max Delbrück Center for Molecular Medicine, Berlin, Germany)

Bożena Kamińska (Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw)
Good, bad and ugly – unraveling signalling pathways and role of microglia in brain pathology

Marcel Leist (University of Konstanz, Germany)
Anti-inflammatory treatent of microglia and astrocytes

Anna Członkowska (Institute of Psychiatry and Neurology, Warsaw)
Anti-inflammatory drugs in neurologic diseases

Krzysztof Selmaj (Medical University of Lodz)
Death receptor-induced oligodendrocyte loss during neuroinflammation

10:30 A.M. – 12:30 P.M. **Symposium III: Circadian rhythms and sleep**
Organiser and Chair: Elżbieta Pyza (Jagiellonian University, Krakow)

Kazue Semba (Dalhousie University, Halifax, Canada)
Direct and indirect links between circadian and sleep-wake systems

Amita Sehgal (University of Pennsylvania, Philadelphia, USA)
Cellular and molecular analysis of sleep in *Drosophila*

Teiichi Tanimura (Kyushu University, Fukuoka, Japan)
Pharmacology and neurogenetics of sleep in *Drosophila*

Jerzy Z. Nowak and Jolanta B. Zawilska (Medical University, Lodz)
The art of working together but independently: A lesson from the avian retina and pineal gland

12:30 P.M. – 1:00 P.M. Coffee Break

1:00 P.M. – 1:45 P.M. **Plenary Lecture**
Disruption of nucleolar activity in dopaminergic neurons leads to progressive parkinsonism
Günther Schütz (German Cancer Research Center, Heidelberg, Germany)

1:50 P.M. – 2:10 P.M. Young Investigator Lecture

2:10 P.M. – 3:30 P.M. Lunch

3:30 P.M. – 5:30 P.M. **Symposium IV: Development and plasticity of neuronal circuits in the sensory pathways**
Organiser and Chair: Kalina Burnat (Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw)

Leo M. Chalupa (University of California, Davis, USA)
Development and plasticity of retina and retinal projections.

Kalina Burnat (Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw)
Experience dependent developmental plasticity of the visual system

Lutgarde Arckens (Katholieke Universiteit Leuven, Belgium)
Molecular mechanisms of organizational plasticity in the sensory neocortex of mammals

Elżbieta Pyza (Jagiellonian University, Krakow)
Circadian clock and plasticity in the visual system of insects

3:30 P.M. – 5:30 P.M. **Symposium V: Novel data processing algorhithms in clinical neurophysiology**
Organiser and Chair: Wojciech Jernajczyk (Institute of Psychiatry and Neurology, Warsaw)
and Bruce J. West (U.S. Army Research Office, Research Triangle Park, USA)

Bruce J. West (U.S. Army Research Office, Research Triangle Park, USA)
Variability: The new paradigm for medicine

Paolo Grigolini (University of North Texas, Denton, USA)
Renewal and non-renewal events in the brain

Mirosław Łątka (Technical University of Wroclaw)
Cerebral hemodynamics in stroke patients: Is it possible to predict stroke?

Jakub Jernajczyk (Technical University of Wroclaw)
Influence of focal epilepsy on stage 2 NREM sleep EEG dynamics

3:30 P.M. – 5:30 P.M. **Symposium VI: Molecular mechanisms of dendrite and synapse formation**
Organiser and Chair: Jacek Jaworski (International Institute of Molecular and Cell Biology, Warsaw)

Maria Passafaro (CNR Institute of Neuroscience, Milan, Italy)
Extracellular domain of AMPA receptor GluR2 subunit interacts with N-cadherin to regulate spines

Casper C. Hoogenraad (Erasmus University, Rotterdam, Holland)
Activity-dependent regulation of liprin-alpha1 degradation by calcium/calmodulin-dependent protein kinase II

Grzegorz Wilczyński (Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw)
Synaptic metalloproteinases in plasticity and epileptogenesis

Jacek Jaworski (International Institute of Molecular and Cell Biology, Warsaw)
Role of mTOR regulated proteins in dendritic branching

5:30 P.M. – 5:50 P.M. Coffee Break
5:50 P.M. – 7:00 P.M. **Poster Session I**
7:30 P.M. Welcome Reception (Collegium Maius)

Wednesday, September 26th

9:00 A.M. – 9:45 A.M. Plenary Lecture
Multiple sclerosis – inflammation or neurodegeneration?
Krzysztof Selmaj (Medical University of Lodz)

9:50 A.M. – 10:10 A.M. Young Investigator Lecture

10:10 A.M. – 10:30 A.M. Coffee Break

10:30 A.M. – 12:30 P.M. **Symposium VII: The role of endosomes in physiology and neuropathology**
Organiser and Chair: Jacek Kuźnicki and Marta Miączyńska (International Institute of Molecular and Cell Biology, Warsaw)

Marta Miączyńska (International Institute of Molecular and Cell Biology, Warsaw)
The dual role of endosomes in trafficking and signaling

Kai Simons (Max Planck Institute for Molecular Cell Biology and Genetics, Dresden, Germany)
Lipid rafts in amyloid-precursor protein processing

Rafał Butowt (University of Nevada, Reno, USA and Nicolaus Copernicus University, Torun)
Sorting of trophic factors in neurons: Role of multivesicular bodies

Sandrine Humbert (Institut Curie CNRS, Paris, France)
Huntington disease: Huntingtin and the control of intracellular dynamics

10:30 A.M. – 12:30 P.M. **Symposium VIII: Cytokines and brain – implications for pathology and treatment of central nervous system disease**
Organiser and Chair: Anna Członkowska and Iwona Kurkowska-Jastrzębska (Institute of Psychiatry and Neurology, Warsaw)

Grażyna Gromadzka (Institute of Psychiatry and Neurology, Warsaw)
Cytokines, genes, and gender: Interrelationships in predicting stroke course and prognosis

Naoto Kawakami (Max Planck Institute of Neurobiology, Martinsried, Germany)
Reactivation in the CNS is crucial for the pathogenic potential of encephalitogenic T cells

Marta Kubera (Institute of Pharmacology, Polish Academy of Sciences, Krakow)
Role of cytokines in pathogenesis of depression

Iwona Kurkowska-Jastrzębska (Institute of Psychiatry and Neurology, Warsaw)
Therapeutic approaches in neurodegenerative diseases: A role of cytokines

Jacek Losy (University School of Medicine, Poznan; and Institute of Experimental and Clinical Medicine, Polish Academy of Sciences)
Cytokines in the pathogenesis of multiple sclerosis

10:30 A.M. – 12:30 P.M. **Symposium IX: Behavioral genetics**
Organiser and Chair: Artur Świergiel (Institute of Genetics and Animal Breeding, Polish Academy of Sciences, Jastrzebiec)

Marek Konarzewski (University of Bialystok)
Spontaneous locomotor activity of mice divergently selected for basal and summit metabolic rates

Andreas Reif (University of Wuerzburg, Germany)
The psychobiology of nitric oxide: Animal and human studies – do they translate?

Rafael Maldonado (University Pompeu Fabra, Barcelona, Spain)
Involvement of the endocannabinoid system in behavior of mice

Marek Wieczorek (Laboratory of Neurophysiology, University of Lodz, Lodz)
Cytokines-induced activation of the HPA axis in mice – neurophysiological and genetic studies

12:30 P.M. – 1:00 P.M. Coffee Break

1:00 P.M. – 1:45 P.M. **Plenary Lecture**
Genetic control of prefrontal cortex development
John L.R. Rubenstein (University of California at San Francisco, USA)

1:45 P.M. – 3:30 P.M. Lunch

3:30 P.M. – 5:30 P.M. **Symposium X: Metalloproteinases-dependent extracellular signalling in native and injured CNS**
Organiser and Chair: Teresa Zalewska (Medical Research Center, Polish Academy of Sciences, Warsaw)

Santiago Rivera (CNRS – Universite de la Mediterranee, France)
TIMP-1, a candidate plasticity protein running for election

Yvan Gasche (Geneva University Medical Center, Switzerland)
Multiple levels of matrix metalloproteinase involvement in ischemic brain injury

Leszek Kaczmarek (Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw)
MMP-9 in synaptic plasticity

Teresa Zalewska (Medical Research Center, Polish Academy of Sciences, Warsaw)
Ischemia modulates signal transduction from extracellular matrix

3:30 P.M. – 5:30 P.M. **Symposium XI: Mechanisms of drug dependence**
Organiser and Chair: Edmund Przegaliński (Institute of Pharmacology, Polish Academy of Sciences, Krakow)

Rainer Spanagel (Central Institute of Mental Health, Mannheim, Germany)
The role of glutamatergic signaling in drug abuse

Kathryn A. Cunningham (University of Texas Medical Branch, Galveston, USA)
Serotonergic mechanisms in extinction and reinstatement of conditioned drug reward

Charles Marsden (University of Nottingham Medical School, UK)
Does long-term cannabis use disrupt brain function – what can we learn from animal studies?

Małgorzata Filip (Institute of Pharmacology, Polish Academy of Sciences, Krakow)
GABA(B) receptor ligands as a pharmacotherapy for cocaine addiction-preclinical studies

Jan Rodriguez-Parkitna (German Cancer Research Center, Mannheim, Germany)
Drug-induced gene transcription and the development of addiction

3:30 P.M. – 5:30 P.M. **Symposium XII: Neuroimmunology of remyelination**
Organiser and Chair: Krzysztof Selmaj (Medical University of Lodz)

Ralf Gold (Ruhr University, Bochum, Germany)
The role of BDGF in tissue repair in multiple sclerosis

Wolfgang Bruck (University of Gottingen, Germany)
Immunopathology of MS lesion repair

Mariusz Stasiołek (Medical University, Lodz)
Mesenchymal stem cells in tissue repair in MS

Maciej Juryńczyk (Medical University, Lodz)
Inhibition of Notch/Jagged pathway enhances tissue repair in experimental autoimmune encephalomyelitis

5:30 P.M. – 5:50 P.M. Coffee Break

5:50 P.M. – 7:00 P.M. **Poster Session II**

8:00 P.M. Gala Dinner (Auditorium Maximum)

Thursday, September 27th (Joint meeting of Polish Neuroscience Society and Polish Chapter of IASP)

9:30 A.M. – 10:15 A.M. **Plenary Lecture**
Modern imaging of structure and function in human brain
Richard Frackowiak (University College London, UK)

10:15 A.M. – 10:40 A.M. Coffee Break

10:40 A.M. – 1:00 P.M. **Symposium XIII: Spinal cord plasticity: From adaptation to repair**
Organiser and Chair: Julita Czarkowska-Bauch and Małgorzata Skup (Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw)

James W. Fawcett (Cambridge University, UK)
The role of the extracellular matrix in the control of axon regeneration and plasticity

Julita Czarkowska-Bauch (Nencki Institute of Experimental Biology, PAS, Warsaw)
What locomotor training brings to intact and injured spinal cord

Thomas Misgeld (Technical University Munich, Inst. of Neurosciences, Muenchen, Germany)
In vivo imaging of spinal cord injury

Barbara Przewłocka (Institute of Pharmacology, Polish Academy of Sciences, Krakow)
Spinal peptidergic mechanisms of neuropathy after nerve injury

Daniel Zarzycki (Jagiellonian University, Collegium Medicum, Krakow)
Spinal cord injury, background, epidemiology and treatment

1:00 P.M. – 2:00 P.M. Lunch

2:00 P.M. – 2:45 P.M. **Plenary Lecture**
Elements of a neurobiological theory of hippocampal memory processing
Richard G.M. Morris (Centre and Division of Neuroscience, University of Edinburgh, UK)

2:45 P.M. – 3:00 P.M. Closing of the Congress

KONORSKI'S LECTURE

K1 Multifarious aspects of antidepressant drugs
Vetulani J.

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

Abstract not received

PLENARY LECTURES

L1 Properties of glia
Kettenmann H.

Max Delbrueck Center for Molecular Medicine, Berlin, Germany

In this lecture I will focus on two types of glial cells, namely astrocytes and microglial cells. Studies in the last year have established the concept that astrocytes are in intimate contact with neurons and can sense neuronal activity and can feedback on the neuronal system. Astrocytes also have an intrinsic form of cellular communication termed calcium waves. We provided evidence that this form of communication is not only found in cultured astrocytes but can also be recorded in tissue. Moreover, we found that astrocytes in a white matter tract, the corpus callosum communicate differently as astrocytes in the cortex. Microglial cells are the immunocomptent cells of the brain and respond to any type of pathology with an activation process. This microglial activation results in the migration to the site of injury or to the release of many cytokines. As cells related to the monocyte lineage they express a variety of different cytokine and chemokine receptors. We now could demonstrate that they also express receptors for neurotransmitters and that these receptors control microglial functions. We found a complex interplay with purinergic and adenosine receptors to control migration or adrenergic and GABAB receptors influencing cytokine release. Transmitter receptors in microglial cells could thus represent a functional crosslink between the immune and the nervous system.

L2 Disruption of nucleolar activity in dopaminergic neurons leads to progressive parkinsonism
Schutz G., Parlato R., Rieker C., Kreiner G.

German Cancer Research Center, Heidelberg, Germany

Disruption of nucleolar activity following the inactivation of the TIF-IA gene, a transcription initiation factor for polymerase-I, in dopaminergic neurons, induces their progressive loss. This is mediated by p53 upregulation and associated with a symptomatology strikingly similar to that observed in toxin-induced models of parkinsonism and Parkinson´s disease patients. Following nucleolar disruption we early see mitochondrial dysfunction leading to oxidative stress which eventually leads to impaired survival of dopaminergic neurons. Furthermore, loss of TIF-IA and impaired nucleolar function sensitizes dopaminergic neurons to complex I inhibition by MPTP treatment. These results demonstrate a crucial role of the nucleolus in control of survival of dopaminergic neurons and provide a novel perspective of the proces of neurodegeneration.

L3 Multiple sclerosis – inflammation or neurodegeneration?
Selmaj K.

Medical University of Lodz, Poland

Abstract not received

L4 Genetic control of prefrontal cortex development
Rubenstein J.L.R.

University of California at San Francisco, USA

I will review our studies on the mechanisms that control patterning and differentiation in the mouse cerebral cortex. Our work has focused on the role of FGF-signaling in controlling the size and nature of the frontal cortex. We have evidence that Fgf8 has a global role in patterning rostral parts of the cortex, whereas other Fgf genes, such as Fgf17, have a selective role in regulating development of the frontal cortex subdivisions. For instance, Fgf17 mutants have hypoplasia of the dorsomedial frontal cortex, whereas the orbital frontal cortex appears normal. In addition, Fgf17 mutant mice exhibit deficiencies in social interactions. These studies provide insights into a signaling pathway that is required for generation of part of the brain that regulates important executive functions, and suggests that defects in this pathway could underlie some forms of human neuropsychiatric disorders.

L5 Modern imaging of structure and function in human brain
Frackowiak R.

University College London, UK

Voxel based morphometry (VBM) is a univariate way of analysing structural MR images. VBM can characterise differences in structural MRI scans of diseases influenced by genetic variation. In X-linked Kallmann's syndrome there is selective hypertrophy of the pyramidal tract in patients with mirror movements compared to those without. In a dominantly inherited, dyspraxic, language-impaired family, gene penetrance is full and associated with abnormal structure and function of the caudate nucleus and other areas. Atrophy of the caudate in affected family members is associated with task-related hyperactivity, suggesting functional compensation. Presently unaffected individuals from families of Huntington's patients show caudate atrophy that correlates with genetic status. Caudate atrophy correlates with clinical score and CAG codon repeats on chromosome 4. Studies with Turner's and partial Turner's patients have identified focal structural brain abnormalities. Candidate regions on the X-chromosome have been found that influence amygdala and orbital frontal cortex development. A structural amygdala abnormality in patients predicts failure to recognise fear in photographs of faces; a prediction that is now confirmed. These studies suggest that imaging is an efficient way of associating candidate genes with quantitative measures of brain structure and function. More recently we have been experimenting with multi-variate image classification methods based on machine learning methods such as SVMs in order to classify individuals into pre-clinical diagnostic categories on scan images alone. The accuracy and sensitivity and specificity are remarkably high in these early phases. i will discuss this translational imaging research with reference to HD and AD and that informative intermediate phenotypes can be described that predict future disease in asymptomatic at-risk individuals.

L6 Elements of a neurobiological theory of hippocampal memory processing

Morris R.G.M.

Centre and Division of Neuroscience, University of Edinburgh, UK

Abstract not received

SYMPOSIUM I
NEUROVISION

S1.1 Dendritic processing in the direction-selective circuitry of the retina

Euler T.[1,2], Hausselt E.S.[1], Detwiler P.B.[2], Denk W.[1]

[1]Biomedical Optics, Max-Planck Institute for Medical Research, Heidelberg, Germany; [2]Physiology & Biophysics, University of Washington, Seattle, WA, USA

While retinal direction selectivity (DS) has been studied for over 40 years the mechanisms underlying the computation of the direction signal is still not fully understood. DS signals are first generated presynaptic to the DS ganglion cells in starburst amacrine cells (SACs). To study the computation of DS in SAC dendrites, electrical responses to centrifugal (CF) and centripetal (CP) visual stimuli were measured via somatic whole-cell recordings in rabbit retina and quantified using spectral analysis. Fundamental and harmonic frequency components were larger in response to CF than to CP motion. DS signals in SACs persisted in the presence of GABA and glycine antagonists, showing that SAC dendrites generate DS in the absence of lateral inhibition. The presence of harmonics indicates non-linearity, which, as the relationship between harmonic amplitudes and holding potential indicates, might be due to the activation of voltage-gated channels. [Ca^{2+}] changes in SAC dendrites evoked by voltage steps and monitored by two-photon microscopy suggest that the distal dendrite is tonically depolarized relative to the soma, due in part to tonic glutamatergic synaptic input, and that high-voltage Ca^{2+}-channels are active at rest. Supported by compartmental modeling, we conclude that DS computation in SACs is a dendrite-autonomous process, relying on voltage-gated channels and a dendritic voltage gradient, which provides the spatial asymmetry necessary for direction discrimination.

S1.2 Two streams of attention related beta activity in the visual system of the cat

Wrobel A.

Dept. of Neurophysiology, Nencki Institute of Experimental Biology, Warsaw, Poland

Areas and subdivisions of the cat's lateral posterior-pulvinar complex of the thalamus (LP-P) were recorded during a behavioral task based on delayed spatial discrimination of visual or auditory stimuli. During visual but not auditory attentive tasks we observed an increase of beta activity (12–25 Hz) as calculated from signals recorded from the LGN, the caudal part of the lateral zone of the LP-P (LPl-c) as well as from cortical areas 17, 18 and the sites located at the middle suprasylvian sulcus (MSS). This beta activity appeared only in the trials that ended with a successful response proving its relation to the mechanism of visual attention. Two subregions of LPl-c (ventromedial and dorsolateral) were distinguished by visually related, attentional beta activity of low (12–18 Hz) and high (18–25 Hz) frequencies, respectively. At the same time LGN and area 17 exhibited attentional activation in the whole beta range, an increase of power in low frequency beta was observed in the medial bank of MSS whereas cortical area 18 and the lateral bank of the MSS were activated in the high beta range. Phase correlation analysis revealed that two distinct cortico-thalamic systems were synchronized by the beta activity of different frequencies. One comprised the cortical area 17, ventromedial region of LPl-c and medial MSS, the second involved area 18 and the dorsolateral LPl-c. Our observations suggest that during attention related behavior the visual network is functionally segregated by distinct streams of beta activity.

S1.3 Mechanisms and neuropharmacology of visual attention in the primate

Thiele A.[1], Delicato L.S.[1], Roberts M.J.[1], Dayan P.[2]

[1]University of Newcastle upon Tyne; [2]Gatsby Computational Neuroscience Unit, UCL, UK

Attention can selectively enhance neuronal responses and exclude external noise, but the neuronal computations underlying these effects and the neuropharmacology involved remain unknown. We hypothesized that the effects of attention are mediated by increasing the synaptic efficacy of feed-forward connections while simultaneously reducing the synaptic efficacy of lateral connections, possibly through contributions from cholinergic mechanisms. We tested this proposal by recording neuronal activity and length tuning in macaque V1 when attention was directed towards or away from stimuli presented in the neuron's classical receptive field. For cells with central/parafoveal receptive fields, attention indeed reduced spatial integration demonstrated by a reduction in preferred length and in the size of the spatial summation area. Conversely, in cells representing more peripheral locations, attention increased spatial integration by increasing the cell's summation area. This previously unknown dichotomy between central and peripheral vision could support accurate analysis of attended foveal objects and target selection for impending eye-movements to peripheral objects. We further tested whether cholinergic mechanisms are an integral part of attentional modulation in V1. Cholinergic antagonists reduced the attentional modulation in the attend RF condition. Conversely acetylcholine application mimicked the effect of attention in the attend away condition. These data demonstrate that the cholinergic system plays an important role in mediating effects of attention in V1 of the macaque monkey.

Supported by: Wellcome Trust, BBSRC; MRC; Gatsby.

S1.4 Multisensory space and motion encoding

Bremmer F.

Dept. of Neurophysics, Philipps-University Marburg, Germany

The neural circuits underlying normal spatial vision and attentive sensorimotor behavior of primates have been most intensively studied in macaque monkeys. Both electrophysiological and anatomical studies have highlighted the importance of the posterior parietal cortex for the integration of neural signals from different sensory modalities and its use for guiding and controlling action in space. In the monkey, a highly modular structural and

functional specialization has been demonstrated within this part of the brain. One such functionally specialized area is the ventral intraparietal area (VIP) located in the fundus of the intraparietal sulcus (IPS). I will review a number of recent studies where we have shown that area VIP contains many neurons that show multisensory directionally selective discharges, i.e. these neurons respond to moving visual, tactile, vestibular or auditory stimuli. The functional properties imply that area VIP is involved in the encoding of self- and/or object motion in near-extrapersonal space. In addition, many VIP neurons also encode sensory information from different modalities in a common frame of reference. Although many specific human behaviors necessitate the convergence and integration of information conveyed through anatomically distinct sensory pathways, to date little is known about multisensory motion processing and integration in humans. To test for equivalencies between macaques and humans, we used functional MRI in normal human subjects while presenting moving visual, tactile, or auditory stimuli. Our functional imaging data reveal a confined network of cortical areas that respond to multisensory stimuli conveying motion information. One of these regions of activation is located in the depth of human IPS. Accordingly, we suggest that this area constitutes the human equivalent of monkey area VIP.

SYMPOSIUM II
MICROGLIA IN BRAIN PATHOLOGY

S2.1 Good, bad and ugly – unraveling signalling pathways and role of microglia in brain pathology

Kaminska B.

Nencki Institute, Warsaw, Poland

Microglia are highly plastic and multifunctional immune cells of the brain that execute various functions from immune surveillance to neuroprotection, and rapidly respond to pathological insults. In neurodegenerative diseases and acute brain injuries, activated microglial cells express pro-inflammatory cytokines and release toxic factors. Inhibition of acute microglia activation attenuates brain injury. The most dangerous brain tumors gliomas recruit microglia to the tumor site, and transform into tumor-supportive cells with no typical features of inflammation. We hypothesized that microglia activated under various conditions may be functionally different. Inflammatory microglial signaling involves activation of NFκB and MAP kinases as critical signal transducers. We demonstrated that in contrast to LPS-induced activation, glioma-derived factors induce: p38 MAPK and JNK signaling, release of factors promoting glioma invasiveness without pro-inflammatory responses. Erk signaling was down-regulated under those conditions. Instead, glioma-exposed microglial cells produced anti-inflammatory mediators: TGFβ1 and IL-10, which stimulate cell motility, invasiveness and phagocytosis. Gene expression profiling revealed differences in profile and extent of gene expression in microglia exposed to LPS- or glioma-derived factors. We demonstrate that while morphologically similar, glioma-associated microglia differs from inflammatory microglia and differences in signal transduction may be responsible for distinct functions. Identification of specific features of microglia in brain pathology will allow fine-tuning of its functions.

S2.2 Anti-inflammatory treatment of microglia and astrocytes

Leist M.

University of Konstanz, Chair of in-vitro alternative Methods, Konstanz, Germany

Microglia are found to be activated or amplified in all major neurodegenerative diseases. Although it is unclear, whether glia play a causal role in human neuropathology, they are certainly major players of the tissue response, and their modulation may be of therapeutic benefit. In order to better understand differential modulation of glia activation, we characterized the overall inflammatory response of astrocytes, primary microglia, the microglia cell line BV-2 and murine brain *in vivo*. Upon cytokine stimulation, astrocytes showed an amazing capacity to mount a full and long-lasting innate immune response, similar to macrophages in the periphery, and a number of interesting new markers such as interferon-inducible protein with tetratricopeptide motif-3 (IFIT-3) or thymidylate kinase (TYKi) were found to correlate with the strength of activation. The genes up-regulated *in vivo* in microglia were nearly all found to be regulated *in vitro*. However, a large number of additional genes appeared to be regulated *in vitro*, possibly due to a lack of counter-regulation. As we found that the MAP kinase pathway was stimulated in all glial cells upon activation, we explored potential upstream targets for a differential modulation of the inflammatory response. Inhibition of the class of mixed lineage kinases (MLK) had a dampening effect on inflammation without affecting central effector systems, such as NF-kB, and without direct inhibition of important immune response kinases such as p38 and JNK. Thus, inhibition of MLKs may be an interesting new approach to modulate the effector functions of glia in neurodegenerative diseases.

S2.3 Anti-inflammatory drugs in neurologic diseases

Czlonkowska A.

Institute of Psychiatry and Neurology, Warsaw, Poland

The mechanisms of neuronal-glial interaction play the main role in the maintenance of environment in the central nervous system during physiological condition and pathology. Any insult to the neurons produces abundant glial reaction and inflammation providing to repair. However, when inflammation is not well controlled it loses its function and may aggravate neuronal damage. Activated microglia produces cytokines and neurotoxic molecules, such as: free radical species, nitric oxide, complement activators, glutamate. Astrocytes, glial progenitors, lymphocytes also may play a dual role in repair and recovery. In mouse model of Parkinson's disease evoked by MPTP intoxication, inhibition of inflammatory reaction by anti-inflammatory agents showed a protective effect towards dopaminergic neurons. Dexamethasone, indomethacin or ibuprofen when administered shortly after or prior to MPTP, resulted in about 15–25% better neural preservation in the substantia nigra pars compacta. On the other hand, prolonged rofecoxib treatment after MPTP administration had no protective effect and produced even deeper dopamine decline. Retrospective and prospective observations have also shown that anti-inflammatory agents users have diminished risk of Parkinson's and Alzheimer's diseases. Nevertheless more recent data show conflicting results, anti-inflammatory agents give a hope for slowing progression of neurodegenerative diseases. Till now, we haven't had enough data to determine their mechanism of action, and we need more clinical trials before its use in neurodegeneration may be established.

S2.4 Death receptor-induced oligodendrocyte loss during neuroinflammation

Selmaj K.

Medical University of Lodz, Poland

Abstract not received

SYMPOSIUM III
CIRCADIAN RHYTHMS AND SLEEP

S3.1 Direct and indirect links between circadian and sleep-wake systems

Semba K.

Department of Anatomy and Neurobiology, Faculty of Medicine, Dalhousie University, NS, Canada

Daily cycles of sleep and wake – or rest and activity – characterize the lives of virtually all animals on Earth. The mammalian master circadian clock is housed in the suprachiasmatic nucleus (SCN). Lesions of the SCN disrupt the circadian pattern of sleep-wake, but do not substantially affect the amount of sleep or wake. Thus, sleep-wake states are regulated by a neural system distinct from the circadian timing mechanisms, and in fact this regulation involves several neuronal groups spread widely throughout the brain. How then do the two systems interact? This talk will review recent evidence suggesting that both direct and indirect neural pathways exist to form a link between the circadian and sleep-wake systems. Although there are a small number of direct pathways, most of the output pathways of the SCN appear to be indirect and relayed. The circadian clock also receives feedback from the sleep-wake system, and anatomical and functional evidence for this feedback will be discussed. These bidirectional links between the SCN and the sleep/wake-regulatory system allow for circadian influence on behavioral states, and modulation of circadian timing mechanisms by the sleep-wake system. This neuronal network, along with humoural messengers, is likely to play an important role in integrating circadian and homeostatic control of sleep and wakefulness.

S3.2 Cellular and molecular analysis of sleep in *Drosophila*

Sehgal A.

Howard Hughes Medical Institute/University of Pennsylvania School of Medicine, Neuroscience, Philadelphia, PA, USA

Drosophila has proved to be an extremely useful model for the study of circadian behavior, prompting researchers to consider it as a model for other behaviors. We showed that the rest state in flies shares behavioral, pharmacological and physiological attributes with mammalian sleep. We are using this fly model for sleep to understand the regulation and function of sleep. Based upon our earlier studies showing that the cAMP-PKA pathway affects sleep in *Drosophila*, we used a transgene encoding constitutively active PKA (PKA*) to map sleep-regulating areas in the fly brain. Thus, we targeted expression of this transgene to different areas of the fly brain and assayed the effect on sleep. The most dramatic sleep phenotypes resulted from expression of the transgene in a structure of the fly brain called the mushroom bodies (MBs) which are the site of learning and memory in *Drosophila*. We verified the role of MBs in sleep by ablating them and also by inducibly expressing transgenes to either promote or suppress electrical activity in selected MB neurons. In other work we showed that serotonin promotes sleep and consolidation of sleep in Drosophila and it does so by acting through a serotonin receptor (d5-HT1A) located in mushroom bodies. This further affirms the importance of the MBs in regulating sleep. The importance of the PKA-CREB pathway, and of the MBs, for learning and memory in all organisms examined, strongly supports the hypothesis that sleep is required for the consolidation of memory.

S3.3 Pharmacology and neurogenetics of sleep in *Drosophila*

Tanimura T.

Kyushu University, Biology, Fukuoka, Japan

Much attention has been paid to have a good sleep in modern societies, but we have not yet fully understood molecular and neural mechanisms of sleep. Recent studies revealed that a rest state in *Drosophila* could be regarded as a sleep-like state as it shares features with those in mammals. Several common molecules are shown to be implicated in the regulation of sleep in mammals and *Drosophila*. We used a computer-video system to track movement of a group of a single fly and analyzed various parameters of movement of flies to examine the effect of chemicals on sleep. Caffeine has a sleep deprivation effect on *Drosophila* as have been well known in mammals. In mammals caffeine is thought to act on the adenosine receptor. We found that a gene coding for the putative adenosine receptor is present in the fly genome and studied the function of the receptor using a cell culture system. The adenosine receptor is highly expressed in mushroom bodies in the fly brain and we showed that interference of function of those neurons resulted in reduction of amount of sleep. These results indicate that the similar neural mechanism of sleep is present both in *Drosophila* and mammals.

S3.4 The art of working together but independently: A lesson from the avian retina and pineal gland

Nowak J.Z.[1,2], Zawilska J.B.[2,3]

[1]Department of Pharmacology Medical University; [2]Center of Medical Biology Polish Academy of Sciences; [3]Department of Pharmacodynamics Medical University of Lodz, Poland

The avian pineal gland and retina synthesize melatonin (MEL) in a light-dependent circadian rhythm with high levels at night. The rate of MEL formation is regulated primarily by serotonin N-acetyltransferase (AANAT). Circadian oscillations in AANAT act. were found in pineal glands and retinas of chickens and turkeys kept for several days in constant darkness, or continuous light (pineals only). Exposure of birds to white and UV-A light acutely suppressed nocturnal AANAT act. and MEL. This light action was mediated by D4-DA receptors in retina and α2-adrenoceptors in pineal, and involved a decrease in cAMP, ultimately leading to destruction of AANAT protein. In addition, light resets circadian pacemaker generating MEL rhythm, producing phase-dependent shifts, advances or delays, in the circadian rhythm of MEL synthesis in pineal gland and retina. Although the avian pineal gland is a photosensitive organ, retinally perceived light has been shown to regulate pineal MEL synthesis in the chick. White light and UV-A,

acting on the eyes only, suppressed nocturnal MEL and resetted phases of circadian rhythm of pineal AANAT act. It is suggested that regulation of MEL synthesis in chick pineal by retinally perceived white and UV-A light may involve input from different photoreceptors. Cascade of events triggered by white light and UV-A includes stimulation of retinal D1-DA and NMDA receptors, respectively. Supported by MNSW grant 2PO6D 02529 and funds from Med Univ 503-1023-1 & 502-13-409.

SYMPOSIUM IV
DEVELOPMENT AND PLASTICITY OF NEURONAL CIRCUITS IN THE SENSORY PATHWAYS

S4.1 Development and plasticity of retina and retinal projections

Chalupa L.M.

University of California, Davis, USA

In all mammalian species the projections from the two eyes to the dorsal lateral geniculate nucleus of the thalamus terminate in separate layers or territories. This mature projection pattern is refined early in development from an initial state where the inputs of the two eyes are overlapping within the lateral geniculate nucleus. I will discuss the results of recent studies designed to test the hypothesis that retinal waves of neuronal activity, which occur spontaneously early in development of the visual system, play an instructive role in the formation of segregated eye-specific retinogeniculate projections. This will include work done in my laboratory in which waves of retinal activity were perturbed by depletion of cholinergic amacrine cells in developing ferrets as well as the results of multi-array microelectrode recordings from prenatal monkey retinas and β2-/- mouse retinas. Collectively, the results of these experiments challenge the prevalent notion that retinal waves of activity play an instructional role in the formation of segregated retinal projections. Rather, neuronal activity appears to be required for the normal elaboration of axonal projections in conjunction with the expression of target specific molecular cues. Supported by grants from the National Eye Institute of the NIH and Research to Prevent Blindness.

S4.2 Experience dependent developmental plasticity of the visual system

Burnat K.

Nencki Institute of Experimental Biology, Warsaw, Poland

What is the main factor delineating the mammalian visual system – visual experience or genetic fingerprints? This talk will vote for visual experience. Alternated visual stimulation is known to induce plasticity early in life. It regulates not only the development of visual cortex but also the development of the retinal circuitry. Effect of deprivation can persist even if normal visual experience follows for years initial period of 6 months patterned binocular deprivation in cat. We examined the expression profile of neurofilament protein in discrete cell types in adult cat retina using a monoclonal antibody SMI-32. A population of large retinal ganglion cells exhibited neurofilament expression in their soma and the proximal parts of their dendritic arbors. In the retinas of control cats the immunoreactive dendrites branched specifically into OFF inner plexiform sublamina. While, in the retinas of deprived cats, the dendrites branched throughout the entire inner plexiform layer showing a multi-stratification pattern, and the diameter of these large ganglion cells was significantly larger. The fact that final maturation of large, motion sensitive Y-type neurons occurs later compared to form sensitive X and modulatory W type cells seems to make them more susceptible to visual manipulation. The perceptual deficits are less pronounced in the form tasks than in the motion tasks in binocularly deprived cats, as is also the case for humans after early removal of bilateral congenital cataract.

S4.3 Molecular mechanisms of organizational plasticity in the sensory neocortex of mammals

Arckens L.

Laboratory of Neuroplasticity and Neuroproteomics, Department of Animal Physiology and Neurobiology, Katholieke Universiteit Leuven, Belgium

The visual cortex of mammals remains vulnerable to changes in visual input throughout the entire lifespan. Monocular and binocular deprivation or retinal lesions induce specific alterations of function and connectivity. Although several molecules have been implicated in driving the growth and refinement of the cortical circuitry, the exact molecular framework that directs age- and experience-dependent plasticity remains to be determined. We exploit proteomics technology to compare protein expression profiles in cat visual cortex at eye opening [postnatal day 10 (P10), when experience-dependent plasticity starts], the peak of the critical period (P30), and in adulthood. Upon comparison of normal control subjects with monocularly or binocularly deprived animals or animals with retinal lesions it became clear that every manipulation has its characteristic molecular fingerprint. Moreover, the expression of molecules involved in developmental plasticity does not always follow the course of the critical period as previously acknowledged, and developmental and adult plasticity involve different protein expression patterns. With this presentation we want to demonstrate the power of 2-D DIGE as a tool toward understanding the molecular basis of nervous system plasticity during development and adulthood.

S4.4 Circadian clock and plasticity in the visual system of insects

Pyza E.

Department of Cytology and Histology, Institute of Zoology, Jagiellonian University, Krakow, Poland

In the visual system of insects circadian rhythms have been described in several processes in the compound eye, including changes in the amplitude of electroretinogram, in the photoreceptor structure and in migration of the screening pigment granules. In addition to changes in the retina, we detected circadian rhythms in synaptic plasticity, and in plasticity of neurons and glial cells in the first optic neuropil, the lamina, in the fly's optic lobe. In the lamina the first order interneurons, L1 and L2 monopolar cells, which receive photic and visual inputs from the retina photoreceptors swell during the day and shrink during the night. This rhythm is maintained in constant darkness and in continuous light indicating on their endogenous, circadian control. The rhythm in neurons is

offset by changes in the lamina epithelial glial cells which are larger during the night than during the day. Using *Drosophila melanogaster* transgenic line expressing green fluorescent protein (GFP) in L2 cells we also found circadian changes in the sizes of nucleus and dendritic tree. While the L2 nucleus is larger during the whole day than at night, its dendrites are largest only at the beginning of the day. The morphological circadian plasticity of L2 was not observed, however, in the clock gene period null (per0) mutant. The structural circadian rhythms in interneurons and glial cells are correlated with daily changes in the number of synaptic contacts between the photoreceptor terminals and L1 and L2 cells.

SYMPOSIUM V
NOVEL DATA PROCESSING ALGORITHMS IN CLINICAL NEUROPHYSIOLOGY

S5.1 Variability: The new paradigm for medicine

West W.J.

U.S. Army Research Office, Research Triangle Park, USA

At the turn of the century it was apparent that the traditional dynamical models were not adequate to capture the complexity of biomedical phenomena. However, a few new ideas involving fractal time series, non-stationary processes, nonlinear dynamics, chaos and the fractional calculus had emerged, offering the hope that the ubiquitous complexity in medicine could be quantified and used diagnostically. We review this history using data sets and pictures rather than presenting the mathematical formalism. To begin we note that a biological signal carries information about the complex biological phenomena being measured and is typically a time series having both a regular and random component. We focus on dynamic physiologic phenomena, in order to reduce the number of time series we discuss to manageable size. The output of dynamical physiologic systems, such as the cardiac system, the respiratory system and the motor control system, have all been shown to be fractal and/or multifractal statistical time series. Such properties can be determined by studying the scaling behavior of the time series, which we do graphically. Consequently, the fractal dimension turns out to be a significantly better indicator of health than the more traditional measures; all average quantities. Fractal physiology, focuses on the complexity of the dynamic systems within the human body and the characterization of that complexity through fractal measures. These new measures reveal that the traditional interpretation of disease as the loss of regularity is not adequate and a better interpretation of disease is the loss of variability.

S5.2 Renewal and non-renewal events in the brain

Grigolini P.

Center for Nonlinear Science of the University of North Texas, Denton, Texas, USA

We analyze EEG dynamics by means of the minimal spanning tree technique, and we define as an event the abrupt change of tree topology determined by the dynamics of this network. We prove that the occurrence of one of these events erases the memory of the earlier events and we find that the corresponding survival probability, namely the probability that no new event occurs up a time t from an earlier event, is a stretched exponential function. We account for this behavior by means of a model of many Poisson constituents that through spontaneous cooperation generates a global and anomalous diffusion process. With the help of this model we show that the brain renewal events are non-Poisson and non-ergodic. We argue that the presence of renewal events is essential for the brain function and that departing from this condition may be the signature of a pathological condition. We confirm this conjecture by analyzing the EEG data of epileptic patients, and proving that in this case the condition of non-ordinary statistical physics is determined by the periodic modulation of Poisson processes, with no significant occurrence of non-Poisson renewal events. We prove that the renewal character of the healthy brain makes it insensitive to periodic perturbation, but very sensitive to perturbations of even very weak intensity, if these perturbations host renewal events of the same non-ergodic nature as the brain renewal events.

S5.3 Cerebral hemodynamics in stroke patients: Is it possible to predict stroke?

Latka M.[1], Turalska M.[1], Czosnyka M.[2], Glaubic-Latka M.[3], Pierzchala K.[4], West B.J.[5]

[1]Wroclaw University of Technology, Poland; [2]Cambridge University, UK; [3]Opole Regional Medical Center; [4]Silesian Medical University, Poland; [5]Army Research Office, USA

Carotid artery stenosis is generally thought to induce stroke by either compromising cerebral perfusion or inciting embolic phenomena. The interplay of cerebral perfusion pressure (CPP) and cerebral autoregulation (CA) determines perfusion of the brain. Under physiological conditions, the CPP dynamics is essentially associated with changes in arterial blood pressure (ABP). The significance of the autonomous nervous system in systemic blood pressure control can hardly be overestimated. For example, baroreflex stabilizes blood pressure fluctuations with characteristic frequencies in the neighborhood of 0.1 Hz. On the other hand, changes in CPP may be compensated by local adjustments of the tone of the cerebrovascular bed – a mechanism known as cerebral autoregulation (CA). Using the mathematical framework of dynamical synchronization, we demonstrate that regulatory effects of baroreflex and CA are comparable. Moreover, we find that the baroreflex strength is significantly impaired both in stroke and transient ischemic attack patients. We emphasize that carotid baroreceptors and chemoreceptors are evolutionarily maladapted to modern diseases such as atherosclerosis or hypertension. Furthermore, the efficacy of carotid stenting or endarterectomy may be limited by inadvertent injury to these receptors.

S5.4 Influence of focal epilepsy on stage 2 NREM sleep EEG dynamics

Jernajczyk J.[1], Krause M.[2], Latka M.[1], Waliniowska E.[3], Terczynska I.[4], West B.J.[5], Jernajczyk W.[3]

[1]Wroclaw University of Technology; [2]Marciniak Regional Medical Center, Wroclaw; [3]Institute of Psychiatry and Neurology; [4]Institute of Mother and Child, Warsaw Poland; [5]Army Research Office, USA

It has been argued that the neural mechanisms involved in the generation of sleep spindles facilitate production of interictal epileptic discharges (IED) in childhood partial epilepsies. To shed new light on this hypothesis we analyze the statistical properties of stage 2

sleep EEG recordings in children with focal epilepsy and in the sex- and age-matched controls. From the standard EEG recordings, we extracted 10 min segments of initial stage 2 sleep and for each channel examined the frequency dependence of the continuous complex Morlet wavelet power normalized by the total power of the corresponding channel. In the centro-parietal area, in 12–14 Hz frequency range characteristic of sleep spindles, the relative wavelet power was on average three times greater in focal epilepsy patients than in the control cohort. Interestingly enough, the amplitude of slow wave oscillations (theta band) in frontal channels, quantified by the wavelet power normalized by the average power per channel, could be as much as four times lower in the epileptic patients than in controls. Previous studies have suggested that appearance of sleep spindles boosts EEG slow waves. Thus, the observed elevated cortical arousal in epileptic patients may trigger increased sleep spindle activity and indirectly exacerbates generation of IEDs.

SYMPOSIUM VI
MOLECULAR MECHANISMS OF DENDRITE AND SYNAPSE FORMATION

S6.1 Extracellular domain of AMPA receptor GluR2 subunit interacts with N-cadherin to regulate spines

Passafaro M.

DTI, CNR-Institute of Neuroscience, Department Pharmacology, University of Milan, Italy

AMPA-type glutamate receptors mediate the majority of excitatory synaptic transmission in the mammalian brain and changes in the number of synaptic AMPA receptors contribute to long-lasting changes in synaptic strength and dendritic spine enlargement. Moreover, abundance of postsynaptic AMPA receptors is correlated with the size of the synapse and dendritic spine head. *Via* the first N-terminal 92 amino acids of the extracellular domain (NTD), the AMPA receptor subunit GluR2 promotes the formation and growth of dendritic spines in cultured hippocampal neurons. Moreover, overexpression of this extracellular domain increases the frequency of miniature excitatory postsynaptic currents (mEPSCs). Biochemically the NTD of GluR2 can interact directly with the cell adhesion molecule N-cadherin, in cis or in trans. N-cadherin-coated beads recruit GluR2 on the surface of hippocampal neurons and N-cadherin immobilization decreases GluR2 lateral diffusion on the neuronal surface. RNAi knockdown of N-cadherin prevents the enhancing effect of GluR2 on spine morphogenesis and mEPSC frequency. Our data indicate that in hippocampal neurons N-cadherin and GluR2 form a synaptic complex that stimulates presynaptic development and function as well as promoting dendritic spine formation.

S6.2 Activity-dependent regulation of liprin-alpha1 degradation by calcium/calmodulin-dependent protein kinase II

Hoogenraad C.C.

Department of Neuroscience, Erasmus Medical Center, Rotterdam, The Netherlands

Activity-dependent calcium entry into neurons induces a variety of changes, ranging from transient post-translational modifications of synaptic proteins to altered gene expression. At excitatory synapses Ca^{2+} influx through voltage-gated Ca^{2+} channels and ionotropic glutamate receptors (particularly NMDA receptors) triggers biochemical cascades that regulate synaptic function. Calcium signaling pathways also control neuronal differentiation, axon pathfinding and dendrite morphogenesis. A major player in all these processes is calcium/calmodulin-dependent protein kinase II (CaMKII), a calcium-activated serine/threonine kinase that is abundant in neurons, especially at postsynaptic sites. By functioning as a local calcium sensor, CaMKII exerts a critical influence on the architecture of the developing and plastic brain. Here we describe a novel activity-dependent regulation of liprin-alpha1 by CaMKII, in which liprin-alpha1 is degraded in response to CaMKII phosphorylation. Liprin-alpha1 levels in hippocampal neurons are additionally regulated by the UPS *via* the E3 ubiquitin ligase APC (Anaphase Promoting Complex). Expression of liprin-alpha1 mutants insensitive to CaMKII degradation specifically inhibits the dendritic targeting of receptor tyrosine phosphatase LAR and leads to reduced dendrite arborization and synapse number. These findings provide a molecular basis for activity-dependent regulation of local dendrite and synapse development by CaMKII, liprin-alpha1 and LAR.

S6.3 Synaptic metalloproteinases in plasticity and epileptogenesis

Wilczynski G.M.

Nencki Institute, Warsaw, Poland

Structural remodeling of dendritic spines is essential for both physiological and pathological synaptic plasticity. Matrix metalloproteinases (MMPs) play pivotal roles in tissue structural remodeling throughout the body. Among those, a major role in synaptic plasticity has been ascribed to MMP-9, a synaptic MMP, that was implicated in long-term potentiation and some forms of learning. In addition, several lines of evidence indicate that MMP-9 plays a key role in aberrant plasticity underlying epilepsy. In particular, kainate-induced status epilepticus, an event triggering epileptogenesis, strongly increases synaptic MMP-9 amount and activity. Moreover, studies performed in organotypic hippocampal cultures, and KO- and transgenic animals indicate that the enzyme is involved in seizure-induced spine loss and mossy fibers sprouting. Importantly, chemical kindling-induced epileptogenesis is retarded in MMP-9-deficient animals whereas it is accelerated in MMP-9-overexpressing animals. It is hypothesized that the enzyme may act, at least in part, by increasing dendritic spine flexibility thereby facilitating plasticity- and epilepsy-related changes in spine size and shape.

S6.4 Role of mTOR regulated proteins in dendritic branching

Jaworski J., Swiech L.

International Institute of Molecular and Cell Biology, Warsaw, Poland

Dendrites are the main site of information input onto neurons, and different neurons have distinctive and characteristic dendrite branching patterns. Diffusible cues, cell-cell interactions and neuronal activity have been shown to regulate various steps of dendrite development. Among the proteins that transduce these signals into changes in dendritic arbor shape are several protein kinases. Recently we showed that mammalian target of rapamycin (mTOR) kinase is crucial for growth and branching of dendrites in cultured hippocampal neurons.

Both basal and induced growth of dendrites was blocked by mTOR inhibitor rapamycin and small interfering RNA directed against mTOR. In search for molecular targets of mTOR activity in the dendritic branching process we focused on its best known effectors – eIF-4E binding protein1 (4E-BP1) and p70S6K. Both these proteins are regulators of protein synthesis. Overexpression of 4E-BP1 or its active mutant as well as knock-down of p70S6K by RNA interference, led to simplification of dendritic arbor both under basal and dendritic branching promoting conditions. I will also present preliminary results showing that mTOR regulated microtubule dynamics by means of controlling CLIP-170 might be involved in shaping dendritic arbor. These results strongly suggest that by regulating global and/or local protein translation, as well as microtubule dynamics and as a convergence point for multiple signaling pathways, mTOR could play a central role in the control of dendrite growth and branching during development and in response to activity.

SYMPOSIUM VII
THE ROLE OF ENDOSOMES IN PHYSIOLOGY AND NEUROPHYSIOLOGY

S7.1 The dual role of endosomes in trafficking and signaling

Miaczynska M.

International Institute of Molecular and Cell Biology, Warsaw, Poland

Accumulating evidence indicates that the processes of endocytic trafficking and intracellular signal transduction are tightly linked and mutually dependent on each other. Transduction of extracellular signals is initiated at the plasma membrane and proceeds through cytoplasm to the nucleus. Initially endocytosis was considered merely a mechanism for signal termination by degradation of receptors activated at the plasma membrane. More recent studies argue, however, that transduction of signals from the cell surface can continue within the endocytic pathway, in part *via* endosome-specific signalling complexes, different from those assembled on the plasma membrane. Therefore, endosomes constitute intracellular platforms for active signal propagation, enabling a precise spatial and temporal control of cellular responses. We identified a subpopulation of early endosomes marked by the presence of APPL1 and APPL2 proteins. These compartments play a role in endocytic trafficking of cargo such as epidermal growth factor or transferrin. At the same time, APPL proteins act as signal transducers by cycling between the endosomes and the cell nucleus in response to certain extracellular stimuli. Cumulatively, the data indicate that both in non-neuronal and neuronal cells, APPL-positive endosomes may represent a specialised endosomal compartment devoted to signalling, further underscoring the link between intracellular signalling and endocytic trafficking.

S7.2 Lipid rafts in amyloid-precursor protein processing

Simons K.

Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany

Our working hypothesis is that raft clustering plays a crucial role in the pathogenesis of Alzheimer's disease. During intracellular transport APP undergoes a series of proteolytic cleavages that lead to the release of an amyloidogenic fragment, β-amyloid (Aβ). It is Aβ that accumulates in the brain lesions that are a hallmark of the disease. While inside raft clusters APP seems to be cleaved by β-secretase, APP outside rafts undergoes cleavage by alpha-secretase. Thus, access of alpha- and beta-secretase to APP, and therefore Aβ generation, may be determined by dynamic interactions of APP with lipid rafts. We have also demonstrated that β-cleavage is occurring in early endosomes and we have recently obtained evidence that a fraction of Aβ leaves the cell associated with exosomes. We are now reconstituting amyloidogenic processing in liposomes. We have established a novel *in vitro* assay to study how the membrane lipid composition influences beta-cleavage of APP. For this, we expressed and purified APP and BACE and reconstituted them into lipid bilayers of specific lipid composition. Our data show the enzyme is activated by sphingolipids, cholesterol and acidic phospholipids *in vitro*. We have also shown that BACE partitions with liquid-order phases in giant unilamellar vesicles and that this partitioning is augmented by raft clustering. All these results support our model for APP processing.

S7.3 Sorting of trophic factors in neurons: Role of multivesicular bodies

Butowt R.

Department of Physiology and Cell Biology, School of Medicine, University of Nevada, Reno, NV-89557, USA

The survival of neurons depends on the retrograde axonal transport of trophic signals. Neurons of the chicken isthmo-optic nucleus (ION) and rodent hypoglossal nucleus (HyN) retrogradely transport trophic factors, and this transport is essential for survival and differentiation of these neurons. Internalized trophic factors may be degraded, or they may be recycled and transferred to other neurons. This transfer is similar to the well-known route of internalized tetanus toxin. In the present study, we address the basic question whether differences in function and signaling of different trophic factors in ION and in HyN are reflected by differences in their subcellular sorting pathways after internalization. In particular we examined the role of multivesicular bodies in sorting of different trophic factors in both *in vivo* model systems. This is of particular interest since (1) to date, the fate of different retrogradely transported trophic factors has not been compared at the ultrastructural level *in vivo*, and (2) current data indicate that targeting to neuronal trafficking and sorting organelles may represent a regulatory mechanism that activates different signal transduction pathways complexes within specific neuronal compartments.

S7.4 Huntington disease: Huntingtin and the control of intracellular dynamics

Humbert S., Saudou F.

Institut Curie, CNRS UMR146 Orsay, France

Huntington disease (HD) is a fatal neurodegenerative disorder that affects 1 in 10 000 individuals of european origin. The neuropathology of HD involves neuronal dysfunction and the selective death of striatal neurons. The mutation that causes disease

is an abnormal expansion of a polyglutamine (polyQ) stretch in the N-terminus of the 350 kD protein huntingtin. The mechanisms by which huntingtin induces dysfunction and death of neurons in the brain are not clearly understood. These could involve the gain of new toxic functions and the loss of the beneficial activities intrinsic to wild-type huntingtin. Indeed, as observed in cell culture and animal models, huntingtin possesses anti-apoptotic properties. We have used 3D fast video microscopy techniques to study the intracellular dynamics in normal and pathological situations. Using this approach we have unravelled a function of huntingtin in the microtubule-based transport of neurotrophic factors such as BDNF. In the pathological situation, huntingtin-stimulated BDNF transport is altered. Reduced BDNF transport leads to a decrease in neurotrophic support and to neurotoxicity that are both rescued by wild-type huntingtin. Our results demonstrate that the anti-apoptotic properties of huntingtin are linked to the ability of huntingtin to promote transport of BDNF in the brain. We will report recent findings that identify compounds and/or signaling pathways that inhibit the toxicity of mutant polyQ-huntingtin in disease but also regulate huntingtin normal function in the control of intracellular dynamics.

SYMPOSIUM VIII
CYTOKINES AND BRAIN – IMPLICATIONS FOR PATHOLOGY AND TREATMENT OF CENTRAL NERVOUS SYSTEM DISEASE

S8.1 Cytokines, genes, and gender: Interrelationships in predicting stroke course and prognosis

Gromadzka G.

Institute of Psychiatry and Neurology, Medical University, Warsaw, Poland

Experimental and clinical data strongly support a causal role of proinflammatory cytokines (PC) in the clinical course of stroke. In experimental animals up-regulation of PC occurred rapidly in the brain following stroke; injections of PC resulted in increased infarct size, more intensive leukocyte recruitment, enhanced apoptosis of neurons, increased neurological deficits and higher mortality. In some clinical studies increased PC concentrations in cerebrospinal fluid and/or serum of stroke patients was associated with poor prognosis of the disease. Gender effects on stroke pathology, mortality, as well as on functional outcome have been well documented in animal studies. Gender impact on clinical stroke course and prognosis has also been described, and is still under exploration. Some data indicates that cytokines – sex hormones interactions may represent a possible background mechanism for the link: gender – stroke outcome. On one hand sex hormones influence PC synthesis as well as modulate adverse cytokine effects. On the other hand, PC may regulate the sex hormones production. Until now only few experimental and clinical stroke studies were published focused on this issue. In humans, the PC production is genetically controlled and dependent on functionally significant PC gene polymorphisms. We recently documented, that gender may modify the effects of functionally significant PC gene polymorphisms on the outcome of stroke in terms of neurological/functional impairment and mortality.

S8.2 Reactivation in the CNS is crucial for the pathogenic potential of encephalitogenic T cells

Kawakami N., Odoardi F., Wekerle H., Flügel A.

Department of Neuroimmulonogy, Max-Planck-Institute for Neurobiology, Martinsried, Germany

We studied the locomotion and function of autoaggressive T cells during Experimental Autoimmune Encephalomyelitis (EAE). Genetically labeled fluorescent myelin antigen-specific CD4+ T cells transferred to syngeneic healthy recipients induced a severe paralytic disease after a prodromal period of at least 3 days. The onset of disease coincided with massive invasion of autoaggressive T cells into the CNS. Using 2-photon imaging we observed three distinct phases of effector cell invasion into the CNS: (1) the first invading T cells were found in vicinity of meningeal vessels crawling along the vessel walls. (2) within hours, their numbers increased dramatically and single cells proceeded to the perivascular space of the CNS white and grey matter. (3) during established disease, the cells distributed throughout the CNS parenchyma. The T cells displayed characteristic locomotion patterns: the majority of cells moved through the tissue seemingly randomly with episodes of fast locomotion interchanging with phases of slower migration or brief halts. The other part of the cells became tethered forming synapse-like contacts to MHC class II positive cells indicating that effector T cells recognize their antigen in midst of their target organ. Accordingly, brain-specific but not control antigen-specific T cells became stopped and up-regulated proinflammatory cytokines within the CNS. Further, soluble antigen infusion during acute EAE induced tethering and activation of autoaggressive T cells, followed by a strong disease exacerbation.

S8.3 Role of cytokines in pathogenesis of depression

Kubera M.

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

Inflammatory responses play an important role in the pathophysiology of depression. Depressed patients were found to have higher level of proinflammatory cytokines and acute phase proteins. Therapeutic administration of interferon a or interleukin-2 leads to depression in up to 50% of patients. Proinflammatory cytokines in experimental animals induced behavioral and pathophysiological changes typical for depression. Depression has been associated with inflammatory markers in the course of several medical illnesses, including cardiovascular disease, autoimmune disease, infection, cancer. Chronic stress, which can induce state of depression, can also promote inflammatory responses. Cytokines access the brain by several routs including entry through leaky regions in the BBB and activation of vagal afferent fibers. It is highly significant that that the major mechanism by which immune stimuli activate the hypothalamic-pituitary-adrenal axis is the same as that involved in the response to psychological stressors, namely activation of CRF-containing neurons in the paraventricular nucleus of the hypothalamus. An infection, especially viral one, induces IFN-g, which is a potent inducer of indoleamine 2,3-dioxygenase. This enzyme degrades tryptophan and limits the availability of tryptophan for serotonin synthesis. Cytokines can also activate the serotonin transporter, which could decrease extracellular serotonin. The increased cytokine production may induce depression in some patients although action of cytokines cannot account for all cases of depression. This study was supported by grant 2074/P01/2007/32 from the Ministry of Sciences and High Education.

S8.4 Therapeutic approaches in neurodegenerative diseases: A role of cytokines

Kurkowska-Jastrzebska I.

Institute of Psychiatry and Neurology, Warsaw, Poland

In neurodegenerative diseases increased level of proinflammatory cytokines and altered level of trophic factors, indicate that active process of neuroinflammation accompany neuronal death. The lack of trophic factors during this process may aggravate primary damage. Changes of not all but specific trophic factors are important for the particular population of neurons. In AD brain, there is observed an increase in NGF and decrease in BDNF in surviving neurons of hippocampus and certain neocortical regions and decrease in TrkA in cortex and nucleus basalis. In PD brain, decreases in neuronal content of GDNF and bFGF in surviving substantia nigra dopaminergic neurons have been observed. In experimental MPTP model of PD we showed that levels of various cytokines (i.e., IL6 and TNFα) increases twice in acute and regeneration stages of injury, indicating its role in recovery process. GDNF and TGFbeta mRNA expressions increase also from the early stages of injury and more intensely, in the situation of immune system activation, which results in dopaminergic neurons protection. In model of hippocampal injury caused by trimethyltin, NGF expression is increased in preserved pyramidal neurons together with increased TrkA expression in astroglia, and with enhanced neurogenesis in subgranular zone. Neuroprotection induced by the activation of immune system in TMT model, is suggested to be mediated by NGF dependent mechanism. As cytokines may promote signals that either lead to cell death or to neuroprotection they are a good target for therapeutic strategies.

S8.5 Cytokines in the pathogenesis of multiple sclerosis

Losy J.

University School of Medicine, and Institute of Experimental and Clinical Medicine, Polish Academy of Sciences, Poznan, Poland

Multiple sclerosis ia an immune mediated demyelinating disease of the central nervous system. Cytokines are important components of the immune inflammatory process and are implicated in oligodendrocyte cell death, demyelination and axonal degeneration. Increased production of both pro- and anti-inflammatory cytokines is a common finding in MS. These cytokines are found in MS tissue as well as in the CSF from patients with MS. Th1 cytokines (IL-2, TNF-alpha, IFN-gamma) are proinflammatory, whereas Th2 cytokines (IL-4, IL-5, IL-6, IL-10) as well as TGF-beta are anti-inflammatory. Another important cytokines in the pathogenesis of MS are IL-12, IL-15 and IL-18. The proinflammatory cytokines play a role in activation of microglia cells, astrocytes, macrophages and endothelial cells stimulating production of adhesion molecules. Cytokines like TNF-alpha may induce the direct injury to myelin as well as damage of oligodendrocytes through induction of apoptosis *via* Fas, TNF-related apoptosis-inducing ligand (TRAIL) or TNF-receptors. Intravenous administration of IFN-gamma induces MS relapses. Th2 cytokines inhibit Th1 differentiation and limit proinflammatory Th1 responses. Some Th2 cytokines stimulate also B cells and antibody production. Some cytokines in MS may have both detrimental and beneficial effects. For example IL-1β can contribute to CNS injury by action on metalloproteinases but is also involved in the production of the nerve growth factor CNTF. TNF is cytotoxic acting *via* TNFR1 but can also support remyelination *via* TNF R2. So far, no pattern of cytokine expression specific for multiple sclerosis has been found. Immunomodulatory therapy may influence synthesis of several cytokines. A good example is an increase of TGF-β levels after therapy with IFN-β in relapsing remitting multiple sclerosis what may decrease ongoing inflammation.

SYMPOSIUM IX
BEHAVIORAL GENETICS

S9.1 Spontaneous locomotor activity of mice divergently selected for basal and summit metabolic rates

Konarzewski M., Gebczynski A.

Institute of Biology, University of Bialystok, Bialystok, Poland

Endotherms (birds and mammals) are able to maintain a relatively constant, elevated body temperature by means of a high rate of basal energy metabolism (BMR). Although the advantages of high BMR are easily recognised, selection forces, which initiated its evolution are still debatable. We tested two alternative hypotheses suggesting that the evolution of high BMR was driven by selection favouring (1) high long-term locomotor activity/metabolism (2) short bouts of summit activity/metabolism. We measured voluntary activity of laboratory mice evaluated as the daily total distance travelled in freely accessed wheels. Mice came for artificial divergent selections for either high/low BMR or high summit metabolic rate. Mice selected for high/low BMR exhibited significant, between line differences in voluntary activity, with mice of high-BMR line being significantly more active. In contrast, the selection for summit metabolism did not result in correlated response in an increased voluntary activity. Thus, our results support the notion that high BMR evolved as a result of selection on long-term, rather than summit locomotor activity.

S9.2 The psychobiology of nitric oxide: Animal and human studies – do they translate?

Reif A., Lesch K.P.

Molecular and Clinical Psychobiology, Department of Psychiatry and Psychotherapy, University of Würzburg, Germany

The gaseous messenger nitric oxide (NO) has been implicated in a wide range of behaviors, including aggression, anxiety, depression, and cognitive functioning. Behavioral phenotyping of mice lacking the neuronal isoform of nitric oxide synthase (NOS-I), the major source of NO in the central nervous system, revealed no differences in activity-related parameters; depression-related tests but a subtle anxiolytic phenotype. The most prominent feature however was cognitive impairment in spatial learning and memory. A set of >120 differentially expressed genes was identified in a gene chip study. Among the most significantly up-regulated genes were CCAAT/enhancer binding protein alpha, 3 genes involved in GABA(B) signalling and the glucocorticoid receptor. In contrast to rodent behavior, little is known about the function of NO in humans. We could identify an promoter polymorphism in the transcriptional control region of exon 1f of the human NOS1 gene. By

a reporter gene assay, we could show that this polymorphism alters the expression of the gene; furthermore, it impacted on the transcriptome of postmortem brain tissue (*n*=105). Human and mouse expressional profiling revealed genes which were regulated in both species. We then aimed to investigate this polymorphism for an association with behavioral traits. Emphasis was placed on outward-bound, disruptive behaviors. Four different samples have been ascertained for this study: a sample of patients with adult ADHD (*n*=383), a sample of subjects with Cluster B and Cluster C personality disorder (*n*=403), a sample of prison inmates stratified for violent and non-violent criminality (*n*=233) and, finally, a control sample of 469 subjects. Short NOS1 exon 1f alleles were significantly associated with Cluster B personality disorder, especially histrionic personality disorder. Likewise, short alleles were found significantly more in patients suffering from adult ADHD. Finally, short allele carriers were more prone to commit violent crime, when childhood adversity was controlled for. Collectively, these data argue for a role of NOS-I in human behaviour. It appears that especially interpersonal, disruptive, outward bound behaviours are influenced by NOS1 genotype. These findings correlate to previous animal studies where NOS1 knockout mice were found to be highly aggressive; whether or not cognitive dysfunctioning, as found in our NOS1 knockout colony, is also a function of exon 1f VNTR, has yet to be investigated. Further analyses on the impact of NOS1 genotype on personality dimension in control populations therefore are under way, which will clarify the role of NO in human behaviour.

S9.3 Involvement of the endocannabinoid system in behavior of mice

Maldonado R.

University Pompeu Fabra, Barcelona, Spain

Abstract not received

S9.4 Cytokines-induced activation of the HPA axis in mice – neurophysiological and genetic studies

Wieczorek M.[1,3], Swiergiel A.H.[2,3], Dunn A.J.[3]

[1]Laboratory of Neurophysiology, Univ. of Lodz; [2]Dept. of Animal Behavior, Inst. of Genetics and Animal Breeding, Polish Academy of Sciences, Jastrzebiec, Poland; [3]Dept. of Pharmacology, Louisiana State Univ. Health Sciences Center, Shreveport, LA, USA

Cytokines have been implicated in mediating behavioral changes in animals and, possibly, depression in humans. The evidence comes from numerous studies in animals, including mouse genetic models. However, despite their potential importance in mediating behavior, the mechanisms of action of peripherally secreted cytokines on the CNS have not been definitely established. Vagal innervation plays a role in conveying immune signals from the periphery to the brain. It is also known that cyclooxygenases (COX) mediating prostaglandin synthesis are involved. We examined the effects of subdiaphragmatic vagotomy of mice along with pretreatment with the COX inhibitor, indomethacin, on the brain responses to peripherally administered interleukin-1 (IL-1). Vagotomy did not alter the reduction in feeding and body weight induced by IL-1. However, while vagotomy or indomethacin alone did not significantly alter basal plasma concentrations of ACTH and corticosterone, the combination of vagotomy and indomethacin treatment almost completely blocked the increases that followed IL-1 treatment. Moreover, whereas basal concentrations of brain norepinephrine (NE) and serotonin (5-HT) were not altered, the increases in NE and 5-HT metabolism, and in brain tryptophan induced by IL-1 were also attenuated or even blocked by the combined treatments. These results suggest that the reductions in feeding are not closely related to the changes in hypothalamo-pituitary-adrenal (HPA) axis activity, nor the changes in catecholamines and indoleamines. These results resemble those we obtained previously in rats, although in that species vagotomy more or less prevented the noradrenergic response as assessed by microdialysis, whereas the IL-1 induced activation of the HPA axis was only partially inhibited. Thus, although the vagus may be involved in the endocrine, and catecholamine, and indoleamine responses to IL-1, it appears unlikely that the neurochemical or endocrine changes are directly responsible for the anorexia induced by IL-1. The results also suggests that these two mechanisms by which IL-1 can affect the brain are probably among the most important ones by which the immune system influences the nervous system. Supported by a grant from the National Institutes of Health (NINDS).

SYMPOSIUM X
METALLOPROTEINASES-DEPENDENT EXTRACELLULAR SIGNALING IN NATIVE AND INJURED CNS

S10.1 TIMP-1, a candidate plasticity protein running for election

Rivera S.

CNRS – Universite de la Mediterranee, France

Tissue inhibitor of metalloproteinases (TIMP-1) is one of the four-member families (TIMPs-1-4) of multifunctional proteins that inhibit matrix metalloproteinases (MMPs). TIMP-1 was first identified in the brain as a candidate plasticity protein highly induced by stimuli leading to LTP. Since then, growing experimental evidence indicates that indeed TIMP-1 modulates neuronal plasticity. Accordingly, TIMP-1 KO and overexpressing mice show impaired and improved learning, respectively. In the seizing and ischemic brain, excitotoxic stimuli trigger rapid induction of TIMP-1 expression that persists over time in resistant to degeneration neurons that undergo axonal sprouting, and in reactive astrocytes, the main source of TIMP-1 in pre-scarring lesioned zones. Seizures-mediated upregulation of TIMP-1 is accompanied by upregulation of MMP-9 and MMP-2 in wild type mice, whereas no changes are observed in TIMP-1 KO mice. In the latter, astrocyte reactivity to inflammatory stimuli is attenuated, supporting the idea that TIMP-1 may also play a pro-inflammatory role. These data provide clues to interpret why TIMP-1 KO mice, unlike wild type mice, are resistant to seizures-mediated neuronal death and do not exhibit mossy fibre sprouting. Further support for TIMP-1 implication in neuronal plasticity comes from recent data showing that TIMP-1 deeply affects neurone morphology, and inhibits neurite outgrowth and growth cone dynamics through mechanisms likely to involve both MMP-dependent and -independent pathways. Altogether, these findings support TIMP-1 election as a plasticity protein.

S10.2 Multiple levels of matrix metalloproteinase involvement in ischemic brain injury
Gasche Y.

Geneva University Medical Center, Switzerland

Abstract not received

S10.3 MMP-9 in synaptic plasticity
Kaczmarek L.

Dept. of Molecular & Cellular Neurobiology, Nencki Institute, Warsaw, Poland

Recently, an extracellular proteolytic system, composed of tissue inhibitor of matrix metalloproteinases, TIMP-1 and matrix metalloproteinase-9, MMP-9, has emerged as a major AP-1 transcription factor target in hippocampal neurons responding to enhanced neuronal activity. Structural remodeling of the dendritic spines and synapses is essential for synaptic plasticity, underlying learning and memory. Matrix metalloproteinases are pivotal for tissue remodeling throughout the body, especially during development. We have recently found, using a variety of morphological and biochemical techniques that in the rodent hippocampus both MMP-9 protein and its transcript are associated with a subset of dendritic spines, bearing asymmetric, excitatory synapses. Moreover, in a model of excitotoxin-mediated plasticity, MMP-9 immunoreactivity, enzymatic activity and mRNA content increased in the dendrites/synapses. Furthermore, functional inactivation of MMP-9 by means of either gene knockout or specific chemical inhibitor or TIMP-1, delivered by an adenoviral vector, affected neuronal plasticity and blocked late phase of long-term potentiation as well as hippocampus dependent learning. In aggregate, these results point to a novel molecular mechanism of synaptic function that operates extracellularly and hence might be particularly amenable for therapeutic interventions aiming at preventing of cognitive declines.

S10.4 Ischemia modulates signal transduction from extracellular matrix
Zalewska T.

NeuroRepair Dept., Medical Research Institute, Warsaw, Poland

Focal adhesion kinase (FAK) is thought to play a major role in transducing extracellular matrix (ECM)–derived survival signals into cells. The function of FAK is linked to its autophosphorylation at Tyr-397 and then recruitment of several effector molecules. Thus, modulation of FAK activity may affect several intracellular signaling pathways and may participate in a variety of pathological settings. In the present study, we investigated the effect of short-term 5 minutes forebrain ischemia on levels and Tyr-397 phosphorylation of focal adhesion kinase and the interaction of this enzyme with Src protein tyrosine kinase and adapter protein p130Cas, involved in FAK-mediated signaling pathway in gerbil hippocampus. The total amount of focal adhesion kinase as well as its Tyr-397 phosphorylation declined substantially between 24 and 48 h after the insult, particularly in CA1 region of hippocampus. Concomitantly, a decreased amount of FAK/Src kinase complex has been observed. These data indicate that inhibition of FAK/Src-coupled signaling pathway may participate in the ischemia-induced neuronal degeneration in gerbil hippocampus. The temporal profile of FAK down-regulation in CA1 area coincides with metalloproteinases (MMPs) activation. These results suggest that extracellular proteolysis might belong to the mechanisms which govern the FAK-coupled pathway in ischemic hippocampus.

SYMPOSIUM XI
MECHANISMS OF DRUG DEPENDENCE

S11.1 The role of glutamatergic signaling in drug abuse
Spanagel R.

Central Institute of Mental Health, Mannheim, Germany

Abstract not received

S11.2 Serotonergic mechanisms in extinction and reinstatement of conditioned drug reward
Cunningham K.A., dela Cruz A.S., Herin D.V., Nic Dhonnchadha B.Á.

Center for Addiction Research and Department of Pharmacology and Toxicology, University of Texas Medical Branch, Galveston, Texas, 77555-1031, USA

Abuse of psychostimulants, especially cocaine and methamphetamine, present an alarming global challenge for which effective and accessible treatment options are limited. Serotonin neurotransmission provides integral control over brain circuits critical in addiction and may be a useful target in the quest to ameliorate and/or reverse the neuroplasticity resident in addicted brains that control drug-seeking behaviors. Relapse to stimulant use is a considerable deterrent in the quest to maintain abstinence in addicts and is often triggered by exposure to cues conditioned to stimulant use. We will present the results of studies in which the oppositional control of the 5-HT_{2A}R and 5-HT_{2C}R over reinstatement of drug-seeking behavior in rodents has been analyzed in two medium-throughput models [conditioned hyperactivity, conditioned place preference (CPP)] as well as the low-throughput self-administration assay. The environmental conditions under which this oppositional control are established will be discussed as well as the utility of individual and combined treatment with a selective 5-HT_{2A}R antagonist and/or 5-HT_{2C}R agonist to suppress responses to stimulant-associated cues. As a greater degree of knowledge of the modulatory role of serotonin in addictive processes is established, new doors will open for the future development of innovative and novel means to treat stimulant addiction. Supported by NIDA DA 00260, DA 16905, DA 06511 (KAC), DA 07287 (DVH), DA 020314 (ASD).

S11.3 Does long-term cannabis use disrupt brain function – what can we learn from animal studies?
Marsden C., Moklas M., Kendall D., Pardon M.C.

Institute of Neuroscience, School of Biomedical Sciences, University of Nottingham Medical School, UK

There is increasing concern about the long-term effects of cannabis use and in particular its potential role in the development of schizophrenia though there is limited experimental data available. The present study investigated the effects of repeated delta 9-tetrahydrocannabinol (THC) in the rat on reward [conditioned place preference (CCP)], novel object discrimination, striatal dopamine turnover and

the expression of Brain Derived Neurotrophic Factor (BDNF) and its receptor TrkB in the hippocampus and frontal cortex. THC (0.5, 2 and 5 mg/kg i.p.) was given every 48 hours for 21 days. Chronic treatment had no effect on CCP although striatal dopamine turnover was increased. In contrast novel object discrimination was impaired at the two higher doses of THC. BDNF levels, measured by Western blotting, were significantly enhanced by chronic treatment in both the cortex and hippocampus while TrkB levels were decreased. The impairment of novel object discrimination was reversed by co-treatment with the CB1 receptor antagonist Rimonabant but the effects on BDNF and TrkB were not. The results suggest that chronic THC impairs working memory through a CB1 receptor mediated process and this effect may have a contributory role in the adverse effects of long-term cannabis use. In contrast the effects of chronic THC on BDNF are not mediated by CB1 receptors and further studies are required to identify the mechanism involved.

S11.4 GABA(B) receptor ligands as a pharmacotherapy for cocaine addiction-preclinical studies

Filip M., Frankowska M., Przegalinski E.

Institute of Pharmacology Polish Academy of Sciences, Krakow, Poland

Drug dependence is characterized by relapse to drug-seeking and drug-taking behavior following periods of abstinence and drug detoxification. Recent behavioral findings point to significance of GABA in the cocaine addictive behaviors. We found that pharmacological stimulation of GABA(B) receptors by agonists or allosteric positive modulators reduces cocaine reinforcement while this property of cocaine is not related to tonic activation of GABA(B) receptors. The GABA(B) receptor stimulation-induced reduction of cocaine reinforcement is separated from its subjective effects. Moreover, a dissociation between effects of a GABA(B) allosteric positive modulator and direct GABA(B) receptor agonists on cocaine and food-maintained responding and on the locomotor activity was demonstrated. On the other hand, tonic activation of GABA(B) receptors is required for the cocaine seeking behavior since a GABA(B) receptor antagonist selectively altered motivated drug-seeking behavior at doses that failed to alter reinstatement of food-seeking behavior, cocaine and food self-administration, or basal locomotor activity. Agonists at GABA(B) receptors inhibited cocaine-seeking but also caused decreases in cocaine or food self-administration, indicating their non-specific effects on relapse. In conclusion, a GABA(B) receptor antagonist seems to be a good therapeutic choice for reducing craving and preventing relapse in cocaine addicts, while a GABA(B) receptor allosteric positive modulator attenuates cue-evoked relapses to cocaine as well as the direct rewarding properties of cocaine.

S11.5 Drug-induced gene transcription and the development of addiction

Rodriguez Parkitna J.[1], Engblom D.[1], Lemberger T.[1], Bilbao Leis A.[2], Spanagel R.[2], Schütz G.[1]

[1]German Cancer Research Center, [2]Central Institute of Mental Health, Mannheim, Germany

Induction of specific gene expression patterns in response to activity confers functional plasticity to neurons. These processes are regulated by signaling cascades, initiated G protein-coupled receptors or calcium ion influx that converge on cAMP response element binding protein (CREB) and serum response factor (SRF). Activation of these transcription factors induces the transcription of immediate-early genes, like Fos or Arc, which are believed to underlie neuronal plasticity. In order to precisely define the roles of transcription factors in induction of immediate-early genes we have generated animals with neuron-type specific ablations of CREB and SRF. We have performed gene expression profiling in the striata of control and mutant animals injected with cocaine. Loss of CREB or SRF produced specific deficits in induction of immediate-early genes, indicating a more narrowly defined function of these proteins in regulation of transcription. Interestingly, despite difference in cocaine-induced gene expression, there were no clear differences in cocaine-induced behaviors. These results indicate no simple correlation between immediate-early gene transcription and drug-induced behaviors.

SYMPOSIUM XII
NEUROIMMUNOLOGY OF REMYELINATION

S12.1 The role of BDGF in tissue repair in multiple sclerosis

Gold R.

Ruhr University, Bochum, Germany

Abstract not received

S12.2 Immunopathology of MS lesion repair

Bruck W.

University of Gottingen, Germany

Abstract not received

S12.3 Mesenchymal stem cells in tissue repair in MS

Stasiolek M.[1], Matysiak M.[1], Orlowski W.[1], Jurewicz A.[1], Janczar S.[1], Raine C.S.[2], Selmaj K.[1]

[1]Department of Neurology, Medical University of Lodz, Poland; [2]Departments of Pathology, Neurology and Neuroscience, Albert Einstein College of Medicine USA

Stem cells (SC) have been recently shown to ameliorate experimental autoimmune encephalomyelitis (EAE), a model of multiple sclerosis (MS). However, little is known about mechanisms underlying this beneficial effect. In order to characterize the function of SC in immune demyelination, syngeneic pluripotent Lin^-Sca1^+ bone marrow stem cells (Lin^-Sca1^+BMSC) were transferred at peak of disease to mice with $PLP_{139-151}$-induced EAE. Animals were studied by immunopathologic, immunologic and molecular technologies. Mice which received Lin^-Sca1^+BMSC transfer showed faster improvement of clinical symptoms compared to control EAE mice. Moreover, during 90 days of observation period the transplanted animals did not show any additional exacerbation whereas the control EAE mice experienced at least one second relapse of disease. In contrast to control EAE, transferred animals displayed diffuse remyelination in the spinal cord. Interestingly proliferation of spleen T cells in response to $PLP_{139-151}$ was strongly inhibited in mice transferred with Lin^-Sca1^+BMSC. Thus, beneficial effect of SC in EAE depends on the induction of immunoregulatory mechanisms leading to the inhibition of T cell reactivity to myelin antigens.

S12.4 Inhibition of Notch/Jagged pathway enhances tissue repair in experimental autoimmune encephalomyelitis

Jurynczyk M.[1], Jurewicz A.[1], Bielecki B.[1], Raine C.S.[2], Selmaj K.[1]

[1]Department of Neurology, Medical University of Lodz; [2]Department of Pathology, Albert Einstein School of Medicine, New York, NY, USA

Spontaneous remyelination in multiple sclerosis (MS) lesions is limited and decreases as the disease progresses. Loss of oligodendrocytes (OLs) in MS does not account for the limited myelin repair as lesions contain considerable numbers of OL precursor cells (OPC). Activation of the Notch pathway has been shown to hamper the ability of OPC to produce myelin during CNS development. In this study we examined whether γ-secretase inhibition of Notch signaling within CNS of mice with experimental autoimmune encephalomyelitis (EAE) influences EAE clinical course and tissue repair. Intraventricular injections with the γ-secretase inhibitor (GSI) or vehicle were performed at the peak of clinical manifestation. 5–10 days post treatment, brain and spinal cords were removed and assessed by histopathology. To prove the effect of γ-secretase inhibitor on Notch1 activation we performed real-time PCR and Western Blotting on OLs isolated from GSI-treated mice. Animals treated with GSI displayed significantly faster recovery from the disease. Differences in recovery became apparent (2.7 vs. 1.8) within 5 days after treatment ($P<0.01$). γ-secretase inhibition promoted remyelination and reduced axonal damage in the CNS. The active form of Notch1 was absent and the expression of a Notch target gene, Hes1, was markedly reduced within OLs of GSI-treated group. In conclusion, γ-secretase inhibition of Notch signaling led to clinical improvement and enhancement of lesion repair within the CNS. Our results suggest that inhibiting the non-myelin permissive environment maintained by Notch pathways within the mature CNS is a promising therapeutic target in MS.

SYMPOSIUM XIII
SPINAL CORD PLASTICITY: FROM ADAPTATION TO REPAIR

S13.1 The role of the extracellular matrix in the control of axon regeneration and plasticity

Fawcett J.W.

Cambridge University Centre for Brain Repair, Robinson Way, Cambridge CB2 2PY, UK

Recovery of function in the damaged CNS is limited due to the absence of axon regeneration and relatively low levels of plasticity. Studies on the adult spinal cord and visual cortex showed that treatment with chondroitinase, which removes glycosaminoglycan (GAG) chains from chondroitin sulphate proteoglycans (CSPGs), leads to a rapid recovery of function, through stimulating plasticity and axon regeneration. Axon regeneration is permitted by the digestion of inhibitory proteoglycans in the glial scar. Plasticity in the adult CNS may be restricted by perineuronal nets (PNNs) around may neuronal cell bodies and dendrites. These contain inhibitory CSPGs, hyaluronan, link protein and tenascin-R. There are also changes in the sulfation patterns of brain CSPGs. The components of PNNs are produced either by the neurones themselves or by surrounding glial cells. All neurones with PNNs express both a hyaluronan synthase enzyme and a link protein, and these are probably the key components that trigger the formation of the structures. A link protein knockout animal lacks normal PNNs on its dendrites. The GAGs within PNNs have a different sulphation pattern to those in the general CNS matrix, giving them high affinity for binding molecules which may affect plastic behaviour in neurons. This may be a mechanism that concentrates active molecules in the regions of synapses.

S13.2 What locomotor training brings to intact and injured spinal cord?

Skup M., Macias M., Dwornik A., Ziemlinska E., Strzalkowski R, Czarkowska-Bauch J.

Nencki Institute of Experimental Biology, Warsaw, Poland

The inseminating study by Barbeau and Rossignol (1987, Brain Res), who described recovery of locomotion after chronic spinalization in the adult cats trained to walk, opened a field of investigations on anatomical and molecular background of such improvement. Whereas twenty years later locomotor training became rehabilitation procedure for enhancing the recovery of walking in persons with spinal cord injuries, underlying mechanisms are only emerging. Acute and chronic voluntary periods of exercise cause in intact rats up-regulation in genes involved with synaptic trafficking and signal transduction pathways. Genes associated with excitatory neurotransmission are up-regulated while genes related to the gamma-aminobutyric acid system are down-regulated. Our studies and those of others suggest also a central role for brain-derived neurotrophic factor (BDNF) in spinal cord plasticity. Locomotor exercise is a powerful means to increase spinal BDNF and its signal transduction receptor TrkB mRNA levels. Long- and short-term locomotor activity of moderate intensity produces stimuli sufficient to recruit a majority of spinal cells to increased BDNF synthesis, suggesting that continuous tuning of BDNF levels permits spinal networks to undergo trophic modulation. Improvement of motor abilities of spinalized animals after training is accompanied by remodeling of neuronal network caudal to the transection as indicated by altered neurotransmitter levels, higher density of BDNF labeled processes and increased expression of synaptic markers. Support: MSE/BMBF grant.

S13.4 *In vivo* imaging of spinal cord injury

Misgeld T.

Technical University Munich, Inst. of Neurosciences, Munich, Germany

Spinal cord injury is a common cause of neurological disability. The lasting impact of spinal trauma, which contrasts to the reversibility of many peripheral nerve injuries, is due to the fact that axons in the central nervous system lack the ability to regenerate. Since Cajal's days, many hypotheses have been put forward to explain this regenerative failure and many strategies have been devised to remedy it. Despite impressive progress, the methods to assess the success or failure of such experimental therapies remain limited, even in animal models. We have developed tools to address these limitations. In my presentation, I will describe these methods, which center around attempts to visualize axon degeneration and regeneration in the spinal cords of living mice with transgenically labeled axons. I will exemplify the versatility of this approach by illuminating the mechanisms that underlie a common sequel of axon transection, acute post-traumatic degeneration or 'die-back'.

S13.5 Spinal peptidergic mechanisms of neuropathy after nerve injury
Przewlocka B.

Department of Pain Pharmacology, Institute of Pharmacology, Krakow, Poland

The etiology of neuropathic pain is still poorly understood and no effective methods for its therapy have been developed. We studied the importance of endogenous peptide families: opioids, melanocortins and nociceptin in the development and maintenance of neuropathic pain which was induced by sciatic nerve injury. In order to establish how the endogenous peptide systems contribute to the neuropathic pain, we have measured the expression of prodynorphin, proenkephalin, proopiomelanocortin and pronociceptin as well as the μ, δ, κ, ORL-1 and melanocortin MC4 receptors in the spinal cord and dorsal root ganglia (DRG). In the spinal cord of neuropathic rats, opioid receptor mRNA showed no change, however, the MC4R immunoreactivity was increased in the spinal cord. In the DRG, the lower expression of all opioid receptors and an increase in MC4R receptor mRNA level were observed. Studying the expression of prohormon molecules, we found higher expression of prodynorphin, and a slight increase in proopiomelanocortin mRNA abundance, and lower expression of proenkephalin in the DRG. The ligation of the sciatic nerve did not change the levels of pronociceptin and ORL1 receptor mRNAs in the dorsal horn, but in the ventral horn ORL1 receptor was increased. These changes in the activity of endogenous peptide systems reflect a complex and adaptive response to neuropathic conditions and might contribute to a plausible explanation of the different symptoms associated with neuropathic pain Acknowledgement. Supported by a grant for Polish MNSW Scientific Network.

S13.6 Spinal cord injury, background, epidemiology and treatment
Zarzycki D.

Jagiellonian University, Collegium Medicum, Krakow, Poland

Spinal cord injury (SCI) often results in significant neurologic dysfunction and disability. An annual incidence of 15 to 40 traumatic SCI cases per million has been reported worldwide. Approximately 85% of spinal cord injures occur in men. Of these, approximately 61% occur in 16 to 30 year old and fewer 15% in people older than 46 years old. Motor vehicle accidents constitute 48% of all injures, 21% from falls, 15% from violence, and 14% from sport injures. Today although 5% to 10% of patients die in the early stages of the injury, most of these deaths can be attributed to concomitant injures, age, and preexisting medical problems, rather than the cord injury. Surgical treatment decompression of spinal cord and stabilization is recommended in 100% cases. Regeneration in the adult CNS following injury is extremely limited. Traumatic spinal cord injury causes a permanent neurological deficit followed by a very limited recovery due to failed regeneration attempts. In fact, it is now clear that the spinal cord intrinsically has the potential to regenerate, but cellular loss and the presence of an inhibitory environment strongly limit tissue regeneration and functional recovery. They have been very successful in several animal models, although results are still controversial in humans. Nonetheless, novel experimental approaches hold great promise for use in humans. The current focus of treatment is on restoring activities of daily living and quality of life.

SATELLITE SYMPOSIA

SYMPOSIUM I
Clinical aspects of neuroscience

Session I
Small vessel disease of the brain

SA1.1 Pathogenesis of lacunar stroke
Markus H.

St George's University of London, UK

Abstract not received

SA1.2 Radiological spectrum of small vessel disease
Enzinger Ch.

Medical University Graz, Austria

Abstract not received

SA1.3 Single-gene ischemic small vessel diseases of the brain
Chabriat H.

CHU Lariboisiere, Assistance Publique des Hopitaux de Paris, Paris, France

Abstract not received

SA1.4 Genetic risk factors of silent and symptomatic small vessel disease
Słowik A.

Jagiellonian University, Collegium Medicum, Krakow, Poland

Abstract not received

Session II
Perspectives of neurodegenerative diseases

SA1.5 Inherited mitochondrial DNA variation and Alzheimer's disease
Zekanowski C., Maruszak A.

Dept. of Neurodegenerative Disorders, Medical Research Center, Polish Academy of Sciences Warsaw, Poland

It has been proposed recently that mitochondrial dysfunction is the primary event in the Alzheimer's disease (AD) etiology. Both mitochondrial and nuclear genomes' variation influence functioning of electron transfer chain subunits and other mitochondrial proteins. Specific mtDNA SNPs and/or their clusters defined as haplogroups have been proposed to affect susceptibility to AD, at least in specific environmental conditions. We studied the association of nine major European

mtDNA haplogroups (H, I, J, K, T, U, V, W and X), and haplogroup clusters (HV, JT, KU, and IWX) with AD in a sample of Polish AD patients and control group. Multivariate regression analysis showed that HV cluster increases 1.7-fold the risk of AD regardless of the APOE4 status, age, and gender. The obtained data strongly suggest further study of haplogroup H as a AD susceptibility factor.

SA1.6 MRI biomarkers of dementia

De Leon M.J.

New York University School of Medicine, New York, USA

Abstract not received

SA1.7 Therapeutic approaches for prion diseases: Can immunization or drugs be effective?

Sadowski M.

Departments of Neurology and Psychiatry, New York University School of Medicine, New York, USA

Currently there is no effective treatment for prion diseases (prionoses). The pathological mechanism of this group of diseases is related to a conformational transformation of cellular prion protein (PrP^{C}) into a toxic, infectious, and self-replicating conformer termed PrP^{Sc}. Following extracerebral inoculation, PrP^{Sc} replicates within the lymphoreticular system for months to years prior to secondary involvement of the CNS. Therefore, PrP^{Sc} formation can be targeted in the early stages of infection, by treatment approaches, which do not have to be capable of penetrating the BBB. Reports from several laboratories including ours have demonstrated that immunization can significantly prolong the incubation period in acquired prionoses or even potentially prevent occurrence of disease symptoms. The effectiveness of these approaches appears to be strictly related to humoral immunity. Therefore, we have identified several anti-PrP monoclonal antibodies (Mabs), which are highly effective in clearing PrP^{Sc} from prion infected cell lines. We have demonstrated that administration of 6D11 (one of our Mabs) to CD-1 mice, following prion exposure prevents accumulation of PrP^{Sc} within the lymphoreticular system. Thus, passive immunization with therapeutically effective anti-PrP Mab may provide a clinically relevant approach applicable to individuals accidentally exposed to prions. Immunization appears to be ineffective once the PrP^{Sc} spreads to the CNS since the effect of Mabs is hampered by their limited BBB permeability. To target the PrP^{Sc} in the CNS and arrest or reverse course of the disease other approaches are being investigated. They include pentosan polyphosphate administered intraventricularly and Quinacrine and Amphotericin B administered systemically. The efficacy of Quinacrine on survival in sporadic CJD is now being investigated in multicenter phase II clinical trial.

SA1.8 Mild cognitive impairment

Barcikowska M.

Medical Research Center, Polish Academy of Sciences, Warsaw, Poland

Abstract not received

SYMPOSIUM II
Newest translational approaches to clinical sciences of neuromuscular diseases

Session I

SA2.1 Molecular basis of muscular dystrophies

Karpati G.

Montreal, Canada

Definition of molecular myology
[Characterizing, understanding, classifying, diagnosing, preventing, and treating muscle diseases in terms of genomic and proteomic features.]
Dividends and impact of molecular myology
[Understanding disease pathophysiology / Rational classification / Investigation and precise diagnosis / Therapy and prevention / Genetic counseling / Ethical aspects / Research]
Understanding genetic disease pathophysiology
[Gene defect (mutation) / Consequence of the above: either absent or defective protein (i.e DMD) or pathogenic RNA transcripts causing splicing aberrations (i.e. MyD 1)]
Molecular therapeutics: Recessive diseases
[Gene replacement / Upregulating a compensating molecule / Gene correction (DNA editing) / Primary transcript correction ("exon skipping") / Translational manipulation ("read-through") / Myostatin inhibition]
Molecular therapeutics: dominant diseases
[Blocking the mutant mRNA by antisense oligonucleotides
Inactivation of the mutant mRNA by RNAi / Upregulating muscle blind protein 1 (MyD 1) to restore splicing accuracy]

SA2.2 Future of stem-cell therapy for neuromuscular diseases

Braun S.

Association Française contre les Myopathies, France

The history of cell therapy is very recent compared to conventional medicine that took centuries to evolve. The first bone marrow transplantations were carried out in the late 70s. As an alternate source, cord blood transplantations are now being investigated. The pluripotency of those cells remains to date confined to mainly blood cells. To date, the vast majority (about 95%) of the 630 clinical trials are aimed at treating blood cell-related diseases. With the higher plasticity of multipotent mesenchymal stem cells from umbilical cord blood or bone marrow, broader applications in regenerative medicine including neuromuscular diseases are now being envisaged. Skeletal muscles have the ability to regenerate through the presence of specialized adult stem cells (satellite cells). The first clinical trials using satellite cells were launched in the early 90s, with little if no benefit so far in the two main targeted applications (genetic muscle diseases and heart infarction). Satellite cells may originate from healthy donor (application Duchenne muscular dystrophy) or autologous muscle biopsies (applications FSH, OPMD). Further developments include the use of genetically-modified autologous stem cells (example: DMD). New adult "plastic" stem cells are being in tissues. Among them blood vessel-derived mesangioblasts seem to be most promising for neuromuscular diseases, as demonstrated

in a dog model of dystrophinopathy. However, the available techniques require time-consuming culturing of limited amounts of tissue. Whether hematopoietic, mesenchymatous, foetal or isolated from adult tissues, non-embryonic stem cells do not bear unlimited capacity for cell division and differentiation. The pluripotency and virtually infinite proliferation properties of embryonic stem cells remains a major advantage. They represent pure lineages which would be easier to manage, provided conditions of respect for elementary and specific safety and ethical rules are met.

SA2.3 Molecular aspects of metabolic myopathies and their treatments
Corrado A.

Padova, Italy

Abstract not received

SA2.4 Pathogenesis and prospects of treatment of mitochondrial myopathies
Schapira A.H.

London, UK

Multiple independent factors may influence clinical expression of a mutation including tissue distribution, levels of heteroplasmy, nuclear background and the varying dependence of organs on OXPHOS for energy. Inevitably, these account for only some of the variation seen and much remains to be discovered on the molecular pathogenesis of these mutations. Defects of the respiratory chain result in the increased production of free radicals and so the administration of anti-oxidants has some scientific basis. N-acetylcysteine and co-enzyme Q10, both antioxidants, improved OXPHOS function and reduced free radical production in cybrid cells carrying the T8993G mutation that causes NARP or MILS. However, the use of antioxidants in mtDNA disease has yet to be tested in a clinical trial. Various strategies are being evaluated to modify the mtDNA mutant load in cells and tissues in patients. An obvious target would be the preferential expansion of wild type mtDNA or the suppression of mutant mtDNA expansion. It appears that the recruitment of skeletal muscle satellite cell expansion can shift heteroplasmy in favour of wild type as mutant mtDNA is absent or at low level in these cells. Satellite cells can be provoked to expand by vigorous exercise regimens or toxic damage, although for obvious reasons both have some practical limitation in patients. Manipulating mtDNA replication by the import into mitochondria of endonucleases that might selectively destroy a specific mutant sequence has been possible in vitro, but presents many challenges to transfer this to an in vivo application. An alternative mechanism for salvaging OXPHOS function in cells with tRNA mutations of mtDNA is the import of normal tRNAs from the cytosol to mitochondria. The import of nuclear encoded RNAs into the mitochondrial matrix has been demonstrated. The import of normal tRNAlys from cytosol to mitochondria improved OXPHOS function in cybrid cells bearing the tRNAlys A8344G mutation that causes MERRF.

Session II

SA2.5 Breaking the magic circle: From mitochondrial myopathies to mitochondrial medicine
Zeviani M.

Milano, Italy

Abstract not received

SA2.6 Autoimmune, viral and inflammatory aspects of inclusion-body myositis: New data and therapies
Dalakas M.C.

Bethesda, USA

Muscle biopsies from patients with sporadic Inclusion Body Myositis (s-IBM) consistently demonstrate that the inflammatory T cells almost invariably invade intact (not vacuolated) fibers whereas the vacuolated fibers are rarely invaded by T cells indicating two concurrently ongoing processes, an autoimmune mediated by cytotoxic T cells, and a degenerative manifested by the vacuolated muscle fibers and deposits of amyloid-related proteins. The autoimmune features of IBM are highlighted by the strong association of the disease with: (1) HLA I, II antigens, in frequency identical to classic autoimmune diseases; (2) other autoimmune disorders in up to 32% of the patients, autoantibodies, paraproteinemias or immunodeficiency; (3) HIV and HTLV-I infection with increasingly recognized frequency (up to 13 reported cases). New data from these cases demonstrate that viral-specific T cells bearing the gag HIV peptide are clonally expanded and invade muscle fibers; (4) the CD8+ autoinvasive T cells have rearranged T Cell Receptor genes, expand in situ, are cytotoxic and antigen-driven, and persist over time, even in different muscles; and (5) muscle fibers express MHC-I antigen and co-stimulatory molecules and form immunological synapses with the autoinvasive CD8-positice T cells. In contrast to IBM, in various dystrophies the inflammatory cells are clonally diverse and the muscle fibers do not express MHC-I or co-stimulatory molecules in the pattern seen in IBM. Like other chronic autoimmune conditions with co-existing inflammatory and degenerative features (i.e. primary progressive multiple sclerosis), IBM is resistant to conventional immunotherapies. Recent data suggest that strong anti-T cell therapies resulting in T cell depletion, such as the one using the monoclonal antibody against CD52, can be promising. Data from this study provides a proof of principle conclusion that the inflammation in IBM is clinically relevant.

SA2.7 Molecular pathogenesis and prospects of treatment of inclusion-body myositis
Askanas V., Engel W.K.

Department of Neurology, USC Neuromuscular Center, University of Southern California Keck School of Medicine, Good Samaritan Hospital, Los Angeles, CA

Sporadic inclusion-body myositis (s-IBM) the most common progressive muscle disease of older persons, leads to severe disability. It is of unknown etiology, and its pathogenetic steps are just beginning to be understood. Pathologically, s-IBM muscle biopsies are characterized by the vacuolar degeneration of muscle fibers and mitochondrial abnormalities accompanied by inflammatory T-cell infiltration. The vacuolar degeneration of

s-IBM muscle fibers is associated with intra-muscle-fiber accumulation of proteinaceous aggregates, most of which are congophilic. Several of the accumulated proteins, including amyloid-β (Aβ), p-tau and α-synuclein, have a propensity to unfold. Recently we demonstrated that s-IBM muscle fibers contain several important features indicative of protein misfolding and proteasomal (Ps) abnormalities. They include: (1) Ps inhibition; (2) ER stress and the unfolded protein response; (3) accumulation of (a) components of the protein surveillance machinery, including ubiquitin, (b) mutant ubiquitin (UBB+1), an inhibitor of the 26S Ps, (c) Ps subunits, (d) aggresomes, and (e) HSP70. Because of the above, s-IBM joins a group of "conformational disorders", probably caused by the unfolded/misfolded proteins and formation of the proteinaceous aggregates. The unfolded proteins disturb functions of normal cell proteins. Another important aspect of s-IBM pathogenesis are oxidative stress and mitochondrial abnormalities. Because various treatments against the inflammatory component of s-IBM pathology so far remain largely unsuccessful, we propose that the degenerative aspects are more important in s-IBM pathogenesis and progression. Their prevention, although challenging, might provide a new treatment approaches for s-IBM, and will be discussed.

SA2.8 Novel pathogenic aspects of myostatin and therapeutic potential in human neuromuscular diseases

Wojcik S., Engel W.K., Askanas V.

USC Neuromuscular Center, Department of Neurology, University of Southern California Keck School of Medicine, Good Samaritan Hospital, Los Angeles, CA, USA; Department of Anatomy and Neurobiology, Medical University of Gdansk, Gdansk, Poland

Myostatin, also called growth and differentiation factor-8 (GDF-8), is a member of the transforming growth factor-β (TGF-β) superfamily. Myostatin is a secreted protein considered to be a negative regulator of muscle growth during development and of muscle mass during adulthood. In mouse models, knocking out the myostatin gene, overexpressing proteins neutralizing myostatin, or natural mutations of the myostatin gene cause increased muscle mass. In humans, myostatin protein was reported increased in muscle tissue of patients with HIV-associated muscle wasting, and myostatin-precursor protein (MstnPP) mRNA was reported increased in muscle wasting associated with osteoarthritis. Recent studies from our laboratory demonstrated that: (1) MstnPP/myostatin is increased in sporadic inclusion-body myositis (s-IBM) muscle fibers, where it accumulates in the form of aggregates co-localizing with amyloid-β (Aβ); (2) MstnPP (but not myostatin) physically interacts with Aβ, (3) Overexpression of Aβ-precursor protein into cultured human muscle fibers (CHMFs) increases MstnPP protein (but not mRNA) in them; (4) ER-stress-induced NFkB activation in CHMFs increases MstnPP mRNA and protein; and (5) MstnPP/myostatin are increased in type II atrophy in human neuromuscular disorders. Although inhibition or removal of myostatin by various experimental means in several animal models has led to increased muscle mass, whether or not the increased muscle mass results in increased muscle strength remains uncertain, as does any role of anti-myostatin therapy in humans.

SA2.9 Role of endoplasmic reticulum stress in muscle diseases

Nogalska A., Engel W.K., Askanas V.

USC Neuromuscular Center, Department of Neurology, University of Southern California Keck School of Medicine, Good Samaritan Hospital, Los Angeles, CA, USA; Department of Biochemistry, Medical University of Gdansk, Gdansk, Poland

The endoplasmic reticulum (ER) plays a critical role in processing, folding and exporting newly-synthesized proteins into the secretory pathway. Accumulation of unfolded and misfolded proteins inside the ER lumen leads to ER stress (ERS). This elicits the unfolded protein response (UPR), a functional mechanism by which cells attempt to protect themselves against ERS. The UPR involves attenuating translation to reduce both the protein overload and the subsequent accumulation of unfolded proteins, and transcriptional induction of ER chaperone proteins, which increase folding capacity of the ER and prevent protein aggregation. While ERS has been extensively studied in various neurodegenerative disorders, its role in human muscle disease has just begun to be elucidated. Our laboratory has shown that: (1) ER stress occurs in sporadic inclusion body myositis (s-IBM) muscle fibers, where it triggers the UPR, as evidenced by increased expression of the main ER chaperones; (2) a novel ER protein HERP, proposed to be linked to both the UPR and the ER-associated degradation, is highly increased in s-IBM muscle fibers, both on the protein and mRNA levels; (3) ERS-mediated NF-κB activation induces upregulation of the myostatin precursor protein mRNA and protein. Because NF-κB activation occurs in s-IBM muscle fibers, this mechanism might contribute to our demonstrated increase myostatin in s-IBM. Some aspects of the ER stress were also demonstrated by others in muscle disease containing abnormal tubular aggregates and in polymyositis. Understanding the role of ER stress in various muscle diseases might provide new therapeutic possibilities.

Session III

SA2.10 Neuromuscular junction biology, its disorders and newest aspects of treatment

Newsom-Davis J.

Department of Clinical Neurology, University of Oxford, West Wing, John Radcliffe Hospital, Oxford, UK

The neuromuscular junction (NMJ) is a well characterised synapse. Spontaneous presynaptic release of a single quantum of acetylcholine (ACh) generates a postsynaptic miniature endplate potential (MEPP). Invasion of the nerve terminal by a nerve action potential opens voltage-gated Ca^{++} channels (VGCCs), allowing the local influx of Ca^{++} and triggering the release into the synaptic cleft of ~30 quanta of ACh (in man). Binding of ACh to the α-subunits of the pentameric ACh Receptor (AChR; α_2, β, δ, ε) leads to brief channel opening and the influx of small cations (mainly Na^+). The consequent endplate potential (EPP) triggers an action potential that activates muscle contraction. Two main pathological processes affecting neuromuscular transmission will be discussed: antibody-mediated autoimmune attack and genetic mutation. In myasthenia gravis (MG) 85% of patients have IgG antibodies to the AChR causing receptor loss. 7% have antibodies to Muscle Specific Kinase (MuSK), crucial for AChR clustering. Antibodies to VGCCs underlie the Lambert-Eaton Myasthenic Syndrome (LEMS). Antibodies to voltage-gated K^+ channels

(VGKCs) underlie neuromyotonia (peripheral nerve hyperexcitability). Congenital Myasthenic Syndromes are typically recessively inherited. Targets include the five genes encoding the AChR subunits, CHAT, acetylcholinesterase, MuSK, and the intracellular subsynaptic proteins Rapsyn and Dok-7 that are important in AChR clustering. The clinical features of these diverse disorders will be outlined.

SA2.11 Motor neuron biology, pathology and treatment of amyotrophic lateral sclerosis

Appel S.H.

Methodist Neurological Institute, Houston, Texas, USA

Amyotrophic lateral sclerosis is an inexorably paralytic disorder with progressive loss of motoneurons. Spinal cord pathology in ALS patients and the mouse model of familial ALS over-expressing mutant superoxide dismutase (mSOD1) is characterized by site-specific neuroinflammation with activated microglia, dendritic cells, and adaptive immune cells. Over-expression of mSOD1 *in vitro* activated microglia and induced more motoneuron death than wild-type (WT) microglia. To determine whether the innate immune microglia influence disease progression and motor neuron loss *in vivo* we bred mSOD1 mice with PU.1$^{-/-}$ mice that are unable to develop myeloid and lymphoid cells. WT bone marrow transplants (BMT) into mSOD1\PU.1$^{-/-}$ mice repopulated the central and peripheral immune systems, slowed the loss of motoneurons, prolonged survival, and increased disease duration by 40 percent compared to mSOD1 BMT. To determine whether the peripheral adaptive (T- and B-cells) immune system regulates microglia-mediated motor neuron injury, we bred mSOD1 mice with RAG2$^{-/-}$ mice that are unable to develop mature and functional T- and B-cells. Although age of onset was not different, mSOD1\RAG2$^{-/-}$ mice died earlier and were lacking T cells and activated microglia compared to control mSOD1\RAG2$^{+/-}$ mice. Following irradiation and BMT, survival was extended in mSOD1\RAG2-$^{/-}$ mice, to ages identical to mSOD1\RAG2$^{+/-}$ mice, and T-cells and increased CD11b expression were restored at sites of injury. These data emphasize the importance of T-cell modulation of microglia in mediating neuroprotection and suggest that enhanced communication between peripheral immune cells and central microglia might provide an opportunity for therapeutic intervention in ALS.

SA2.12 Molecular pathogenesis and treatment of immune-mediated neuropathies

Engel W.K.

Department of Neurology, Univ. of Southern California Keck School of Medicine, USC Neuromuscular Center, Good Samaritan Hospital, Los Angeles, CA

Rx rationale: some malfunctioning cells – schwannocytes, lower-motor and sensory neurons – can recover if freed from "immuno-oppression". Ideal Rx: (1) Selectively kill pathogenic B-cells secreting toxic immunoglobulins (Ig's) or fragments. (2) Kill dyscrasic T-cells. (3) Block expression of foreignized auto-antigens or foreign antigens. (4) Stop cytokine toxicity. (5) Thereby, stop pathogenic mechanisms, to permit maximal enduring autogenous recovery (repair, regeneration). Longer the cells with recoverable biochemical functions remain non-functioning, greater the likelihood they will die: [normal ↔ malfunctioning → dead]. An anti-Di Rx, of which IVIG (i.v. IgG) is best and safest, must be individualized to aggressively minimize cellular down-time, lest it fester irreversibility (death). With intermittent anti-Di Rx, e.g. IVIG, monitor closely for rise and fall of benefits *vis-a-vis* dosings. IVIG, prednisone and cytotoxins shield from immuno-oppression, allowing repair of cell damage (but pain and cramp meds don't). My CIDP (chronic immune dysschwannian polyneuropathy), ± diabetes-2 (2001, Lancet 358: 2086), patients, >200, on IVIG Rx are very sensitive to IVIG scheduling. To optimize long-term benefit in each, I maximally reduce symptoms as long as possible – oft-hallowed IVIG inflexible protocols are not designed to do this. Common Rx's: IVIG, Plasmaphereses, Corticosteroids (Cst's), Cytotoxins (Azathioprine; Cyclophosphamide; Rituximab or Thalidomide for some high IgM's). Other Rx's: Interferon α-2A (for fever-responsive CIDP); [Total-Body Irradiation (TBI), targeting lymphocytes, benefits DM and MG]. Futuristic Rx's: Make focusing drugs with anti-Di properties of Cst or cytotoxin, without side-effects. Make a small-volume micro-IVIG for sq. use. Cocktails of less-toxic doses of anti-Di's. Other interferons. Photopheresis. Add selective neurotrophins to promote repair/regeneration. Ideally, patients should not be compromised by inflexible protocols/guidelines or payer restrictions offering less than maximal benefit. Restriction-of-benefit is unacceptable in anti-pathogenic Rx of infectious diseases and should not be in DysN's.

SA2.13 Myelin and axons: Lessons learned from inherited peripheral neuropathies

Suter U.

Institute of Cell Biology, ETH Zürich, Switzerland

We are in interested in the cellular and molecular basis of inherited Motor and Sensory Neuropathies (HMSN), also called Charcot-Marie-Tooth disease (CMT). In particular, we have a major interest in the mechanistic basis of these genetically heterogeneous diseases since axon-Schwann cell interactions in myelinated peripheral nerves are heavily affected. By examining the structure and function of the proteins involved, and the miss-function of these proteins in disease, we anticipate that we will obtain eventually a comprehensive picture of which pathways are crucial for the development, maintenance, and regeneration of peripheral nerves. At the same time, we are studying also candidate proteins that might contribute to the functional integrity of peripheral nerves. Our findings appear to demonstrate that both approaches are beneficial, in synergetic ways, to our basic understanding of the biology of peripheral nerves as well as its associated diseases.

SA2.14 Spastic paraparesis – gene abnormalities and potential treatments

Martinuzzi A.[1], Orso G.[1], Pendin D.[1], Rossetto M.[1], Bassi M.T.[1], Daga A.[2]

[1]"E. Medea" Scientific Institute, Conegliano and Bosisio Research Centre, Italy; [2]Dulbecco Telethon Insitute

Hereditary spastic paraplegias (HSPs) are a group of diseases of growing clinical importance. No effective therapy, specific treat-

ment or rehabilitative management is presently available. To date 31 different loci are listed (SPG1-33) with 80% of the reported cases due to mutations in dominant loci, namely SPG4, SPG3a and possibly SPG31. Recessive forms are rarer. Among the genes for recessive forms (SPG7, 11, 20, 21) paraplegin (SPG7) is the most frequent, but still can account only for <5% of families. The genes associated with HSP code for proteins belonging to different families (AAA superfamily, GTPase, Heat shock proteins, kinesins, metalloproteases. The involvement of many of these genes in microtubule metabolism, mitochondrial function, endosomal trafficking and intracellular motion provide a unifying link between disruption of endosomal trafficking and axonal transport. Animal models offer insight into the function that HSP proteins, and into the biological consequences of their abnormal expression. Our group has developed Drosophila models of spastin and atlastin disregulation from which we obtained important contributions to the understanding of the pathophysiology of neuro-axonal dysfunction. Over expression of normal D-spastin leads to destabilization of axonal microtubule scaffold, while its silencing causes a dramatic increase in stabilized microtubules. Expression of mutated D-spastin results in phenotypes indistinguishable from those observed in Knock-down animals. In both lines the exposure to the destabilizing agent vinblastin substantially ameliorates the phenotypes. Three important elements arise from this study: (1) spastin main function is microtubule severing; (2) mutant spastin can act through a dominant negative mechanism; (3) agents modifying microtubule dynamics may counteract the cellular abnormalities due to loss of spastin activity, and may represent a worth exploring therapeutic strategy.

SA2.15 Genetic studies in a patient with Cap disease
Fardeau M.

Paris, France

Abstract not received

SYMPOSIUM III
The progress of neurological sciences improves clinical practice

Session I

SA3.1 Molecular mechanisms modifying the course of genetic diseases
Jedrzejowska M.

Medical Research Center PAS, Neuromuscular Unit, Warsaw, Poland

Advances in molecular biology resulted in better understanding of the patophysiology of SMA. SMA is even hoped to be one of the first inherited diseases that may be treated by transcriptional activation or splicing correction. However, many questions remain unclear. In patients with SMA, the SMN1 gene is mutated (a biallelic deletion is found in more than 95% of patients) and small amounts of SMN protein are produced by SMN2 gene. The number of the SMN2 copies is undoubtedly the major SMA phenotype modifier. The higher the number of SMN2 copies, the more benign is the course of the disease. Some reports suggest that the presence of five SMN2 copies compensates the loss of both SMN1 gene alleles. Indeed, one of our three asymptomatic carriers of biallelic SMN1 deletion had five SMN2 copies, and in two others four SMN2 copies were found. However, increased number of SMN2 copies does not explain the phenomenon of intrafamilial heterogeneity. We observed 5 siblings with identical changes in SMA region with marked phenotypical discrepancies, suggesting that other factors also affect the SMA phenotype. Interestingly, all of these cases suffered from chronic forms of SMA and women were less affected, suggesting gender influence.

SA3.2 SMN and SMA how does it all fit together
Burghes A.H.M., McGovern V.L., Gavrillina T.O., Butchbach M.E.R., Workman E.L, Le T.L.

Ohio State University, USA

MA is caused by loss of the SMN1 and retention of SMN2. We have replicated this situation in the mouse by placing 2 copies of SMN2 onto a null background (SMN2+/+; Smn-/-). These mice show severe atrophy of muscle and die at 5 days. One copy of SMN2 results in embryonic lethality whereas 8 copies of SM2 rescues SMA. The introduction of SMN lacking exon 7 extends survival of SMA mice to 14 days whereas introduction of a mild missensse mutation SMNA2G extends survival beyond a year and results in mice with mild SMA. Interestingly SMNA2G cannot rescue embryonic lethality of SMN null animals but can rescue the lethality of single copy SMN2 animals indicating the function of heteromers in SMNs essential function. We have crossed SMA carrier mice so as to label their motor axons with GFP throughout development. We find that severe SMA mice have completely normal motor neuron outgrowth and pathfinding. This contrasts to what is observed in cultures of motor neurons from these mice or knockdown of SMN in fish. However we do observe a significant increase in unoccupied synapses of SMA embryos at day 18.5 but not at embryonic day 17 indicating denervation occurs prior to birth in these animals. Over expression of SMN in neurons off SMA animals rescues the SMA phenotype. However over expression in just muscle fibers has no impact on survival of SMA animals. We are taking a genetic approach to dissect out the critical function of SMN in SMA. In addition we have tested drug compounds in the mice we have developed.

SA3.3 Spinal muscular atrophy a defect of muscle maturation
Vrbova G.

Autonomic Neuroscience Centre, Royal Free and University College London Medical School, UK

The importance of the muscle for the unique involvement of the motoneurone in spinal muscular atrophy (SMA) has often been considered. The critical dependence of the developing motoneurone on interaction with the muscle has also been documented. However, it is difficult to establish whether the involvement of the muscle is an important contributor for the symptoms of the disease and responsible for loss of motoneurones. Although there are many reports of immaturity of skeletal muscles both in SMA patients and animal models of SMA they have often been considered to be the consequence of loss of motoneurones, rather then the primary cause of motoneurone death. This view has gained support from he finding on transgenic mice, where the smn gene was selectively reduced in neu-

rones only and caused modest motoneurone death (1). However, there is a large body of experimental evidence from normal animals to show that arrest of muscle development has a devastating effect on motor units. A recent finding from animal models of SMA shows that SMN protein is interacting with the structural proteins of the muscle fibres (2). Moreover, increasing the levels of SMN protein in a mouse model of SMA ameliorates the symptoms of disease by acting on muscle (3). References: 1. Frugier et al. (2000) Hum Mol Genet 9: 849–858; 2. Rajendra et al. (2007) J Cell Biol 176: 831–641; 3. Avila et al. (2007) J Clin Invest 117: 659–671.

SA3.4 Spinal muscular atrophy (SMA) – a neurodevelopmental disorder

Hausmanowa-Petrusewicz I.

Neuromuscular Unit, Institute of Experimental and Clinical Medicine PAS, Warsaw, Poland

We present the data collected during many years on SMA patients which striked us by (1) the specific character of this disease different from the other degenerative disorders of nervous system, (2) the morphology of the skeletal muscle, motor roots and peripheral nerves had a foetal appearance. The large body of electrophysiological findings (both emg and nerve conduction velocity study) confirmed the delayed motor unit maturation. The paralel studies on human foetuses affected by SMA confirmed the prenatal changes of motoneurons. On basis of those findings we proposed hypothesis on the role of immaturity in the pathogenesis of SMA. This hypothesis has been accepted only by a few authors. However the interest in SMA pathogenesis revived newly due to search for a rational therapy. Our previous hypothesis has been partly supported by molecular findings, and particularly by the data on the role of SMN protein in the development. Many questions however remain still unanswered, e.g., (1) why the main target of SMA or at least the most sensible structure seems to be motoneuron, (2) what is the link between this target and muscle, the axonal transport and destabilized motor-end junction, (3) why reduction of such ubiquitous protein as SMN affects only some tissues sparing others. Of some importance are the data indicating that the SMN protein promotes maturation but does not promote regeneration. Hopefully the search for rational therapy will enhance the knowledge on SMA pathogenesis.

SA3.5 Paradoxonase gene polymorphism and sporadic ALS

Slowik A.

Jagiellonian University, Collegium Medicum, Krakow, Poland

Abstract not received

Session II

SA3.6 Nonmuscle myosins in muscle pathologies

Redowicz M.J.

Nencki Institute of Experimental Biology, Department of Biochemistry, Warsaw, Poland

Myosins, the actin-based molecular motors were originally isolated from skeletal muscle as early as in 1939 and until 1973 it was believed they were muscle specific proteins. They are ubiquitously expressed in all eukaryotic cells and are involved in a panoply of cellular functions, including cell migration, the processes employing intracellular trafficking such as endo- and exocytosis, cytokinesis, and gene transcription. Based on the differences in the sequence of a motor domain, myosins are classified into about 30 distinct families. Muscle isoforms and those resembling them both structurally and functionally are termed as conventional myosins, and the other ones are referred as unconventional myosins. Despite the fact that several non-muscle myosins (I, IIA, IIB, V, VI and XVIIIB) have been detected in skeletal, cardiac and smooth muscles, there are only few reports on their possible roles in muscle tissues. However, it has been shown so far that a point mutation in nonmuscle myosin II or myosin VI genes lead to cardiac dysfunction; lack of nonmuscle myosin IIB causes severe cardiac and neuronal defects in mice, resulting in death up to 2 days after birth. Moreover, nonmuscle conventional myosin II may partially substitute smooth muscle myosin in transgenic mice. Our own studies indicate that expression of myosins I, IIB, V and VI seems to be enhanced in denervated skeletal muscles.

SA3.7 Molecular mechanisms of cardiac muscle contraction control

Wrzosek A.

Nencki Institute of Experimental Biology PAS, Department of Biochemistry, Warsaw, Poland

The sympathetic nervous system, force-frequency relationship, and Frank-Starling effect are the basic mechanisms that regulate contractile force of the heart intact muscle. An increase in the length of cardiac muscle produces an immediate potentiation of twitch force (Frank-Starling relationship) which, at least, partially can be explained by increase in myofilament Ca^{2+} sensitivity with possibly small change in the filament overlap, but not by the changes in the calcium transient. However, how does the myofilament can sense the changes in the muscle length still remain unclear? The steep change in force, after the increase in heart muscle length, is followed by a further slow force augmentation over the next 20 min. The delayed increase in force is at least partially due to an increase in intracellular Ca^{2+} transient, but its underlying mechanisms in slow force responses are still not fully understood. Some published data suggest involvement of the sarcolemmal Na^{+}/H^{+} exchanger which can be activated by local autocrine/paracrine systems releasing angiotensin II and endothelin-1 in slow force responses. In the intact heart and in the neonatal cardiac cell culture prolonged heart muscle mechanical stretch induces hypertrophy which may involve Ras proteins, extracellular signal-regulated kinases 1/2 (ERK1/2) and protein kinase B. It is also suggested that mechanical stress signal may be directly transmitted to the nucleus by cytoskeleton or *via* the calcium/clmodulin-calcineurin dependent pathway.

SA3.8 Structure of nucleus and its abnormalities in some neuromuscular disorders

Fidzianska-Dolot A.

Neuromuscular Unit, Institute of Experimental and Clinical Medicine PAS, Warsaw, Poland

Ultrastructural and immunohistochemical studies were performed on nuclear abnormalities in muscle from patients with envelopathies as well as with sporadic inclusion body myositis

(sIBM). The diagnosis was based on clinical and molecular findings. Different degree of nuclear architecture remodeling ranging from misshapen nuclei, nuclear chromatin condensation, decondensation and focal loss to complete nuclear fragmentation were detected in investigated cases. Most interesting finding was the appearance of tubulofilamentous inclusions inside of nuclear matrix of X-linked EDMD patients. This phenomenon prompted us to evaluate nuclear proteins in sIBM. Relocalization of lamin A from nuclear rim to nuclear matrix as well as formation of emerin decorated inclusions within nuclear interior were surprising findings in sIBM patients. All these nuclear aberration seems to be structural indicators of nuclear dysfunction evoked by nuclear envelope proteins abnormality.

SA3.9 Mutation or polymorphism in neurodegenerative disorders?

Kochanski A.

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

A question whether a certain variant of the DNA sequence is a pathogenic-causative mutation or harmless polymorphism has arisen in the recent years due to the accumulation of contradictive data. Some mutations reported earlier to be pathogenic in neurodegenerative disorders have been shown not to have any role in the pathogenesis, whereas numerous "silent polymorphisms" were proved to be pathogenic mutations. There is no problem to distinguish causative mutations from polymorphisms in case of DNA variants occurring in different populations, segregating with "clear" phenotype in multigenerational families and showing deleterious effect in the functional studies. In fact, vast majority of the patients diagnosed with Parkinson's, Alzheimer's or ALS originate from small families or represent sporadic cases. To prove that a new gene variant is pathogenic is a challenge for neurogenetics. At the other hand the "silent" polymorphisms have been also shown to contribute to pathogenesis of neurodegenerative disorders. In the postgenomic-"SNP era", characterized by expotential rise of DNA variants with unknown pathogenic status, a comprehensive set of criteria should be proposed to asses their pathogenic effect.

SA3.10 Molecular aspects of two myotonic dystrophies

Zaremba J.

Institute of Psychiatry and Neurology, Warsaw, Poland

Myotonic dystrophy type 1 (DM1) is a multisystem disorder that affects skeletal and smooth muscle as well as the eye, heart, endocrine system, and central nervous system. The clinical findings have been categorized into three overlapping phenotypes: mild, classic, and congenital. Myotonic dystrophy type 2 (DM2) is characterized by myotonia and muscle dysfunction (weakness, pain, and stiffness) and less commonly by cardiac conduction defects, iridescent posterior subcapsular cataracts, insulin insensitive type 2 diabetes mellitus, and testicular failure. Both DM1 and DM2 are inherited as autosomal dominant traits and are caused by dynamic mutations: DM1 by an expansion of a CTG trinucleotide repeat in DMPK gene whereas expansion of a CCTG repeat in ZNF9 gene causes DM2. CTG repeat length in range from 5 to 37 is normal; 38–49 is the range of premutation without clinical findings and the number above 50 CTG correlates with the disease. The polymorphic trait in the intron 1 of ZNF9 gene consists of motif: (TG)n(TCTG)n(CCTG)n. The loss of interruptions TG or TCTG may cause the instability and expansion. The number of CCTG repeats in expanded alleles ranges from approximately 75 to more than 11 000. Similarly to other diseases caused by dynamic mutations the anticipation in DM1 is observed, whereas in DM2 cases anticipation was not described up to the date. Molecular genetic testing is available for patients with clinical symptoms as well as predictives and prenatal cases from families with confirmed DM1 or DM2.

POSTER SESSION I
AUTONOMIC AND UNSPECIFIC SYSTEMS

P1.01 Cross-talk between leptin and glucagon-like peptide-1 (GLP-1) in the control of food intake

Bojanowska E., Nowak A.

Department of Behavioral Pathophysiology, Medical University of Lodz, Poland

Using exendin-(9-39), a GLP-1 antagonist, and exendin-4, a GLP-1 agonist, we investigated whether leptin interacts with this peptide in the regulation of food intake. In the first series, rats were injected intraperitoneally (i.p.) with either 50 μg exendin-(9-39) alone, 100 μg leptin alone or exendin-(9-39) and then with leptin. In the second series, rats were injected i.p. with exendin-4 alone (0.1, 2 or 10 μg per rat), leptin alone (0.1, 2, 10 or 100 μg per rat) or exendin-4 and leptin together (0.1 + 0.1, 2 + 2, 10 + 10, or 2 + 100 μg per rat, respectively). Exendin-(9-39) did not affect markedly food intake whilst 100 μg leptin alone significantly reduced a 24-hour food ingestion. On the other hand, the inhibitory effect of leptin on food intake was completely abolished when exendin-(9-39) was injected prior to leptin. At the lowest dose used, leptin and exendin-4 injected together but not separately reduced significantly a 24-hour food intake. When used in higher doses, however, leptin did not change the exendin-4-dependent suppressory effect on food consumption. It is concluded that GLP-1 mediates the effect of leptin on food intake. Leptin and exendin-4 may act additively to inhibit appetite when present in low concentrations. The lack of synergistic effects of exendin-4 and high leptin concentrations on food intake may explain, at least in part, mechanisms responsible for leptin resistance in subjects with hyperleptinaemia. This work was supported by the Medical University of Lodz, grant No. 502-16-509 and 503.

P1.02 Respiratory hypoxic response to adenosine A2 antagonist in adult rats

Ilasz R., Budzinska K.

Department of Respiratory Research, Medical Research Center, Warsaw, Poland

Adenosine may be a factor to respiratory depression that appears in the late phase of hypoxic exposure. Peripherally, adenosine activates the carotid bodies through A2 receptors. We studied whether selective adenosine A2a receptor antagonist 3,7-dimethyl-1-propargylaxanthine (DMPX) modulates biphasic respiratory response to hypoxia. Anesthetized adult rats were vagotomized, paralyzed and artificially ventilated. Amplitude and frequency of integrated activity of phrenic and hypoglossal nerve and minute phrenic (PHR) and hypoglossal (HG) activity were calculated as indices of the respiratory output. In baseline condition and 20 minutes after DMPX (5 mg/kg, i.p.) injection a mixture of 11% O_2, in N_2 enriched with 1.7% CO_2 was applied as long as a the respiratory excitatory response changed into respiratory depression or apnoea. DMPX alone augmented respiratory activity. The respiratory response to hypoxic exposure remained biphasic after DMPX injection, however maximal stimulation of respiration attained lower level after DMPX in comparison to baseline response (133.6 ± 8.1%, vs. 173.2 ± 8.1%; $P<0.05$ for PHR). This difference came from a significantly smaller increment of respiratory frequency. Second phase of the response to hypoxia appeared earlier after DMPX (17.5 ± 1.2 s vs. 28.0 ± 1.4 s, $P<0.00002$). The results suggest that DMPX antagonism to adenosine A2a receptors may decrease peripheral chemoreceptors sensitivity and promote a second depressant phase of the respiratory response to hypoxia.

P1.03 Blood immune cell distribution after chronic electrical lateral hypothalamic stimulation in rats

Jankowski M., Ignatowska-Jankowska B., Glac W., Wrona D.

Department of Animal Physiology University of Gdansk, Poland

Previously, we found that chronic electrical stimulation of the representative structure of the so-called “brain reward system” such as the lateral hypothalamus (LH) enhances natural killer cell cytotoxicity and large granular lymphocyte number in rats. The present study extends our previous findings on non-specific to specific immune response. Using the same experimental design (21 consecutive days, 7:00–11:00 AM), in the present study the total and percentage blood leukocyte, lymphocyte and their subsets were determined by flow cytometry. Three-color immunofluorescent antibody staining procedure was used: CD3-FITC/CD45RA-PC7/CD161A-APC and CD3-FITC/CD4-PC7/CD8-APC for determination of T/B/NK and T cell subpopulations (CD3+CD4+, CD3+CD8+), respectively. As compared to the sham controls, chronic LH stimulation significantly ($P<0.05$) increased T cell percentage number (56.4 ± 7.5% vs. 65.0 ± 2.5%) and their CD3+CD8+ subset (14.8 ± 3.1% vs. 20.1 ± 3.4%). In contrast, a marked decrease ($P<0.01$) in B cell number in the LH stimulated group (15.5 ± 4.2% vs. 27.2 ± 7.4%) was observed. However, there was no significant change in the total and percentage number of leukocytes (11 940 ± 3 448/μm vs. 12 205 ± 3 106) or lymphocytes (7 698 ± 2 732 vs. 8 359 ± 2 205 or 64.2 ± 8.8% vs. 68.5 ± 2.9%) between stimulated and control groups. These and previously obtained results indicate that positive reinforcement-related area LH influences the immune cell distribution including increase the numbers of lymphocytes connected with non-specific and specific cytotoxic anti-tumor and anti-viral activity.

P1.04 Immune effect of electrical stimulation on the bed nucleus of stria terminalis in rats

Myslinska D., Glac W., Ignatowska-Jankowska B., Jankowski M., Trojniar W.

Department of Animal Physiology, University of Gdansk, Poland

In our previous study we found that electrolytic lesion of the bed nucleus of the stria terminalis (BNST) caused depression of the peripheral blood natural killer cell cytotoxicity (NKCC) and the number of large granular lymphocytes (LGL). In the respective sham operated group mere insertion of electrodes into the BNST evoked transient enhancement of NKCC, probably resulting from mechanical stimulation of the BNST tissue. To check this effect in the present study, we evaluated both spleen and blood NKCC (51Cr-release assay) and 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 BNST in conscious, freely

behaving rats (n=7). Additionally, peripheral blood leukocyte, lymphocyte and neutrophile number was measured. Chronic stimulation of the BNST caused significant augmentation of blood NKCC (P<0.01) and LGL number (P<0.01), as well as slight enhancement of leukocyte, lymphocyte and neutrophile number in comparison to the sham group (n=6). No such effect was found in the spleen. A week after termination of the stimulation procedure all measured parameters returned to the baseline. The results obtained indicate that BNST belongs to the limbic structures involved in the regulation of immune responses: its lesion causes depression and stimulation causes enhancement of cellular immunity.

CIRCADIAN RHYTHMS AND SLEEP

P2.01 The effect of obesity on sleep quality in patients treated for the sleep related breathing disorders

Antczak J.[1], Richter A.[2], Horn B.[2], Bodenschatz R.[2], Schmidt E.W.[2]

[1]Department of Clinical Neurophysiology, Institute of Psychiatry and Neurology, Warsaw, Poland; [2]Sleep Centre at Hospital Kuchwald, Chemnitz, Germany

There is a number of studies convincing that obesity is an independent factor (irrespective to sleep related breathing disorders – SRBD) for a disordered sleep architecture. The aim of the study was to investigate, if the obesity affects the sleep quality in SRBD patients, who are treated with continuous positive airway pressure (CPAP). The investigated groups included 17 severely obese and 18 nonobese SRBD patients matched for age and gender with respiration normalized under CPAP. The polysomnographic recordings from the night before CPAP, from the second night under CPAP and from the control night – three months after CPAP start, were compared between groups. The differences in sleep pattern before CPAP included decreased REM sleep and decreased Non-REM sleep stages 2 and 4 (S2, S4) as well as increased Non-REM sleep stage 1 (S1) in severely obese patients. At the second night under CPAP the severely obese group presented increased REM sleep and decreased S1 when compared to the nonobese group. There were no differences in sleep pattern between both groups at control night. According to our data the severe obesity seem not to affect the sleep quality in SRBD patients under long term (three months) CPAP therapy. The increased REM sleep and decreased S1 (light sleep) at second night under CPAP suggest more pronounced sleep-rebound in severely obese patients with SRBD.

P2.02 Effect of 8-OH-DPAT on the phase shift of locomotor activity rhythm in constant light in mice

Bartoszewicz R., Barbacka-Surowiak G.

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

The PRC of the effect of 8-OH-DPAT, a serotonin agonist, on the locomotor activity rhythm in golden hamster kept in LL was constructed by Tominaga. Other work showed that the differential response to systemic 8-OH-DPAT in mice and hamsters likely reflects a species difference in functional responses to 8-OH-DPAT. Moreover, experiments on the influence of serotonin upon the locomotor activity rhythm of nocturnal rodents kept under constant light conditions are very scarce. Therefore, we have decided to examine the effect of 8-OH-DPAT on the phase of locomotor activity rhythm in constant light in mice. Eight week old C57BL/10 inbred male mice, were kept in sound-proof room in individual cages with free access to the running wheel, recording the wheel-running activity rhythm in LD 12:12 and then in succeding LL conditions. In the LL condition 8-OH-DPAT was intraperitoneally injected every two hours during twenty-four hours. The analysis of the phase shifts of the locomotor activity rhythm of mice in the LL condition indicates that between 8.00–18.00 CT phase advances occurred. In the subjective night between 22.00 CT–2.00 CT phase delay was observed. At 4.00 CT–6.00 CT and at 20.00 CT no phase shifts were seen. It is very interesting that our PRC is very similar to PRC for dark pulses in LL in mice described by Barbacka-Surowiak.

P2.03 Daily expression of synaptic proteins in the visual system of *Drosophila*

Gorska-Andrzejak J.

Department of Cytology and Histology, Jagiellonian University, Krakow, Poland

The first visual neuropile or lamina in Diptera exhibits several structural circadian rhythms. One of them are oscillations in the number of tetrad synapses formed between the photoreceptor terminals (R1-R6) and monopolar cells L1 and L2, which receive photic information from the photoreceptors (Pyza and Meinertzhagen 1993). Looking for mechanisms involved in synaptic plasticity controlled by a circadian system we examined the expression of two proteins important for assembling multiprotein complexes at synaptic contacts, a presynaptic active zone protein Bruchpilot and a postsynaptic site protein Disc large (Dlg). In our experiments, wild type and transgenic lines of *Drosophila* that were reared under light/dark (LD 12:12) and constant darkness (DD) regimes were decapitated at different times of the day and processed for confocal microscopy. The results revealed that the expression of both studied proteins changes during the day, and is highest at ZT 13 (one hour after lights-off) for Dlg, and at ZT 1 (one hour after lights-on) for Bruchpilot. Interestingly, the oscillations in the level of expression of the postsynaptic protein (Dlg) are more pronounced than the ones in the presynaptic protein (Bruchpilot). The observed changes clearly indicate the cyclic remodeling of synaptic contacts in the lamina by a circadian clock input, especially at the postsynaptic sites.

P2.04 The effects of ACh agonist and antagonists on isoperiodic oscillations in the IGL neurons activity

Siejka S., Lewandowski M.H.

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

The mammalian circadian timing system consists of number of interconnected neuronal centres. The most important part of this system are suprachiasmatic nuclei (SCN) of the hypothalamus. Intergeniculate leaflet (IGL) constituted a second element of this system. IGL receives photic information directly from retinal ganglion cells. At the same time IGL neurones receive non-photic influences mainly from nonspecific structures located in brain stem. One of them is a cholinergic projection from laterodorsal (LDT) and pedunculopontine tegmental nucleus main sources of cholinergic afferents

in the mammalian brain. Our previous experiments showed that electrical stimulations of LDT inhibited neuronal firing of IGL cells. The purpose of the present study was to determine an influence of locally applied acetylocholine agonist and antagonists on the isoperiodic oscillations in the IGL neurones activity. The experiments were performed on Wistar rats, using standard surgical and stereotaxic procedures used in our laboratory. The multiple-unit neuronal activity was extracellularly recorded in the IGL with glass micropipettes which were assembled with three-barrelled drug delivery capillaries. Two barrels were filled with drugs (carbachol and gallamine or pirenzepine) and the last one contained 165 mM NaCl for current balancing. Our preliminary results show that cholinergic agonist – carbachol inhibited neuronal firing in the numerous of cases. This effect is probably modified by specific cholinergic antagonists. Obtained data suggest that cholinergic projection may play a role in modulation of neuronal activity of a population of IGL cells.

P2.05 Effects of constant light and the clock genes on circadian plasticity of neurons in *Drosophila*

Weber P., Pyza E.

Department of Cytology and Histology, Institute of Zoology, Jagiellonian University, Krakow, Poland

In the visual system of *Drosophila melanogaste*r the first order interneurons, L1 and L2, monopolar cells, reveal circadian rhythms in changes of the girth of axon and in morphological plasticity of the dendritic tree. In our previous study, using a transgenic line 21D-GAL4xUAS-mCD8-GFP which expresses green fluorescent protein (GFP) targeted to cell membrane in L2, we found that the perimeter of dendritic tree is the largest at the beginning of the day in a day/night (LD12:12) cycle. This rhythm is maintained in constant darkness (DD) indicating on its circadian origin and controlled by clock genes because it was not detected in the clock gene period mutant (per01; 21D/TM2) in LD and DD. In the present study we examined this rhythm under continuous light conditions (LL) and in the Drosophila primary circadian photoreceptor cryptochrome mutant (cryb21D/TM6B) in LD and DD. The obtained results showed no statistically significant differences in the perimeters of dendritic trees in flies fixed at different time points in LL, suggesting that like in case of other circadian rhythms, LL seems to abolish the circadian rhythm in the L2 dendrite plasticity. In case of cryb mutant this rhythm was detected in LD but the L2 dendritic tree was the largest in the middle of the day. In DD the rhythm was detected only in males, however. In addition we found the effect of cryb mutation on the morphology of L2 dendrites. Supported by grant no. BW/IZ/16b/2004.

P2.06 Electrical properties of morphologically characterized neurons in the IGL of the rat

Szkudlarek H.J.[1], Raastad M.[2]

[1]Institute of Zoology, Jagiellonian University, Poland; [2]Institute of Basic Medical Sciences, University of Oslo, Norway

The intergeniculate leaflet (IGL) is a thin layer of cells, located between dorsal and ventral part of lateral geniculate body, involved in regulation of circadian rhythmicity. Immunohistochemical and tract tracing studies suggest existence of distinct classes of neurons within the IGL. Although, electrophysiological extracellular studies also support this statement, the IGL cells were not investigated with intracellular methods and therefore it was not known whether this diversity is also reflected in membrane properties. In this study we have characterized basic membrane properties of IGL neurons and labeled them with biocytin, included in the recording pipette. The results showed that most of IGL cells (77%) was spontaneously active with predominant regular firing pattern, characterized by coefficient of variation of the spike intervals <0.2. Input resistance was higher in active neurons (1.3 ± 0.1 GΩ) than in silent ones (0.7 ± 0.2 GΩ) but membrane time constant, resting membrane potentials and spike thresholds overlapped between these two groups. 81% of the IGL neurons generated rebound depolarization in response to hyperpolarizing pulse with one or a few fast action potentials on top of it. Morphological parameters show that IGL cells form a continuum and silent cells do not differ from active neurons. The spontaneous spiking, rebound depolarization and morphology of IGL cells are therefore features that distinguish them from interneurons in dorsal and neurons in ventral lateral geniculate nuclei.

P2.07 Control of melatonin rhythm in chick pineal by UV-A and white light: Role of retinal NMDA and D1-dopamine receptors

Zawilska J.B.[1], Lorens A.[2], Berezinska M.[2]

[1]Dept. of Pharmacodynamics; [2]Dept. of Pharmacology, Medical University Lodz, Poland

Light is the dominant environmental factor controlling rhythmic melatonin (MEL) synthesis in pineal gland of vertebrates. Although the avian pineal gland is a directly photosensitive organ, it has recently been demonstrated that light perceived by the eyes only regulates its activity.This work was aimed to investigate whether retinal illumination alone was capable of resetting the biological oscillator generating circadian rhythm of AANAT (a key regulatory enzyme in MEL biosynthesis) activity in the chick pineal gland. Ocular exposure of chicks to UV-A or white light during the second half of the subjective night markedly decreased AANAT activity in the pineal gland, and produced a significant phase advance of the circadian rhythm of AANAT activity. The suppressive and phase-shifting effects of UV-A were antagonized by intraocularly given MK 801 (a selective blocker of NMDA glutamate receptors), but were not modified by SCH 23390 (a selective antagonist of D1-dopamine receptors). Effects of retinally perceived white light were antagonized by SCH 23390, and not affected by MK 801. It is suggested that ocular illumination with UV-A and white light, by activating, respectively, NMDA glutamate and D1-dopamine receptors-coupled transduction pathways in the retina, provide powerful signals controlling circadian MEL rhythm in the pineal gland. Supported by Med Univ Lodz, Poland (502-13-409).

P2.08 The effect of obesity on sleep quality in patients treated for the sleep related breathing disorders

Antczak J.[1,2], Richter A.[2], Horn B.[2], Bodenschatz R.[2], Schmidt E.W.[2]

[1]Department of Clinical Neurophysiology, Institute of Psychiatry and Neurology, Warsaw, Poland; [2]Sleep Centre at Hospital Kuchwald, Chemnitz, Germany

There is a number of studies convincing that obesity is an independent factor (irrespective to sleep related breathing disorders

– SRBD) for a disordered sleep architecture. The aim of the study was to investigate, if the obesity affects the sleep quality in SRBD patients, who are treated with continuous positive airway pressure (CPAP). The investigated groups included 17 severely obese and 18 nonobese SRBD patients matched for age and gender with respiration normalized under CPAP. The polysomnographic recordings from the night before CPAP, from the second night under CPAP and from the control night - three months after CPAP start, were compared between groups. The differences in sleep pattern before CPAP included decreased REM sleep and decreased Non-REM sleep stages 2 and 4 (S2, S4) as well as increased Non-REM sleep stage 1 (S1) in severely obese patients (4.7 ± 5.2 vs. 8.3 ± 4.2 – in % of the total sleep time, $P<0.02$; 26.5 ±10.9 vs. 35.5 ± 8.9, $P<0.03$; 3.5 ± 6.0 vs. 8.1 ± 6.6, $P<0.01$; 33.2 ± 16.4 vs. 19.2 ± 9.5, $P<0.01$) respectively. At the second night under CPAP the severely obese group presented increased REM sleep and decreased S1 when compared to the nonobese group (21.6 ± 7.0 vs. 11.8 ± 4.0, $P<0.01$; 7.8 ± 3.8 vs. 13.3 ± 6.7, $P<0.01$; 24.3 ± 7.9 vs. 31.1 ± 9.5, $P<0.04$). There were no differences in sleep pattern between both groups at control night. According to our data the severe obesity seem not to affect the sleep quality in SRBD patients under long term (three months) CPAP therapy. The increased REM sleep and decreased S1 (light sleep) at second night under CPAP suggest more pronounced sleep-rebound in severely obese patients with SRBD.

P2.09 The influence of orexin-A on spontaneous activity of the intergeniculate leaflet neurons – *in vitro* studies

Pekala D., Blasiak T., Lewandowski M.H.

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

The best known function of the thalamic intergeniculate leaflet (IGL) is its involvement in the regulation of biological rhythms. However, there are suggestions indicating that IGL might also play a role in other non-circadian systems (visuomotor, vestibular and sleep/arousal). The latter function is strongly supported by the anatomical data showing the reciprocal connection between the IGL and orexin (hypocretin) synthesizing cells in the lateral hypothalamus. Widespread efferents of the orexinergic cells form an extensive projection system that terminates in divers brain regions. Orexinergic Orexinergic system is involved in the control of the food intake and maintenance of wakefulness. The purpose of present study was to investigate the effect of orexin-A on the spontaneous discharge rate of the IGL neurons. Experiments were performed on rat brain slices using the extracellular, single-unit recording technique. After stable baseline activity had been recorded for at least 300 seconds, orexin-A was administered locally, by pressure injection. Preliminary results indicate that orexin-A increased the firing rate of the IGL neurons. Moreover, we have observed that orexin-A exerts a modulatory effect on the basic firing pattern of the recorded, IGL cells. Our data are in agreement with other studies showing the excitatory properties of orexin-A in other brain nuclei. Obtained results reinforce the possibility that IGL can redistribute the signal, coming from the orexinergic system, to its efferent projection targets. Supported by MNiSW grant PB 0972/30.

P2.10 Correlation between ZT and the spontaneous activity of 5-HT cells of the rat median raphe nucleus

Werhun K. [1,2], Raison S. [2], Pevet P. [2], Lewandowski M.H.[1]

[1]Department of Neurophysiology and Chronobiology, Jagiellonian University, Krakow, Poland; [2]Department of Neurobiology of Rhythm – INCI, CNRS–ULP, University of Strasbourg, France

The suprachiasmatic nuclei (SCN) is the major center for generation and regulation of mammalian circadian rhythms. They are synchronized primarily by photic information relayed directly from the retina to the SCN *via* the retinohypothalamic tract and indirectly, by projection from the intergeniculate leaflet, the geniculohypothalamic tract. A third major input to the SCN is provided by serotoninergic (5-HT) afferences arising from the median raphe nucleus (MRN). The functioning of 5-HT neurons has been shown to be rhythmic in LD and DD conditions. There are evidences suggesting that tryptophan hydroxylase (TpH) protein and TpH mRNA levels exhibit circadian variations. Also 5-HT release within the SCN is circadian and correlated with its synthesis in the serotoninergic terminal field. Another parameter that has not been studied as yet is neuronal firing of 5-HT MRN cells. Therefore the aim of the present study was to determine, by using *in vivo* measurement of extracellular single-unit activity, if there are any relationship between zeitgeber time (ZT) and firing of the 5-HT neurons within the rat MRN. We have characterised the 24-h profile of spontaneous discharge of 5-HT cells in anesthetized rats. Our results indicate that activity of 5-HT neurons display daily fluctuation. The mean activity of 5-HT neurons reveals two peaks at ZT 2 and 12 and reach minimum at the middle of day and night. Our data confirm the rhythmic functioning of 5-HT cells within the circadian system.

DEVELOPMENT AND ADULT NEUROGENESIS

P3.01 Fibronectin stimulates the MMPs activity during neural stem cells development *in vitro*

Dragun P., Sypecka J., Zalewska T.

NeuroRepair Dept, Medical Research Institute, Warsaw, Poland

Neural stem cells development is regulated by the signals from extracellular matrix (ECM) and therefore seems to depend on the activity of the matrix metalloproteinases (MMPs). The aim of our study was to investigate whether the selected ECM components (laminin, fibronectin, collagen and poly-L-lysine) influence simultaneously the neural stem cells differentiation and the MMPs activity *in vitro*. Methods. The cells of HUCB-NSC line were seeded on the ECM components-coated plates. On the 4th, 8th and 14th day in culture, *in situ* zymography was performed, followed by the immunocytochemistry with the neural markers. Results. Among all of the examined ECM components, the fibronectin stimulated MMPs activity most intensively (~20% increase in the 2-weeks culture), as revealed by *in situ* zymography. Co-localization studies shown that in the developing neural stem cells MMPs were localized predominantly within the nucleus, while in the differentiated (TuJ+, GalC+) cells their activity is observed mainly within the cytoplasm. Conclusions. ECM components might be the potent factors in promoting of the cell proliferation and/or differentiation. As it was shown in the present study, the fibronectin stimulates the MMPs activity *in vitro*. It also coincidences with the MMPs

translocation within the cell during the differentiation process. Observed mechanism can be the important part of signaling pathway governing the cell development. Supported by MSRHE grants: 1266/P01/2006/31 and N40101832 /0296.

P3.02 Building the central complex of the grasshopper *Schistocerca gregaria*

Herbert Z., Williams L., Gebhardt S., Boyan G.

Division of Neurobiology, Department of Biology II, Ludwig-Maximilians-Universitat Munich, Munich, Germany

The central complex is a major neuropilar structure in the insect brain whose distinctive, modular, neuroarchitecture in the grasshopper is exemplified by a bilateral set of four fibre bundles called the w, x, y and z tracts. These columns represent the stereotypic projection of axons from the pars intercerebralis into commissures of the central complex. Each column is established separately during early embryogenesis in a clonal manner by the progeny of a subset of four identified protocerebral neuroblasts. We report here that dye injected into identified pioneers of the primary brain commissure between 31–37% of embryogenesis couples to cells in the pars intercerebralis which we identify as progeny of the W, X, Y, or Z neuroblasts. These progeny are the oldest within each lineage, and also putatively the first to project an axon into the protocerebral commissure. The axons of pioneers from each tract do not fasciculate with one other prior to entry into the commissure, thereby prefiguring the modular w, x, y, z columns of the adult central complex. Within the commissure, pioneer axons from columnar tracts fasciculate with the growth cones of identified pioneers of the existing primary fascicle and do not pioneer a separate fascicle. The results suggest that neurons pioneering a columnar neuroarchitecture within the embryonic central complex utilize the existing primary commissural scaffold to navigate the brain midline.

P3.03 Thalamic dissociated culture as a model to investigate Lef1/Tcf target genes in postmitotic neurons

Misztal K.[1], Wisniewska M.B.[1], Kuznicki J.[1,2]

[1]International Institute of Molecular and Cellular Biology; [2]Nencki Institute of Experimental Biology PAS, Warsaw, Poland

Wnt signaling is one of the most important mechanisms engaged in embryonic development. The key mediator in the signaling is beta-catenin. Activated beta-catenin translocates to the nucleus and triggers the transcription of Lef1/Tcf target genes. Recently several papers have shown that some nuclear components of Wnt pathway are specifically present in the adult thalamus. According to our observations, there is nuclear accumulation of beta-catenin in thalamic neurons *in vivo*. To explain the possible role of nuclear beta-catenin in the thalamus we set up *in vitro* culture. The thalamus was isolated from a rat embryo at day 19 (E19) and from a newborn rat at day 0 (P0). Cortical conditioned medium and increased concentration of potassium ions were essential for long-term culture. After 3 weeks, the neuronal survival in E19 was 40% whereas in P0 it was only 15%. To induce beta-catenin nuclear translocation we successfully used either Wnt3a factor or lithium ions. We also observed nuclear accumulation of beta-catenin in Lef1 overexpressing neurons. Beta-catenin localization was visualized by immunocytochemical staining. This model will be further used to reveal the physiological role of beta-catenin in thalamic neurons. This work is supported by EU FPVI Promemoria Grant contract no 512012.

P3.04 Timing of neurogenesis in the hippocampal formation of the opossum *Monodelphis domestica*

Olkowicz S., Glowacz A., Turlejski K., Djavadian R.

Department of Molecular and Cellular Neurobiology, Nencki Institute of Experimental Biology PAS, Warsaw, Poland

We examined the time of hippocampal and retrohipocampal cell generation during postnatal development of the Monodelphis opossum. Opossum pups were injected with bromodeoxyuridine (BrdU) at various days after birth and then survived until postnatal day 35 or P90. BrdU is incorporated into DNA during the S-phase of mitosis and may be detected immunohistochemically in brain sections. Like in other mammalian species, in the opossum neurogenesis in the dentate gyrus persisted beyond the time of development. At all time points studied labeling was strongest in the granule cell layer. However, the hilar region of the dentate was also heavily labeled during development, ceasing after P9. In the Ammon's horn, at P5 labeling was visible in all layers, but it was most numerous in the pyramidal cell layer. In the area CA3 BrdU labeled nuclei only until P9. In contrast, in the area CA1 neurogenesis lasted until P17. In the entorhinal cortex neurogenesis was almost completed at P14, but in the presubicular layer II it ceased only at P17. These results suggest that the hippocampal formation in the opossum shares temporal and spatial dynamics with other mammals but the whole development takes place postnatally.

P3.05 Function of the b-subunit of Na^+/K^+-ATPase in developing *Drosophila* eye disc

Radowska A., Gorska-Andrzejak J., Pyza E.

Institute of Zoology, Jagiellonian University, Krakow, Poland

The Na^+/K^+-ATPase serves to maintain low cytosolic Na^+ and high K^+ concentrations in almost all animal cells. It consists of at least two subunits, a catalytic a-subunit and a regulatory b-subunit. In the nervous system, the regulatory b-subunit appears to be essential not only in the ion transport but also in the cell recognition processes (Gloor et al. 1990). We studied its function in development of Drosophila eye disc with the use of a transgenic line that expresses green fluorescent protein (GFP) under control of transcriptional regulatory element for the b-subunit gene of Na^+/K^+-ATPase, Nervana 2. Our studies revealed that this subunit is important in functioning of the retinal basal glia (RGB), the cells that originate in the optic stalk and migrate into photoreceptor axon layer of an eye disc in the third larval instar to play a role in axon guidance processes. Interestingly, they don't express Nervana 2 (Nrv2) protein in that instar. In brains or eye discs isolated from the late third instar larvae and kept in tissue culture conditions the first Nrv2 expressing RGB cells appear only after 12–16 hours of culturing. In the eye disc fragments showing neurite outgrowth in the culture, RGB cells that express Nrv2 are localized predominantly in sites of axon outgrowth. These results indicate the RGB cells have to undertake an additional function during development of the visual system in the pupal stage, for which expression of the b-subunit of the Na^+/K^+-ATPase is required.

P3.06 CLIP170 role in dendritic arbor development

Swiech L.[1], Dortland B.[2], Hoogenraad C.C.[2], Jaworski J.[1]

[1]International Institute of Molecular and Cell Biology, Warsaw, Poland; [2]Erasmus Medical Center, Rotterdam, The Netherlands

The precise control of the microtubule (MT) polymerization dynamics is crucial for formation of dendritic arbor. Microtubule dynamics depends on activity of microtubule plus ends binding proteins. CLIP170 was a first discovered protein in this group and it is believed to regulate MT dynamics by promoting rescue-phase. It was shown that activity of CLIP170 depends on its phosphorylation status. Mammalian target of rapamycin (mTOR) kinase, is one of the kinases capable of regulating CLIP170 activity. Recently, we presented evidence that mTOR is one of the kinases crucial for proper dendritic arbor development of hippocampal neurons. These findings raise interesting question if CLIP170 activity is necessary for dendritic arbor development in mTOR dependent fashion. To answer this question we first studied effects of CLIP170 removal by RNA interference from developing hippocampal neurons in culture. Introduction of small interfering RNA (siRNA) against CLIP170 into rat hippocampal neurons in the dissociated and organotypic primary cultures resulted in the significant reduction of the number of primary and secondary dendrites. Sholl analysis revealed also decrease in the complexity of dendritic arbors and shrinkage of dendritic fields of neurons lacking CLIP170. Surprisingly, siRNA treatment of more mature neurons resulted rather in substantial decrease in number and severe changes in morphology of dendritic spines. Taken together these data prove the role of CLIP170 in the development of dendritic arbor and spinogenesis.

P3.07 Neuropathological findings in Dandy-Walker malformation: A case report

Tsamis K.[1], Mavroudis I.A.[1], Mytilinaios D.[1], Safouris A.[1], Glaftsi S.[2], Njau S.N.[2], Baloyannis S.J.[1]

[1]Laboratory of Neuropathology, First Department of Neurology, AHEPA Hospital; [2]Laboratory of Forensic Medicine and Toxicology, Aristotelian University of Thessaloniki, Greece

Dandy-Walker malformation is a congenital malformation that involves mainly the cerebellum and fourth ventricle. It is characterized by hypoplasia of the cerebellar vermis, cystic dilatation of the fourth ventricle, and enlargement of the posterior fossa. We studied by Golgi method and routine techniques the alterations in the brain of a 23-year-old case of Dandy-Walker malformation that died accidentally. In the cerebellum the morphological study apart from gliosis revealed Purkinje cells with normal appearance intermixed with others having diminished size of the cell body – especially in the tonsil and in the vermis – and decreased thickness of dendritic arborization with loss of dendritic branches and spines. In the cerebral cortex and the hippocampus the most prominent findings were the tortuous configuration of the apical dendrites of the pyramidal neurons, the focal swellings of the axons and the dendrites and significant gliosis. The blood vessels and the capillaries of the cerebral and the cerebellar cortex appeared to have abnormal course, focal dilations and strictures. All the above findings are presumably related not only to the developmental alterations caused by the Dandy-Walker malformation, but also to the increased intracranial pressure due to the hydrocephalus.

P3.08 Control of microphtalmia transcription factor localization through regulated isoform-specifif nuclear shuttling

Dziembowska M.[1,2], Cigna N.[2], Anezo O.[2], Cordelieres F.P.[2], Klein Ch.[3], Saule S.[2]

[1]Nencki Institute of Experimental Biology PAS, Warsaw, Poland; [2]Institut Curie, CNRS UMR 146, Orsay; [3]Institut Biomedical des Cordeliers, Paris, France

Mitf encodes a basic helix-loop-helix transcription factor essential for eye development and the differentiation of neuroretina, retinal pigmented epithelium and of neural crest-derived melanocytes. Expression of Mitf results in at least nine different MITF isoforms that differ at the N-terminus, each corresponding to the use of different promoter. The A-Mitf protein (eye-specific) bears the largest N-terminus and is localized in both the nucleus and cytoplasm. Here we show that a leptomycin B-sensitive protein export system recognizes the A-Mitf protein due to a nuclear export signal sequence (NES) that we have identified downstream from the leucine zipper domain. Despite containing the NES, the M-Mitf isoform is not efficiently exported from the nucleus and exhibit strictly nuclear localization in the cell. Using MITF-EGFP proteins we performed FRAP experiments demonstrating that A-Mitf nuclear export is more active than its import. In addition, glycogen synthase kinase 3beta (GSK3beta)) was found to phosphorylate Ser298 (Ser399 in A-Mitf) adjacent to the NES increasing the cytoplasmic relocation of A-Mitf, suggesting that this kinase affects the nucleo-cytoplasmic repartition of the Mitf proteins.

P3.09 Adult neurogenesis and exploratory behavior of the laboratory opossum after buspirone treatment

Grabiec M., Djavadian R., Turlejski K.

Lab.of Neurobiology of Development and Evolution, Nencki Institute of Experimental Biology PAS, Warsaw, Poland

The serotoninergic 5-HT1A receptor regulates among others the rate of adult neurogenesis and spatial memory. We investigated exploratory behavior of the adult and aged opossums and the influence of injections of the partial 5-HT1A agonist, buspirone, one month prior to the behavioral tests on their exploratory behavior and the number of newly generated neurons. Exploratory behavior was evaluated in the spatial novelty test according to Husnaker (2005). Seven six-minute sessions were performed in one day. In session 1 the exploration field was empty. In session 2–4 five objects were present there, always in the same places. In session 5–6 placement of two objects was exchanged. In session 7 one old object was replaced with a new one. All animals intensely explored new objects and then habituated. Aged opossums explored more than adults. Control opossums approached all new objects, while the buspirone-pretreated explored more but selectively. Object displacement increased exploration only in the adults injected with buspirone, while introduction of a new object increased its exploration in both adult and aged buspirone-treated groups. Injections of buspirone raised numbers of newly incorporated neurons in the dentate DG and OB. These results suggest that injections of buspirone may have long-time effects on both adult neurogenesis and selectivity of the exploratory behavior in the opossum.

P3.10 Involvement of estrogen receptors in the neurogenesis in ovariectomized female rats

Malinowska-Kolodziej I.[1], Wlaszczuk A.[1], Jedrzejowska-Szypulka H.[1], Larysz-Brysz M.[1], Marcol W.[1], Viau V.[2], Lewin-Kowalik J.[1]

[1]Department of Physiology, Medical University of Silesia, Katowice, Poland; [2]Department of Cellular and Physiological Sciences, University of British Columbia, Vancouver, Canada

The aim of this work was to show estrogen influence on neurogenesis in hippocampus with peripheral nerve grafting. We used two main experimental groups of female rats: with ovariectomy and with estradiol substitution after ovariectomy. In brains we did bromodeoxyuridine (BrdU)- labeling of proliferating cells and counted proliferation rate in dentate gyrus. Immunohistochemistry of markers: early neuroepithelial cells-nestin and astroglia-GFAP, as well as estrogen receptors alfa and beta showed changes depending on estradiol plasma level. Receptors were found in different subcellular localizations. In conclusion, the study prove positive influence of 17-beta-estradiol in physiological high concentrations on neurogenesis mainly in ipsilateral side in hippocampus, together with neurotrophic effect of nerve grafting in rats. Changes in receptors distribution can be connected with their different function and mechanisms of action.

P3.11 Hippocampal microenvironment instructs NG2 precursors to become neurons

Sypecka J., Sarnowska A., Domanska-Janik K.

NeuroRepair Dept, Medical Research Institute, Warsaw, Poland

The fate choice of neural progenitors could be dictated by local cellular environment of adult CNS. The aim of our study was to investigate the effect of hippocampal slice culture on the differentiation and maturation of the oligodendrocyte NG2 precursors. Methods. The hippocampal slice culture was established from the brains of 7-days old rats. The NG2 precursors, obtained from old mixed primary culture of neonatal rat hemispheres, were labeled with CMFDA and seeded on hippocampal slices. After 7–14 days in co-culture, the cells were stained with neural markers. Results. The NG2 cells differentiated predominantly into oligodendrocytes, presenting various stages of maturation: progenitors (NG2), pre-oligodendrocytes (O4) and finally mature GalC-positive cells. However, a considerable number of the cells differentiated into neurons: TuJ+ and even MAP-2+ cells were frequently observed. Moreover, a certain population gathered the proliferative properties, as revealed by Ki67 expression. Conclusions. Neuronal microenvironment provided by the culture of hippocampal slices is potent to induce neurogenesis from oligodendrocyte progenitors and promotes their differentiation not only into OLS but also into neurons. It also supports their proliferative capacity. The results indicate the crucial role of local cellular environment in fate-decision of neural progenitor cells and thus may affect their differentiation after transplantation into CNS. Supported by MSRHE grant: N40101832 /0296.

LEARNING, MEMORY AND PLASTICITY

P4.01 Interhemispheric separation by corpus callosum transection preserved the spatial cognition in rats

Adamska I.

Nencki Institute of Experimental Biology PAS, Warsaw, Poland

We found in previous investigation that cutting of the ventral hippocampal commissure (VHC), partial cutting of the corpus callosum (CC) and adjacent cortex (CTX) does not affect spatial learning and cognitive coordination in active place avoidance (Room+/Arena-) task. In this task rats on a continuously rotating arena (1 rpm) must avoid an unmarked foot-shock zone. Avoidance of the to-be-avoided place demanded segregation of relevant distal Room+ cues from irrelevant proximal cues from Arena–. Leaving out of misleading information and use of relevant one is hippocampal dependent and is named cognitive coordination. We hypothesized that the remaining intact CC fibers allowed effective transmission of information between both hemispheres that supported cognitive coordination. To verify this hypothesis ten rats were trained in the Room+/Arena– task after total corpus callosum (TCC) incision. The rats learned place avoidance which was measured by a number of entries to the shock zone (E) and time to the first entry (T1). We found that number of E in TCC rats decreased in consecutive days of training likewise VHC, CC and CTX rats ($F_{4,164}$=59.6; P<1.0–10) and T1 increased ($F_{4,164}$=21.6; P<2.7–14). We conclude that either separation of hippocampi by VHC transection or separation of hemispheres by total corpus callosum transection preserves the cognitive coordination requiring segregation relevant distal room cues from irrelevant proximal arena cues in rats.

P4.02 Investigations of spatial task performance strategies in case of brain local lesions in rats

Beselia G.

I Beritashvili Institute of Physiology, Tbilisi, Georgia

We assessed the effects of electrolytic lesions of nucleus accumbens and medial septal nuclei on spatial learning and memory in the Morris Water maze on the basis of the investigation of learning and performance of defensive motivation-based spatial task. In these task, both groups of animals reached the criterion in similar time – approximately in 7–9 days, although the significant difference reveal in probe trials. The escape latency from novel start position in medial septal lesioned group significantly exceeded the results of same group in basic trials and the results obtained in same type probe trials of rats of second groups. In the landmark-poor environment the escape latency in nucleus accumbens lesioned group exceeded the results of same group in basic trials and the results obtained in same type probe trials of rats of second groups. Getting results give us ground to consider nucleus accumbens in that functional system, which participate in motor response learning processes and it is one of the functional unit of neuroanatomical substrate of nondeclarative memory. On the second hand medial septal nuclei must participate in place learning processes and assumptionally it is one of the links of neuroanatomical substrate of declarative memory.

P4.03 The effects of baclofen, LY367385 and MPEP on spatial memory and the activity of MMP-2 and MMP-9

Car H.[1], Michaluk P.[2], Wisniewska R.J.[1], Kaczmarek L.[2]

[1]Department of Pharmacology, Medical University of Bialystok, Poland, [2]Laboratory of Molecular Neurobiology, Department of Molecular and Cellular Neurobiology, Nencki Institute of Experimental Biology PAS, Warsaw, Poland

The balance between the GABAergic and glutamatergic systems in the hippocampus is one of the most important factors in learning and memory processes. The aim of the present study was to determine the effects of joint administration of baclofen, agonist of GABAB receptors and LY367385, antagonist of mGluR1a or MPEP, antagonist of mGluR5 on spatial memory in the water maze, and on the activity of MMP-2 and MMP-9 in the hippocampus of rats. We observed that LY367385 impaired acquisition in water maze, while MPEP and baclofen did not exert any influence on spatial learning. Interestingly, LY367385 and MPEP changed the activity MMP-2 and MMP-9 in the hippocampus but baclofen did not. Administered together with LY367385, baclofen prolonged the latency escape in the first session, but it did not influence acquisition in the water maze when administered with MPEP. Joint administration of baclofen with antagonists of mGluRs did not change reference memory in the Morris maze. Gel zymography showed elevations of MMP-2 and MMP-9 activity in the hippocampus after administration of baclofen with LY367385 or with MPEP. Concluding, the activity of MMP-2 and MMP-9 in the hippocampus does not correspond with the influence given by baclofen combined with LY367385, or with MPEP, on acquisition in the water maze.

P4.04 Role of perirhinal cortex in the short-term auditory recognition memory in rat

Jakubowska-Dogru E.[1], Wesierska M.[2], Elibol B.[1], Guven S.[1]

[1]Middle-East Technical University, Ankara, Turkey; [2]Nencki Institute of Experimental Biology PAS, Warsaw, Poland

It has been postulated that perirhinal cortex is involved in higher order processing of polymodal sensory information and is important for the familiarity discrimination aspect of recognition memory. However, the recent dog and monkey data indicate that damage to the perirhinal cortex do not affect auditory recognition memory what may suggest that auditory memory is organized differently from the memory in other modalities. The aim of the present study was to examine the effects of bilateral neurotoxic lesions of perirhinal cortex on the auditory recognition memory in rat. Before the sound recognition training, an object recognition task was carried out as a behavioral test of lesion location. Visual recognition was tested in the delayed nonmatching to sample task with trial unique junk objects and variable delays (10 s and 15 min). Sound recognition was tested in a runway according to the matching to sample procedure with short, 3 s, delay between trial unique sample and test stimuli. In the present study, lesions within the medial temporal lobe destroying perirhinal cortex had no effect on simple discrimination of visual stimuli but disturbed short-term object recognition memory at longer (15 min) delay. Lesioned animals did not show impairment in the sound recognition task with trial – unique auditory stimuli at the tested short delay. However, overall response latencies (both on positive and negative trials) were significantly longer in Lesion than in Control Group.

P4.05 Impairment in spatial but not social learning of APP-V7171 mice

Kiryk A., Wawrzyniak M., Lioudyno V., Kaczmarek L.

Nencki Institute of Experimental Biology, Warsaw, Poland

Social learning is well known adaptive behaviour allowing an animal to base its response on the experience of others, and avoid risk connected with individual learning. We have examined the learning of APP-V717I transgenic mice of different ages: 5, 12 and 18 months. All the transgenic mice showed memory impairment, either in spatial tasks for an individual mouse (Morris Water Maze), or for the whole group (IntelliCage). In IntelliCage tests, the mice were supposed to choose one correct corner out of the 4 available (the same corner for all the mice) where they had access to sugar water (place preference task), and 5 days later to relearn to avoid this corner because of getting a punishment there (place avoidance task). Surprisingly, when mixed with their non-transgenic siblings, APP-V717I transgenic mice were able to fulfill the same tasks in the IntelliCage. The ability to learn from others is an important adaptation that allowed APP-V717I transgenic mice to acquire, at low cost, information concerning reward and avoidance responses to an aversive stimulus. It seems that this type of learning remains unaffected in the genesis of Alzheimer's disease.

P4.06 c-Fos activation in mouse auditory cortex in response to acoustic stimuli

Lioudyno V., Nikolaev E., Kiryk A., Kaczmarek L.

Nencki Institute of Experimental Biology PAS, Warsaw, Poland

In the present study we have used the expression of the c-Fos protein as a marker of neuronal activation in the cortical fields of mice. Two experiments were carried out. The first experiment involved classical cue conditioning. The aim was to differentiate the neurons activated by sound alone from those activated by sound followed by a foot-shock. The separate group of mice was exposed to tone during five consequent days. We observed the c-Fos activation in visual, auditory and limbic parts of cortex in all experimental groups when compared to the home cage controls. The strongest activation in response to tone alone was observed in the ventral part of secondary auditory fields. The second experiment was performed to check the hypothesis that appearance of novel frequency in auditory signal can be recognized by mouse auditory system and it would induce the different pattern of c-Fos activation in auditory fields. Two groups of mice were exposed to "standard" sound during 5 days, once a day, but on the 5th day the animals from one group were treated also with the sound of higher frequency ("deviant"). Our preliminary results suggest that new frequency could be recognized by mouse auditory system and changing of c-Fos activation in cortical auditory fields could reflect this reaction to novelty.

P4.07 Epileptogenesis and fear conditioning-related genes in the basolateral amygdala of rat

Majak K.[1,2], Dabrowski M.[3], Pitkanen A.[1]

[1]A.I. Virtanen Institute for Molecular Sciences, University of Kuopio, Finland; [2]Department of Anatomy and Neurobiology, Medical University of Gdansk; [3]Laboratory of Transcription Regulation, Nencki Institute PAS, Warsaw, Poland

During development of temporal lobe epilepsy the basal and lateral nuclei of the amygdala (BLA) are severely affected.

These nuclei are also critically involved in fear conditioning. We aimed at revealing differences in gene expression pattern in rat BLA during emotional learning and epileptogenesis. Electrical stimulation of the lateral nucleus induced epileptogenesis. Then rats received training session composed of two presentations of either paired or unpaired tone and footshock and were sacrificed 2 h later. BLA were microdissected using Leica AS LMD Laser System, isolated RNA was amplified and hybridized to GeneChip® Rat Genome 230 2.0 Array (Affymetrix). Statistical analysis revealed a large set of candidate "true positive" epileptogenesis-related gene. Semi-quantitative RT-PCR confirmed the array result for selected genes. Epileptogenesis also induced significant changes in gene expression within a subset of GO terms (groups of functionally linked genes). We also observed change in expression of alpha-2-macroglobulin that was specific for the fear-conditioning in control rats. We could not demonstrate a statistical significance of this change after correction for the multiplicity of testing using the microarray data alone, but it was confirmed by semi-quantitative RT-PCR. The analysis highlights the differences in molecular processing of physiological and pathological conditions within the BLA in rat.

P4.08 Passive avoidance and flexibility impairment in a rat model of hepatic encephalopathy

Mendez M.[1], Mendez-Lopez M.[1], Lopez L.[1], Aller M.A.[2], Arias J.[2], Arias J.L.[1]

[1]Laboratory of Psychobiology, School of Psychology, University of Oviedo; [2]Surgery Chair, School of Medicine, Complutense University of Madrid, Spain

Hepatic encephalopathy (HE) is a CNS disorder associated with liver disease and portosystemic shunting. This serious disorder is characterized by a large variety of symptoms including cognitive deficit such a spatial disorientation, memory and attention impairment. There are several animal models to study HE and portacaval anastomosis (PCA) model is a specific model in type B HE. Our objective is to evaluate passive avoidance behavior and behavioral flexibility using a reversal task in the Morris Water Maze (MWM) in a rat PCA model of hepatic dysfunction. There are not differences between groups in anxiety and activity tasks. 6 PCA and 6 sham-operated controls SHAM were tested in a shuttle box under a passive avoidance paradigm. On session one subjects received a mild shock when crossing from the illuminated chamber to the dark compartment. After a 24 hours delay, animals were introduced again in the maze and latency to crossing to the dark chamber was measured. Only SHAM remembered the shock ($F_{1,10}$=13.201, P=0.005). 8 PCA and 6 (SHAM) were used. The spatial reference memory task shows no differences between groups in escape latencies ($F_{4,41}$=1.469, P=0.229). No-platform probe test shows that PCA and SHAM have learned the location of platform. PCA group is unable to learn the new location of platform in reversal test. This specific model of type B HE produces memory and flexibility impairment. This work was supported by grant MEC SEJ2004-07445, Spain.

P4.09 Transfer of information between both hippocampi in a passive avoidance task

Mendez-Lopez M.[1], Cimadevilla J.M.[2], Mendez M.[1], Arias J.L.[1]

[1]Laboratory of Psychobiology, School of Psychology, University of Oviedo; [2]Dept. of Neuroscience, University of Almeria, Almeria, Spain

The hippocampus is a critical structure for memory. Hippocampal unilateral blockage impairs acquisition, consolidation and retrieval of memories. It was shown that the information of one hippocampus can be transferred to the other. This was not tested in a passive avoidance task. Wistar rats were tested in a Shuttle box passive avoidance paradigm. On acquisition rats received a shock when crossing to the dark compartment. After a 24 h delay, rats were introduced again in the maze (reexposed 1 min) and 15 min later retested. Tetrodotoxin was applied unilaterally. Group 1 received TTX in the right hippocampus (RH) in acquisition and the same treatment in retrieval. Group 2 received TTX into the RH in acquisition and TTX into the left hippocampus in retrieval. Group 3 received the same treatment as group 2 except that during the reexposition to the context both hippocampi were intact. Groups 1 and 2 were injected with TTX before the reexposition to the context. Results showed that animals that acquired the task with one hippocampus can perform correctly if the trained hippocampus is available. When the trained hippocampus is blocked, subjects are impaired in the task. When the rats were reexposed to the context with both hippocampi intact, unilateral blockade of the trained hippocampus has not effect on retrieval. Results suggest a kind of transfer of information between hippocampi, a fact previously showed in other tasks. Supported by SEJ2005-05067/PSIC and SEJ2004-07445 MEC Spain.

P4.10 Retrosplenial cortex (RS) participates in segregation of spatial information in the rat

Wesierska M., Adamska I., Malinowska M.

Nencki Institute of Experimental Biology PAS, Warsaw, Poland

The hippocampus is crucial for spatial place avoidance task that requires selective use of relevant but not irrelevant information. However, the role of the RS, involved in hippocampal circuitry, in coordinating the use of relevant and irrelevant information is not clear. The RS lesioned and control (C) rats were trained in place avoidance task. We found that RS and C rats avoided shock zone in (Room&Arena)+ and then in A+ task. In the (R&A)+ task the arena was stable and the room was lit so the shock zone could be identified by distal Room (R) and local Arena (A) cues. In the A+ task the arena rotates and darkness hides room cues leaving only rotating arena cues to guide avoidance of a rotating part of the arena. However, the RS rats were impaired in avoidance in the (R+A–) task, in which the arena rotated in lit room so only distal cues define the shock zone and rotating cues must have be ignored. The problem is if the RS lesion impaired avoidance because arena cues are not available or there is a conflict between (R+A–) cues. In present study the RS and C Long-Evans rats were trained in (R&A)+ and then in R+ task. In the R+ task the room was lit, the rotating arena was covered by water so distal cues defined the shock zone. RS rats learned (R&A)+ place avoidance, measured by reduction of entering the shock zone in following days of training ($F_{5,70}$=8.4; P<1.0–4) and transferred avoidance to the R+ task ($F_{5,70}$=12.4; P<1.0–5). We concluded that the retrosplenial cortex participates in segregation of relevant and irrelevant spatial cues for navigation.

P4.11 The influence of t-ADA on behavioral activity of rats with experimental chronic hyperammonemia

Wisniewska R.J., Bycul I., Car H.

Department of Pharmacology, Medical University of Bialystok, Poland

The exact role of group I metabotropic glutamate receptors (mGluRs) in causing hyperammonemia injury is not clear. The influence of t-ADA [trans-azetidine-2,4-dicarboxylic acid], an agonist of mGluR1/5, on behavioral activity of rat control group and with experimental chronic hyperammonemia (chHA) in the open field and the passive avoidance tests was assessed. Experimental chHA was induced by intraperitoneal injection of ammonium acetate (12 mmol/kg) for five consecutive days. The blood ammonia level in rats treated with ammonium acetate was 417.56 mmol/l, in the control rats it was 75.04 mmol/l. The chHA significantly decreased the number of crossings, rearings and bar approaches in the open field test, and impaired acquisition, consolidation and retrieval in the passive avoidance situation. In rats without hyperammonemia, t-ADA (100 nmol i.c.v.) did not influence locomotor and exploratory activity in the open field test but it markedly improved consolidation in the passive avoidance situation. In rats with chHA, t-ADA significantly improved locomotor activity in the open field test, improved acquisition and consolidation processes, but it did not affect retrieval in the passive avoidance test. The obtained results suggest that t-ADA, the agonist of group I mGluRs, had beneficial effects on acquisition and consolidation in the passive avoidance test and on locomotor activity in rats with chronic hyperammonemia. This compound presents a possible protective activity against chronic hyperammonemia induced in rats.

P4.12 Influence of dopaminergic system injury on working memory abilities

Zaremba M.M.[1], Kurkowska-Jastrzebska I.[2], Joniec I.[1], Piechal A.[1], Blecharz-Klin K.[1], Balkowiec-Iskra E.Z.[1], Czlonkowski A.[1]

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

Changes of neurotransmitters level in the central nervous system is associated with cognitive impairment observed during various neurodegenerative disorders, such as Parkinson's disease. It has been shown that dopamine may influence the proper functioning of hippocampus and frontal cortex, which are critically involved in spatial learning and memory processes. In the present study the influence of dopaminergic neurons injury, provoked by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) administration on memory impairment has been studied. Four months old C57/BL/10Tar male mice, received MPTP 40 mg/kg or 80 mg/kg, and control group received 0.9 % NaCl. To evaluate learning and memory abilities, the Morris water maze behavioral test was twice provided when the dopamine level was the lowest. We noticed the differences in the mean latency of reaching the platform, the swimming distance, the speed of swimming and the time spent in the goal quadrant Southeast between MPTP and the control animals indicated impaired spatial memory in animals with injured dopaminergic system. More investigation are necessary to check if the observed memory deficits are caused by interactions between neurotransmitters or by neurodegenerative changes in distant structures of the brain induced by nigrostriatal degeneration.

P4.13 Nuclear localization of matrix metalloproteinases in neurons after stroke in the rat cortex

Aleksy M., Rogozinska K., Skangiel-Kramska J.

Nencki Institute of Experimental Biology PAS, Warsaw, Poland

Metalloproteinases (MMPs) are known for their role in extracellular matrix remodelling in physiological and pathological conditions, including ischemia. The aim of this study was to examine the expression and activity of MMP-2 and MMP-9 and their natural tissue inhibitors (TIMPs) in the rat brain after focal cortical stroke. For this purpose unilateral phototrombotic stroke was performed in the primary somatosensory cortex in the rat using bengal rose. MMPs and TIMPs were studied over the time course ranging from 1 hour to 4 days after infarct. *In situ* zymography revealed that as early as 1 hour after stroke there was gelatinotitic activity in the infarct core. MMPs activity colocalized with fluorescences for NeuN and DAPI suggesting their neuronal and nuclear localization. Such expression in neurons was still observed 4 and 24 h after stroke. Four days after ischemia the MMPs activity was found only in debris and blood vessels, but not in astrocytes at the rim of infarct cavity. Gel zymography and Western blot analysis showed that gelatinolitic activity was exclusively due to MMP-9. Extracts of nuclear fraction prepared from lesioned tissue revealed the presence of MMP-9 proenzyme dimer form. This form of a MMP-9 was detected at 4 and 24 hours after stroke. Preliminary results showed that TIMP-1 was not present in nuclear extract. In non-nuclear fractions at all investigated time points the active monomeric form of MMP-9 was detected. These results indicate the possible role of MMP-9 in degradation of neuronal nuclear proteins after stroke.

P4.14 Effect of stroke on experience dependent plasticity in the intact hemisphere

Jablonka J., Kossut M., Nosecka E.

Nencki Institute of Experimental Biology PAS, Warsaw, Poland

Reciprocal interactions between cerebral hemispheres are important for recovery after stroke. Inhibition of one hemisphere by the other can influence its excitability and capacity for plastic changes. Experimental data on the effect of interhemispheric interactions upon cortical plasticity is conflicting. We addressed this problem in rat model of focal cortical stroke, using sensory deprivation of vibrissae to induce the plasticity. In rats, a month of trimming all whiskers except one row, results in expansion of cortical representation of the spared row, as visualized by [14C]2-deoxyglucose (2DG) brain mapping. If a focal stroke is placed behind the barrel field, it impairs this plastic change in the same hemisphere (Jablonka et al. 2007). We investigated the effects of stroke situated close to the barrel field and stroke destroying the barrel field upon the deprivation-induced plastic changes of whiskers representation in the opposite, intact hemisphere. Stroke was induced by phothotrombosis. Whiskers deprivation sparing row B started on the day of stroke and lasted for one month. During 2DG brain mapping bilateral stimulation of rows B whiskers was applied. The width of 2DG labelling of cortical representation of row B whiskers was measured on autoradiograms. Stroke outside of the barrel field did not affect deprivation – induced plasticity in the opposite hemisphere. Stroke in the barrel field decreased the plastic change in the contralateral barrel field by 15%. The results support data by Rema (2003) showing negative influence of stroke on experience dependent plasticity in the intact hemisphere.

P4.15 Whisker stimulation leads to the formation of excitatory synapses on spines of the barrel field

Jasinska M.[1], Siucinska E.[2], Glazewski S.[3], Pyza E.[1], Kossut M.[2]

[1]Department of Cytology and Histology, Institute of Zoology, Jagiellonian University, Krakow; [2]Nencki Institute of Experimental Biology, Warsaw, Poland; [3]Keele University, Staffordshire, UK

We have previously reported that short-lasting classical conditioning involving stimulation of a row of whiskers (conditioning stimulus – CS) paired with mild electric shock (unconditioned stimulus – UCS) to the tail (Siucinska and Kossut 1996) results in the formation of new symmetric (GABA-ergic) synapses on spines of the cognate barrel hollows. The formation of new inhibitory synapses was found to be correlated with the increase in the density of spines containing one excitatory and one inhibitory synapse (double-synapse spines) and with no change in the density of the single-synapse spines. In the present study, we found that the density of asymmetric (excitatory) synaptic profiles in response to short-lasting CS application (three daily sessions lasting 10 min each, 40 applications of CS consisting of 3 slow strokes) was significantly enhanced, while CS+UCS treatment produced only a small increase. Moreover, CS but not CS+UCS resulted in up-regulation of single-synapse spines with no changes in the density of synapses on dendritic shafts. This strongly suggests that the increase in the density of excitatory synapses after CS is due to the formation of new excitatory synapses on spines. Taken together, the data suggests that the CS-UCS leads to the formation of the double-synapse spines by selective addition of inhibitory synapses to the pre-existing single-excitatory synapse spines.

P4.16 Neuronal activity-driven synaptic localization of matrix metalloproteinase-9 mRNA

Konopacki F.A.[1,2], Rylski M.[1], Wilczek E.[3], Amborska R.[1], Detka D.[1], Kaczmarek L.[1]

[1]The Nencki Institute; [2]School of Molecular Medicine; [3]Medical University of Warsaw, Warsaw, Poland

The phenomenon of dendritic transport and local translation of mRNA is considered to be one of the most fundamental mechanisms underlying long-term synaptic plasticity. Matrix metalloproteinase 9 (MMP-9) is implicated in synaptic long-term potentiation and hippocampus-dependent memory. It was recently shown to be prominently upregulated in the rat hippocampal dentate gyrus (DG) upon kainate-mediated neuronal hyperexcitation. Here, using a high resolution nonradioactive in situ hybridization at the light- and electron-microscopic levels, as well as subcellular fractionation followed by reverse-transcription PCR, we provide evidence that in the rat hippocampus, MMP-9 mRNA is associated with dendrites and dendritic spines bearing asymmetric (excitatory) synapses. The number of MMP-9 mRNA-positive dendritic and synaptic compartments increases dramatically after kainate seizures, suggesting its activity driven dendritic transport. Our work links the phenomenon of dendritic translocation with a proteolytic enzyme, thus pointing at a novel mechanism regulating synaptic proteolysis. It also provides further support to the concept of dendritic mRNA translocation being a key mechanism of long-term synaptic modifications.

P4.17 Are early immediate genes activated in learning-induced plasticity?

Radwanska A., Filipkowski R.K., Konopacki F.A., Wilczynski G.M., Zakrzewska R., Kossut M.

Nencki Institute of Experimental Biology PAS, Warsaw, Poland

In rodents inputs from whiskers terminate in the layer IV of the cortex forming distinct clusters of neurons, termed barrels. Every whisker is anatomically and functionally represented by a distinct barrel. Training mice in a paradigm of classical conditioning – in which stroking the row of whiskers with a brush (conditioned stimulus) is associated with an tail shock (unconditioned stimulus) – elicits broadening of the functional representation of the stimulated whiskers, as measured by uptake of radioactive 2-deoxyglucose. In order to gain insight into molecular processes underlying the observed learning-induced plastic change, expression of early immediate genes, reported to be involved in learning, will be analyzed. There are findings suggesting a relationship between c-fos expression and some forms of aversive learning in rats. Experiments with Arc knockout mice demonstrated that Arc plays a pivotal role in durable forms of learning. One of the aims of this study is to examine differences in number of cells expressing c-Fos protein in stimulated barrels in mice subjected to conditioning and tactile stimulation only. To analyze Arc activation catFISH (cellular compartment analysis of temporal activity by fluorescence in situ hybridization) will be used. This will enable visualization of neurons activated by two discrete experiences, tactile stimulation and conditioning, within the same animal. Number and distribution of neurons labeled in studies concerning c-fos and Arc expression will be compared.

P4.18 Beta-dystroglycan as a target for MMP-9, in response to enhanced neuronal activity

Michaluk P., Kolodziej L., Mioduszewska B., Wilczynski G.M., Dzwonek J., Kaczmarek L.

Nencki Institute of Experimental Biology PAS, Warsaw, Poland

Matrix Metalloproteinase-9 has recently emerged as an important molecule in control of extracellular proteolysis in the synaptic plasticity. However, no synaptic targets for its enzymatic activity had been identified before. In this report, we show that beta-dystroglycan comprises such a neuronal activity-driven target for Matrix Metalloproteinase-9. This notion is based on the following observations: (i) recombinant, autoactivating Matrix Metalloproteinase-9 produces limited proteolytic cleavage of beta-dystroglycan; (ii) in neuronal cultures beta-dystroglycan proteolysis occurs in response to stimulation with either glutamate or bicuculline and is blocked by Tissue Inhibitor of Metalloproteinases-1, a metalloproteinase inhibitor; (iii) beta-dystroglycan degradation is also observed in the hippocampus *in vivo* in response to seizures but not in the Matrix Metalloproteinase-9 knockout mice; (iv) beta-dystroglycan cleavage correlates in time with increased Matrix Metalloproteinase-9 activity and (v) beta-dystroglycan and Matrix Metalloproteinase-9 co-localize in postsynaptic elements in the hippocampus. In conclusion, our data identify the beta-dystroglycan as a first Matrix Metalloproteinase-9 substrate digested in response to enhanced synaptic activity. This demonstration may help to understand possible role of both proteins in neuronal functions, especially in synaptic plasticity, learning and memory.

P4.19 Rats with overexpression of MMP9 show impairment in long term memory

Wawrzyniak M., Kiryk A., Owczarek D., Lioudyno V., Meyza K., Konopacki F.A., Michaluk P., Majczynski H., Balcerzyk M., Kaczmarek L.

Nencki Institute of Experimental Biology PAS, Warsaw, Poland

Matrix metalloproteinases are a major group of enzymes regulating cell-matrix composition by using zinc for their proteolytic activities. They are essential for many biological processes. Matrix Metalloproteinase-9 (MMP9) has recently emerged as an important molecule in control of extracellular proteolysis in the synaptic plasticity. The authors have created rats with overexpression of MMP9 limited to the brain (hippocampus, cortex and cerebellum; MMP9-gene is under control of synapsin-1 promoter). Transgenic rats were indistinguishable from their wild type siblings with regard to general motor function (Foot Printing, Rotarod and Open Field tests). The memory of rats was examined using a battery of behavioral tests. In Morris Water Maze transgenic rats acquired memory as well as wild type rats during training. However, tested 24 and 96 hours later, transgenic rats showed memory impairment measured as time (%) spent in the quarter of pool where the platform was located in training. Transgenic rats spent in target quadrant only 27% of time while wild type rats were looking for a platform in proper quadrant for more than 50% of swimming time. In Novel Object Recognition test, transgenic rats were able to recognize new object after 1 hour but not after 24 hours from last training. Electrophysiological test showed that rats with overexpression of MMP9 had a higher hippocampal LTP level than wild type rats. Our behavioral and electrophysiological results suggest that MMP9 may influence on a synaptic plasticity.

P4.20 Investigation of drosophila mushroom body physiology: New method and data

Tsydzik V., Wright N.J.

Eastern New Mexico University, Portales, NM, USA

The fruit fly *Drosophila melanogaster* has proved to be an excellent model system for studying many areas of biology. Fruit flies can exhibit olfactory associative learning, produces different phases of memory lasting from minutes to days. Olfactory associative learning in drosophila is one main physiological function of Mushroom Bodies (MBs). The new technique for preparing of fly before pharmacological and physiological manipulation of the MBs in the intact living adult flies was described earlier (2006, J Neurosci Methods, 155: 77-80). Intracellular level of calcium was quantitative measured by monitoring endogenous expression fluorescent calcium reporter camgaroo. We were obtained earlier an increase in intracellular calcium level after discrete application of acetylcholine (ACh) as possible equivalent of principal input from the antennal lobe. That effect was reversibly eliminated in calcium-free Ringers as well as blocked by the nicotinic antagonist d-tubocurarine. Dopamine (DA) also reversibly increased calcium level after adding in Ringer solution similar as serotonin, octopamine, histamine. Last year we were using iontophoretic application Ach and DA into MBs as more reliable and quantifiable. We are attempting to mimic a protocol that produces a memory by applying pulses of ACh for 1 min = antennal lobe input (application rate of 1 Hz) and DA every five second = electric shock input (application rate of 0.2 Hz). ACh does produce the big increase in calcium-level. DA-induced increasing was also measurable. Co-application Ach and DA under the above-stated conditions repeatable produce much larger response. Probably, our preliminary result does indicate about significant role of intracellular calcium in olfactory associative learning.

MOTOR SYSTEMS

P5.01 Calbindin-D28K is critical factor of precise motor coordination in cerebellar Purkinje cells

Barski J.[1,2], Hartmann J.[2], Rose Ch.[3], Hoebeek F[4]., Noll-Hussong M.[3], de Zeeuw Ch.I.[4], Meyer M.[3]

[1]Institute of Biotechnology and Environmental Sciences, University of Zielona Gora, Poland; [2]Max-Planck-Institute of Neurobiology, Martinsried, Germany; [3]Institute of Physiology, Ludwig-Maximilians-University of Munich, Germany; [4]Department of Neuroscience, Erasmus University Rotterdam, The Netherlands

Mice nullmutant for calbindin-D28k (Cb) are indistinguishable from wildtypes with respect to behavior in standard cage environments and brain histology. Specific tests of motor co-ordination and adaptation such as the elevated runway, however, revealed a significant impairment of nullmutant mice with concomitant alterations of calcium handling in Purkinje cells (Airaksinen et al. 19971). Since in the cerebellum, Cb is exclusively and abundantly expressed in Purkinje cells, we hypothesized that motor disturbance is due to the absence of Cb specifically in this cell population. To directly address the role of Purkinje cells and Cb for motor co-ordination we generated a Purkinje-cell specific conditional nullmutant using Cre/loxP methodology.

P5.02 Influence of location of single motor unit within a muscle on shape of a mechanomyographic signal

Celichowski J.[1], Kaczmarek P.[2], Drzymala H.[1], Kasinski A.[2]

[1]Department of Neurobiology, University School of Physical Education in Poznan; [2]Institute of Control and Information Engineering, Poznan University of Technology, Poland

The activity of isolated motor units in the rat medial gastrocnemius muscle was evoked by a stimulation of thin filaments of ventral roots. The motor unit force, action potentials and mechanomyograms (MMGs) were recorded. The MMG was recorded with a laser distance sensor. The profiles of MMG signals were categorized into three classes. Class P (positive) comprised motor units with the MMG recordings similar to force recording profiles, i.e., when the distance between the muscle surface and the laser sensor increased during the contraction. Class N (negative) comprised motor units with the MMG recordings inverted in comparison to force recording, i.e., when the distance decreased during the contraction. The third class M (mixed) is formed by motor units with MMG profiles first increasing in parallel with the beginning of the twitch force increase, but then decreasing to negative values when the twitch force reached the peak. The semi-pennate muscle model enabling estimation of the MMG generated by a single motor unit in relation

to localization of its muscle fibers has been proposed. The analysis have shown that the localization of motor unit in relation to the laser sensor explains variability of observed MMG profiles. Concluding, the MMG signal profiles provide the information concerning an architecture of motor unit in pennate skeletal muscles.

P5.03 Differences between motor unit properties in distal and proximal muscles of rat hind limb

Ciechanowicz I., Celichowski J.

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

The study concerned differences between motor unit (MU) properties in distal (flexor digitorum brevis, FDB) and proximal (medial gastrocnemius, MG) muscles of Wistar rats. Contractile parameters: the force of twitch and tetanus, the contraction and the half-relaxation times (CT and HRT), the fatigue index, the relation between force and stimulation frequency were analysed. Activity of studied motor units was evoked by the stimulation of filaments of the L5–L6 and L4–L5 ventral roots for FDB and MG, respectively. The mean muscle mass amounted to 83 mg for FDB and 895 mg for MG. In the MG muscle three types of MUs were found and sag was observed in all fast units in 40 Hz unfused tetani. In FDB, only FF and FR MUs were found and sag was absent. In the MG muscle the fatigue index had bimodal distribution, whereas in FDB the distribution was continuous. The most prominent differences were observed in MUs force and twitch time parameters. The twitch and tetanus forces were significantly lower in FBD, whereas CT was significantly longer. Moreover, differences between shapes of MU twitch recordings were found. These differences were expressed by a CT/HRT ratio which was lower for MG motor units. The steep parts of force-frequency curves of fast motor units in FDB were shifted towards lower frequencies in relation to MUs of MG. In conclusion, the differences between MU properties in FDB and MG concern all studied contractile parameters and likely reflect different motoneuronal firing properties.

P5.04 Nonlinear summation of motor unit forces in rat medial gastrocnemius muscle

Drzymala H., Celichowski J., Krutki P.

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

The mechanism of the muscle force regulation is controlled by the central nervous system in two ways: by the motoneuronal firing rate and the recruitment of motor units. The purpose of this study was to examine summation of forces of two or four individual motor units and four groups of units in the medial gastrocnemius muscle during parallel stimulation of motor axons. The experiments were performed on Wistar rats under pentobarbital anesthesia. Activity of groups of units was achieved by division of L5 ventral root into four bundles of axons. The effects of summation were presented as differences between the recorded force and the algebraic sum of individual forces. For pairs of motor units, variable effects of summation were observed, more-than-linear or less-than-linear. For four motor units or groups of units, less-than-linear summation was noted. The more units were simultaneously active, the smaller effects of summation were observed. Moreover, the force summation of unfused tetani was more effective than summation of single twitches or fused tetani. In conclusion, forces produced by motor units during simultaneous stimulation summate nonlinearly. This observation implies that motor units work less effectively during voluntary activity than during their isolated contractions.

P5.05 Force generated by fast motor units during stimulation with pulses at variable intervals

Krutki P.[1], Pogrzebna M.[1], Drzymala H.[1], Raikova R.[2], Celichowski J.[1]

[1]Department of Neurobiology, University School of Physical Education, Poznan, Poland; [2]Centre of Biomedical Engineering, Bulgarian Academy of Sciences, Sofia, Bulgaria

The aim of the study was to simulate natural conditions of motor unit contractions (MU) during voluntary activity. Experiments were performed on functionally isolated fast MUs in the rat medial gastrocnemius (MG) muscle. 5 different patterns of regular frequencies were compared to 5 random patterns of irregular stimulation with the same mean values of interpulse intervals, between 10 and 75 ms, and variability of these intervals of ± 50% in each case. These values cover natural range of the preferred firing rates of MG motoneurones from. Analysis of changes in the tetanic force indicated the linear relationship between the interpulse interval as well as the initial level of the force and the amplitude of the force increaseof the following contraction. It was demonstrated that variability of the instantaneous tetanic force during irregular discharge pattern depends on a level of tetnic fusion. Moreover, it was demonstrated that for low and moderately-fused tetani, effectiveness of a MU contraction (expressed as the force-time area) is considerably higher for contractions evoked by irregular stimulation patterns. Conclusions: (1) during voluntary contractions, the influence of changes in the motoneuronal firing rate on the MU force depends on the initial level of force; (2) irregular stimulation pattern produces higher output of MUs than regular stimulation pattern during tetanic contractions used for motor tasks requiring low or moderate level of force.

P5.06 Division of motor units into fast and slow on the basis of profile of 20 Hz unfused tetanus

Krysciak K.[1], Krutki P.[1], Celichowski J.[1], Slawinska U.[2], Majczynski H.[2]

[1]Department of Neurobiology, University School of Physical Education, Poznan; [2]Nencki Institute of Experimental Biology PAS, Warsaw, Poland

In the medial gastrocnemius muscle of intact rats, division of motor units into slow or fast type is typically based on presence of a sag phenomenon in 40 Hz unfused tetanic contraction. Motor units with sag are classified as fast (F) while those without sag as slow ones (S). However, it has been observed that in rats after spinal cord injury this phenomenon almost totally disappears and cannot be used as a basis for differentiation. Moreover, in intact animals, ranges of contraction times for F and S motor usually do not overlap, but in spinal animals considerable overlapping of these values has occurred. Therefore, the aim of this study was to propose a method for division of

motor unit into types that might be applied for contractions with absence of sag. It was observed after spinal cord injury that characteristic shape of 20 Hz tetanus of S motor units remained unchanged and could be reflected as a ratio of the force of the last contraction within this tetanus to the force of the first contraction. This ratio for S motor units in intact rats was comprised in a range 2.3–8.5, whereas for F units between 0.8 and 1.5. Similar results were obtained in groups of animals after the total spinal cord transection and spinal cord hemisection. We conclude, that this 20 Hz index appears to be an alternative method for division of motor units into S and F types.

P5.07 Interhemispheric relations in the motor function of the subthalamic region

Ledochowski P., Grembecka B., Jerzemowska G., Trojniar W.

Department of Animal Physiology, University of Gdansk, Poland

The subthalamic region (STR), the subthalamic nucleus (STN) in particular, is part of the basal ganglia motor circuitry, and its overactivity is suppose to be the cause of main symptoms of Parkinson's disease. Both stimulation and lesion of STN alleviate parkinsonian symptoms, although mechanism of these effects is unknown. In the present work we studied the effects of unilateral electrical stimulation (current intensity: 80–260 μA, impulse width: 1 ms, frequency: 50 or 130 Hz) and contralateral electrolytic lesion (1 mA, 15 s) of STR in healthy rats. It was found that STN stimulation caused contralateral forelimb dyskinesias or rotations. High frequency current (130 Hz) induced less pronounced dyskinetic responses then the low frequency (50 Hz) one. Contralateral STR ablation involving the zona incerta resulted in a decrease of these dyskinesias (manifesting as a decrease of response duration under electrical stimulation) while lesions restricted only to the STN caused an increase or had no effect. The results obtained indicate that the zona incerta area is involved in the regulation of motor function of the subthalamic nucleus and that it concerns both hemispheres.

P5.08 Tetanic depression of fast motor units during gradually increasing frequency of stimulation

Lochynski D.[1,2], Celichowski J.[1]

[1]Department of Neurobiology; [2]Department of Kinesiotherapy, University School of Physical Education, Poznan, Poland

During isometric voluntary contractions motor units modulate discharge rates whenever increase or decrease of muscle force is required. The present study aimed to describe the differences in the force development during tetanic contractions of fast motor units evoked at constant versus gradually, ramp increasing (20–150 Hz) and decreasing (150–20 Hz) frequencies of stimulation. We compared the tetanic force obtained at constant 60 Hz frequency of stimulation and the force obtained at the same instantaneous stimulation frequency during ramp increasing and decreasing contractions. In FF and FR motor units the mean values of force measured during constant frequency contraction were 85.8 ± 39.7 and 45.1 ± 17.6 mN, respectively. We found that the mean values of force obtained during ramp increasing contraction were decreased (tetanic depression) by 12.8% (74.8 ± 39.8 mN, $P<0.01$, Student's t-test) and 7.7 % (41.6 ± 19.0 mN, $P<0.05$), in FF and FR units, respectively, in comparison to constant frequency contraction. On the other hand the mean values of force obtained during ramp decreasing contraction were increased (catch effect) by 10.8% (96.2 ± 44.3 mN, $P<0.05$) and 13.4% (52.1 ± 21.4 mN, $P<0.05$) in FF and FR units, respectively, in comparison to constant frequency contraction. At voluntary contractions, the tetanic depression may be important physiological phenomenon which limits the development of force during increase of motoneuronal firing rates.

P5.09 Changes in afterhyperpolarization after double stimuli in rat motoneurones

Mrowczynski W., Krutki P., Celichowski J.

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

The influence of a pair of stimuli running in time sequence between 5–10 ms (a doublet) on the basic parameters of antidromic action potentials was studied in rat motoneurones. Electrophysiological experiments were based on stimulation of axons in the sciatic nerve and intracellular recording of antidromic action potentials from individual motoneurones located in L4–L5 segments of the spinal cord. The following parameters were analyzed after application of the single stimulus and the doublet: amplitude and duration of the antidromic spike, amplitude, total duration, time to peak, half-decay time of the afterhyperpolarization (AHP). It was demonstrated that application of a pair of stimuli resulted in: (1) a prolongation of action potentials, (2) a prolongation of the total duration and half-decay time of the AHP, (3) a decline of the time to peak of the AHP, d) an increase of the AHP amplitude. Significant differences in AHP parameters were found either in fast or slow motoneurones. We suppose that doublet-evoked changes in the AHP amplitude and duration are linked to intrinsic properties of individual motoneurones and may lead to the prolongation of the time interval to subsequent motoneuronal discharges during voluntary activity.

P5.10 The successive contractions subtracted from unfused tetanus of fast and slow motor units

Pogrzebna M.[1], Raikova R.[2], Drzymala H.[1], Celichowski J.[1], Aladjov H.[2]

[1]Department of Neurobiology, University School of Physical Education, Poznan, Poland; [2]Centre of Biomedical Engineering, Bulgarian Academy of Sciences, Sofia, Bulgaria

The course of successive contractions subtracted from motor unit unfused tetanus was studied. For this purpose, fast and slow motor units of rat medial gastrocnemius muscle were stimulated with progressively increasing number of impulses, from one (i=1) to sixteen (i=16) at the frequency evoking well fused tetani, i.e. 15 Hz for slow motor units and 60 Hz for fast motor units. The individual contractions were calculated by subtracting the (i)-th tetanus recording from the (i+1)-th one. The contractions obtained following subtraction were modeled using a 6-parameter analytical function. The successive contractions within the tetanus are considerably different for slow and fast motor units.

The force and time parameters of subtracted contractions change in a different manner for these two motor unit types. For slow motor units, the maximal forces and the time parameters increase considerably to the 4th contraction, after which they remain nearly constant or show only a slight increase. For fast motor units, the maximal forces and the total durations also increase, whereas the remaining time parameters initially increase and then maintain a constant level or decrease, which explains the sag phenomenon visible in unfused tetani of fast motor units. The study indicated that although the successive motoneuronal action potentials are of "all-or-none" type, the following contractions within the tetanus are variable.

P5.11 Influence of repetitive transcranial magnetic stimulation on motor function in Parkinson's disease

Rakowicz M., Antczak J., Derejko M., Rola R., Inglot E., Tarnecka B.

Institute of Psychiatry and Neurology, Warsaw, Poland

The motor cortical hyperexcitability found in Parkinson's disease (PD) results in increased motor evoked potential (MEP) and shortened central silent period (CSP). This condition leads to motor dysfunctions in PD. We investigated the influence of high-frequency repetitive transcranial magnetic stimulation (rTMS) on motor signs, MEP and CSP in PD patients. Ten PD patients in mean age: 59.8 ±10.2 years; disease duration: 45.0 ± 37.4 months and Hoehn&Yahr stage: 1.7 ± 0.8 were treated with 10 sessions of rTMS of the left and right primary motor cortex. The stimulation was performed at 5Hz frequency, 3 000 pulses per session, 120% of motor cortex threshold (MT) intensity of the abductor digiti minimi muscle (ADM). The MEP and CSP were recorded from ADM after a single magnetic stimulus at 125% of MT intensity. The clinical effect of rTMS was evaluated using the Unified Parkinson's Disease Rating Scale (UPDRS). Results obtained during the 10th session revealed no significant changes in the cortical excitability compared to the first session. The MEP amplitude from the ADM was 0.77 ± 0.5 mV vs. 0.87 ± 0.6, and the CSP duration was 69.5 ± 32.6 ms vs. 73.7 ± 28.1. However, the UPDRS improved significantly (P<0.04), from 25.9 ± 15.9 points in the first session to 17.9 ± 7.5 points in the 10th session. The reduced intensity of the resting tremor, bradykinesia, postural and gait disturbances were observed. The modulation of motor cortical excitability may be responsible for the better motor performance of the PD patients treated with rTMS.

NEUROBIOLOGY OF GLIA

P6.01 STAT oligodeoxynucleotide decoys efficiently modulate gene expression in glial cells

Adach A., Kaminska B., Dabrowski M.

Nencki Institute of Experimental Biology PAS, Warsaw, Poland

JAK/STAT pathway transduces signals initiated by several growth factors and cytokines. STAT transcritpion factors regulate gene expression and mediate many processes, including cell proliferation, survival, apoptosis and differentiation. The expression of target genes may be modulated by synthetic, double-stranded oligodeoxynucleotide (ODN) decoys with a high affinity consensus binding sequence for transcription factors. When introduced into cells, ODN decoys bind endogenous transcription factors and block activation of target genes. The effectiveness of this strategy was tested in C6 glioma cells, where ODN decoy against STAT were co-transfected with a plasmid carrying luciferase reporter gene under the control of STAT-dependent promoter. ODN decoys were tested at various concentrations to confirm that the effect of the decoy was due to sequence-specific inhibition of transcription factor rather than a non-specific effect. ODN decoys with the mutated STAT motifs were used as a control for specificity. Further, we determined the decoy effects on endogenous gene expression. The evaluation of target genes expression was performed using real-time quantitative RT-PCR. We observed that ODN decoy against STAT, but not mutated ODN, was able to reduce the expression of several STAT-dependent genes. Our results suggest that STAT oligodeoxynucleotide decoys efficiently and selectively modulate expression of several STAT-dependent genes, indicating a considerable potential of such approach in modulating gene expression.

P6.02 Ammonia-induced alteration in S100B secretion in astrocytes is not reverted by creatine addition

Brolese G., Leite M., Almeida L., Goncalves C.A.

Universidade Federal do Rio Grande do Sul, Brasil

Hyperammonemia is a major element in the pathogenesis of hepatic encephalopathy (HE) and ammonia neurotoxicity involves an effect on the glutamatergic neurotransmitter system. Astrocytes are intimately related to glutamatergic neurotransmission and, in fact, many specific glial alterations have been reported as a result of ammonia exposure. S100B protein, particularly extracellular S100B, is used as a parameter of glial activation or commitment in several situations of brain injury. However, there is little information about this protein in ammonia toxicity and none about its secretion in astrocytes under ammonia exposure. In this study, we investigated S100B secretion in rat cortical astrocytes acutely exposed to ammonia, as well astrocyte morphology, glial fibrillary acidic protein (GFAP) content and glutamine synthetase (GS) activity. Moreover, we studied a possible effect of creatine on these glial parameters, since this compound has a putative role against ammonia toxicity in cell cultures. We found an increase in S100B secretion by astrocytes exposed to ammonia for 24 h, accompanied by a decrease in GFAP content and GS activity. Since elevated and persistent extracellular S100B plays a toxic effect on neural cells, altered extracellular content of S100B induced by ammonia could contribute to the brain impairment observed in HE. Creatine addition did not prevent this increment in S100B secretion, but was able to prevent the decrease in GFAP content and GS activity induced by ammonia exposure.

P6.03 Expression of chemokine proteins in the rat model of temporal lobe epilepsy

Lukasiuk K., Guzik A., Sliwa A.

Nencki Institute of Experimental Biology PAS, Warsaw, Poland

The abstract will be presented as P7.09

P6.04 Induced glia activation is harmful for neurogenesis of human umbilical cord blood neural stem cells

Markiewicz I., Winiarska H., Domanska-Janik K., Lukomska B.

NeuroRepair Department, CMDiK PAS, Warsaw, Poland

Brain inflammation contributes to the propagation of the neuropathological events that involves activation of astrocytes and microglia. It remains obscure how activated glial cells affect the survival and differentiation of NSC. The aim of the study was to analyse neuronal commitment of HUCB-NSC cultured in the presence of normal and LPS or TMT activated glial cells. Labeled HUCB-NSC were seeded on confluent monolayer of normal or stimulated astrocytes and microglial cells isolated from neonatal rat brain and cultured for 7DIV. Normal rat astrocytes induce HUCB-NSC to differentiate mostly into neurones (75% TUJ1+; 65% MAP-2+), microglia stimulate HUCB-NSC to differentiate into neurons (45% TUJ1+) as well as into astrocytes (56% S100B+). Induced astrocytes diminish neurogenesis of HUCB-NSC (29% and 33%, respectively, vs. 75% TUJ1+) and increase astrocyte differentiation (52% and 53%, respectively vs. 1% S100B+) comparing to non-stimulated astrocytes. Microglia activation decreases HUCB-NSC differentiation into neurons (27% and 26%, respectively, vs. 45% TUJ1+) but enhances oligodendrogenesis (9% and 7% respectively vs. 1% O4+) compared to normal microglia. Activation of microglia and astrocytes induced by LPS and TMT attenuate proneural effect of non-stimulated (resting) glia co-culture. Interaction with glial cells modyfied by inflammation is crucial for NSC survival and differentiation after brain insult. Supported by grants: 2P05/A177/29; 1309/P01/2006/31.

P6.05 Response of glia in hippocampus of immature rat brain after prolonged Pb exposure

Struzynska L., Dabrowska-Bouta B., Koza K.

Laboratory of Pathoneurochemistry, Department of Neurochemistry, Medical Research Centre, PAS, Warsaw, Poland

Lead (Pb) is an environmental pollutant considered as a dangerous neurodevelopmental toxin causing cognitive and behavioral deficits and leading to the neurodegenerative changes. Both astro- and microglia are cell populations very sensitive to pathogenic stimuli, including toxic ones. The aim of the study was to examine the response of glia in hippocampus of immature rats brains after prolonged lead exposure (15 mg/kg of lead acetate i.p. for two weeks). Features of activation were found both in microglial and astroglial cells using microscopic and immunohistochemical methods. Additionally, overexpression of proteins connected with astrocitic cells – S100beta and gap junctions' protein – connexin 43 was observed in the examined brain region after Pb treatment. Activated microglia and astrocytes may generate and/or maintain the inflammatory reaction in brain by producing cytokines. The enhanced level of the main proinflammatory cytokines – interleukine-1 beta and TNF-alpha were noticed in hippocampus. As it was suggested purinergic receptor P2X7 may take part in inflammatory processes. The enhanced expression of receptor's protein was also seen in the conditions of Pb toxicity. The results showed that hippocampus of immature rats is a structure especially sensitive to Pb exposure that leads to the complex, proinflammatory in nature, activation of glial cells.

P6.06 Morphological and quantitative analysis of hippocampal microglia in endotoxemic mature rat brain

Wojcik A., Ziaja M.

Department of Neuroanatomy, Institute of Zoology, Jagiellonian University, Krakow, Poland

LPS injection directly into the brain develops acute inflammation that activates microglial cells. LPS given peripherally may cause multiorgan response and mimics septic shock. During septic encephalopathy, sequences of neurological changes are observed, which suggests disturbances in different structures of CNS. The purpose of our study was to estimate the changes of morphology and quantity of microglial cells in hippocampus evoked by peripheral application of LPS. 30-day-old rats were given i.p. injection of LPS (*E. coli* serotype 027:B8; 10 mg/kg b.w.). At different times following injection the animals were anaesthetised and perfused by saline and paraformaldehyde. The brains were frozen, cut on a microtome and 20 mm slices containing hippocampus were stained with BSI-B4 isolectin. The number and morphology of microglial cells were analysed microscopically. There were few types of lectin positive cells distinguished, i.e. microglia with thin branched processes, microglia with increased cell body and stout processes, and finally macrophage-like cells with big, oval cell bodies, sometimes with short, thin processes. The study shows that microglial cells number in hippocampus changes quickly, similarly in both hemispheres and the highest number was noted at 12 and 24 hours following LPS injection. In the same time there were observed macrophages, which could also be founded as late as 8 day after LPS application. Our study clearly indicates that peripherally injected LPS may affect the number and morphology of microglia in hippocampus.

P6.07 Glutathione and homocysteine toxicity in rat astrocytes *in vitro*

Zieminska E.[1], Wegrzynowicz M.[2], Dybel A.[2], Albrecht J.[2], Lazarewicz J.[1]

[1]Department of Neurochemistry; [2]Department of Neurotoxicology Medical Research Centre PAS, Warsaw, Poland

Homocysteine (Hcy) is a sulphur-containing amino acid normally present in the blood and cerebrospinal fluid at low micromolar concentration. Hcy neurotoxicity is known to be mediated by disturbances in methylation processes, by the NMDA and group I metabotropic glutamate receptor-mediated excitotoxicity and/or by oxidative stress. The aim of this study was to evaluate the role of intracellular glutathione in homocysteine toxicity in rat astrocytes. Primary cultures of rat astrocytes were incubated for 24 h in the presence of 5 mM D,L-Hcy, where after their viability was assessed using the MTT test. The results demonstrated a significant Hcy toxicity, which was neither related to activation of glutamate receptors, nor to mitochondrial disturbances. Alterations in intracellular glutathione levels were measured after 4 and 24 h incubation with 250 μM or 5 mM Hcy. The total glutathione decreased dose-dependently during 4 h and increased after 24 h incubation with 5 mM Hcy. The GSH/GSSG ratio increased after 4 h incubation with both Hcy concentrations. Addition of 50 μM–2.5 mM buthionine-sulfoximine (BSO) decreased cells viability, while 50 μM – 1 mM N-acetyl-cysteine (NAC) had not effect. These results suggest that the increased accumulation of the GSSG form of glutathione has compensatory character, but it is not sufficient to protect astrocytes against death induced by 5 mM Hcy.

NEUROCHEMISTRY AND NEUROANATOMY

P7.01 Brain expression and subcellular localization of Calmyrin 2, a novel Ca-binding protein

Blazejczyk M.[1], Sobczak A.[1], Jaworski J.[1], Kuznicki J.[1,2], Wojda U.[2]

[1]Laboratory of Neurodegeneration, IIMCB; [2]Laboratory of Calcium Binding Proteins, Nencki Institute of Experimental Biology PAS, Warsaw, Poland

Ca signals in neurons are transmitted by EF-hand Ca-binding proteins including Neuronal Ca-Sensor Proteins (NCS). Common property of the NCS proteins is myristoylation that determines anchoring to membranes. Some of the NCS protein such as VILIP1 in response to Ca signal exhibits redistribution from the cytoplasm to membranes by so called Ca/myristoyl switch. Recently, a new similar to the NCS family named calmyrins (CaMy, CIB) was described. The only characterized protein of this family is calmyrin1 (CaMy1). Here we report the first data regarding distribution and subcellular localization of calmyrin2 (CaMy2) in neurons of the rat brain. CaMy2 transcript and protein were detected mainly in the hippocampus and cortex. Immunocytochemistry revealed significant differences in the cellular and subcellular distribution of CaMy2 protein in comparison to CaMy1. In contrast to mostly nuclear localization of CaMy1, CaMy2 is partially associated with Golgi membranes and dendrites. Incorporation assay of 3H-myristic acid proved that CaMy2 is myristoylated, similarly as CaMy1 and VILIP1. However, while VILIP1 exhibited Ca/myristoyl switch, both calmyrins were constitutively associated with membranes without Ca-dependent translocation. Observed differences in the localization of calmyrins suggest they function in different Ca signaling pathways at their specific sites in the cell. Supported by the Polish grant N30110932/3854.

P7.02 Cytochrome P450-mediated synthesis of dopamine in rat brain

Bromek E, Haduch A., Daniel W.A.

Institute of Pharmacology PAS, Krakow, Poland

The CYP2D subfamily of cytochrome P450 in the rat consists of six isoforms (CYP2D1-5 and CYP2D18), whereas in man it has only one isoform CYP2D6. CYP2D4 is a key CYP2D isoform in rat brain. CYP2D is able to metabolize a number of neuroactive drugs and toxins and to catalyze the formation of dopamine from tyramine. The aim of the present study was to characterize CYP2D isoforms involved in the hydroxylation of tyramine to dopamine and to assess the CYP2D activity in the brain (HPLC). Of the CYP2D isoforms tested (c-DNA-expressed CYPs), only CYP2D1 displayed no activity towards dopamine formation. The efficacy of all CYP2Ds involved in dopamine formation was higher for m-tyramine than for p-tyramine. The affinity of tyramine for the tested CYP2Ds (Km) was as follows: CYP2D6 > CYP2D2 > CYP2D18 > CYP2D4 for m-tyramine, and CYP2D18 > CYP2D4 > CYP2D2 > CYP2D6 for p-tyramine. Rat brain microsomes were able to catalyze the hydroxylation of tyramine to dopamine. Quinine (a specific CYP2D inhibitor) inhibited the above-described reaction. The activity of CYP2D (1`-hydroxylation of bufuralol) varied throughout the brain and was as follows: the cerebellum > substantia nigra > truncus cerebri > nucleus accumbens > striatum > frontal cortex > the remainder. Our study offers the first direct evidence that dopamine can be formed from tyramine by CYP2D isoforms in rat brain, which may provide the neurotransmitter in degenerative diseases. Grant 2PO5F00229, ME&S, Warszawa.

P7.03 NAPE-PLD, 12-LOX and TRPV1 immunoexpression in the hippocampus and cerebellum of the mouse brain

Cristino L.[1], Starowicz K.[1], De Petrocellis L.[1], Guglielmotti V.[1], Di Marzo V.[1,2]

[1]Endocannabinoid Research Group, Inst. of Cybernetics; [2]Inst. of Biomol. Chem. CNR, Pozzuoli, Italy

Anandamide (AEA) is an endogenous hybrid; agonist of CB1 and TRPV1 receptors in the brain. FAAH (fatty acid amide hydrolase) hydrolyzes AEA to arachidonic acid and in previous studies we have found a strong overlap between TRPV1- and FAAH-immunoreactivity in hippocampal pyramidal neurons, whereas a complementary pattern of expression was observed in cerebellar Purkinje neurons. Aim of this study was to localize in the hippocampus and cerebellum the immunohistochemical expression of the two enzymes, N-acyl-phosphatidylethanolamine-selective phospholipase D (NAPE-PLD) and 12-lypoxygenase (12-LOX), which are involved in the biosynthesis of AEA and the other proposed TRPV1 ligand, 12-hydroperoxyeicosatetraenoic acid (12-HPETE), respectively. We used polyclonal specific antibodies for single immunoperoxidase labeling and double TRPV1/NAPE-PLD and TRPV1/12-LOX immunofluorescence, all performed on cryostated brain sections. Both methods point to the same results. TRPV1/NAPE-PLD-ir was detected in hippocampal pyramidal neurons and in the somata of the vast majority of Purkinje cells. TRPV1-ir and 12-LOX-ir co-expression was observed in pyramidal neurons of Ammon horn whereas, in cerebellar cortex, intense 12-LOX-ir was found only in the granular layer. These data seem to suggest that while NAPE-PLD products (including AEA) might act as TRPV1 agonists in both the hippocampal pyramidal cells and Purkinje cells, 12-LOX products (i.e., 12-HPETE) are likely to act as endovanilloids only in the former neurons.

P7.04 Structural and biochemical properties of Calmyrin 2, a novel calcium binding protein in the brain

Sobczak A.[1], Blazejczyk M.[1], Debowska K.[1], Cymerman I.[1], Kirilenko A.[3], Pikula S.[3], Wojda U.[1]

[1]Laboratory of Neurodegeneration; [2]Laboratory of Bioinformatics; [3]Laboratory of Biochemistry of Lipids IIMCB, Warsaw, Poland

Major role in transduction of Ca signals in neurons is assigned to a superfamily of proteins coordinating Ca in the helix-loop-helix motives called EF-hands. Recently, a new gene family coding several EF-hand proteins named calmyrins was described. The only characterized protein of this family is calmyrin 1 (CaMy1, called also CIB1 or KIP1), implicated in Alzheimer's disease. CaMy1 shares 59% sequence similarity with Calmyrin 2 (CaMy2). We have demonstrated that CaMy2 is expressed in brain (poster by Blazejczyk et al.). Here we report CaMy 2 homology modeling, cloning and the first biochemical characterization of rat CaMy2. Homology modeling, 45Ca gel overlay, gel

filtration and spectral characteristics indicate that CaMy2 transmit Ca signals by the Ca-induced conformational switch in the monomeric molecule, but not by oligomerization. Comparison to CaMy1 reveals significant differences between both proteins. EF-2 motif of CaMy2 binds Ca, whereas that of CaMy1 is not functional. Moreover, the proteins exhibit differences in their Ca-dependent conformations. These differences between two homologous CaMy proteins suggest that they play a different role in Ca-signaling. Supported by the Polish research grant N30110932/3854.

P7.05 Looking for beta-catenin target genes in mature neurons

Wisniewska M.B.[1], Klejman M.[1], Michowski W.[1], Dabrowski M.[2], Filipkowski R.K.[2], Kuznicki J.[1,2]

[1]International Institute of Molecular and Cellular Biology; [2]Nencki Institute of Experimental Biology PAS, Warsaw, Poland

Beta-catenin is the key mediator of the Wnt signal, implicated in cell proliferation and differentiation and playing crucial role in the central nervous system development. Activation of the signaling pathway leads to stabilization of beta-catenin in the cytosol and its transfer into the nucleus. Beta-catenin interacts with Lef1/Tcf transcription factors and converts it from a transcription repressor to an activator. We examined beta-catenin protein expression pattern in the forebrain of adult mice by immunochemistry and Western blot methods. We found that the protein is stabilized in thalamic neurons and is present in the nuclei of the cells. It suggests that beta-catenin-dependent gene transcription plays a role in the adult brain. A genetic program activated by beta-catenin in mature neurons is not known. We did in silico inquire to identify potential neuronal Lef1/Tcf target genes. We screened homology regions in promoters of human and rat orthologs and found conserved Lef1/Tcf binding sites in 21 neuron specific genes. Expression level of three out of five tested candidates, analyzed by Real Time PCR, correlated with nuclear localization of beta-catenin in the brain. They encode synaptic proteins, namely L1cam and neurofascin cell adhesion molecules, as well as a subunit of T-type calcium channel. We are going to investigate further the regulation of the putative targets by beta-catenin and Lef1/Tcf transcription factors. This work was supported by EUFPVI Promemoria Grant contract no. 512012.

P7.06 Role of brain dopaminergic pathways in the regulation of cytochrome P450 in the liver

Wojcikowski J.

Institute of Pharmacology PAS, Krakow, Poland

Cytochrome P450 (CYP) is implicated in the metabolism of endogenous substances (e.g., steroids, neurotransmitters) and the majority of clinically important drugs. Genes encoding CYP isoforms are regulated by endogenous hormones (e.g., pituitary hormones, thyroid hormones, glucocorticoids) which are all under control of the central nervous system. The aim of the present study was to investigate the influence of lesions of brain dopaminergic pathways on the level and the activity of CYP isoforms (1A, 2A, 2B, 2C6, 2C11, 2D, 3A) in rat liver. At 48 h after lesion of the tuberoinfundibular pathway, only the activity and the protein level of CYP2B were significantly decreased. 7 days after lesion of the above-mentioned pathway, significant inhibition of CYP2B, CYP2C11 and CYP3A activities and a decrease in CYP protein levels were observed. At the same time, the activity and the protein level of CYP1A considerably increased. 14 days after damage of the mesolimbic pathway, the activity and the protein level of CYP3A were significantly reduced, while those of CYP1A were substantially elevated. In contrast, lesion of the nigrostriatal pathway did not affect any CYP isoforms studied. The obtained results provide the first direct evidence for the influence of brain dopaminergic system on the level and the activity of CYP in the liver, which is pathway- and isoform-dependent. Acknowledgments: The study was supported by grant no. 2 P05F 013 27 from the Ministry of Science and Higher Education, Warszawa, Poland.

P7.07 Double modifications of RAS protein change the DNA-binding activities of transcription factors

Zhuravliova E.[1], Narmania N.[1,2], Barbakadze T.[1], Ramsden J.[2], Mikeladze D.[1]

[1]Institute of Physiology, Tbilisi, Georgia; [2]Collegium Basilea, Basel, Switzerland

Several types of cellular proteins can be modified by farnesylation and nitrosylation, among which probably the most significant is Ras. We used manumycin, a farnesyl transferase inhibitor and L-NAME (Nω-nitro-L-arginine methyl ester), a NOS inhibitor for characterization of the activities Ras-dependent downstream targets activities. Our results suggest that changing farnesylation inhibition and the steady-state level of nitric oxide modified the activities of several transcription factors. Inhibition of farnesylation by manumycin decreased the DNA-binding activity of NF-kB, did not change the DNA-binding activities of STAT, Sp1, ATF-2, and CREB, and increased the activities of c-Fos, JunD and c-Jun. Under such conditions, phosphorylation of Akt was decreased, whereas phosphorylation of ERK was increased and phosphorylation of JNK did not change. The diminution of the intracellular concentration of nitric oxides by L-NAME causes an increase in the activities of c-Fos, ATF-2 and JunD, and decreases the activities of CREB, STAT, Sp1, and c-Jun. The activities of all of these transcription factors are restored to normal levels in the presence of manumycin, suggesting that the simultaneous modifications of proteins by farnesylation and nitrosylation change the direction of Ras-controlled down stream pathways. Our results provide further evidence for the significance of post-translational modifications of Ras on the transducing cascade networks' specificity and physiological outcomes.

P7.08 AdTx1, novel selective modulator of alpha1a adrenoceptor subtype isolated from snake venom

Gilles N.[1], Quinton L.[2], Girard E.[3], Ciloek J.[4], Mourier G.[1], Molgo J.[3]

[1]CEA, IBITECS, Gif sur Yvette; [2]Ecole Polytechnique, Palaiseau; [3]CNRS, UPR 9040, France; [4]Nicolaus Copernicus University, Torun, Poland

Animal venoms are a stupendous source of new pharmacologically active peptides. Mostly directed against ionic channels, venoms contain also peptides active on G-protein coupled recep-

tors (GPCRs). Their receptors are the target of 50% of drugs and the need for highly selective ligands is critical. We have established an experimental strategy, based on the monitoring of biological activities associated with highly accurate mass analysis, of extracting peptides specific to GPCRs from mamba venom. We discovered AdTx1, a 65-residue peptide present as 0.5% of the crude venom, which antagonizes epinephrine activation by binding to an allosteric site physically distinct from the orthosteric one. It has a nanomolar range affinity for alpha1a adrenoceptor subtype and micromolar affinities for the other subtypes. This unique high-affinity modulator for alpha1a-adrenoceptor opens the possibility to AdTx1 to be a drug candidate against benign prostatic hyperplasia. Conjugated with radioactive iodine or a fluorophore, 125I-AdTx1 can be used to gain insight into the structure of the allosteric site and AdTx1-CY3B is the first fluorescent ligand able to specifically label the alpha1a-adrenoceptors on live cells.

P7.09 Expression of chemokine proteins in the rat model of temporal lobe epilepsy

Lukasiuk K., Guzik A., Sliwa A.

Nencki Institute of Experimental Biology PAS, Warsaw, Poland

Chemokines are small secreted proteins originally identified as chemoatractants and activators of immune cells. Our recent metaanalysis aiming at identification of common features in the molecular response across different animal models of epileptogenesis indicated that expression of genes coding for chemokines is frequently altered by epileptogenic stimuli. Here we present changes in expression of MIP-1alpha, MIP-1beta and MIP-2 proteins in the rat model of temporal lobe epilepsy caused by amygdala stimulation induced status epilepticus (SE). Expression of MIP-1alpha in the control brain was present in neurons in several brain areas. One day following SE the high level of expression appeared in glial cells in selected brain areas including medial nucleus of the amygdala and piriform cortex, while by 4-d following SE its expression decreased. MIP-1beta was expressed at low level in neurons in control brain. Following SE its expression increased at 1-d in cortex in glial cells. Expression of MIP-2 in the control brain was negligible, but increased in the glial cells and neuropil in selected areas, including retrosplenial, entorhinal and piriform cortices at 1-d following SE and decreased later on. These findings indicate potential, but yet unknown function of chemokine signaling in the brain reaction to SE and/or epileptogenesis.

P7.10 Alterations of the dendrites and dendritic spines of the human Purkinje cells during normal aging

Mavroudis I.A., Tsamis K., Safouris A., Mytilinaios D., Njau S.N., Costa V.

Laboratory of Neuropathology, First Department of Neurology, Aristotelian University of Thessaloniki, Greece

In senile brains there is a decline in the thickness of the cerebellar cortex as well as loss of neurons, hypertrophy and hyperplasia of the astrocytes and progressive defoliation of dendrites in the ageing Purkinje cells. Furthermore qualitative and quantitative changes were found in the cerebellar circuitry of old rats as compared to young ones, such as reduced number of synapses, prominent lipofuscin bodies in Purkinje cell somata, tortuous Purkinje cell dendrites in a thinned molecular layer, and abundant vacuolar profiles and membrane swirls in small and intermediate-sized dendrites. In this study we attempted to describe the morphological and morphometric alterations of the dendrites of the human Purkinje cells and their dendritic spines. We studied the cerebellar cortex of 4 senile and 4 younger individuals, by Golgi technique. We excised small parts of the cerebellar cortex from the nodule, the floccules, the pyramid, the tonsils, the clivus, and from the cerebellar hemispheres at the level of the superior surface of the cerebellum. The morphological and morphometric estimation revealed substantial alterations, such as loss of tertiary and final dendritic branches, decrease in the thickness and the height of the dendritic arborization. Furthermore many of the dendrites appear to be degenerating, fracted and swollen, while significant loss of dendritic spines has also been noticed.

P7.11 Stereological approach to seasonal volume changes of telencephalic structures in the common shrew

Michalski A., Bartkowska K., Turlejski K.

Nencki Institute of Experimental Biology PAS, Warsaw, Poland

Mechanisms of seasonal variation of brain volume in Soricidae, an aspect of the Dehnel effect, remain insufficiently elucidated. Previous work showed that change in the number of cells is not sufficient to explain the variation. It seems important to determine whether the volume of brain structures in shrews changes proportionally or which structures change most. We approached the problem using stereological methods. We investigated difference in the volume of telencephalic structures in two groups of the common shrews (*Sorex araneus*): young animals caught in summer and the wintering ones. On the Nissl-stained sections we measured stereologically the volume of neocortex, hippocampi, olfactory areas and the whole telencephalon in each individual. In agreement with the results of other authors we found the telecephalon to be significantly smaller in the wintering animals. The groups differed in the volume of neocortex and olfactory areas but not of the hippocampi. While the first finding accords with results published by Yaskin (1994), the last does not. These results support the hypothesis that reduction of the brain volume in the Dehnel effect involves a major reorganisation of the brain rather than its general dehydratation.

P7.12 Differences in the ponto-cerebellar projection to the vermis: Double labeling method in the rabbit

Mierzejewska-Krzyzowska B., Bukowska D., Zguczynski L.

Department of Anatomy in Gorzow and Department of Neurobiology in Poznan, University School of Physical Education, Poznan, Poland

Topography of projection from the pontine nuclei (PN) to the caudal vermis was examined by microinjections of two fluorescent tracers to the pyramis and uvula, and mapping of retrogradely labeled neurons in PN. PN, the major structure which transmits

information from the cerebral cortex comprises: the paramedian (PM), peduncular (PD), lateral (L), dorsolateral (DL) and ventral nuclei. The data indicate that connections are fourth times more numerous to the uvula than pyramis. The strongest projections arise from DL. These from PM, L and PD are weaker or absent from V. Regarding the pyramis projecting neurons in PM constitute dorsally two populations, medial and lateral. In PD they are present dorsomedially, but in the L dorsolaterally. In DL projection originates from entire caudal part and more rostrally is restricted to the dorsolateral region. As concerns the uvula projecting neurons in the dorsal PM cluster in one (caudally) or two groups (centrally and rostrally). In PD they lie ventrolaterally and in L dorsolaterally. In the rostral DL main projection comes from the lateral half. The findings show that the projection is differentiated: the caudal PN aspect supplies the uvula and rostral PN aspect – pyramis. Projection from the central PN is partially common for these two targets. Thus, functionally various pyramis (receives spinal afferents) and uvula (is interconnected with the vestibular nuclei) are influenced by information from different PN regions.

P7.13 Amacrine cells of the mouse retina

Perez de Sevilla L.M.

University of Oldenburg, Neurobiology, Oldenburg, Germany

Amacrine cells (ACs) are inhibitory interneurons in the retina that modulate the information flow from bipolar cells to ganglion cells in the inner plexiform layer (IPL). There are many different types of ACs, which vary in size, morphology and function. The number of AC types may come in 30–40, based on differences in dendritic architecture, retinal distribution, and neurotransmitter used. In addition, many of the subtypes express connexins and form homologous or heterologous, coupled networks. The aim of this study was to characterize and classify the ACs in the INL of the mouse retina. They were classified according to their horizontal and vertical stratification patterns, general morphology, dendritic field size, soma size and coupling patterns. We classified twenty different types of Acs into four groups: Narrow-field (dendritic field less than 150 µm), small-field (between 150–300 µm), medium-field (between 150–300 µm), and wide-field ACs (bigger than 500 µm). The most frequently-labeled AC types were the starburst ACs, bow-tie cells and the AII cells. We found that the narrow-field ACs are the predominant group. Such ACs transmit or modify the synaptic input from bipolar cells to ganglion cells and therefore need to be reduplicated many times within the ganglion cell`s receptive field. We show that ACs display a rich variety of stratification and branching patterns and form specific networks which surely reflect the wide range of their functional roles in the processing of visual signals in the inner retina. Supported by Deutsche Forschungsgemeinschaft.

P7.14 Alterations of the human visual cortex during normal aging, revealed by silver staining methods

Safouris A.[1], Tsamis K.[1], Mavroudis I.A.[1], Mytilinaios D.[1], Njau S.N.[2], Costa V.[1]

[1]Laboratory of Neuropathology, First Department of Neurology, AHEPA Hospital; [2]Laboratory of Forensic Medicine and Toxicology, Aristotelian University of Thessaloniki, Greece

The visual cortex undergoes age related changes that have been studied mainly in rats, *Macaca mulatta*, and human beings. Despite the fact that there is no extensive neuronal loss in aged brains, a lot of important pathological changes are found in the morphology of the neurons. In pyramidal cells which represent the majority of cortical neurons, age related pathology can be noticed in cell somata as well as, most importantly, in dendrite number and morphology. This study is based on the morphological analysis of ten brains, 5 senile without recorded signs of dementia and 5 younger individuals, by Golgi method, Golgi- Nissl staining and Gallyas technique. Even if some pyramidal cells retain high spine density, in most of the cells studied there seems to be important spine loss, and extensive dendrite pathology. Apical dendrites are distorted and tortuous. Horizontal dendritic arborization is severely decreased leading to an amputated, bell shaped cell soma. Although senile plaques have been often revealed, only occasionally do cells exhibit neurofibrillary changes.

P7.15 Synaptic localisation of NPY Y1 receptors in the rat cingulate cortex

Sichardt K.[1], Beck-Sickinger A.G.[2], Nieber K.[1]

[1]Institute of Pharmacy, Dept. of Pharmacology; [2]Institute of Biochemistry, University of Leipzig, Germany

Neuropeptide Y (NPY) is one of the most abundant neuropeptides in the CNS and exerts various functions on at least six receptor subtypes (Y1R-Y5R, y6R). Activation of Y1Rs results in inhibition of excitatory synaptic transmission whereas a presynaptic influence on cortical neurons is postulated. Previous studies of our group confirmed the inhibitory effect of NPY *via* Y1Rs on pyramidal cells of the rat cingulate cortex but did not differentiate between a pre- and/or postsynaptic side of action. Therefore the aim of the present study was to elucidate on which side the inhibition occurs. Intracellular recordings were performed in rat brain slice preparations containing pyramidal cells of the cingulate cortex using glass microelectrodes placed in layer V. Superfusion of NPY and the selective Y1R agonist [F7, P34]pNPY caused inhibition of postsynaptic potentials (PSPs) but had no significant influence on membrane potential and input resistance. In the presence of CNQX, a non-NMDA receptor antagonist, the inhibitory effect of [F7, P34]pNPY on PSPs was present. When preincubation with APV, a NMDA receptor antagonist, occurred, inhibition of PSPs failed. Furthermore, the influence of [F7, P34]pNPY on glutamate induced depolarisation of the cell membrane and attenuation of input resistance was investigated. [F7, P34]pNPY decreased depolarisation as well as changes of input resistance. The data indicate an interaction with NMDA receptors and reveal that Y1Rs are not exclusively located presynaptically.

P7.16 Kir4.1, Kir2.1 and aquaporin-4 mRNA level in the brain and retina of rat with toxic liver failure

Obara M.[1], Pannicke T.[2], Bringmann A.[3], Reichenbach A.[2], Albrecht J.[1]

[1]Dept. of Neurotoxicology, Medical Research Centre PAS, Warsaw, Poland; [2]Dept. of Neurophysiology, P. Flechsig Institute of Brain Research; [3]Dept. of Ophthamology and Eye Clinic, Medical Faculty of the University of Leipzig, Germany

Cerebral and retinal edema is a complication of hepatic encephalopathy (HE) and retinopathy (HR), respectively, but the underlying mechanism is not understood in detail. This study addresses a hypothesis linking cerebral and retinal edema in a rat HE model with deregulation of potassium and water transport. Three thioacetamide (TAA) administrations (250 mg/kg, i.p.) at 24 h intervals induce liver failure associated with edematous changes in the cerebral cortex and retina, but not in the striatum (Hilgier and Olson 1994, Albrecht et al. 1998). Real-time PCR analysis revealed a markedly decreased expression of Kir4.1 mRNA in all the three structures of the TAA-treated rats. This observation is consistent with previous observations of a decreased Kir4.1 expression in retinal edema associated with ischemia (Iandiev et al. 2006). TAA treatment specifically decreased Kir2.1 mRNA expression in the retina, which contrasts with unchanged expression of this channel in retinal edema triggered by other causes, and may reflect a compensatory response of the cells to avoid intracellular K^+ overload. A decrease of aquaporin 4 mRNA observed in the striatum, but not in the other regions, may have counteracted striatal edema by limiting water inflow. Supported by the Ministry of Science and Education, grant no 2P05A06628.

P7.17 Procaine injections into the PPN suppress behaviour evoked by stimulation of the VTA

Jerzemowska G.

Department of Animal Physiology, University of Gdansk, Poland

The pedunculopontine nucleus tegmental (PPN) is anatomically and functionally connected with the dopaminergic ventral tegmental area (VTA). In the present experiment, we studied a possible involvement of the PPN in feeding and exploration reaction elicited by stimulation of the VTA. The effect of temporary inactivation of the PPN by mean of direct procaine injection (water injection as a control) on VTA-stimulation elicited feeding or exploration was tested in male Wistar rats with the use of reaction latency/stimulation frequency curve-shift paradigm witch allows separate assessment vs motor aspects of appetitive behaviour. Ipsilateral and contralateral VTA-PPN relationships were analyzed. The effect of contralateral injection was much more pronounced in the eating group (n=6). On the contralateral side, there was an increase in the reaction threshold by about 20% in comparison to the water and the rightward and upward shift of the latency/frequency curve. On the ipsilateral side, the threshold increased by about 12% and curve shifts were less evident. In the exploring group (n=5) the effect of contralateral and ipsilateral injection wasn't significant and the values were 11.69% and 17.39%, respectively. The tested reaction maintained destabilized for several days following procaine injections in both groups. The results indicate that the PPN and VTA belong to the same central circuitry involved in the regulation of motivational and motor aspect of food intake as well as psychomotor activation manifestly as exploratory behaviour.

P7.18 A new, precise method of bregma detection

Blasiak T., Ignaciak A., Czubak W., Lewandowski M.H.

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

Stereotaxy is commonly used to implant microelectrodes or microprobes in the particular structure of the brain *in vivo*. In this technique the position of the brain nuclei is given as the distance from the reference points on the skull and thus it is crucial to correctly locate this points on the bone. On the rodent skull cap this landmarks are called bregma and lambda and are marked out by the bone sutures. Classically the position of these points is roughly evaluated by the experimenter – approach being the source of potential error. In the recent experiments we have developed and tested the alternative method of locating the reference points on the skull of the mature Wistar rat. In this method a digital picture of the exposed skull cap is fed to the computer and curves mathematically fitted to the image of the skull cap sutures are used to trace the bregma and lambda points. Our observations are that in many cases the position of bregma points located on the skull by two methods varied by hundreds of microns. Systematic, experimental testing of the new method revealed that its use yields significantly smaller stereotaxic error in the anterior-posterior and mediolateral direction (Mean error $AP_{new} = 0.2 \pm 0.08$ mm vs. Mean error $AP_{clasical} = 0.5 \pm 0.12$ mm; Mean error $ML_{new} = 0.1 \pm 0.04$ mm vs. Mean error $ML_{clasical} = 0.4 \pm 0.07$ mm; n=9; P<0.01). This results confirm that new method of locating the stereotaxic reference points improves the precision of the *in vivo* electrode implantation. Supported by IZ UJ grant no. BW/47/2006.

SENSORY SYSTEMS

P8.01 Evidence for diverging projection from the dorsal column nuclei to the cerebellar pyramis and uvula

Bukowska D.[1], Zguczynski L.[2], Mierzejewska-Krzyzowska B.[2]

[1]Department of Neurobiology, University School of Physical Education, Poznan; [2]Department of Anatomy, University School of Physical Education, Gorzow Wlkp., Poland

The mammalian dorsal column nuclei (DCN), composed of the gracile (Gr) and cuneate (Cu) nuclei, are essential in relay and modulation of somatosensorimotor information. Gr and Cu receive afferents from the hindlimbs and lower trunk, and the forelimbs and upper trunk, respectively. They project mainly to the thalamus, but direct connections to the cerebellum also exist. Using a retrograde axonal transport we show DCN projections to the cerebellar vermis by axonal collaterals. Such study has not been undertaken in any species. Microinjections of two fluorescent tracers in the pyramis (with inputs from the spinal cord) and uvula (cooperating with the vestibular system) revealed in DCN certain number of double labeled neurons (n=175) among many single labeled. Double labeled neurons were found in the lateral cuneate nucleus (CuL; n=153) and in the complex of Gr and medial cuneate nucleus (Gr+CuM; n=22). No double labeling was observed in Gr and CuM. In CuL double labeled cells occupied mainly the ventrolateral region throughout the rostral and central parts, but more caudally they clustered in the ventromedial region. In Gr+CuM they lay laterally, adjoining CuL. These data indicate that there is divergent projection to the cerebellar vermis from determined regions of DCN. This way information from the limbs and trunk mediated *via* DCN can reach simultaneously two functionally different cerebellar targets and exert modulatory effect on their neuronal activity.

P8.02 Aspects of testing in the human chromatic memory

Georgescu C.[1], Catalin B.[2], Grigorie D.[2], Grigorie O.[2], Nicorescu A.[2], Ciorbagiu M.[2], Velicu N.[2]

[1]National College Carol I; [2]University of Medicine and Pharmacy, Craiova, Romania

Our study has intended to test the memory in the chromatic system, given the lack of information in the field. We have conducted our tests on a group, without ophthalmologic pathology or having the refraction modifications corrected. As stimulus source, we used a LCD monitor, placed 60 cm away from the eyes. The subject received the stimulation color in full screen mode for 90 seconds. Each color (red, blue, green) were separated in 256 nuances, measuring the difference between the nuance recognized by the subject and the one proposed for memorizing. We had two daily recognitions, one immediately after the stimulus and the other five minutes after. The processing of the results of these tests was made by computing standard deviation, regression slope, difference between the averages, surfaces report, correlation recognized color-stimulus color, in order to distinguish the decrease in differences between the color stimulus and the recognized color, we have observed: the regression graphic dragged during the whole period of the study (even if there are some important daily variations) scores a negative slope, with few exceptions. Our research will be extended on more subjects, for a longer period of time. The results of the tests were not correlated with the daily schedule.

P8.03 Electrophysiological testing of coloured sight through visual evoked potentials

Iancau M., Georgescu D., Catalin B., Sfredel V., Romanescu F., Georgescu M., Nestianu V.

University of Medicine and Pharmacy of Craiova, Romania

Our study was imposed as a necessity in widening the range of stimulation methods in visual electrophysiological testing, in which we can use both the LED matrix and the TV monitor, with luminous stimuli of various wavelengths. The tested groups consisted of 12 volunteers, ophtalmologicaly healthy or with corrected refraction pathologies (5 boy, 7 girls, ages 19–22). VEP recording was made through pattern-reversal stimulation, narrow vertical bars, yellow-green LED and TV monitor, with stimulation in red, yellow, green and blue. The visual field of stimulation was of 15 degrees 30 min, and 100–150 successive monocular stimulations were made, with a random frequency between 0.7 and 1.2 Hz, to avoid habituation. The recorded data were processed with an original software, measuring latency, amplitude, duration, surface, abruptness of the waves of the complex N75-P100-N135. From the abundance of data, only the latency of the waves was retained and statistically analyzed. Data processing revealed that P100 waves latency has the highest value at blue light stimulation, next is the one obtained at red light stimulation and the lowest obtained at green light stimulation. Through comparative analysis of the P100 latencies, highly significant values of *P* were obtained for blue-green, significant between red–green, blue–yellow and blue–green and insignificant for yellow–red and yellow–green. Some of the revealed aspects can be explained through the characteristics of the receiving cones, but others can't.

P8.04 Electrophysiological correlates of auditory perception of temporal order

Lewandowska M.[1], Bekisz M.[2], Szymaszek A.[1], Szelag E.[1,3]

[1]Laboratory of Neuropsychology; [2]Laboratory of Visual System, Nencki Institute of Experimental Biology PAS; [3]Warsaw School of Social Psychology, Warsaw, Poland

The perception of temporal order (TO) of two successive acoustic stimuli is only possible if they are separated by an Inter-Stimulus-Interval (ISI) of ca. 30 ms. Previous studies shown that patients with language disorders require longer ISIs to identify the TO correctly than normal subjects. It supports the notion of relation between temporal processing skills and language competences. However, any evidence on the cortical activity associated with the TO detection remains unknown. The aim of this study was to investigate electrophysiological correlates of TO perception. Nineteen subjects were presented binaurally with pairs of 400 Hz and 3 000 Hz tones with an ISI of 10 ms and 60 ms. The task was to report the TO of two tones (high-low vs. low-high). Subjects were classified into two groups on the basis of their performance level, specifically: Skilled Group (SG, 75% of correctness) and Non-skilled Group (NG, at a chance level). The Auditory Evoked Potentials (AEPs) were analyzed within 10 time windows of ca. 100 ms during 1s period after the onset of the first tone. The SG displayed more positive late AEPs than NG at the Fz and Cz electrodes. No differences were observed in the amplitude and latency of early AEPs (N1, P2) that are strongly dependent on physical properties of processed stimuli. Supported by the grant MNiSW no 1082/P01/2006/31.

P8.05 Variability of visual responses of superior colliculus neurons depend on their velocity preferences

Mochol G., Wypych M., Wojcik D.K., Wrobel A., Waleszczyk W.J.

Nencki Institute of Experimental Biology PAS, Warsaw, Poland

We studied trial-to-trial reliability of visual responses of neurons in the cat superior colliculus (SC). Extracellular single unit activity was recorded from superficial, retinorecipient layers of the SC of anesthetized and paralyzed cats during visual stimulation by moving light spot. On the basis of velocity preferences recorded cells were separated into four class. The first two groups encompassed cells activated by stimuli moved within a band of velocities which tended to be either low or high. The neurons in the remaining groups responded to broad range of velocities. All were excited by slow motion but some were activated and some were suppressed by high velocities. To quantify the regularity of analyzed signals the time of responses was divided into bins and in each bin the mean number of spikes and its variance across trials were computed. The ratio of these two quantities known as Fano factor (FF) was used as a measure of spike count variability. Time evolution of the FF was distinct depending on the collicular neurons velocity response profile. For all responses in the low-velocity range we observed the increase in FF along with augmentation of rate. The responses to fast stimuli in both 'high speed' and 'broad band' neurons showed, however, different relation. Changes of activity during excitation or suppression evoked by fast stimuli anti-correlated with FF. One of the possible reasons of differences in response variability can be specific convergence from retinal, cortical and local inputs to different types of collicular cells.

P8.06 Modelling the neural network of the upper layers of the superior colliculus

Wypych M., Mochol G., Waleszczyk W.J, Wrobel A.

Nencki Institute of Experimental Biology PAS, Warsaw, Poland

Superior colliculus (SC) is the principal, retinorecipient nucleus of the extrageniculate visual pathway involved in visuo-motor behaviour. Neurons in the superficial layers of the SC are extremely sensitive to moving visual stimuli. The SC is a structure with one of the highest number (40%) of GABAergic interneurons in the mammalian brain. The role of interneurons in processing visual information about motion is not resolved. To address this issue we constructed a simplified model consisting of a widefield collicular cell and horizontal inhibitory interneurons. Both types of cells receive inputs from the retina and interneurons additionally contact dendrites of the widefield cell with dispersed (and delayed) connections. Our model shows that: (1) spatially limited inhibition suppresses the response of widefield collicular cell to stationary or slowly moving stimuli; and therefore (2) the inhibition causes the cell to be more sensitive to rapid changes in its receptive field (e.g., fast moving stimuli); (3) directional preference of SC model neurons can be realized by the spatial asymmetry of inhibitory connections. These results suggest that inhibitory interneurons can play a crucial role in processing information about visual motion in the upper layers of the SC. Supported by MNISW grant no. N303 046 31/1483.

P8.07 Pupil diameter oscillations in the rat

Czubak W., Blasiak T., Lewandowski M.H.

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

Slow, rhythmic bursting has been show to be present in some nuclei of the subcortical visual system in rodents (e.g. intergeniculate leaflet IGL or olivary pretectal nuclei). This neuronal oscillations (frequency ca. 0.01 Hz) so far lack any link to the physiological function or phenomenon. The presented study has been designed to check the existence of the slow oscillations in the diameter of the rat pupil and, if present, to test their relationship to the slow bursting activity in the IGL. All the experiments were performed on the anaesthetized Wistar rats in the constant light conditions. Obtained results show the presence of the slow, rhythmic oscillations in the rat pupil diameter. Preliminary analysis indicate that rhythmic oscillation of the pupil diameter has the same period as the rhythmic bursting simultaneously recorded in the contralateral IGL. Observed oscillations (MUA in the IGL and the pupil diameter) had the opposite phase, that is the maxima were about 180 degrees shifted. The amplitude of the pupil diameter oscillations was higher during the spontaneous or paw pinch induced desynchronization of the EEG. The pupil dilatation induced by the topical application of the atropine into the eye did not influence the oscillatory neuronal activity in the contralateral IGL. Results reported here, for the first time show the existence of the slow oscillations in the diameter of the rat pupil. Moreover is has been shown that this physiological phenomenon may be linked to the slow bursting activity of the subcortical visual system neurons.

SYNAPTIC TRANSMISSION AND EXCITABILITY

P9.01 A high-threshold heat-activated channel in cultured rat dorsal root ganglion neurons resembles TRPV2

Babes A.[1,2], Leffler A.[3], Linte R.[1,2], Nau C.[3], Reeh P.[1]

[1]Department of Physiology; [3]Department of Anesthesiology, Friedrich-Alexander University Erlangen-Nuremberg, Erlangen, Germany; [2]Department of Animal Physiology and Biophysics, Faculty of Biology, University of Bucharest, Bucharest, Romania

Of the four heat-activated ion channels from the vanilloid-type TRP group (TRPV1-4), the least is known about TRPV2. Expressed in a variety of neuronal and non-neuronal tissues, TRPV2 is a high threshold (>52 C) heat receptor channel, blocked by ruthenium red and activated by 2-aminoethoxydiphenyl borate, and proposed to act as a sensor for intense noxious heat in mammalian sensory neurones. In this study we propose a new pharmacological tool to distinguish between the heat responses of TRPV2 and the closely related channel TRPV1: the trivalent cations lanthanum and gadolinium show opposite effects on the two channels, blocking TRPV2 and sensitising TRPV1 to heat. We also demonstrate that TRPV2 can be activated by heat in cell-free membrane patches in the outside-out configuration. Recordings from rat dorsal root ganglion cultures reveal that medium and large capsaicin-insensitive neurones express a heat-activated current that closely matches the temperature dependence, self-sensitisation and pharmacological properties of TRPV2 in a heterologous expression system. Taken together our results provide new evidence for a role of TRPV2 in mediating high-threshold heat responses in a subpopulation of mammalian sensory neurones.

P9.02 Bistability of frog tectal pear-shaped neurons: Modeling and experimental study

Baginskas A., Kuras A.

Laboratory of Neurophysiology, Institute for Biomedical Research, KMU, Kaunas, Lithuania

Dendrites of neurones from many regions of nervous system contain voltage-sensitive channels generating persistent inward currents. We have recently suggested that a slow negative wave (sNW), extracellularly observed in the frog tectum during the burst discharge of a single retinal ganglion cell, can be generated by persistent inward current in dendrites of tectal pear-shaped neurons. The aim of the present study was to prove this hypothesis by simulation on quasi-reconstructed pear-shaped neuron with the bistable dendrites and experimental investigation of the nature of sNW. Simulation was based on the compartmental model of dendrites, membrane of which has N-shaped current-voltage relation. Experiments were done *in vivo* on frogs. During the experiments, discharge of a single retinal ganglion cell was elicited by electrical stimulation of the retina using multi-channel electrode. Evoked electrical activity of the tectum was recorded by the carbon-fiber microelectrode brought into the tectum layer F. We show that: (1) Slow dendritic inward current or plateau potentials in bistable dendrites are reflected in the extracellular space as a slow negative wave or plateau. (2) The sNW, seen in the recordings of evoked minimal activity of the frog tectum, is generated by activation of L-type calcium channels in dendrites of pear-shaped neurons. (3) The burst discharge of a single retinal ganglion cell projecting to the tectum layer F can lead to a long lasting suprathreshold excitation of up to 5 recurrent pear-shaped neurons due to activation of dendritic slow inward current.

P9.03 Influence of capsaicin on spontaneous activity of GABAergic hippocampal neurones

Drebot I.

Bogomoletz Institute of Physiology, Department of General Physiology of Nervous System, Kiev, Ukraine

For a long time, vanilloid receptors 1 (VR1) were thought to be selectively expressed in a subpopulation of sensory neurons responsible for processing of chemical and thermal noxious stimuli, where its role has been studied in detail. More recently, it has been shown that VR1 receptors are also expressed in neurons of several brain structures including the hippocampus, cortex, locus coeruleus, and cerebellum, where they are involved in the regulation of glutamatergic synaptic transmission. In this study, we started to investigate a possibility that VRs1 are also involved in the regulation of GABAergic synaptic transmission. For this purpose, the effect of a VR1 agonist, capsaicin, on spontaneous GABAergic IPSCs was studied in hippocampal cell cultures using a patch-clamp technique. It was found that capsaicin (10 μM) decreased both the frequency and amplitude of spontaneous IPSCs. This finding suggests the involvement of VRs1 in the regulation of neuronal firing in some GABAergic interneurons and in the modulation of the GABAergic synaptic transmission efficacy. However, considering the direction of the effect (a decrease in the IPSC frequency) and lack of its desensitization, the involvement of other receptor(s) also cannot be currently ruled out.

P9.04 Both glutamate and ATP mediate evoked excitatory transmission in CA3 neurons of rat hippocampus

Kondratskaya E., Akaike N.

Research Division for Life Sciences, Kumamoto Health Science University, Kumamoto, Japan

It was recently shown that P2X2 and P2X4 receptors are expressed in the CA3 hippocampal neurons. However, the existence of functional purinergic component eliciting fast synaptic transmission in rat CA3 pyramidal cells was not shown. The main objective of this study was to explore a further role of ATP-mediated signalling and/or modulating role in CA3 rat hippocampal neurons by characterizing the profiles of spontaneous and evoked excitatory postsynaptic events. Fast spontaneous postsynaptic currents in CA3 neurons are mediated by glutamate receptors, since the most of mEPSCs were completely blocked by bath application of AMPA/kainate and NMDA receptor blockers (NBQX and D-APV). It was found two populations of evoked postsynaptic currents (eEPSCs) in CA3 neurons recorded in slices that are mediated by glutamate receptors, since they were blocked by the mixture of AMPA/kainate and NMDA receptor blockers (NBQX, 50 μM and D-APV, 25 μM) and those that contain non-glutamatergic component. Thus, in about 50% of estimated cells the residual non-glutamatergic current was sensitive to ionotropic P2X receptor antagonists suramin (25 μM) and NF023 (2 μM). In conclusion, ATP-mediated excitatory synaptic response in CA3 pyramidal cells in slices might be triggered by multiple presynaptic inputs firing during electrical stimulation.The pharmacological analysis of eEPSCs recorded has clearly revealed two-componential character of postsynaptic current mediated by both ionotropic glutamate (AMPA/kainate) and P2X postsynaptic receptors.

P9.05 Aconitum alkaloid songorine acts as a potent $GABA_A$ receptor agonist in the rat brain *in vivo*

Koszegi Zs.[1], Atlasz T.[1], Csupor D.[2], Hohmann J.[2], Hernadi I.[1]

[1]Dept. of Experimental Zoology and Neurobiology, University of Pécs; [2]Dept. of Pharmacognosy, University of Szeged, Hungary

Songorine (SON) is a diterpenoid alkaloid from plants of the *Aconitum* genus. Preparations of *Aconitum* roots are employed in Chinese and Japanese traditional medicine as antirheumatics, analgesics, anaesthetics, and in the treatment of various neurological disorders. SON was first isolated from *Aconitum soongaricum*. According to previous studies SON acts as a GABA receptor antagonist in rat hippocampal slices. In the present article, we investigated: (1) the effects of microiontophoretic application of SON on neurons in various brain areas (cortex, hippocampus, thalamus), *in vivo*; (2) the combined effects of SON and a $GABA_A$ receptor antagonist picrotoxin (PIC). We used a combined method of microiontophoresis and extracellular single unit recording. Four-barrelled micropipettes were used for recording neuronal activity and for microiontophoretic application of SON, PIC and control drugs. Spike sorting routines were performed on-line and off-line to ensure that data were always recorded from a single neuron. Our results show that SON caused inhibition (n=138/276, 50%) on neurons in the cortex, hippocampus and in the thalamus and PIC could block this inhibitory effects of SON (n=23/35, 66%). The effects of SON was not blocked by, a well known $GABA_B$ receptor antagonist Saclofen (n=20/27, 74%). Our results suggests, that SON seems to be a potent $GABA_A$ receptor agonist in the rat brain, *in vivo*.

P9.06 17beta-estradiol alters tonic and phasic GABAergic currents during development *in vitro*

Wojtowicz T.[1], Pytel M.[1], Mercik K.[1], Sarto-Jackson I.[2], Sieghart W.[2], Ikonomidou C.[3]

[1]Lab. of Neuroscience, Dept. of Biophysics, Wroclaw Med. Univ., Wroclaw, Poland; [2]Div. Biochem. Mol. Biol., Center for Brain Res., Vienna Med. Univ., Vienna, Austria; [3]Dept. of Pediatric Neurol., Carl Gustav Carus, Tech. Univ. Dresden, Dresden, Germany

Estrogens synthesized in the gonads and brain exert a variety of modulatory effects on structure and function of the nervous system. 17beta-estradiol (BE) affects GABAergic inhibition in adult animals but its action on GABAergic currents during development has not been elucidated. For this purpose we have investigated the impact of BE on GABAergic currents in hippocampal neuronal culture developing *in vitro*. In this model, kinetics of mIPSCs showed acceleration with age along with increased GABAAR alpha1 subunit expression, similarly as *in vivo*. Long term treatment with BE increased mIPSCs amplitudes in neurons cultured for 6–8 and 9–11 days *in vitro* (DIV) and prolonged the mIPSCs decaying phase in the 9-11 DIV group. The time needed for BE to exert its effect was approximately 48 h. Interestingly, in neurons cultured up to 11 days, treatment with this drug strongly reduced tonic conductance activated by low [GABA]. Western blotting revealed no change in expression of the considered GABAAR subunits (alpha1-3, alpha5-6, gamma2) while alpha4 and delta subunits were at detection limit. We provide evidence that BE differentially affects the phasic and tonic components of GABAergic currents in neurons developing *in vitro*. Support: PBZ-MIN-001/P05/28.

P9.07 Molecular and morphological basis of neuronal excitability in the visual cortex of albino rats

Blaszczyk W.M.[1,4], Neuhaus E.M.[2], Epplen J.T.[3,4], Hoffmann K.P.[1,4]

[1]General Zoology & Neurobiology, [2]Cell Physiology, [3]Human Genetics, [4]International Graduate School of Neuroscience, Ruhr-University Bochum, Germany

Albinism results in anatomical abnormalities like differences in retinal cell number, misrouting of visual pathways and abnormal representations of the visual field in higher areas of visual processing. Additionally, *in vitro* electrophysiological recordings imply an altered chloride reversal potential in the visual cortex of the albino Wistar rat at P28 (Barmashenko et al. 2005). This shift of the intracellular chloride concentration might lead to alterations in the $GABA_A$ mediated signalling and information processing in albinism. To explain these physiological changes, an altered expression of the inwardly directed cation-chloride cotransporter (CCC) NKCC1 or the outwardly directed CCC KCC2, or both has been suggested. In this study, the molecular basis of CCC expression has been examined by RNA expression analysis using quantitative real-time RT-PCR and quantification of protein levels by Western blot. These molecular approaches do not reveal expression differences between albino and pigmented rats in the visual cortex at P28 and P1. In addition, immunohistochemical stainings were performed on P28 rats in order to examine the allocation of CCC protein. Qualitative analysis of the staining pattern in the visual cortex as well as quantitative analysis of prelabelled pyramidal cells in layer V is applied in order to reveal if any cell specific distribution of KCC2 and NKCC1 might explain the physiological differences present in the visual cortex of albino rats.

P9.08 The gap junction mediation of hippocampal theta rhythm in anasthetized rats

Bocian R., Posluszny A., Kowalczyk T., Golebiewski H., Konopacki J.

Department of Neurobiology, University of Lodz, Poland

Gap junctions (GJs) are specialized membrane areas that serve as sites of fast intercellular communication. Numerous examples of this kind of communication have been described in different mammalian brain regions, for example, in the hypothalamus, cerebellar cortex, olfactory bulb, the retina, and hippocampus (HPC). We have demonstrated earlier the gap junction mediation of cholinergically induced theta in the hippocampal formation slices. We have showed also that electrical couplings were involved in the production of the hippocampal theta in freely behaving cats. In the present study we extended our previous observations concerning GJ involvement in mechanisms responsible for generation of theta oscillation in the limbic cortex. Specifically, we analyzed the effect of gap junction blocker quinine infusion, on the hippocampal theta rhythm recorded in urethanized rats. Quinine was administrated intrahipocampally in concentration of 50 μg/1 μl. The inhibitory effect on HPC theta amplitude and power was observed after four hours after injection. The effect was found to be partially reversible. We observed the recovery of theta rhythm with decreased amplitude and power seven to eight hours after quinine administration. Obtained results, provide strong evidence for the contribution of gap junctions communication in mechanisms of neuronal synchrony, underlying the production of the hippocampal theta in urethanized rats.

P9.09 EEG activity during intermittent hypoxia in the rat

Budzinska K., Ilasz R.

Department of Respiratory Research, Medical Research Center, Warsaw, Poland

The relation between cortical activity and respiratory response to intermittent hypoxia, a component of sleep apnea syndrome was studied in anaesthetized rats subjected to vagotomy, paralyzed and artificially ventilated. Integrated phrenic nerve activity (PHR) indicated the central respiratory activity. EEG signal was obtained from the frontal cortex. Five episodes of breathing with 11% hypoxia lasting 1.5 min were introduced every 3 minutes. Respiratory response to hypoxic exposure consisted of stimulation of PHR and subsequent decline of the response up to apnoea. The excitatory phase of respiratory response to hypoxia was accompanied by lowering of the total power of EEG. Relative EEG power increased in the delta frequency range and decreased in remaining frequency ranges examined. During strong depression of the respiratory activity or hypoxic apnoea the total power of EEG decreased strongly or almost ceased. Each episode of hypoxia caused similar respiratory and cortical effects, however in comparison to the baseline total power of EEG decreased gradually while power of the delta frequency range increased in subsequent hypoxic episodes. Recovery of EEG activity after the last hypoxia reached or exceeded the original level during about 40 minutes. It is concluded that mild hypoxia induces only modest changes in the cortical activity that increase with severity of hypoxia. Intermittent hypoxia induces long lasting changes in the cortical activity that may contribute to disorders present in sleep apnea syndrome.

P9.10 Cholinergic/GABAergic interaction in hippocampal theta production in freely moving cats

Golebiewski H., Eckersdorf B., Kowalczyk T., Bocian R., Posluszny A., Konopacki J.

Department of Neurobiology, University of Lodz, Poland

It has been histochemically revealed that in addition to cholinergic component (50%) approximately 30% of fibers forming septo-hippocampal projections are GABAergic. Cholinergic nature of hippocampal formation (HPC) theta field activity is well documented both *in vivo* and *in vitro* preparations. Furthermore, there is accumulating evidence for GABAergic involvement in neuronal mechanisms responsible for generation of HPC theta rhythm. In the present study intraseptal injection of procaine (local anesthetic) reversible eliminated carbachol induced theta activity registered from HPC in freely moving cats. Similar effect was observed when intrahippocampal carbachol injection was pretreated by intraseptal infusion of procaine. In contrast, well-synchronized theta was registered from HPC as a result of local injecion ofcarbachol and bicuculline ($GABA_A$ antagonist). The participation of cholinergic/GABAergic interaction in generation of hippocampal theta field activity in freely moving cats is discussed.

P9.11 Spectral analysis of rat cerebellar activity in an animal model of epilepsy

Grbic G.[1], Culic M.[1], Martac Lj.[1], Sokovic M.[1], Spasic S.[2]

[1]Institute for Biological Research; [2]Center for Multidisciplinary Studies, University of Belgrade, Belgrade, Serbia

We investigated spectral characteristics of cerebellar electrocortical activity in the rat model of epilepsy induced by intraperitoneal

administration of camphor oil (225–500 ml/kg). Our recent study (Grbic et al. 2006, Acta Physiol Pharmacol Serb, 42: 233–238) describes that this acute model was characterized by seizure activity (single and multi high amplitude spikes) in parietal cortex, partially suppressed in anesthetized states. Electrophysiological recordings of paravermal cerebellar activities were performed in anesthetized rats (Nembutal 35 mg/kg) before and after camphor oil administration. Spectral analysis of cerebellar signals was performed by fast Fourier Transformation after analog to digital conversion of signals at the sampling rate of 256 Hz. There was a moderate increase of the total mean power spectra of cerebellar signals (not so prominent as at the cerebral level) and the modest increase of the relative power spectra in delta frequency range, within first hour after camphor administration. The spectral changes of cerebellar activities occurred at even lower doses (225 ml/kg), also. The general importance of the cerebellar involvement in epilepsy is far from being complete, but these results widen the areas of competence of the cerebellum. This work was supported by Serbian Ministry of Science and Environmental Protection (project 143021).

P9.12 Is the dental gyrus an independent generator of *in vitro* theta rhythm?

Kowalczyk T., Golebiewski H., Bocian R., Posluszny A., Konopacki J.

Department of Neurobiology, University of Lodz, Poland

Since our first demonstration of cholinergically induced theta in the slice preparation obtained from the hippocampal formation (HPC), this *in vitro* model has been successfully used in a number of investigations concerning physiology and pharmacology of theta rhythm. In our previous studies the laminar profile of hippocampal theta rhythm recorded *in vitro* was investigated. According to these studies we hypothesized that dentate area is not capable of generating *in vitro* theta rhythm independently. In the present study we attempted to verify this hypothesis using the transected hippocampal slice preparations. The EEG activities of DG, CA1 and CA3c transected slices were induced by the continuos bath perfusion with cholinergic agonist – carbachol. The data obtained revealed that dentate area separated from CA1 and CA3c regions of hippocampal formation was not capable of generating theta rhythm during continuos cholinergic stimulation. The mechanisms underlying the generation of hippocampal theta rhythm recorded *in vitro* are discussed.

P9.13 Effects of bicuculline and muscimol injection into the area of A10 cells on hippocampal theta rhythm

Orzel-Gryglewska J., Kusmierczak M., Trojniar W.

Dept. Animal Physiology, University of Gdansk, Poland

As we found previously, inactivation of the ventral tegmental area (VTA) supresses hippocampal theta rhythm. The aim of the present study was to evaluate a possible role of GABA, one of the main transmitter in VTA, in the regulation of hippocampal activity. In the separate groups of urethane-anasthetized rats, $GABA_A$ antagonist bicuculline (50 ng/0.5 µl, n=9), or $GABA_A$ agonist, muscimol (100 ng/0.5 µl, n=10) was unilaterally injected into the VTA. Spectral analysis of hippocampal EEG was performed on 5-s samples taken from theta episodes. Peak power (Pmax) [mV, rms] and corresponding peak frequency (Fmax) were determined for standard EEG bands, delta and theta. Intra-VTA bicuculline evoked in both hippocampi continuous theta trains lasting for 35 ± 3.5 min, usually with 0 latency. In the muscimol group, in which 60-s theta episodes were elicited by tail-pinch, muscimol caused a significant decrease in the Pmax for the theta (from 115 to 57 mV) lasting up to the end of 80-min EEG recording, with simultaneous increase in delta Pmax (from 27 to 75 mV). The depression of theta Pmax was initially small but became more pronounced with every subsequent episode of theta elicited by tail-pinch and it became stronger in the ipsilateral hemisphere compared to the contralateral one 60–80 min after muscimol injection. The results indicate that VTA influence on hippocampal field activity is at least in part GABA-ergic. It may be either direct or consist in GABA modulation of activity of A10 dopaminergic neurons which project directly or *via* the medial septum to the hippocampal formation.

P9.14 The effects of muscimol and bicuculline on slow bursting activity of OPT neurons in the rat

Szkudlarek H., Zakiewicz I., Lewandowski M.H.

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

Olivary pretectal nucleus (OPT) is an important visual processing center receiving inputs not only from retina, but also from other visual system structures. The OPT is involved in coding information about illumination level and conveying this information to the pupil and probably circadian timing system. Certain OPT neurons were previously described as a slow oscillatory cells, electrophysiologically characterized by intervals of high and low activity. These cyclic changes can be observed in light and darkness. The purpose of the present study was to determine whether decreased interburst activity of the slow oscillatory OPT neurons is mediated by the GABAergic inhibitory system. Experiments were performed on Wistar rats with multibarrel electrode technique. Iontophoretic application of muscimol, $GABA_A$ receptor agonist, into the OPT caused pronounced inhibition of the electrical activity of oscillatory neurons. In the same oscillatory cells iontophoretic application of $GABA_A$ receptor antagonist – bicuculline reversed the inhibitory effect of muscimol and greatly increased the firing rate. Additionally we investigated the effect of these drugs in dark conditions and on the light-evoked responses (5 s long light pulses). Results showed that muscimol inhibited and bicuculline attenuated these responses. Obtained results clearly suggest that $GABA_A$ receptors modulate firing frequency and light-evoked responses of oscillatory neurons in the rat OPT.

P9.15 Modulation of neuronal activity in the pre-Bötzinger complex by medullary raphe neurons

Ptak K.[1], Milescu L.S.[1], Yamanishi T.[1], Zhang R.[1], Richerson G.B.[2], Smith J.C.[1]

[1]Cellular and Systems Neurobiology Section, NINDS-NIH, Bethesda, MD 20892; [2]Dept. of Physiology, Yale University School of Medicine, New Haven, CT, USA

Medullary raphe nuclei (RN) project to respiratory neurons and contain serotonin (5-HT), thyrotropin-releasing hormone (TRH)

and Substance P (SP), any of which stimulate respiration. It has been postulated that 5-HT raphe neurons are sensitive to pH and CO_2 and have been proposed to be central chemoreceptors. Here, we are investigating modulation of respiratory network by raphe neuron in 350 μm *in vitro* slice preparations from neonatal rats. We have mapped neurons immunoreactive for 5-HT. These neurons are located along the midline corresponding to n. raphe obscurus (RN). The inspiratory rhythm is perturbed by blocking 5-HT receptors, suggesting endogenous release of 5-HT and modulation of rhythm generation. Extracellular and whole-cell recordings from 5-HT neurons revealed spontaneous activity, consisting of a regular spiking pattern (1–2 Hz). Local acidification (pH 6.8) or alkalinization (pH 7.6) of RN respectively elevates or depresses the spontaneous spiking frequency of these neurons and inspiratory frequency. We determined that microinfusion of AMPA elevated the spiking activity of raphe neurons and potently increased inspiratory frequency. This augmentation of inspiratory was only partially attenuated by blocking 5-HT receptors. The elevation of inspiratory frequency that occurs under these conditions is three fold greater acidic solution has been applied. We observed that 5-HT depolarizes pre-BötC neurons, increasing their excitability and rhythmic bursting frequency. Additionally, following block of glutamatergic transmission, these neurons still depolarized due to the endogenous release of 5-HT after excitation of the RN region by elevated K^+. We conclude that: (1) endogenously released 5-HT is regulating excitability of the pre-BötC; (2) raphe neurons in the *in vitro* slices have chemosensory properties; and (3) at high levels of raphe neuron excitation, SP or possibly TRH in addition to 5-HT is released and modulate of rhythm generation.

POSTER SESSION II
ADDICTION

P10.01 Effects of delta-9-tetrahydrocannabinol on natural killer cell cytotoxicity

Badtke P., Glac W., Orlikowska A., Stojek W., Tokarski J.

Department of Animal Physiology, University of Gdansk, Poland

Delta-9-tetrahydrocannabinol (THC) is the main psychoactive component of marijuana and hashish. It is generally believed that the effects of THC and other drugs of abuse on the immune system are mainly suppressive. Here we present data indicating that in some condition THC can act not only as an immunosuppressive agent. Rats received THC by i.p. injection in a dose of 0.5 or 1 mg/kg. Natural killer cell cytotoxicity (NKCC) (51Cr - release assay), number of NK cells (identified as large granular lymphocytes, LGL) (Timonen method), leukocytes, lymphocytes, monocytes and granulocytes were evaluated in the peripheral blood and spleen. It was found that THC caused an increase of NKCC in the blood (+81%) while in the spleen NKCC remained unchanged. The number of LGL and monocytes increased in the blood (+86% and +129%, respectively) and decreased in the spleen (+39% and 34%, respectively). THC also increased the number of leukocytes, lymphocytes and granulocytes in the blood (+40%, +24% and +104%, respectively) and spleen (+24%, +34% and +31%, respectively). These results indicate that THC can evoke an increase of NK activity (cytotoxicity) as well as NK cells number (cellularity) in the peripheral blood and that recirculation of cells from the spleen not fully explains THC-induced increase in number of cells in the peripheral blood.

P10.02 Morphine regulates the level of kinesin light chain 1, a molecule involved in neuronal trafficking

Bilecki W., Wawrzczak-Bargiela A., Przewlocki R.

Dept. of Molecular Neuropharmacology, Institute of Pharmacology PAS, Krakow, Poland

Intracellular traffic is organized by a family of molecular motors, kinesins that also contribute to neuronal events required for learning and memory. A conventional kinesin is composed of two kinesin heavy chains (KHC) and two kinesin light chains (KLCs) and binds directly to microtubules. In particular KLC1 seems to be essential for proper KHC activation and targeting. Although it was known that KLC1 was expressed in neurons the specific pattern of KLC1 distribution in the brain is largely unknown. Recent data provide evidences suggesting that neuronal trafficking (e.g., axonal transport) may represent a previously unanticipated mechanism contributing to the neuronal alterations after chronic opioid abuse in humans. We have found that KLC1 level is enriched in sub-cortical regions of the brain especially in the striatum, hippocampus and amygdala that are known to be engaged in drug addiction. We have also discovered that chronic, but not single, morphine treatment lowered KLC1 level in the amygdala, frontal cortex and hippocampus but increased the expression of KLC1 mRNA as well as protein level in the striatum of C57black mice. Withdrawal from morphine elevated the KLC1 in the brain but the hippocampus (where KLC1 remained reduced) and augumented the effect of morphine on striatal KLC1 mRNA and protein. Our results provide the first evidence that chronic morphine treatment and withdrawal may specifically affect dendritic and axonal transport in the brain. Supported by KBN grant N401 066 3/1682.

P10.03 Cross-reinstatement of nicotine-conditioned place preference in rats

Budzynska B., Kruk M., Biala G.

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

In the present experiments, we employed this paradigm to study the extinction and reinstatement of extinguished nicotine conditioned place preference (CPP). In our investigations animals were initially trained to associate one distinctive environment, initially less-preferred with nicotine injection (0.5 mg/kg, i.p.) and the different environment, initially more-preferred with saline injection during three drug sessions. Following the training, animals were given a choice between the two compartments of apparatus on a drug free test day, and typically spent more time in nicotine paired environment. This acquired preference was extinguished by repeated training in the absence of the drug. After the extinction phase, injection of ethanol (0.5 g/kg, 10%, i.p.) or cannabinoid CB1 receptor agonist – WIN 55,212-2 (0.5 mg/kg, i.p.) reinstated the extinguished CPP. In the second step, in order to examine the role of calcium-dependent mechanisms in drug relapse, we investigated the effect of L-type voltage dependent calcium channel blockers: nimodipine (5 and 10 mg/kg) and flunarizine (5 and 10 mg/kg), on the expression of ethanol- or WIN 55,212-2-induced reinstatement of nicotine-conditioned place preference. We found that both antagonists, administered 15 min prior to the priming injections of ethanol or WIN 55,212-2, dose-dependently blocked the reinstatement effect of these drugs. Results of present study may contribute to modern therapy of nicotine addiction and may provide new possibilities of therapeutic use for L-type voltage dependent calcium channel blockers.

P10.04 Involvement of CB1 cannabinoid receptors in memory-related response induced by nicotine in mice

Kruk M., Budzynska B., Biala G.

Departmentof Pharmacology with Pharmacodynamics, Medical University of Lublin, Poland

In the present study, we investigated the possible implication of the cholinergic nicotinic and endocannabinoid systems in memory-related behavior using the elevated plus maze test in mice. In this test the time, in which the mice took to moving from the open arm to the enclosed arm (i.e. transfer latency) was used as an index of memory. Our results revealed that both, an acute administration of nicotine (0.1 and 0.5 mg/kg, s.c.) and an acute administration of CB1 cannabinoid receptor antagonist AM 251 (0.5; 1.0; 1.5; 3.0 mg/kg, i.p.) dose-dependently shortened the transfer latency in comparison with the saline-treated group. In turn, the acute administration of CB1 cannabinoid receptor agonist WIN 55,212-2 (0.25; 0.5; 1.0 mg/kg, i.p.) produced no significant effects in this paradigm. Moreover, we investigated the involvement of endocannabinoid system in nicotine-induced memory-related behavior. Both, AM 251 (0.25 mg/kg, i.p.) and WIN 55,212-2 (0.25 and 0.5 mg/kg, i.p.) injected 15 min prior to an acute injection of nicotine, reversed the improvement of memory induced by nicotine. Our findings indicate that both cannabinoid and cholinergic systems play an important role in consolidation of memory, and confirm the notion that there is a close relationship between these two neuronal pathways. Based on the recent hypothesis that learning and memory are essential for the development of addiction, our investigations may provide new possibilities of therapeutic use for cannabinoid CB1 receptor ligands in the treatment of tobacco addiction.

P10.05 Significance of the CB-1 receptor antagonist SR-141716 in the ethanol drinking in WHP rats

Dyr W., Ligieza J., Wyszogrodzka E., Kostowski W.

Institute of Psychiatry and Neurology, Warsaw, Poland

In this study we investigated the effects of single and repeated injections of CB-1 receptor antagonist, SR 141716 on voluntary intake of ethanol in alcohol-preferring WHP line of rats. First experiment The ethanol and food were available only during the 4 hours per day (the experimental session) while the water was present ad libitum. Rats were divided into 4 groups (n=7–8). The first group was injected (i.p.) with vehiculum (2 ml/kg 0.1% Tween 40), and the next groups were treated with CB-1 receptor antagonist, SR 141716 in the doses 2.5, 5.0 and 10.0 mg/kg. The consumption of 10% ethanol solution, water and food were measured every 60 min during 4-h experimental session. Second experiment. Throughout the duration of the second study, alcohol was available in a free-choice paradigm between the alcohol solution (10%, v/v) and water. SR 141716 in the dose 1 mg/kg was administered twice daily for 10 consecutive days. Alcohol, water and food consumption were assessed once daily before the dark phase throughout the 10 days of administration and for 20 days after the last injection. Results Single injection of all three doses SR 141716 markedly decreased ethanol intake in the WHP rats. Further, SR 141716 given twice daily (at 1 mg/kg) for 10 days, prevented the acquisition of alcohol drinking in WHP rats. The results suggest that activation of cannabinoid CB1 receptors is involved in alcohol intake and acquisition of alcohol drinking behavior in alcohol preferring line of rats.

P10.06 Opioid systems in the brain of inbred mouse strains

Gierek A., Ziolkowska B., Solecki W., Przewlocki R.

Institute of Pharmacology PAS, Krakow, Poland

Systems of endogenous opioids are implicated in the processes of reward and reinforcement, therefore the basal activity of these systems may determine vulnerability to addiction. The aim of our study was to examine association between drug dependence susceptibility and the activity of opioid systems in inbred strains of mice (C57BL/6J, DBA/2J, SWR/J and 129P3/J) which present different behavioral phenotypes in response to morphine. Marked differences in the expression of PENK and PDYN genes were found between the strains. C57BL/6J mice, the most sensitive to opiates in locomotor activity and oral self-administration paradigms, showed higher levels of PENK mRNA and lower levels of PDYN mRNA in the nucleus accumbens than the other three strains. In contrast, the reverse relationship between PENK and PDYN expression levels was observed in DBA/2J mice which are the least sensitive to morphine in behavioral tests mentioned above. The functional importance of the observed differences was further examined in DBA/2J mice. Pretreatment of these animals with the kappa opioid receptor antagonist nor-binaltorphimine, which attenuates the activity of PDYN-derived opioids, increased the locomotor response and enhanced conditioned place preference of DBA/2J mice in response to morphine. Our data suggest that expression of endogenous opioids may influence behavioral effects of the opiate administration. The lack of response to morphine in DBA2/J mice may, in part, result from high basal activity of the dynorphin system in the nucleus accumbens. Supported by EU grant LSHM-CT-2004-005166.

P10.07 Motor activity and the immune response to repeated amphetamine in rats

Glac W., Kloss G., Badtke P., Orlikowska A., Sielicka-Dudzin A., Tokarski J.

Department of Animal Physiology, Institute of Biology, University of Gdansk, Poland

The effect of repeated administration of amphetamine (AMPH) on natural killer cells cytotoxicity (NKCC) and number of large granular lymphocytes (LGL; NK cells) together with the number of lymphocytes and plasma corticosterone level was evaluated in male Wistar rats differing in locomotor reactivity to novelty (high, HR and low, LR responders). 20-day daily administration of 1 mg/kg AMPH evoked an increase in NKCC and LGL numbers in the peripheral blood. Conversely, in the spleen AMPH induced a suppression of NKCC and a decrease in NK cells. These effects showed substantial individual variability. The level of the peripheral blood NK cytotoxicity in HR rats was higher than in LRs, while the spleen NKCC was higher in the latter. The diversity of LGL number changes was similar to NKCC. Repeated AMPH cause a marked decrease in the spleen lymphocytes number, whereas the number of the blood lymphocytes number were unchanged. AMPH-induced plasma corticosterone elevation were higher in HR rats. The results obtained indicate that long-term AMPH administration can evoke an increase in NK-related cytotoxic activity in the peripheral blood. Motor activity differentiates the reactivity of NKCC to AMPH. The high behavioral reactivity rats seems to show more favourable type of the immune reactivity to AMPH than the low motor activity ones, at lease in range of anti-viral and anti-tumour immunity related to NK cells in the blood. It seems that central tone controlling motor activity exerts an essential influence on the immune system.

P10.08 Alterations of the cocaine- or food-induced relapse by serotonin (5-HT)1B receptor ligands in rats
Golda A., Filp M., Przegalinski E.

Institute of Pharmacology PAS, Krakow, Poland

Numerous data indicate that dopamine-dependent cocaine behavioral responses can be modified by serotonin (5-HT) neurotransmission. Recent findings suggest that 5-HT1B receptors are particularly involved in the reinforcing effects of cocaine (Parsons et al. 1998, J Neurosci; Cervo et al. 2002, Psychopharmacology). In the present study we used the 5-HT1B receptor antagonists SB216641 and GR127935 and the selective agonist CP94253 to evaluate the role of 5-HT1B receptors and their pharmacological stimulation, respectively, on the reinstatement of extinguished seeking behavior induced by cocaine or by food delivery in rats. To this end we employed an extinction/reinstatement task in an cocaine and food self-administration model. Pretreatment with GR127935 (10 mg/kg), or SB216641 (7.5 mg/kg), or CP94253 (5 mg/kg) reduced the reinstatement of cocaine-seeking behavior evoked by the priming dose of cocaine. Moreover, CP94253 (7.5 mg/kg) but not GR127935 or SB216641 attenuated food-seeking behavior. Our results point that 5-HT1B receptors or their pharmacological stimulation reduce a cocaine-seeking behavior suggesting that 5-HT1B receptors modulate cocaine-seeking behavior possibly through the differential effects of 5-HT1B agonists and antagonists on pre- and post-synaptic 5-HT1B receptors. Moreover, the 5-HT1B receptor agonist-induced reduction of food-induced reinstatement points to the conclusion that stimulation of these receptors decreases motivation, while the effects of the 5-HT1B receptor antagonists can been distinguished from the other goal-directed responses.

P10.09 Involvement of 5-HT1A receptors in cocaine treatment-related impairment of LTP
Grzegorzewska M.[1], Hess G.[1,2]

[1]Institute of Pharmacology PAS; [2]Jagiellonian University, Krakow, Poland

Changes in the expression of polysialylated neural cell adhesion molecule (PSA-NCAM) may reveal plastic changes occurring in the adult brain. Single administration of cocaine decreases the expression of PSA-NCAM which may, potentially, influence LTP in the dentate gyrus. 5-HT1A receptor may be an important target for the development of pharmacotherapies for the treatment of cocaine abuse. To check the involvement of 5-HT1A receptors in the cocaine effect on LTP, rats aged 6 to 8 weeks received cocaine (15 mg/kg, i.p.) or saline. The third group received N-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-N-2-pyridinyl-cycylhexanecarboxaminde maleate (WAY 100636), an antagonist of 5-HT1A receptors (0.4 mg/kg, i.p.), 20 min before cocaine. Brain slices were prepared 2 days later. Field potentials were evoked by stimulation of the medial perforant path and recorded from the middle molecular layer of the dentate gyrus. LTP was induced by high-frequency stimulation (HFS, 100 Hz, 1 s; 3 × every 5 min). While in slices obtained from control rats (n=30) a stable LTP (128 ± 5%) was maintained for 90 min, in slices from rats receiving cocaine LTP was impaired (109 ± 6%, n=22, P<0.05, ANOVA). WAY 100635 reversed LTP to control level (130 ± 7%, n=28, P<0.05, cocaine vs. WAY 100635+cocaine). Thus, single dose of cocaine attenuates LTP in the dentate gyrus. This effect was reversed by WAY 100635 administration which suggests the involvement of 5-HT1A receptors in the inhibitory effect of cocaine on LTP.

P10.10 Effects of morphine on gene expression in the striatum
Korostynski M., Piechota M., Kaminska D., Solecki W., Przewlocki R.

Department of Molecular Neuropharmacology, Institute of Pharmacology PAS, Krakow, Poland

Whole-genome analysis of morphine-induced changes in brain transcription of mouse strains with different opioid-related phenotypes provides an opportunity to evaluate the associations between gene expression and behavioral response to the drug. We have analyzed transcriptional effects of single (20 mg/kg, s.c.) and repeated (10–40 mg/kg, s.c.) morphine administration in selected inbred mouse strains (129P3/J, DBA/2J, C57BL/6J and SWR/J). Using microarray-based (Affymetrix Mouse Genome 430v2) gene expression profiling in striatum, we found 3457 strain-specific and 661 morphine-responsive genes (FDR<1%). Through ontological classification, we have linked particular groups of genes to biological functions, including metabolism, transmission of nerve impulse and cell-cell signaling. We identified numerous novel morphine-responsive genes (e.g. Sgk3 and Cebpd), as well as a number of transcripts with strain-specific changes in expression (e.g. Hspa1a and Fzd2). Moreover, transcriptional activation of group of genes (e.g. Zbtb16 and Tsc22d3) was identified as mediated *via* glucocorticoid receptor (GR) and associated with behavioral effects of morphine. Using gene expression profiling of morphine effects in striatum, we were able to reveal multiple physiological factors with potential influences on opioid-related phenotypes and to relate gene networks to this complex trait. Furthermore, the results suggest the involvement of GR-regulated genes in mediating response to morphine. Supported by EU grant LSHM-CT-2004-005166.

P10.11 *In vitro* studies of the interactions between opioids and cannabinoids
Kryst J.

Dept. of Molecular Pharmacology, Institute of Pharmacology PAS, Krakow, Poland

Opioid and cannabinoid agonists produce similar intracellular effects by binding to Gi/o protein-coupled receptors. Interactions between opioids and cannabinoids were described in models of drug dependence. The aim of this study was to investigate molecular mechanism underlying cannabinoid-opioid interactions *in vitro*. We used HEK 293 cells double transfected with mu and CB1 receptors to study the influence of pretreatment with morphine or cannabinoid agonist WIN 55,212-2 on cAMP level and activation of MAPK pathway mediated by both receptors. In naive HEK MOR/CB1 cells, acute morphine and WIN 55,212-2 produced 80% inhibition of forskoline-stimulated cAMP level. Short-term (6 h) pretreatment with morphine abolished these acute effects of both agonists, while WIN 55,212-2 preincubation reduced the responsiveness of CB1 receptor. Acute stimulation of mu and CB1 receptors led to activation of MAPK pathway in naive HEK MOR/CB1 cells. Both 6 h and 72 h pre-exposure to morphine completely abolished the effects of acute morphine and attenuated the effects of acute WIN 55,212-2 to about 60%. While 6 h pretreatment of the cells with WIN 55,212-2 reduced only the acute effects of cannabinoid agonist, 72 h preincubation abolished the responsiveness of both receptors. Our data suggest the asymetric cross-desensitiza-

tion in HEK MOR/CB1 cells between opioids and cannabinoids. While short-term exposure to morphine reduced the CB1 receptor responses, cannabinoid agonist influenced the mu opioid receptor activation only after a long-term incubation. Supported by EU grant LSHM-CT-2004-005166.

P10.12 Development of behavioral disorders due to intravenous fentanyl self-administration in rats

Maglakelidze G., Naneishvili T., Noselidze A., Chkhvishvili N., Dashniani M., Burjanadze M., Asanidze T.

I. Beritashvili Institute of Physiology, Tbilisi, Georgia

Determination of the factors for development of compulsive behavior disorders due to intravenous self-administration of psychotropic addictive drugs will promote comprehension of neural mechanisms of addictive behavior. The aim of experiment was to investigate the role of emotion and amount of instrumental responses for acquisition substance-related behavior disorders and development compulsive behavior induced by drug administration. Behavioral model of intravenous fentanyl self-administration has been used under fixed-ratio schedule in 4 groups of rats. Intravenous drug self-administration responses were acquired effectively in rats receiving a relatively higher single doses of fentanyl, while intravenous drug self-administration compulsive behavior was demonstrated better in the rats, which pressed active lever reliably frequently in the first series of experiment. These results indicate the importance of emotion defining drug seeking behavior for the development of behavioral disorders at the initial stage of acquisition induced by intravenous self-administration of psychotropic addictive drugs and the significance of amount of performed active lever responses for developing compulsive behavior. A view has been set forth about the existence of nervous mechanisms for instrumental response performance determining nervous circuits separately from the nervous mechanisms reinforcing incentive salience of environmental stimuli.

P10.13 Effect of alcohol on rat brain MAO and immunity

Moroz V.[1], Tsydzik V.[1,2]

[1]Grodno Medical University, Belarus; [2]Eastern New Mexico University, NM, USA

Brain monoamine oxidase (MAO) plays an important role in amine neuromediators catabolism. We determined histochemically by own method the activity and the isozymic structure of MAO in neurons of various neuromediator systems, and also in number of brain barrier structures as well as in glial cells of not purebred white male rats after single alcohol administration (i.p. in dosage 3.5 g/kg) and long time (5 g/kg i.p. twice daily – 5 day) alcohol administration (according to Majchrowicz 1973). Some data of the immune system condition were determined also. We have found out significant changes of MAO condition after single acute alcohol administration. In 5-HT neurons of rat brainstem MAO B activity was increased and in some noradrenergic neurons (A1) MAO A activity was decreased. We also have found an increase in total amount of leucocytes with a decrease in the relative amount of lymphocytes. After chronic alcoholization changes in MAO activity were much bigger. In some neurons and non-neuronal cells the activity of MAO was decreased. In contrast in ependymocytes of the fourth ventricle it was increased. The depressive effect on MAO activity was evident until the 7th day of withdrawal. MAO A inhibitor clorgyline and MAO B inhibitor deprenyl had protective effect in most cells structures. Thus, single acute alcohol administration results in no significant infringements of brain MAO but strongly expressed peripheral inflammation syndroms. Forced alcohol administration has much bigger depressive effect on MAO condition.

P10.14 Analysis of regulatory elements in promoters of genes regulated by morphine in mouse brain

Piechota M., Korostynski M., Przewlocki R.

Dept. of Molecular Neuropharmacology, Institute of Pharmacology PAS, Krakow, Poland

Acute administration of morphine alters profile of gene expression in the brain. These alterations contribute to neuronal plasticity related to long-term changes in phenotype, including development of opioid dependence and addiction. To identify factors mediating transcriptional response to morphine we have applied an integrated approach combining gene co-expression and evolutionary conservation of regulatory regions. Promoter sequences were identified by bl2seq alignment between 5' upstream sequences of mouse and human orthologous genes. Identification of transcription factor binding sites (TFBSs) was performed using Perl TFBS module and matrices from JASPAR database. Recognition of associative networks between the transcripts was achieved by analysis of co-expression in the panel of BXD mouse strains using WebQTL. Substantial changes in expression of Arc and Sgk in response to morphine were previously reported and these genes were selected as the markers of morphine-activated processes. Using the trait correlation tool we established lists of genes co-expressed with Sgk (e.g., Tsc22d3, Nfkbia and Plekhf1) or Arc (e.g., Fos, Egr1 and Nr3a1). The obtained genes were further inspected for their transcriptional response to morphine using qPCR. Majority of the transcripts were found to be morphine regulated. Analysis of TFBSs in promoters of genes associated with Arc revealed over-representation of binding sites for SRF and CREB. Our results provide insights into the molecular mechanisms of morphine action. Supported by KBN grant PBZ-MNiI-2/1/2005.

P10.15 Behavioral opioid-related phenotype in three inbred strains of mice

Solecki W., Kubik J., Krowka T., Przewlocki R.

Dept. of Molecular Neuropharmacology, Institute of Pharmacology PAS, Krakow, Poland

Sensitivity to several effects of morphine in mice is determined, at least in part, by genotypic factors. Inbred strains of mice exhibit behavioral differences in their response to morphine, providing a useful tool to study the role of genotype in opioid-related phenotype. Here we report a behavioral morphine-related phenotype of three inbred mouse strains. We studied acute effects of morphine measuring its analgestic, hypothermic and locomotor activatory properties in C57BL/6J, DBA/2J and SWR/J mice. In addition, we evaluated long-lasting impact of morphine administrated repeatedly on mouse behavior in locomotor sensitization, tolerance and physical dependence studies as well as morphine rewarding effects in conditioned place preference paradigm (CPP). Our results indicate that there exist strain-specific differences in behavioral effects of morphine. C57BL/6J mice are more sensitive to analgestic, locomotor activato-

ry but not hypothermic properties of morphine in comparison to DBA/2J and SWR/J mice. C57BL/6J mice are also more prompt to develop analgestic tolerance, as well as locomotor sensitization. In addition, C57BL/6J mice develop greater physical dependence, and display morphine CPP which seems to be independent of cognitive load of this task. In conclusion, we propose that the C57BL/6J strain of mice represent a genotype which is more sensitive not only to acute behavioral effects of morphine but also to long-lasting behavioral phenomena associated with adaptation to chronic morphine treatment. Supported by EU grant LSHM-CT-2004-005166.

P10.16 Does pre-exposure to methadone modify locomotor and brain c-Fos responses to morphine in rats?

Taracha E.[1], Chrapusta S.J.[2], Lehner M.[1], Skorzewska A.[1], Maciejak P.[1,3], Szyndler J.[3]

[1]Institute of Psychiatry and Neurology; [2]Medical Research Center PAS; [3]Medical University of Warsaw, Warsaw, Poland

Methadone is commonly used in substitution therapy of heroin addicts, hence its potential for modifying addict reaction to opiates is of clinical interest. We compared the effects of pre-exposure of rats to morphine and methadone on locomotor activity and CNS neuronal activation (as assessed by c-Fos expression) responses to morphine challenge given two weeks after the pretreatment. The morphine challenge revealed sensitization of the effect only in the morphine-pretreated rats. Some brain regions (basolateral amygdaloid nucleus, dorsal striatum) showed robust morphine-induced c-Fos activation that was unaffected by the pretreatments tested. Some other regions (nucleus accumbens shell and core, paraventricular thalamic nucleus, centrolateral striatum and some layers of motor and somatosensory cortices) showed negligible c-Fos activation in drug-naive rats; this response was considerably enhanced by morphine pretreatment only, which effect may be related to the emergence of opiate addiction. A similarly negligible c-Fos response in layers IV and VI of the somatosensory cortex and layer VI of the insular cortex of the drug-naive rats was enhanced both by morphine and methadone pretreatment; the similarity of methadone and morphine pretreatment effects in the latter cortical regions may be of importance for the therapeutic utility of methadone.

P10.17 Regulation of extracellular signal-regulated kinases activity by morphine in mouse brain

Wawrzczak-Bargiela A., Bilecki W., Przewlocki R.

Department of Molecular Neuropharmacology, Institute of Pharmacology PAS, Krakow, Poland

Opioid addiction leads to adaptative molecular changes in the brain that involve the extracellular signal-regulated kinases (ERK1 and ERK2). It was shown recently that ERK1 knockout mouse manifested enhanced behavioural responses to the rewarding properties of morphine. However, the specific pattern of alterations of ERK1 and ERK2 phosphorylation (required for ERKs activity) in the brain following morphine treatment has not been studied yet. We have studied the effect of acute and prolonged morphine administration on phosphorylation changes of ERK1 and ERK2 in the frontal cortex, striatum including nucleus accumbens, hippocampus and the amygdala that are known to be engaged in drug addiction. We observed that chronic administration of morphine increased phosphorylation of ERKs in striatum, frontal cortex and hippocampus and decreased ERK phosphorylation in amygdala of C57Bl/6 mice. Spontaneous withdrawal (24 hours) augmented increased ERK1 and ERK2 activity. Moreover, in the striatum and nucleus accumbens the phosphorylation of ERK1 and ERK2 remained elevated even 10 days after last injection of morphine. Interestingly, the observed changes in phosphorylation for the most part affected ERK2 and altered phosphorylated ERK2/phosphorylated ERK1 ratio. Our results indicate that although activity of both ERK1 and ERK2 are affected during morphine treatment it is the ERK2 that may play the crucial role in opioid addiction. Research supported by statutory funds from the Ministry of Science and Higher Education.

P10.18 Cocaine- and tiagabine-induced changes in alpha-1-adrenoreceptors mRNA

Witarski T., Nawrat D., Roman A., Kowalska M., Nalepa I.

Department of Biochemistry, Institute of Pharmacology PAS, Krakow, Poland

Cocaine is a powerfully addictive stimulant and inhibitor of monoamins reuptake. Our recent data showed that the cocaine-induced behavioral sensitization is accompanied by changes in the alpha-1-adrenergic receptors (alpha-1 ARs) density in some regions of rat brain. The alpha-1 AR family consists of three receptor subtypes, alpha-1A, alpha-1B, alpha-1D. Here we investigate how single doses of cocaine and GABA-mimetic, tiagabine, (injected separately or jointly) affects the alpha-1 AR subtypes mRNA in rat brain. Male Wistar rats received single ip injections of saline, cocaine (10 mg/kg), tiagabine (10 mg/kg) or tiagabine given 2 h before cocaine and 24 h later the expression of alpha-1A, alpha-1B, alpha-1D ARs mRNA was assessed in amygdala, nucleus accumbens (NAc), prefrontal cortex and hypothalamus using quantitative RT-PCR method. Changes in alpha-1 AR were found exclusively in NAc where cocaine increased alpha-1B AR mRNA (by 41% vs. saline) and the effect was annulled by tiagabine. Moreover, the inhibitory effect of tiagabine (administered either separately or jointly with cocaine) was observed in case of alpha-1D AR (decrease by 54% vs. saline) though cocaine alone induced no change. Our results suggest that alpha-1B AR in NAc is specifically engaged in the mechanism of cocaine action and show the interaction between GABA and noradrenergic systems at alpha-1 ARs level. Supported by a grant 24/IV/2005 from POLPHARMA Foundation For Development of Polish Pharmacy and Medicine.

P10.19 Effects of morphine on immediate-early gene expression in C57BL/6 and DBA/2 strains of mice

Ziolkowska B., Korostynski M, Piechota M., Kaminska D., Przewlocki R.

Institute of Pharmacology PAS, Krakow, Poland,

C57BL/6 and DBA/2 mouse strains differ markedly in their behavioural reactions to morphine. In search of molecular bases of these differences, expression of immediate early genes (IEG) in response to morphine was studied in the striatum of C57BL/6 and DBA/2 mice. A time-course experiment using RT-PCR demonstrated greatest changes in IEG expression at 4 h after an acute morphine injection. An analysis of microarray results (Affymetrix GeneChip®Mouse Genome 430 2.0) for striatal tissue was per-

formed in both strains injected acutely or chronically with morphine. Significant effects of the treatment were detected for c-fos, c-jun, junB, egr4, arc, cox-1, β-inhibin, BDNF, mkp-1, homer1, cyclin L1 and L2. Hierarchical clustering analysis suggests that c-fos, junB, egr1, egr2, egr4, NGFIB, arc, homer1, mkp-1 and β-inhibin are regulated similarly in the striatum. After an acute morphine injection, these genes were up-regulated to a greater extent in DBA/2 than in C57BL/6 mice. After the last of the chronic morphine doses, IEG induction was smaller than after a single injection in DBA/2 mice and greater in C57BL/6 mice. These results indicate that morphine causes up-regulation of several IEG in concert in the striatum, which may contribute to long-term neuroadaptations produced by prolonged opiate use. The changes in the response intensity with repeated injections suggest that DBA/2 mice develop transcriptional tolerance to morphine, while in C57BL/6 mice the molecular responses seem to be sensitized. Supported by grant 2 P05A 072 29.

P10.20 Alcohol as a modulator of steroids in peripheral blood in rats

Oczkowski M.[1], Dyr W.[2], Kostowski W.[2], Gromadzka-Ostrowska J.[1]

[1]Dept. of Dietetics, Faculty of Human Nutrition and Consumer Sciences, Warsaw Agricultural University; [2]Dept. of Pharmacol., Institute of Psychiatry and Neurology, Warsaw, Poland

The aim of this work was to evaluate the impact of short-term ethanol intake on peripheral plasma adrenal and gonadal hormone concentrations in two lines of rats: WHP-alcohol preferring and WLP-alcohol non preferring. Adult male rats were divided into two groups (WLP n=9;WHP n=9). Five animals in each group were treated with 10% ethanol for 21 days, using a free choice procedure with water. Plasma cholesterol (Ch) level, testosterone (T), dehydroepiandrosterone (DHEA) and corticosterone (Cs) concentration were determined by enzymatic and radioimmunoassay methods, respectively. There were no differences in food intake and body mass gain between WHP and WLP rats. WHP rats had higher concentration of Ch, T, and DHEA (P<0.05). Cs plasma levels in WHP and WLP rats were similar. The level of Ch was decreased in the WHP-treated rats with comparison to WHP-non treated rats. The same, the level of T was lower in WHP-treated vs. in non treated. Decreasing effect was observed in the level of Cs in alcohol drinking WHP vs. in non-drinking. Ethanol intake had no effect on DHEA. Values of WLP rats drinking and not receiving alcohol were lower, but with no statistical differences. In summary, we have demonstrated that ethanol intake modulates steroid concentrations in WHP line rats, which may be a useful animal model evaluating ethanol's effect on hormonal regulation.

HUMAN BRAIN IMAGING AND NEUROPSYCHOLOGY

P11.01 fMRI study of individual differences in working memory capacity

Binder M., Urbanik A., Sobiecka B., Kozub J.

Jagiellonian University, Krakow, Poland

We studied neural correlates of individual differences in working memory capacity using fMRI and a verbal working memory task. Sixteen subjects took part in this study – eight with high working memory capacity and eight with low working memory capacity. They were selected from 56 subject sample on the basis of the working memory operation span task results. The experimental task used during fMRI session required subjects to decide whether probe letter belonged to the previously presented memory set. In all subjects pattern of activation covered ventrolateral prefrontal cortex (with stronger left activation), lateral temporal regions, primary visual areas, primary motor cortices, and supplementary motor area. Direct comparison of both working memory capacity groups revealed stronger activation in bilateral dorsolateral cortex in the high working memory capacity group, and stronger activation in bilateral motor cortices, visual cortices and left ventrolateral prefrontal cortex in the low working memory capacity group. These results demonstrate that both groups employed different neural mechanisms when performing experimental task. Activation of dorsolateral cortex region suggest that high working memory capacity group relied more on performance strategies. On the other hand, low working memory capacity group relied more on short-term memory processing (activation of visual and motor cortex), and therefore was more prone to the influence of interference (evidenced by the activation of left ventrolateral prefrontal cortex).

P11.02 Attention engagement operation, pulvinar activity, and diurnal variability: fMRI study

Fafrowicz M.[1], Golonka K.[1], Marek T.[1], Mojsa-Kaja J.[1], Tucholska K.[1], Oginska H.[2], Gatkowska I.[3], Kozub J.[4], Sobiecka B.[4], Urbanik A.[4], Orzechowski T.[5]

[1]Dept. of Neuroergonomics, Institute of Applied Psychology; [2]Dept. of Ergonomics and Effort Physiology, Collegium Medicum; [3]Chair of Computational Linguistics ; [4]Chair of Radiology, Collegium Medicum, Jagiellonian University; 5 Dept. of Automatics, University of Science and Technology, Krakow, Poland

The orienting attention network consists of three neural structures. The parietal lobes disengage attention from its current focus. The superior colliculus moves the spotlight of attention to the area of the cue. Finally, the pulvinar engages attention, selects, and enhances the contents of the attended object or area for processing. Effect of the time of day on the attention engagement operation was investigated. The study was performed with 6 right-handed, male volunteers (aged 25–36) who underwent semi-constant routine protocol. The covert orienting paradigm versus rest condition were used. The activity of pulvinar was measured as an indicator of the engagement operation in block design fMRI study. The measures were taken in five different times of day, every four hours starting from 6 AM. The data were acquired with a GE Signa 1.5-T scanner. The eye movement registration Jazz Multisensor system was used to control the position of eye. The diurnal effect on the efficiency of the attention engagement operation for the pulvinar activity was analyzed (SPM software) and discussed. Research was supported by grant from the Polish Ministry of Science and Higher Education (N106 034 31/3110) (2006-2009).

P11.03 Switching handedness: fMRI study of motor function lateralisation

Gut M.[1], Urbanik A.[2], Forsberg L.[3], Binder M.[4], Rymarczyk K.[1], Sowiecka B.[2], Grabowska A.[1]

[1]Nencki Institute of Experimental Biology PAS, Warsaw; [2]Collegium Medicum; [4]Institute of Psychology, Jagiellonian University, Krakow, Poland; [3]Karolinska Institute, Stockholm, Sweden

Some individuals report the experience of pressure to switch their hand preference from left to right. The question follows whether that leads to changes in brain organization toward the pattern shown by right-handers or whether they preserve features of brain organization characteristic of left-handers. Fifty-two subjects participated in fMRI study (consistent right- and left-handers [RH, LH] and two groups of subjects forced to switch their left-handedness toward the right side). Subjects performed two types of tasks with either hand in sequence. The results showed that in RH there was a predominance of the left-hemisphere activation. In LH this pattern was reversed. The switched groups showed no such volumetric asymmetry. Increasing of complexity of motor activity resulted in an increase in the volume of activated areas and involvement of ipsilateral activations especially in the dominant hemisphere. In RH and LH movements of the preferred hand activated mainly the contralateral hemisphere, whereas movements of the non-preferred hand resulted in a higher involvement of the ipsilateral one. This suggests that in strongly lateralized subjects the preferred hand is controlled mainly by the dominant hemisphere, whereas the non-preferred hand is controlled by both hemispheres. In switched subjects the result of handedness shift corresponds with pattern of ipsilateral activation asymmetry. It suggests that ipsilateral control of motor function is critical for hand motor domination.

P11.04 Functional dissociation within the reward system in humans

Jednorog K.[1], Szatkowska I.[1], Marchewka A.[1], Wolak T.[2], Bogorodzki P.[2]

[1]Nencki Institute of Experimental Biology PAS; [2]Institute of Radioelectronics, Warsaw University of Technology, Warsaw, Poland

An important brain function is to evaluate the motivational significance of ongoing events and to use this information to adapt behavior. Functional neuroimaging studies of reward processing have identified a number of brain areas that are activated by primary or secondary reinforces. The present study investigated how activity in these areas is modulated by the rewarding value of cognitive task. Fourteen healthy volunteers were scanned during the performance of a rewarded 2-back task in which the financial reward value varied between task blocks. Two distinct patterns of responding in the reward-sensitive regions were observed. Medial orbitofrontal cortex (BA 11) showed a linear increase in response with increasing reward value. In contrast, several basal ganglia areas (putamen, caudate, globus pallidus), insula, and medial prefrontal regions (BA 10, 32, 24, 25) responded nonlinearly, such that response was enhanced for the lowest and highest reward values relative to midrange. The results show functional dissociation in response patterns within human reward system. Medial orbitofrontal cortex seems to represent absolute magnitude of the rewards, whereas the other reward-sensitive regions seem to respond to relative, rather than absolute, values of rewards. This work was supported by the grant 2 P05B 060 27 from the Polish State Committee for Scientific Research.

P11.05 fMRI study of emotional processing in males and females

Kuniecki M.

Dept. Psychophysiology, Inst. of Psychology, Jagiellonian University, Krakow, Poland

In the following fMRI study, we examined the sex differences in processing of emotionally loaded visual information. In the positive condition, explicit comparison of men and woman yielded more pronounced activation in occipital cortex in the male group, and right superior temporal gyrus with bilateral parahippocampal gyri activation in female group. In the positive condition, comparison of male and female sub samples showed stronger activations in left insula, right temporal gyrus and left medial frontal gyrus in males, and thalamus along with bilateral orbital cortex in females. Activation of bilateral occipital cortex proves enhancement of visual processing of emotional slides as compared to neutral checkerboards. This might be attributed to top–down processes originating in the frontal areas of the cortex. In the case of positive slides, the more pronounced activation of occipital cortex in male subjects indicates that they allocate more attentional resources to the analysis of highly arousing positive stimuli. Common activations of insular cortex in the negative condition are probably related to autonomic arousal accompanying the watching of emotional content, as well as to feelings of disgust. This activation was more pronounced in males. The activation of the thalamus along with orbital cortex in females might be indicative of the stronger activation of the pathway leading from amygdala to the frontal lobes *via* thalamus.

P11.06 The effect of emotion on false recognition: An fMRI study

Marchewka A.[1], Grabowska A.[1], Nowicka A.[1], Brechmann A.[2], Scheich H.[2]

[1]Nencki Institute of Experimental Biology, Department of Neurophysiology, Warsaw, Poland; [2]Leibniz Institute for Neurobiology, Center for Learning and Memory Research, Magdeburg, Germany

False recognition is a common memory distortion which occurs in everyday life and can be induced in laboratories. We investigated neuronal mechanism underlying false recognition of emotionally negative and neutral pictures. The experiment consisted of two phases – a study phase and a test phase. During the study phase subjects were presented with a set of emotionally negative and neutral pictures (International Affective Picture System) in divided visual field paradigm. In the test phase subjects performed an old/new judgment task on stimuli from the study phase mixed with new ones. Behavioural results revealed that subjects significantly more often made false recognition (i.e. called new items old ones) in case of emotionally negative material than for emotionally neutral. This effect was more pronounced when stimuli were addressed to the LVF/right hemisphere in comparison to the RVF/left

hemisphere presentation. In line with those results fMRI data showed greater activation for falsely recognised emotionally negative material in comparison to neutral ones in the right hemisphere (frontal lobe, BA 47, 9). Moreover, significant activation related to false recognition was present only in the case of left visual field presentation. This study showed that emotional valence of stimuli increase the likelihood of making false memory recognition. It revealed also that the right hemisphere is more susceptible to false memory.

P11.07 Region of interest analysis of fMRI studies with fluid dynamics based segmentation

Wolak T.[1,2], Kuriata R.[1], Orzechowski M.[1], Bogorodzki P.[1], Piatkowska-Janko E.[1]

Warsaw University of Technology, [1]Institute of Radioelectronics, Medical and Nuclear Electronics Divi; [2]International Center of Hearing and Speach, Functional Imaging Laboratory, Warsaw, Poland

The strategy for the segmentation of brain structures from MR volumetric images using digital brain atlas and fluid dynamics based transformation will be presented. The purpose of this work is to create a tool for region of interest (ROI) analysis of functional Magnetic Resonance Images. Common used methods of fMRI (functional MRI) data analysis are based on hot spots activations in whole brain after transformation of all patients data into normalized space. This approach do not allow to compare shapes and sizes of anatomical structures as well as do not allow to compare activations within the structures. Method presented in this paper enables to do individual brain segmentation in order to find relations between activations in anatomical or functional regions. This approach provides two main advantages over a normal whole-volume mapping approach, increased statistical power and spatial correspondences across subjects. The ROI-based analysis approach is statistically powerful because only a small number of a priori specified ROIs/voxels are analyzed. In addition, this approach provides homologue anatomical or functional areas across subjects, which allows a statistical analysis across subjects without spatial uncertainties. As a result it is possible to use the regional activation information with SEM (Structural Equation Modeling) or DCM (Dynamic Casual Modeling) methods to find any relations between selected structures.

P11.08 General intelligence and temporal control of motor tasks

Dreszer J.[1,2], Szelag E.[1,3], Osinski G.[2], Gorski D.[2]

[1]Laboratory of Neuropsychology, IBD PAN, Warsaw; [2]Nicolaus Copernicus University, Torun; [3]Warsaw School of Social Psychology, Warsaw, Poland

Temporal information processing seems to be more related to the general intelligence level than classical measures of mental speed. However, the relationship between intelligence and temporal control of movement remains poorly understood. On the basis on this assumption, the present study aimed at testing an association between subjects' intelligence and temporal control of repetitive finger movements. Fifty 15-years-old volunteers were studied and classified into two groups, according to their level of fluid intelligence (The Raven's AMP Test): superior- (SI) and average-intelligent (AI) individuals. Temporal aspects of motor control were investigated with two finger-tapping tasks, performed in a maximum or in personally chosen tempi. Using a standard analysis of linear statistics and nonlinear elements for reconstruction of dynamical properties of hypothetical 'internal clock', the results showed significantly faster performance on the maximum than personal tempo in both groups. Moreover, SI tapped faster than AI. A clear dissociation between groups was found for the maximum tempo. The AI tapped faster and less variable with the right hand than with the left one. This relationship disappeared in the SI. Generally, results support the notion of higher rate of 'internal clock', suggest also the involvement of the right cerebral hemisphere both in information processing and movement control in SI individuals. Supported by grant nr 1H01F09430.

P11.09 Procedural and declarative memory deficits in Parkinson's disease

Gawrys L.[1], Szatkowska I.[2], Grabowska A.[2], Friedman A.[3], Kaczmarek L.[1]

[1]Laboratory of Molecular Neurobiology; [2]Laboratory of Psychophysiology, Nencki Institute of Experimental Biology PAS; [3]Department of Neurology, Medical University, Warsaw, Poland

This study compared the memory functions of 19 patients with Parkinson's disease (PD) with 21 age-matched control subjects in order to investigate if degeneration of basal ganglia observed in PD affects implicit skill learning. To this end two procedural tasks: mirror reading (MR) and serial reaction time (SRT) were employed. We found no differences between PD patients and control group in learning of visuoperceptual skill in MR task. In the case of sequence learning measured in SRT task PD patients showed reduced acquisition of the skill. In the present research we also examined executive functions level in aim to assess possible involvement of prefrontal area dysfunction in the performance of procedural tasks. We found correlation between MR results and perseveratives scores from Wisconsin Card Sorting Test (WCST) in PD patients group. In contrast we did not find any correlation between WCST scores and SRT performance. Those results support hypothesis that implicit sequence learning depends on integrity of basal ganglia.

P11.10 The effects of important stimuli on irrelevant cortical activity

Michalski A., Milner R., Gierych E.

Nencki Institute of Experimental Biology PAS, Warsaw, Poland

Threshold regulation hypothesis (1) was tested using event-related potentials in humans. According to this hypothesis, the income of important stimulus inhibits irrelevant cortical activity. Two stimuli were presented in random order. The subjects were asked to count one of them mentally. Counted (target) stimuli evoked P300 potential. Cortical responsiveness after target and non-target stimuli was evaluated by measuring responses to additional probing stimuli. The experiments showed that responses to probes were inhibited after target presentation (2). This inhibition however, could be observed only when target, non-target and probe stimuli were simple (blinks of LED diodes). When complex images were used as stimuli, no clear

inhibitory interactions were found. In separate series of experiments, additional irrelevant cortical activity was produced with the visual noise or the movie in the background (3). The effects of such stimulation were reduced after presentation of target stimuli. However, the interaction between the effects of the type of incoming stimulus (target or non-target) and the effect of the background stimulation was significant only for the movie and only in Pz recordings (where P300 waves are usually the biggest). The implications of such findings for threshold regulation theory are discussed. 1. Elbert T and Rockstroh B (1987) J Psychophysiol 4: 317–333. 2. Michalski A (2001) Acta Neurobiol Exp (Wars) 61: 93–104. 3. Milner R, Michalski A (2003) Acta Neurobiol Exp (Wars) 63: 337–346.

P11.11 Temporal training as a new method of aphasia therapy

Szymaszek A.[1,2], Szelag E.[1,2], Lewandowska M.[1], Seniow J.[3], Fink M.[4], Ulbrich P.[4], Pöppel E.[4]

[1]Laboratory of Neuropsychology, Nencki Institute of Experimental Biology; [2]Warsaw School of Social Psychology; [3]Institute of Psychiatry and Neurology, Warsaw, Poland; [4]Generation Research Program, Human Science Center, Bad Tölz, Germany

Several studies have reported the deficits in temporal information processing (TIP) in aphasics who displayed elevated temporal order threshold (TOT). The present study aims at testing whether speech comprehension deficits in aphasic patients can be ameliorated by the specific temporal training. In 25 aphasics auditory comprehension was assessed on the phonological level, using Token Test and Phoneme Discrimination Tests, whereas the TOT was measured with (1) monaurally presented two 1 ms clicks and (2) binaurally presented two 10 ms tones (400, 3 000 Hz). All patients participated either in the specific temporal training aimed at improving sequencing abilities or in control training aimed at volume discrimination. The temporal training yielded the improved TIP, moreover, there was a transfer of improvement to the language domain which was untrained during the applied training. In contrast, the control training improved neither sequencing abilities nor speech comprehension in aphasic patients. These results are in accordance with previous data by Paula Tallal's group which showed language improvements in children following the temporal training. Supported by the KBN grant No PBZ-MIN/001/PO5/06 and BMBF grant 01GZ0301.

P11.12 Right and left hand performance in bimanual adaptation to visuomotor rotation in pointing movements

Weigelt C.

German Sport University, Physiology and Anatomy, Cologne, Germany

Objective: To assess potential interlimb differences and coupling during bimanual pointing during a visually induced rotational distortion. Methods: Targets were presented around a start circle in a horizontal plane at eight equidistant locations. Right-handed subjects (n=12) were asked to quickly and directly move their finger(s) from the start position to the shown target and back again. During baseline (BL) and retention (R) condition, pointing performance was assessed separately and simultaneously in both hands (LH, RH) without cursor feedback. Two hundred fifty-six movements were performed under a visual distortion condition (VDC; +30°). Throughout VDC, on-line feedback was provided about the cursor position. Temporal (RT, t2Vmax, MT) and spatial (initial directional error) parameters were determined for all experimental phases. Correlation coefficients were calculated to detect the strength of bimanual coupling. Results: Baseline performance did not differ between hands. During VDC LH achieved higher initial spatial accuracy expressed as constant error of the initial directional error when compared with RH's performance. LH MT was longer than MT for RH. No hand differences emerged for RT. Correlation coefficients were highest in RT, followed by MT and finally by t2Vmax. VDC did not affect the temporal coupling between hands but spatial coupling initially increased during early adaptation and then decreased with practice. Spatial coupling in retention tests was higher than during pretests. Conclusion: Simultaneous bimanual adaptation to visual rotation seems to increase spatial coupling between hands.

P11.13 Neural correlates of the perception of bimodal emotional incongruity

Gierych E., Milner R., Michalski A.

Nencki Institute of Experimental Biology PAS, Warsaw, Poland

The activity of a human brain perceiving cognitive incongruity can be seen at the scalp surface as N300 or N400 potentials appearing 300–400 ms after stimuli. Little is known about the electrophysiological correlates of emotional incongruity. We investigated responses to emotional stimuli presented with congruent and incongruent context of different modality. In the first two experiments, we compared potentials evoked by the pictures of laughing or crying faces while the sounds of human cry or laughter were used as context. In the third, control experiment we analyzed potentials evoked by the pictures of different animals preceded by the voices typical or not for the presented species. Our data showed that N300 amplitudes varied between the congruous and incongruous pairs depending of the emotional valence of presented pictures. In the experiment with crying faces, N300 were more negative for incongruous context (i.e. crying faces preceded by the sound of laughter) than for congruous context (i.e. crying faces preceded by the sound of crying). In the experiment with laughing faces these relations were opposite: more negative N300 components were found for congruous pairs. Further analysis revealed that the differences between congruous pairs (laughing faces preceded with laughter and crying faces preceded with crying) were much smaller that the differences between incongruous pairs. No significant differences in N300 amplitudes were found in the control experiment.

INJURY OF SPINAL CORD AND BRAIN

P12.01 Functional and structural changes in rat medial gastrocnemius muscle after spinal cord transection

Kedziorek-Pietryka A.[1], Lapinska I.[1], Majczynski H.[1], Karczewska E.[1], Redowicz M.J.[1], Krutki P.[2], Slawinska U.[1]

[1]Nencki Institute of Experimental Biology PAS; [2]University School of Physical Education, Warsaw, Poland

In adult rats after a complete spinal cord transection (SCT) at low thoracic level, the locomotor movements of hindlimbs are impaired. Due to reduced activity, the structural and contractile properties of hindlimb muscles are considerably changed. Previous investigations

of effects of the SCT on two antagonistic muscles: fast dorsi flexor (extensor digitorum longus; EDL) or slow extensor (soleus; Sol) demonstrated that contractile properties of both muscles tended to become similar. To verify whether the way of muscle transformation depends on the muscle physiological function or on the primary muscle fiber composition, we investigated rat medial gastrocnemius muscle (fast extensor) that is composed of three different types of motor units. In our study, the changes in EMG activity, contractile properties and the contents of myosin heavy chain (MHC) isoforms in adult rats were studied one, three and six months after SCT at low thoracic level. We demonstrated that just one month of reduced muscle activity evoked by SCT resulted in the significant changes in muscle contractile properties that were maintained later on. The fast gastrocnemius muscle became slower and weaker (as EDL m.) but surprisingly at the same time it became more fatigable and composed of fast MHC isoforms only (as Sol m.).

P12.02 Morphological changes underlying secondary axonal impairment in ganglia and peripheral nerves

Lackova M., Schreiberova A., Kolesar D., Davidova A., Lukacova N., Marsala J.

Institute of Neurobiology, Slovak Academy of Sciences, Kosice, Slovakia

The aim of our study was to perform a comparative analysis of changes in relationship between intraganglionic perineuronal and peripheral axonal gliocytes after spinal cord ischemia in rabbits. The cervical ganglia showed increases in glial count, generally combined with changes in structure and the shapes of neurons. There were three variants after ischemia by which gliocytes increased: (a) uniform increases in numbers of gliocytes forming the capsules, (b)dislocation of nucleus in neurons to the point of ectopy, (c) groupings of gliocytes in interneuronal spaces. Migration of intraganglial gliocytes to the region of axonal fibers resulted in the intensive enlargement of glial count and in changes of axonal perimeters. Morphologically distinct kinds of diaphorase exhibiting axons were identified in sciatic and radial nerve. Considerably tapered diaphorase labeled fibers were seen in nerve bundles corresponding to the femoral and sciatic nerve afflicted by paraplegia as outgrowth of spinal cord ischemia with NO-mobilization. Histomorphometric analysis proved that the process of axonal regeneration after ischemia took places despite ischemic lesion in femoral nerve earlier than in sciatic nerve. The findings suggest the increases in quantities of gliocytes, ectopy of nuclei, migration of gliocytes to the axonal area in ganglia and moderate axonal atrophy in nerves. Supported by APVT 51-013002 and APVV 0314-06 Grants and by VEGA 2/5134/25 Grant from SAS.

P12.03 Positive effects of microcrystalic chitosan application after laminectomy in rats

Larysz-Brysz M., Marcol W., Slusarczyk W., Malinowska-Kolodziej I., Lewin-Kowalik J.

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

The main complication met after spinal cord surgery is the formation of connective tissue scar, which causes pressure on the spinal cord and nerves. This process can be prevented by many substances, but none of them has given fully satisfactory results yet. Microcrystalic chitosan possesses some positive characteristics, e.g. inhibition of fibroblasts proliferation, reduction of the parenchymal bleeding, and antibacterial action. The study examined effect of chitosan application after L1-L2 laminectomy in rats divided into two groups. In the experimental group, meningeal surface was covered with microcrystalic chitosan gel. After 4 weeks, the spinal cord was removed and fixed. Frozen sections histological and immunohistochemical staining was used to analyze the level of scar area on the surface of meninges. The results proved that chitosan can prevent formation of scar in operated area with good influence on neural structures. Moreover, the microcrystalic chitosan gel is a material easy to apply without any special equipment.

P12.04 Noradrenergic contribution in control of hindlimb motor function in adult spinal rats

Maleszak K.[1], Cabaj A.[1], Majczynski H.[2], Slawinska U.[2]

[1]Institute of Biocybernetics and Biomedical Engineering; [2]Nencki Institute of Experimental Biology PAS, Warsaw, Poland

It was shown that an improvement in hindlimb locomotor movement in adult spinal rats could be achieved by grafting serotonergic cells. In present study we verified whether this improvement was also due to any noradrenergic control. Thus the influence of noradrenergic $\alpha 2$ antagonist (Yohimbine) and $\alpha 2$ agonist (Clonidine) on hindlimb motor function in spinal rats (complete transection at thoracic level T9/T10) that received a graft of embryonic raphe nuclei into the spinal cord below the lesion (GR; n=6) and spinal rats without a graft (CN; n= 6) was investigated. Restitution of hindlimb functions was evaluated using neurological tests: tactile and proprioceptive placing, cross reflex and "buttock" reflex. Hindlimb locomotor movements was tested during exploration in open field, on a treadmill and in air stepping. In general, GR rats performed more appropriate hindlimb movements than CN rats. In all animals from both groups the Clonidine treatment (i.p; 25 μg/kg b.w.) deteriorated hindlimb motor functions while Yohimbine application (i.p.; 1.5 mg/kg b.w.) restored them to the level before treatment. Although, in GR rats after Clonidine administration the hindlimb movements in open field and during air stepping were more proper than before treatment. Our results demonstrated that locomotor abilities are control by noradrenergic $\alpha 2$ receptors in grafted and control spinal rats as well. Thus further experiments are needed to verify the existence of noradrenergic innervation in spinal cord of experimental animals.

P12.05 nNOS-immunoreactivity axons in the white matter after ischemia/reperfusion injury of the spinal cord

Schreiberova A., Lackova M., Lukacova N., Jergova S., Marsala J.

Institute of Neurobiology, Centre of Excellence, Slovak Academy of Sciences, Kossice, Slovak Republic

Nitric oxide (NO) is a signaling diffusible biomolecule, which is generated during conversion of L-arginine to L-citrulline. The reaction is catalyzed by the Ca^{2+}-dependent neuronal isoform of nitric oxide synthase (nNOS) and acts as an important non-con-

ventional neurotransmitter in the central and peripheral nervous system. In the present study the changes in distribution of neuronal nitric oxide synthase-immunoreactivity (nNOS-IR) axons in the white matter of the rabbit's spinal cord were examined using transverse sections after ischemia/reperfusion injury. Spinal cord ischemia (15 min) was induced by Fogarty catheter through the femoral artery occlusion of abdominal aorta in the lumbosacral part of spinal cord with a reperfusion period of 7 and 14 days. This ischemia/reperfusion interval caused a marked deterioration of neurological function of hindlimbs and often results into developed paraplegia. The immunohistochemical method was used to label nNOS-IR axons in the lumbar L7 and sacral S1 segments. The recorded results show the changes of nNOS-IR axons distribution pattern in the ventral, lateral and dorsal columns. We detected the intersegmental alternations there as well. This finding may suggest a role of nitric oxide in disorder, its influence on the afferent and efferent innervations in unknown manner, where the white matter is a target of damage, such as ischemic stroke. Supported by the VEGA Grants No. 2/5134/25, APVT No. 51-013002, APVT No. 51-011604 and APVV 0314-06.

P12.06 New approach to spinal cord injury: Non-laminectomy model – pressure impactor

Wlaszczuk A.[1], Marcol W.[1], Slusarczyk W.[1], Gzik M.[2], Tejszerska D.[2], Bobrowski M.[2], Lewin-Kowalik J.[1]

[1]Department of Physiology, Medical University of Silesia, Katowice; [2]Department of Applied Mechanics, Silesian University of Technology, Gliwice, Poland

Traumatic injuries to the spinal cord and their treatment are among the most serious challenges of modern medicine. A uniform model of the spinal cord injury in laboratory animals which would reconstruct the pathomechanism of spinal cord injury in humans in best possible way needs to be developed. The pressure impactor designed by our team allows to bring about a repeatable, fully controlled spinal cord injury without the necessity of an extensive opening of the vertebral canal. By use of our device we are able to induce micro cysts inside the spinal cord with no damage to the meninges. We developed an original set stabilizing the vertebral column so that the impact to the spinal cord surface is precise. In the spinal cord injury models employing laminectomy, the loss of blood and prolonged anaesthesia can cause death of small animals. Therefore our model can be applied successfully in both larger and small laboratory animals.

P12.07 Spinal cord transection alters NGF and its p75 receptor expression: Does exercise counteract it?

Ziemlinska E., Macias M., Sulejczak D., Czarkowska-Bauch J., Skup M.

Nencki Institute of Experimental Biology, Warsaw, Poland

The p75 receptor (p75NTR) binds growth-promoting neurotrophins (NTs) as well as Nogo receptor for growth-inhibiting proteins, so it is well situated to gauge the balance of positive and negative influences on axonal plasticity. In the adult rat spinal cord p75NTR expression is limited to nerve growth factor (NGF)-responsive DRG primary fibres, but motoneurons disclose potential to re-express p75NTR following nerve injury. Since altered p75NTR may affect remodeling of spinal network, here we examined whether complete spinal transection at Th 9 segment (1) changes p75NTR in DRG central processes (2) induces NGF/p75NTR in spinal cells rostrally and caudally to injury. NGF immunoreactivity (IR), undetectable in intact rats, was induced in dorsal horn neuropil at 3 and 10 days after transection. By 6 weeks it tended to decrease whereas p75NTR IR fibers distributed in laminae I–IV and upholstering dorsal funiculus, maintained IR rostrally and caudally to injury. In its proximity their appearance was distorted and p75NTR distribution altered. An induction of IR was found in glia in lateral and ventral funiculi. As long-term locomotor exercise is a potent cue to stimulate spinal NTs and locomotor recovery in spinalized animals, we evaluated in parallel its effect on NGF/p75NTR expression. Training increased p75NTR protein in intact rats by 12%, but did not cause gross change in p75NTR IR pattern in either group of rats. Results suggest lack of modulatory potency of training on NGF/p75NTR signaling following spinalization. Supported by MSE/BMBF grant.

P12.08 Postoperative scarf to the laminectomy which is a source of changes in morphology of somatosensory evoked potentials

Jankowski R[1]., Szukala A.[2], Huber J.[2], Blok T.[1], Szymas J.[3]

[1]Dept. and Clinic of Neurosurgery and Neurotraumatology; [2]Dept. of Pathophysiology of Locomotor Organs; [3]Laboratory of Neurosurgical Pathomorphology, Department of Clinical Pathomorphology; Karol Marcinkowski University of Medicine, Poznan, Poland

Postoperative scarf after laminectomy is unfortunately impossible for excluding element of recovery after neurosurgical treatment. It is also a source of changes in the afferent transmission within dorsal and dorsolateral funiculi of the spinal cord. The aim of this work was to ascertain the range of changes in morphology in somatosensory evoked potentials following the surgery of partial laminectomy (dorsolateral) or total laminectomy at the lumbar level of spine. Beside of the neurophysiological verification with somatosensory evoked potentials recorded from T7-T9 dorsal root entry zone following electrical stimulation of ischiadicus nerve, the histological examination were used to estimated the range of the postoperative scarf (three months after surgery). The somatosensory potential at normal conditions which is recorded at such circumstances as it was described above is consisted of three components which the first showed transmission at the level of the dorsal root entry zone and in dorsal funiculi. The second component reflects the activity of dorsal and ventral horn centers and the intermediate gray matter centers while the third component reflects the transmission in dorsolateral funiculi in the spinal cord. In studies on such effects of laminectomy, the greatest changes were found in morphology of the first component in somatosensory potentials recordings.There is a strict correlation of changes in recordings taken in experimental studies with recordings performed in clinical neurophysiological studies in patients after surgery of tumors and discopathies at the lumbar level. Hence, findings the normal recording has got the clinical consequences in assessment of spinal transmission performed in patients after surgeries.

P12.09 The role of IL-1 in leukocyte migration into the CNS after subarachnoid hemorrhage in rats

Jedrzejowska-Szypulka H.[1], Larysz-Brysz M.[1], Kotulska K.[2], Olakowska E.[1], Woszczycka-Korczynska I.[1], Lewin-Kowalik J.[1]

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

Subarachnoid hemorrhage (SAH) develops when arterial blood enters subarachnoid space and mixes with cerebrospinal fluid (CSF). Many data indicate that the pathologies observed after hemorrhage are linked to imunno-inflammatory reactions following SAH. Early changes include increase of IL-1, IL-6 and TNF-a in CSF. These cytokines may be produced by leukocytes, ischemic neurons and endothelial cells. Increase in cytokines level results in brain-blood barrier injury and leads to migration of leukocytes from peripheral blood. We examined the migration of leukocytes into the CNS after SAH and the role of interleukin-1 (IL-1) in this process. The study was performed in adult rats. SAH was produced by infusion of 150 μl of autologous arterial blood into cisterna magna. IL-1 activity was inhibited by intracerebroventricular administration of anti-rat IL-1 antibodies. Leukocytes migration into the CNS was examined 90 min and 24 h following SAH. Most numerous leukocytes found in the brain were neutrophils; monocytes appeared at lower number and lymphocytes were practically absent. Some lymphocytes were found, strongly attached to the arachnoid. Leukocyte migration into the CNS was limited during first 24 h following SAH. Inhibition of IL-1β by specific antibodies decreased this migration.

P12.10 Retinal ganglion cells changes following complete optic nerve transection

Lewin-Kowalik J., Golka B., Pietrucha-Dutczak M., Olakowska E., Korczynska I., Marcol W.

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

Damage to the optic pathway, and especially to the optic nerve and its disc results in structural changes with consequent vision defect or loss. Despite of recent progress in ophthalmology and neurology, this kind of vision loss remains as a permanent disability. Retinal ganglion cells (RGCs) of adult rats are unable to regenerate their axons after optic nerve injury and soon after they enter the pathway of apoptosis. However a small population of RGCs survives for a relatively long time. This study was undertaken to compare the number of surviving RGCs at 14 days following optic nerve transection with normal eye controls (no optic nerve transection). Wistar rats underwent bilateral stereotactic injection of fluorescent tracer – Fluorogold into the superior colliculus to label RGCs. After one week the right optic nerve was transected. Left eye without optic nerve axotomy was established as control. Fourteenth days following optic nerve transection the total number of FG-positive RGCs was counted in seven radial sections through the optic disk. After axotomy the number of surviving cells was reduced to 20.5% (from 2223.2 ± 100.86 – in control group to 456.8 ± 42.85 – in group after axotomy).The phenotype of surviving cells in retinal radial sections were examined histochemically.

P12.11 NO production after brain injury at different developmental stages examined with EPR spectroscopy

Ziaja M.[1], Pyka J.[2], Ciombor J.[2], Plonka P.[2]

[1]Department of Neuroanatomy, Institute of Zoology; [2]Department of Biophysics, Faculty of Biotechnology, Jagiellonian University, Krakow, Poland

The concentration of the nitric oxide in the tissue is one of the factors determining its influence on inflammatory reaction. It acts as neuroprotectant but also causes secondary damages of the nervous tissue. The inflammatory reaction induced by brain injury seems to be dependent on the CNS maturity. Taking it into consideration it is important to examine whether NO production depends on the age when brain injury was made. We used EPR spectroscopy and spin trapping in order to determine the NO production in the lesioned brain. Brains of 6 or 30-day-old rats were injured and were examined at different time after the injury. Thirty minutes before perfusion animals received spin trap and brains were then frozen in liquid nitrogen and collected in the glass tubes. EPR measurements were performed on an ESP300E Brucker spectrometer or on a Varian E-3 spectrometer. In the mature brains there was fast decrease of the NO concentration in both hemispheres from 2 to 12 hours after the injury. The amounts of NO were also never higher at any studied time point than in control values. Contrary, in the injured brains of neonatal rats the NO concentration increased at 1 and 2 day post injury, but only in lesioned hemisphere. Increase of EPR signal was also observed at 7 d.p.i. The results indicate the significant differences in the course of post-injury NO production in relation to the stage of the nervous system development.

P12.12 Effect of mature brain injury on NADPH-diaphorase expression induced by lipopolisaccharide

Zygmunt K., Ziaja M.

Department of Neuroanatomy, Institute of Zoology, Jagiellonian University, Krakow, Poland

NADPH-diaphorase staining is a technique that facilitate visualisation of cells with an active NO synthase (NOS). It is considered that this method allows to stain mainly neuronal NOS but there is not excluded the possibility that cells with other isoforms of the enzyme, endothelial and inducible are also stained. In previous research we found that after brain injury there is disproportional expression of NOS in comparison to NO generation but after LPS application this expression correspond with the dynamic of NO production changes. The purpose of the study was to qualify if the brain injury can modulate the expression of NOS triggered by LPS application and if changes of this expression can influence on lowering level of NO production after brain injury. LPS injection was made to the 30 day-old animals and 4 hours later brain injury was performed. At different time after injury rats were perfused with saline and paraformaldehyde and brains were frozen and cut. Slices containing the lesion site were stained with NADPH-d method and positive cells were counted microscopically. At first studied period smaller number of positive cells than in the suitable time of endotoxemia was noted and this effect existed in both brain hemispheres. At later time points the number of NADPH-d+ cells was similar to brain following LPS injection but considerably smaller than in lesioned brains. Our results demonstrate that changes of NOS expression are rather not responsible for notified fluctuations of NO concentration in the brain following injury.

P12.13 Influence of bone marrow stromal cells on injury-induced astrogliosis in the rat cerebral cortex

Opydo-Chanek M., Dabrowski Z.

Department of Experimental Hematology, Institute of Zoology, Jagiellonian University, Krakow, Poland

Astrocytes are activated in response to CNS injury and it results in so-called 'reactive gliosis', a reaction with specific structural and functional characteristic. Reactive gliosis contributes to the formation of glial scar, a frontier which isolates the still intact CNS tissue from secondary lesions. Glial scar is also considered to be an impediment to axonal regrowth after the injury. The present study was carried out to examine the temporal response of astrocytes to cerebral cortex injury after intralesional administration of bone marrow stromal cells (BMSCs). BMSCs are the candidate for cellular therapy of brain injury. The latest studies have shown the potential of BMSCs to enhance functional outcome after CNS injury, but precise mechanisms responsible for these finding are still not known. In this study we used GFAP immunocytochemistry for the specific visualization of reactive astrocytes in the damage brain. We found that the treatment of cerebral cortex injury with BMSCs causes statistically significant increase in the number of GFAP-positive cells in comparison with control group (saline only) at 7, 14 and 30 day after injury, without acceleration of glial scar formation. Thus, BMSCs might play an important role in modulation of astrocytic response after brain injury. The therapeutical benefit resulting from these finding should be evaluated. Supported by BW/IZ/11/2005.

P12.14 Brain injury and 3-nitrotyrosine expression induced by peripheral lipopolisaccharide administration

Kulijewicz M., Ziaja M.

Department of Neuroanatomy, Institute of Zoology, Jagiellonian University, Krakow, Poland

Changes in the NO production are good indicator of inflammation intensity. Our previous studies demonstrated differences in NO production in endotoxemic and injured brain. In endotoxemic brain there was fast and considerable but transitory NO increase while in the injured ones there was distinct decrease of its concentration. The mechanism responsible for that diminution may be connected to production of NO derivatives such as peroxynitrites. Occurrence of 3-nitrotyrosine in the tissue is one of the markers of peroxynitrite action and indirectly also NO concentration. We checked if decrease in NO content after brain injury might be the result of increase in peroxynitrite reflected by changes in nitrotyrosine content. We associate brain injury with earlier i.p. LPS injection when NO production in the brain is very vigorous. 30-day-old rats were injected with LPS (10 mg/kg b.w.) and 4 hours later brain injury was performed. At different time after injury the rats were perfused with saline and paraformaldehyde and frozen. Slices with injury site were stained for 3-nitrotyrosine, microscopically analysed and the number of positive cells was counted. Results showed increase in 3-NT+ cells number following LPS application. However, brain injury causes considerable but short-lived decrease and subsequent increase in the number of such cells in the cerebral cortex. This data indicates that peroxynitrite production and subsequently 3-NT may be one of the reasons of the lower NO density in the lesioned brain

NEURODEGENERATION AND NEUROLOGIC DISEASES

P13.01 OPA1 protein influences mitochondrial structure and function

Bulczak A.[1], Fiket M.[1], Schuelke M.[2], Chan D.C.[3], Alexander C.[1]

[1]Neurodegeneration, Max-Delbrueck-Center for Molecular Medicine; [2]Clinic for pediatrics, Charite, Berlin, Germany; [3]Division of Biology, California Institute of Technology, Pasadena, USA

Mutations in the coding region of OPA1 lead to blindness in patients with autosomal dominant optic atrophy (adOA). AdOA symptoms result from a degeneration of retinal ganglion cells followed by a bilateral atrophy of the optic nerve. The OPA1 protein contains a mitochondrial leader sequence, two coil-coiled domains and a GTPase domain and belongs to the large dynamin-related GTPase family. Experiments on OPA1 demonstrate a role in cristae structure maintainance, mitochondrial fusion, respiratory chain function and apoptosis. A mutation screen of the OPA1 gene in adOA patients revealed an accumulation of mutations in the GTPase-domain and in the C-terminus of the OPA1 protein. Mutants of the OPA1-GTPase were generated by *in vitro* mutagenesis. The effects of mutations on GTP-hydrolysis were examined on the isolated proteins after bacterial expression. Moreover, GTP-mutant constructs were used for transfection of HeLa and MEF OPA1(-/-) cells in order to investigate changes in mitochondrial morphology, cristae structure and respiratory complex activities.

P13.02 Progressive motoneurones death in animal model of neurodegenerative disease

Cabaj A.[1], Lapinska I.[2], Majczynski H.[2], Gorlewicz A.[2], Wilczynski G.M.[2], Nestorowicz K. [3], Herbik M.A.[3], Slawinska U.[2]

[1]Institute of Biocybernetics and Biomedical Engineering; [2]Nencki Institute of Experimental Biology PAS; [3]Mossakowski Medical Research Center, Warsaw, Poland

Amyotrophic lateral sclerosis (ALS) is a neurological disorder characterized by the selective loss of upper and lower motoneurones with consequent progressive paralysis and death. Most cases of ALS have an unknown etiology; around 20% of ALS cases are familial due to genetic defects, including mutations in the gene encoding Cu, Zn superoxide dismutase (SOD1). Our study was undertaken to identify disease progression in two types of muscles (slow – soleus, Sol and fast – extensor digitorum longus, EDL) in transgenic rats with SOD1 mutation. The motor unit numbers and contractile properties of both muscles were established in acute experiments in rats divided into 4 groups according to movement deficits. We demonstrated the significant reduction of motor unit numbers in both hindlimb muscles. Moreover the contractile properties were significantly altered and the muscles became progressively weaker. Histological investigation of spinal cord tissue confirmed the presence of degenerating motoneurones (FluoroJade), associated with gliosis (GFAP+S100) and loss of neurofilament protein in ventral horn of SOD1 rats. We concluded that the progressive movement impairment in hindlimbs of SOD1 rats is a consequence of motoneurone death resulting in a gradual reduction of motor units number and decrease of muscles force. Moreover, we demonstrated that in slow muscle (Sol) neurodegeneration appears earlier than in fast muscle (EDL).

P13.03 Systemic inflammation influences brain response to Abeta peptides. The role of COX and LOX

Cakala M., Malik A.R., Strosznajder J.B.

Department of Cellular Signalling, Medical Research Centre PAS, Warsaw, Poland

It is known that chronic neuroinflammation influence Alzheimer disease (AD) pathology. Extracellular deposits of beta-amyloid peptide (Abeta) are thought to be responsible for development of chronic inflammation in AD brain. Little is known how peripheral inflammation affects disease progression. In this study we have investigated the effect of systemic inflammation on Abeta dependent locomotor and memory disturbances. Moreover, the role of cyclooxygenase-2 (COX-2) and 12-lipoxygenase (12-LOX) in Abeta peptide evoked memory impairment in mice was evaluated. 12-month old C57Bl6 mice were injected i.c.v. with Abeta(1-42) alone or simultaneously with lipopolysaccharide (LPS, i.p.). Some mice also received COX-2 or 12-LOX inhibitor. Another group of mice was pretreated with LPS at 4 and 7 month of age and injected with Abeta at 12 month of age. Our data demonstrated that Abeta enhanced COX-2 and 12-LOX protein level in hippocampus and decreased the locomotion and exploration in mice. Systemic inflammation elevated COX-2 immunoreactivity at early time after injection and worsened recognition performance. COX-2 inhibitor protected mice against memory deficit and locomotor disturbances. In LPS pretreated animals Abeta induced locomotor disturbances, but had no effect on memory and COX-2 level. Our results indicated that Abeta evoked enhancement of COX-2 protein level and memory deficit. COX-2 inhibitor protected brain against Abeta induced memory disturbances.

P13.04 17beta-estradiol restores the nigrostriatal function following MPTP intoxication in aged male mice

Ciesielska A.[1,2], Joniec I.[2], Przybylkowski A.[2], Kurkowska-Jastrzebska I.[1,2], Czlonkowska A.[1,2], Czlonkowski A.[2]

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

The neuroprotective action of estrogen (Es) against 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) has been shown in various reports on both female and male mice, however only when given chronically prior to MPTP insult. In this study, we tested the chronic effects of 17β-estradiol (0.25 mg per pellet, 21-d release) in C57Bl male mice (14–16 months old) to function as a neuroprotectant when administered prior to (Experiment 1) or after (Experiment 2) MPTP treatment. Striatal tyrosine hydroxylase (TH) concentrations were measured by Western blot method at 1, 7 and 21 (Exp. 1) and 7 and 21 (Exp. 2) day post MPTP intoxication to assess the neuroprotective action of Es on nigrostriatal system. MPTP treatment reduced striatal TH within 1–21 days following intoxication. We indicated that Es exerted a neuroprotective effect upon nigrostriatal system when administered at 7 days prior MPTP intoxication, consistent with previous reports. Surprisingly, we also observed that Es protected the striatum from MPTP insult when Es administered at 3rd day post MPTP injection. The implantation of Es pellets after intoxication attenuated the MPTP-induced loss of striatal TH at 7 and 21 time-points. We also showed (by Western blot method) that Es decreased the content of specific astrocytic marker – glial fibrillary acidic protein (GFAP) in the striatum, when administered prior as well after MPTP intoxication.

P13.05 Apoptosis and autophagy are induced in hippocampal neurons during systemic inflammatory response

Czapski G.A.[1], Gajkowska B.[2], Strosznajder J.B.[1]

[1]Department of Cellular Signalling; [2]Department of Cell Ultrastructure, Medical Research Centre, Warsaw, Poland

Inappropriate activation of inflammatory system may have extremely destructive effects for adjacent tissues. This is the case in many diseases of CNS, including neurodegenerative diseases, but also in septic shock. Our recent data shown that systemic inflammation evoked by bacterial endotoxin, lipopolysaccharide (LPS), induces programmed cell death pathways in neurones of substantia nigra. The aim of the present study was to analyse whether systemic inflammation affects also hippocampus. Adult mice C57BL6 were treated with LPS at 1 mg/kg b.w., i.p. Hippocampal tissue was analysed up to 48 h after LPS injection. Morphological and immunocytochemical electron-microscopic studies indicated pronounced alterations in many hippocampal neurons. Classic apoptotic execution or autophagy was noticed in several pyramidal neurons and glial cells. The accumulation of autophagosomes and autophagolysosomes, autophagic degeneration of synapses with concomitant activation of cathepsin B was observed. Translocation of apoptosis inducing factor from mitochondria to nucleus was noticed, but in the same conditions caspase-3 activity was not changed. Moreover, the enhanced expression of inflammation related genes for COX-2, TNFalpha and iNOS occurred, suggesting activation of local inflammatory reaction. Our data indicate that mild systemic inflammation induces apoptosis and autophagy-dependent neurodegeneration in hippocampus.

P13.06 Search for diagnostic methods in Alzheimer's disease using human immortalized lymphocytes

Kachamakova N.[1], Zebrowska B.[1], Szybinska A.[1], Kuznicki J.[1,2], Wojda U.[1]

[1]International Institute of Molecular and Cell Biology; [2]Nencki Institute of Experimental Biology PAS, Warsaw, Poland

Alzheimer's disease (AD) is a neurodegenerative disorder leading to progressive dementia. Mutations in three different genes, namely presenilin 1 (PS1), presenilin 2 (PS2) and amyloid precursor protein are associated with early-onset familial Alzheimer's disease (FAD). In a study of Zekanowski et al. (2003, Exp Neurol) several new mutations in presenilin genes were identified in a group of Polish patients. The aim of the current study was to assess the pathological mechanism of these new mutations and to test the usage of FAD lymphocytes as possible diagnostic material in AD. We studied immortalized lymphocytes from five patients with distinct mutations in PS1 or PS2 in comparison to control lymphocytes from healthy individuals. Flow cytometry was used for analysis of the cell cycle progress and for assessment of the susceptibility to apoptotic stimuli. DNA for the cell cycle analysis was stained with propidium iodide (PI). Apoptosis was estimated by two independent methods – Annexin V/PI combination and analysis of sub-G1 DNA content. Most of the lymphocytes with the PS mutations showed increased vulnerability to apoptotic stimuli whereas alteration of the cell cycle was observed in lymphocytes with one of the new PS1 mutations. This suggests that the susceptibility to apoptosis could be further studied as a possible diagnostic tool, whereas

the changes in the cell cycle seem to be limited to specific PS mutations. Work was supported by the Polish ordered grant PBZ-KBN-124/ PO5/2004.

P13.07 Purinergic receptors P2X7 in the course of EAE

Koza K., Sulkowski G., Rafalowska U., Struzynska L.

Laboratory of Pathoneurochemistry, Department of Neurochemistry, Medical Research Centre PAS, Warsaw, Poland

Purinergic P2X7 receptors are ATP-gated ion channels widely distributed in brain. Strong evidence suggests that they are involved in cross-talk between glial and neuronal cells being activated during pathological conditions. The upregulation of P2X7 has been observed in several pathological models like ischemia and epilepsy, where this receptor appeared to contribute to glutamatergic excitotoxicity. Moreover, P2X7 may also participate in regulation of inflammatory response. Recent studies have strongly indicated that glutamate excitotoxicity may contribute to the pathological changes observed in multiple sclerosis. Thus, it was of interest to examine expression of P2X7 protein during experimental autoimmune encephalomyelitis (EAE), an animal model of MS. The profile of P2X7 R expression was examined in homogenates of forebrain cortex in different stages of the disease (4, 8, 10, 20, and 25 days postimmunisation). Overexpression of receptor's protein was seen in late phases of disease. In the peak of the neurological deficits observed in animals (12 days postimmunisation), increased level of P2X7 protein was noticed in homogenates obtained from forebrain cortex and hippocampus, whereas decreased expression of receptor's protein was seen in the cerebellum. To identify the relative importance of the above observations, further studies are needed.

P13.08 Use of an endogenous suicide mechanism to study pathogenesis of neurodegenerative diseases

Kreiner G., Parlato R., Rieker C., Bierhoff H., Grummt I., Schutz G.

German Cancer Research Center, Dept. Molecular Biology of the Cell I, Heidelberg, Germany

We propose a novel approach to generate mouse models of neurodegenerative diseases based on the genetic ablation of the transcription initiation factor IA (TIF-IA) that blocks the synthesis of ribosomal RNA leading to p53-mediated apoptosis. We used the conditional inactivation of the gene encoding TIF-IA by the Cre-loxP system to induce selective loss of different types of neurons in mice. Deletion of the TIF-IA gene leads to rapid loss of neural progenitors and, more interestingly, to progressive loss of postmitotic neurons preceded by p53 upregulation. Here we report that disruption of the nucleoli in dopaminergic neurons and striatal dopaminoceptive neurons results in the generation of mutants showing respectively the typical phenotype of Parkinson's disease (degeneration of dopaminergic neurons in substantia nigra and ventral tegmental area, depletion of dopamine in the striatum and typical motor dysfunctions) or Huntington's disease (loss of striatum, impairment of motion control and clasping behavior). We believe that our mutant mice may become a valuable tool to study the pathogenesis of neurodegenerative diseases regarding the recent data implicating the crucial role of p53 in these processes. In addition, our study indicates that cellular changes associated with nucleolar disruption may recapitulate some changes associated with neurodegeneration in response to oxidative stress.

P13.09 Preliminary results of haplotyping study in a group of Polish patients with SCA2

Krysa W.[1], Sulek-Piatkowska A.[1], Rajkiewicz M.[1], Zdzienicka E.[1], Rakowicz-Raczynska M.[2], Szirkowiec W.[1]

[1]Institute of Psychiatry and Neurology, Department of Genetics; [2]Department of Neurophysiology, Warsaw, Poland

Spinocerebellar ataxia type2 (SCA2) is progressive, neurodegenerative, late onset disorder characterized by progressive cerebellar ataxia, dysarthria, slow saccadic eye movements. It is inherited as autosomal dominant trait and results from dynamic mutation which leads to an expansion of CAG repeats within SCA2 gene. The anticipation phenomenon often appears, and results in increasing disease severity or decreasing age at onset in successive generations. Molecular diagnosis of SCAs has been carried on in The Institute of Psychiatry and Neurology since 1998 year, and diagnostics, predictive and prenatal tests are available. Genetic tests for different types of SCAs performed in over 1 300 patients with diagnosis of ataxia, revealed dynamic mutation in SCA2 locus in 53 individuals of 22 pedigrees. In 14 individuals from these families predictive tests were performed and 4 of them confirmed presence of the pathologic expansion. To determine if SCA2 causative mutation derives from one or more founder chromosomes we performed haplotype analysis of the SCA2 positive families. Microsatellite markers, chosen for this analysis, span a region around the SCA2 CAG repeat in the following order: cen.– D12S105 – D12S1672 – D12S1333 – D12S354 – tel., with intragenic D12S1672 and 20kb centromeric to CAG strech. Preliminary results of the analysis suggest that there is no a single founder of SCA2 in Poland.

P13.10 Homocysteine-induced alterations in tau phosphorylation in cultured rat cerebellar granule neurons

Kuszczyk M., Gordon-Krajcer W., Lazarewicz J.W.

Department of Neurochemistry, Medical Research Centre PAS, Warsaw, Poland

Homocysteine (Hcy) is a sulphur-containing amino acid, which at high concentration may induce excitotoxicity. Hiperhomocysteinemia is a risk factor in Alzheimer`s disease (AD). It has been suggested that in AD patients Hcy blood level may be correlated with formation of neurofibrillary tangles (NFT), the hallmark of AD. The major component of NFT is hyperphosphorylated tau protein. The aim of this study was to evaluate the effect of Hcy on the phosphorylation level of tau in cultured cerebellar granule cells, and to verify a role of glutamate receptors in this process. The initial experiments confirmed the role of N-methyl-D-aspartate (NMDA) receptors and group I metabotropic glutamate receptors (mGluRs) in acute neurotoxicity induced by 30 min exposure of cultures to 15 mM D,L-Hcy. Both, uncompetitive NMDA receptor antagonist 0.5 μM MK-801 and mGlu1 or mGlu5 antagonists 25 μM LY367385 and MPEP, respectively, provided neuroprotection. Western blot analysis showed that acute incubation of cell cultures with 15 mM D,L-Hcy has no significant effect on the expression of tau, but induces a rapid, time-dependent decrease in immunostaining of the phosphorylated form of tau. Application of

NMDA and mGluRs antagonists resulted in inhibition of Hcy-evoked dephosphorylation of tau protein suggesting a glutamate receptor-mediated activation of phosphatase(s). The results of this study demonstrate that Hcy may trigger initial decrease in tau phosphorylation mediated by NMDA and group I mGluRs.

P13.11 BDNF gene polymorphisms and increased risk of MS

Mirowska-Guzel D.[1,2], Mach A.[1], Gromadzka G.[1,2], Czlonkowska A.[1,2]

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

It is hypothesized that genetic factors influencing immune function could determine susceptibility to multiple sclerosis (MS) or the severity of the disease. Brain-derived neurotrophic factor (BDNF), a neurotrophin produced by neurons and immune cells, promotes neuronal survival and repair during development and after nervous system injury. We investigated two single nucleotyde polymorphisms (SNPs) of the human BDNF gene: C270T and G758A, and their impact on the susceptibility to MS. Materials and methods EDTA blood samples were collected from 333 individuals: 186 MS patients and 147 healthy individuals. The genomic DNA was extracted and polymerase chain reaction restriction fragments length polymorphism (PCR-RFLP) was performed to genotype the DNA sequence variants of the BDNF gene. Results Statistically significant differences in frequencies of C270T and G758A BDNF polymorphisms in MS patients and controls were found ($P<0.0001$ and $P=0.0018$, respectively). C/C genotype was determined in 63.4% of patients and 94.6% of controls, C/T genotype in 36.6% of patients *versus* 5.4% of controls. G/G genotype was found in 62.4% of patients and 45.6% of controls, A/G in 37.1% of patients and 54.4% of controls. Conclusion Our results show that both C270T and G758A BDNF polymorphisms affect susceptibility to MS, however none of them have any impact on the form of the disease.

P13.12 Processing of tau in Pick bodies parallels the early processing of tau found in Alzheimer disease

Mondragon S.[1,2], Mena R.[1,2], Binder L.I.[1,2], Perry G.[1,3], Smith M.A.[1,4], Garcia-Sierra F.[1,2]

[1]Department of Cell Biology, Dep. of Neurosciense, CINVESTAV-IPN, Mexico City, Mexico; [2]Dept. of Cell and Molecular Biology, Northwestern University Medical School, Chicago, Illinois; [3]College of Sciences, University of Texas at San Antonio, San Antonio, Texas, [4]Department of Pathology, Case Western Reserve University, Cleveland, Ohio, USA

In AD, a well defined pattern of conformational changes and truncation of tau has been described. In this study, we used laser scanning confocal microscopy to characterize and compare the processing of tau protein during Pick disease with that found in AD. We found that tau protein of Pick disease share a number of epitopes characteristic of AD, the conformational changes recognized by Alz-50 and Tau-66, the cleavage sites D421 and E391, as well as many phosphorylated sites such as Ser199/202, Thr205 and Ser396/404. In Pick disease, we found a strong association between phosphorylation and cleavage at D421, as well as between phosphorylation and the conformational Alz-50 epitope. However, late AD markers such as the conformational Tau-66 epitope and MN423 (cleavage at site E391) showed a significantly minor overlap. Morphological and quantificative analysis indicates that phosphorylation is an early event, likely preceding the cleavage of tau at D421 i.e., paralleling the sequence of events in AD. Despite such consistent with AD, we found a major distinction namely, that PBs lack β-sheet conformation. Taken together, our findings suggest a scheme of early tau processing in Pick disease, which mimics that seen in AD.

P13.13 Neuronal excrescences in CA3 area of human hippocampus in ageing and in Alzheimer's disease

Mytilinaios D.[1], Tsamis K.[1], Njau S.N.[2], Mavroudis J.[1], Safouris A.[1], Baloyannis S.J.[1]

[1]Lab. of Neuropathology, 1st Department of Neurology; [2]Lab. of Forensic Medicine and Toxicology, Aristotle University of Thessaloniki, Greece

Ramon y Cajal was the first who described the existence on CA3 neurons of spines with great size and abnormal shape in rabbit, and named 'thorny excrescence'; other researchers described later neuronal excrescences in rat, cat and primates. However, the first detailed description of these thorny excrescences or branched spines in human brain didn't come earlier than 2006. Studies in rats have shown that these spines exist in most CA3 neurons and in CA4 hilar mossy neurons and they are the sites of input of mossy fiber originating from dentate gyrus granule cells. The initial notion was that pyramidal projection cells receive the major part of afferent fibers from granule cells but recent data suggest that mossy fibers make synapses mainly with GABAergic interneurons. Thus, the functional correlation of CA3 excrescences – that are not found in any other brain region – remains unclear. In addition, recent studies on rats have shown alterations of CA3 dendritic and spinal morphology in different conditions related to sex, hormone levels (thyroxin) and stress. This data suggest that neurons in CA3 area can adapt their morphology, in altering conditions and this is a sign of a kind of plasticity hardly observed in other brain regions. In this study we examined the morphology of CA3 neurons in human hippocampus in healthy individuals of different age and sex (who died accidentally) and in patients with Alzheimer's disease.

P13.14 Modulation of neurological deficits during EAE by glutamate receptors antagonists MPEP and memantine

Sulkowski G.[1], Kwiatkowska-Patzer B.[2], Lenkiewicz A.[1], Struzynska L.[1]

[1]Lab. of Pathoneurochemistry, Dept. of Neurochemistry; [2]Dept. of Neuropeptides, Medical Research Centre PAS, Warsaw, Poland

Recent studies have strongly indicated a role of glutamate transporters and receptors in the glutamate homeostasis during the course of EAE, implicating that excitotoxicity may be involved in the pathogenesis of multiple sclerosis (MS). The current investigation were undertaken to observe if the usage of MPEP – the antagonist of group 5 metabotropic glutamate receptor, and memantine – the uncompetitive NMDA receptor antagonist can modify the neurological course of EAE. Memantine in dose of 10 mg/kg b.w./day; and MPEP in dose of 5 mg/kg b.w./day were administered ip into

EAE rats for 7 days, starting from day 5 to 12 post immunization. During the experiment the body weight and neurological deficits were monitored daily, so as duration of disease phases and the lethality. Additionally, the expression of glutamate receptors was examined by Western blot in brain homogenates. The results showed that especially application of memantine significantly attenuates neurological deficits in rats suffering from EAE, suggesting the predominant role of NMDA receptors in the conditions of experiment. Thus, the results confirmed the contribution of glutamate neurotoxicity to the pathological changes observed in EAE rats.

P13.15 Homocysteine modulates expression of β-APP immunoreactivity and tau protein phosphorylation in the rat brain hippocampus *in vivo*

Gordon-Krajcer W., Filip A., Kuszczyk M., Lazarewicz J.W.

Department of Neurochemistry, Medical Research Centre PAS, Warsaw, Poland

Hyperhomocysteinemia is an independent risk factor in Alzheimer´s disease. Recently we demonstrated, that Hcy excitotoxicity is mediated cooperatively by group I metabotropic glutamate receptors (mGlur I) and NMDA receptor. These receptors are known to modulate differentially β amyloid precursor protein (βAPP) expression, processing and neurofibrillary tangles formation. In this study we examined the effects of intrahippocampal injections of Hcy in adult rats on the immunoreactivity of several βAPP epitopes and tau protein expression and phosphorylation. Microdialisis of the rat hippocampus demonstrated that ^{45}Ca release induced by 5 mM D,L-Hcy is strongly inhibited by 1 mM LY367385, which confirms the stimulatory effect of Hcy on group I mGlurs. As determined by Western blotting, unilateral intracerebral injection of Hcy (0.5 μmol) resulted in the progressive increase in the immunoreaction with antibodies against N- and C-terminal domains of βAPP. These data suggest that Hcy induced enhancement of the expression of βAPP with delayed activation of its processing *via* non-amyloidogenic pathway. D,L-Hcy has weakly effect on the expression of tau, but induces progressive decrease in phosphorylation of tau epitopes. Co-injection of Hcy with MK-801 (uncompetitive NMDA receptor antagonist) and/or LY367385 (mGlu1 antagonists), provided neuroprotection – resulted in significant inhibition of immunoreactivity of βAPP epitopes and inhibition of tau dephosphorylation.

P13.16 Searching for the FMR1 gene premutations in large cohort of Polish ataxia patients and in controls

Rajkiewicz M., Sulek-Piatkowska A., Zdzienicka E., Szirkowiec W., Krysa W., Fidzianska E.

Genetic Department, Institute of Psychiatry and Neurology, Warsaw, Poland

For long time male and female carriers of the premutation in FMR1 gene were considered as asymptomatic. In 2001 Hagermann at al reported the distinct clinical disorders with neurological symptoms, called Fragile X Tremor Ataxia Syndrome (FXTAS). The aim of our study was to examine the presence of the FMR1 gene X premutations in a large group of patients affected with ataxia of unknown origin and in controls. CGG repeats size ranges and alleles frequencies were determined in both groups by PCR and electrophoresis. The first group comprised 366 (143 female, 223 male) with or without other neurological symptoms, in whom molecular tests carried out previously, excluded SCA1,2,3,6,8,12,17, DRPLA and HD. The control group was composed of 512 samples aged 11–91 years, not affected with neurological disorders and screened for the presence of FMR1 premutation. The largest allel found in the first group of the patients with ataxia was 50 CGG repeats (belonging to the grey zone). In the group of controls we determined the FMR1 allele range from 7 do 56 CGG repeats, with the most frequent 29 CGG alleles and 30% homozygosity among females. We also observed rather high proportion of intermediate size alleles (35–60 CGG) in the patients (12.3%) and in controls1 (7.2%); the difference statistically significant. Our study may suggest that Polish population is characterized by rather high level of intermediate size alleles (35–60 CGG). However our data show that premutations in the patients with sporadic ataxia are less frequent than we expected.

P13.17 Status epilepticus (SE) -induced gene expression – new epileptogenesis related genes

Stefaniuk M., Lukasiuk K.

Nencki Institute of Experimental Biology PAS, Warsaw, Poland

In approximately 30% of patients epilepsy develops as a result of brain damaging insult (such as stroke, head trauma or SE). It is believed that alterations in gene expression following such insults are critical for the circuitry remodeling which underlies the appearance of spontaneous seizures. The identification of new epileptogenesis-related genes might contribute to the designation of new targets for intervention during the epileptogenic phase. On the base of microarray analysis candidate genes that might be involved in the development of the disease were identified. These genes included a number of unknown ones (EST-expressed sequence tags). The aim of this study was to validate those data, clone and characterize new epileptogenesis-related genes. For this study rat model of temporal lobe epilepsy (TLE) was used. To induce SE rats were injected with kainic acid or pilocarpine. The brain tissue was collected at 1, 4, 14 day after induction of SE. In situ hybridization was performed using radioactively labeled oligonucleotide probes. The genes that revealed SE-induced alterations in expression were cloned using 5'RACE technique and sequenced. To identify nature of cells that express gene of interest, cRNA probe was synthesized and dual *in situ*/immunohistochemistry staining was performed. Investigated transcripts were detected mainly in neurons. Bioinformatic analysis of sequences revealed their homology to already known genes or predicted ones. Further analysis will be conducted to provide data beneficial for understanding their function. Supported by KBN grant 2P04A05226.

P13.18 Expression of mRNAs coding for phosphate-activated glutaminases (PAG) in human cerebral tumors

Szeliga M.[1], Matyja E.[2], Obara M.[1], Grajkowska W.[3], Albrecht J.[1]

[1]Department of Neurotoxicology; [2]Department of Experimental and Clinical Neuropathology, Medical Research Centre, PAS; [3]Department of Pathology, Children's Memorial Health Institute, Warsaw, Poland

PGA in mammalian tissues occurs in 3 isoforms: LGA, KGA and GAC (Marquez et al. 2006, Neurochem Int). Human malignant gliomas (WHO grades III and IV) overexpress GAC mRNA but

lack expression of LGA mRNA (Szeliga et al. 2005, Neurosci Lett). Here we analyzed the expression of these 3 mRNAs in the biopsy material derived from brain tumors of different malignancies, cellular composition and brain regions: atrocytoma pilocyticum (AP, grade I), ganglioglioma (GG, grades I, II, III or IV), ependymoma (EP, grades I, II or III), subependymal giant cell astrocytoma (SEGA, grade I), a single case of oligodendroglioma (OG, grade II) and in cultured rat astrocytes and neurons. Overexpression of GAC relative to KGA was a feature common to all malignant tissues, but not to cultured cells, consistent with the high GAC expression reported for peripheral tumors. LGA mRNA expression was very low to absent in AP, EP and SEGA, and was significant – though much lower than KGA or GAC, in GG and OG, which contain a discernable proportion of non-astrocytic cells. LGA mRNA expression was very low in cultured astrocytes, but as high as that of KGA and GAC mRNA in neurons. Low LGA appears to be a feature of tumors of predominantly glial origin, but not a marker of tumor malignancy. Supported by Ministry of Science and Education, grant no 2PO5A 08930 and Foundation for Polish Science.

NEUROPROTECTION

P14.01 The role of the inducible cAMP early repressor (ICER) isoforms in neuronal survival

Bieganska K., Klejman A., Kaczmarek L.

Department of Molecular and Cellular Neurobiology, Nencki Institute of Experimental Biology PAS, Warsaw, Poland

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 one of those isoforms, ICER IIγ, is highly expressed in apoptotic neurons *in vitro* and its overexpression evokes neuronal death. We investigated the role of all four ICER isoforms in cortical neurons culture, comparing their expression level in serum-deprived and MK-801-treated neurons. The pro-apoptotic properties of all four ICER isoforms was also tested in transfected cortical neurons. We have found that all four 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 most efficiently induced, as well as the most effective in evoking neuronal cell death were both ICER Iγ and IIγ isoforms. We have also developed siRNA directed specifically against ICER but not CREM sequence to be able to downregulate the expression of ICER isoforms to study the effect of ICER inhibition on neuronal physiology.

P14.02 Effect of delayed postconditioning against kainate induced neurodegeneration in the rat brain

Burda J.[1], Danielisova V.[1], Nemethova M.[1], Gottlieb M.[1], Domorakova I.[2]

[1]Institute of Neurobiology, Slovak Academy of Sciences; [2]Faculty of Medicine, P.J. Safarik University, Kosice, Slovakia

The findings reported in this work show that posttreatment with norepinephrine can provide a potent protection against neurodegeneration in the model of neuronal death-generating treatment such as kainic acid injection. Rats were injected i.p. with 8 mg/kg of kainic acid and postconditioning, an injection of norepinephrine (3.1 μmol/kg i.p.) as long as 2 days after kainic acid, was used. Neuronal degeneration, assessed by analysis of neuronal density on Fluoro Jade B-stained hippocampal sections, was significantly reduced in norepinephrine-treated rats (6%), while kainic acid alone caused 64% neurodegeneration in CA1 subfield. The neuroprotection observed following the injection of norepinephrine 2 days after to induction of a severe epileptic challenge was associated with the learning and memory capabilities when tested using Morris water maze, too. These results strengthen the idea of an interesting potential therapeutical value of postconditioning in neuronal protection. Sponsored by grants APVV 51-021904, VEGA 2/6211/26 a 1/4237/07.

P14.03 Bradykinin postconditioning induces protective effects against transient forebrain ischemia in rats

Danielisova V., Gottlieb M., Nemethova M., Burda J.

Institute of Neurobiology, Slovak Academy of Sciences, Soltesovej 4, Kosice, Slovakia

Bradykinin is considered an important mediator of the inflammatory response in both peripheral and central nervous system and it has attracted recent interest as a potential mediator of brain injury following stroke. Bradykinin, is recognized to play an important role in ischemic brain. We investigated the effects of bradykinin postconditioning on ischemic damage two days after 8 min of ischemia and 3 days of reperfusion. Bradykinin was administered at a dose of 150 μg/kg i.p. The study demonstrated that bradykinin postconditioning induces protection against ischemic brain injury, and this protection is the promotion of neuronal survival and functional outcome. Total activity of SOD 3 days after ischemia was at the control level in both cases, with or without postconditioning. However, analyze of individual SOD show us interesting differences; while CuZnSOD was decreased 3 days after ischemia, activity of MnSOD is significantly increased. Interesting is the fact that MnSOD is mitochondrial enzyme and its activity in cytosol points to possible mechanism of protection provided by postconditioning, which could be prevention of release of mitochondrial proteins to cytoplasm that means protection against mitochondrial pathway of apoptosis. Supported by grants APVV 51-021904, VEGA 2/6211/26 and VEGA 2/6210/26

P14.04 Expression of NPY in gerbil hippocampus after ischemic and hypoxic preconditioning

Duszczyk M.[1], Smialowska M.[2], Gadamski R.[1], Ziembowicz A.[1], Lazarewicz J.W.[1]

[1]Medical Research Centre PAS, Warsaw; [2]Institute of Pharmacology PAS, Krakow, Poland

Preconditioning by a brief ischemia, mild hypobaric hypoxia or sublethal epileptic episode induces tolerance to the subsequent ischemic insult lethal to neurons. There are suggestions that common mechanisms may be involved in such a cross-tolerance. Here we tested a hypothesis based on the role of neuropeptide Y (NPY) in the epileptic preconditioning, that enhanced expression of this peptide might participate in the universal mechanisms of tolerance. Changes in NPY expression in the Mongolian gerbil hippocampus, assessed 2, 4 and 7 days after preconditioning ischemia or with three trials of mild hypobaric hypoxia (360 Torr, 2 h), were compared with the level of

tolerance to test ischemia. Survival of the CA1 pyramidal neurons was studied 14 days after the insult. Three-min test ischemia caused damage to 80% of CA1 neurons. Two-min ischemia was only slightly injurious and mild hypobaric hypoxia was harmless. Both ischemic and hypoxic preconditioning attenuated to about 50% the damage evoked by test ischemia. Preconditioning ischemia resulted 2 days later in 50% rise in the NPY expression that disappeared 4 days after ischemic episode. Mild hypobaric hypoxia induced a twofold increase in the number of NPY-positive neurons, lasting at least 7 days. Although induced tolerance to ischemia two days after preconditioning is accompanied by enhanced NPY immunoreactivity, there is no correlation between intensity of NPY expression and the level of neuroprotection. Supported by MSHE grant 2.P05A.170.29.

P14.05 TNF-alpha receptor 1 regulates the production of BDNF in trimethyltin-treated dentate gyrus cultures

Figiel I.

Nencki Institute of Experimental Biology PAS, Warsaw, Poland

Tumor necrosis factor-alpha (TNF-alpha) is one of the most important, pleiotropic cytokines regulating inflammatory and immune responses in the CNS. Conversely, TNF-alpha has also been reported to exert neuroprotective effects. The biological properties of TNF-alpha are signaled through two distinct cell surface receptors (TNFR1 and TNFR2) that are constitutively expressed on both neurons and glia. In previous studies, performed on mixed neuronal-glial cultures of hippocampal dentate gyrus, we demonstrated that trimethyltin (TMT) induced neuronal apoptosis, which was accompanied by an enhanced production of TNF-alpha in microglial cells. Moreover, TMT led to a strong increase in TNFR1 expression on astrocytes. In order to elucidate the role of TNFR1 in mediating the capacity of TNF-alpha to stimulate neuroprotective effects, I have studied, in cultures treated with TMT, the expression of brain-derived neurotrophic factor (BDNF), implicated in resistance of dentate granule cells. I found dose-dependent increase in BDNF production, mainly in astrocytes, which correlated with the changes in expression of TNFR1. Simultaneous addition of anti-TNFR1 antibody and TMT to the cultures suppressed the synthesis of BDNF. These results indicate that in dentate gyrus cultures TMT stimulates TNF-alpha expression in microglia that subsequently acts upon TNFR1 on astrocytes, leading to enhanced production of BDNF. Therefore it seems that TNF-alpha may, indirectly, provide neurotrophic support to TMT-injured granule neurons.

P14.06 Paradoxical effects of adenosine receptor ligands on L-DOPA-induced generation of free radicals

Golembiowska K., Dziubina A., Kowalska M.

Institute of Pharmacology PAS, Krakow, Poland

Parkinson's disease (PD) is a neurodegenerative disorder associated with a selective loss of DA neurons in SNPC. Among proposed mechanisms of DA cells degeneration, oxidative stress is believed to play an important role. On the other side, L-DOPA used as a main therapy of PD could cause toxicity through formation of free radicals (FR). Adenosine, an endogenous neuromodulator in CNS induces neuroprotection and promotes recovery from FR as shown in various *in vitro* and *in vivo* models. In our study, we investigated the role of adenosine A1 and A2A receptor ligands in FR generation by L-DOPA and a possible mechanism of adenosine neuroprotection in L-DOPA-induced oxidative stress. The level of FR and DA in striatal dialysates was measured with HPLC. L-DOPA (50 μM) infused into striatum markedly increased the level of DA and FR. A selective adenosine A1 receptor agonist N6-cyclopentyladenosine (CPA, 25–50 μM) and a non-selective A1/A2A receptor agonist 2-chloroadenosine (2-CADO, 50–100 μM) decreased both the level of DA and FR, but a selective A2A receptor agonist CGS 21680 (25–100 μM) did neither. Caffeine (50–100 μM), a non-selective A1/A2A adenosine receptor antagonist, had no effect on the level of DA, but decreased formation of FR. All adenosine receptor ligands counteracted production of FR in Fenton reaction *in vitro*. We concluded that L-DOPA-induced oxidative stress is reduced by inhibition of DA synthesis and scavenging properties of adenosine analogs and caffeine.

P14.07 FK506 prevents pro-inflammatory and cytotoxic events associated with reactive astrogliosis *in vitro*

Gozdz A., Kaminska B.

Dept. of Cell Biology, Nencki Institute of Experimental Biology PAS, Warsaw, Poland

Reactive astrogliosis is implicated in many acute and chronic neuropathological conditions and involves astrocyte proliferation, activation and hypertrophy accompanied by production of cytokines, growth factors and metabolic alterations. Astrocyte activation may exert both beneficial and detrimental effects on nervous system cells, therefore its modulation is an attractive target for neuroprotective therapies. We have demonstrated that a widely used immunosuppressant FK506 was a potent inhibitor of gliosis *in vivo* and improved recovery in a rat stroke model (Zawadzka and Kaminska 2005, Glia 49: 36–51). To dissect the mechanism of FK506 action on activated astrocytes, we employed a model of "reactive astrogliosis *in vitro*" based on primary rat astrocyte cultures stimulated with the mix of pro-inflammatory cytokines (IL-1beta, IFN-gamma and TNF-alpha). Cytokine cocktail activated p38 and JNK MAPK signalling pathways followed by cellular hypertrophy, increase of GFAP staining, nitric oxide production and expression of mRNA for pro-inflammatory or cytotoxic molecules (il-6, cox-2 and trail). FK506 treatment reduced the astrocyte hypertrophy, decreased the level of activated p38 MAPK in a dose dependent manner, as well as down-regulated cox-2 and trail mRNA. Our data suggest that FK506 may exert neuroprotective effect partially *via* an inhibition of the pro-inflammatory astroglia activation and implicate a calcineurin as a new candidate for triggering of astrogliosis. Supported by PBZ/MEiN/01/2006/32 (AG).

P14.08 Chemical preconditioning attenuates excitotoxic, but not apoptotic neuronal cell death

Jantas D., Lason W.

Dept. of Experimental Neuroendocrinology, Inst. of Pharmacology PAS, Krakow, Poland

A number of studies have demonstrated that preconditioning of neurons by a sublethal stimulus induces tolerance to a subsequent, lethal insult. In the present study we evaluated the efficacy of chemical preconditioning with 3-nitropropionic acid (3-NPA), a succinate dehydrogenase inhibitor, to protect mouse primary neocortical neurons against excitotoxic and apoptotic cell death. Preconditioning was induced by 1- and 2-hour exposure of neurons to 10–250 μM of

3-NPA in a balanced salt solution, which did not affect cell viability as compared to sham-treated cells, whereas a higher concentration (500 μM) of that compound had a cell-damaging effect. After 24- and 48-hour preconditioning with 3-NPA, glutamate (1 mM), staurosporine (0.5 μM) and doxorubicin (0.5 μM) were added to neurons to induce necrotic and apoptotic cell death, respectively. The 24-hour incubation of cells with glutamate and staurosporine, and the 48-hour incubation with doxorubicin induced moderate but statistically significant cell damage, as estimated by lactate dehydrogenase (LDH) release and MTT reduction assays. Pretreatment with 3-NPA almost completely attenuated the glutamate-evoked cell death. In contrast, no effect of 3-NPA preconditioning on the staurosporine- or doxorubicin-induced cell death was observed. The obtained data indicate that the preconditioning of primary neocortical cells with the 3-NPA decreases the glutamate-induced excitotoxic cell death, but has no effect on the staurosporine- and doxorubicin-induced apoptosis.

P14.09 Neuroprotective effect of 17beta-estradiol administration in murine model of Parkinson's disease

Joniec I.[1], Ciesielska A.[1,2], Przybylkowski A.[1], Kurkowska-Jastrzebska I.[2], Czlonkowska A.[1,2], Czlonkowski A.[1]

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

The potential role for estrogen (Es) acting as a neuroprotectant of dopaminergic system may be supported by the epidemiological reports showing a gender differences in Parkinson's disease (PD). Additionally experimental investigations strongly support a neuroprotective role of Es in numerous neurodegenerative processes. The aim of the present study was to examine the chronic effects of Es (17b-estradiol, 0.25 mg per pellet, 21-days release) on dopaminergic system in female C57Bl mice (12 months old) in a murine model of PD induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). Estrogen was administered 7 days prior to (exp.1) or 3 days after MPTP intoxication (exp. 2). MPTP (40 mg/kg) was injected to the animals in four intraperitoneal (i.p.) injections at 1-h intervals. Striatal dopamine (DA) was quantified by high-performance liquid chromatography (HPLC) at 1, 7 and 21 (exp.1) or 7 and 21 days (exp. 2) post intoxication. MPTP treatment decreased DA concentration within 1-21 days following MPTP injection. Es exerted a neuroprotective effect upon dopaminergic system (less pronounced MPTP-induced decreases of DA) when administered 7days prior MPTP, but not when Es was implanted 3 days after MPTP intoxication.

P14.10 cAMP effects and neuroprotective activity of PACAP in astrocytes and neurons subjected to hypoxia

Jozwiak-Bebenista M.[1], Nowak J.Z.[1,2]

[1]Dept. of Pharmacology, Medical University; [2]Centre for Medical Biology, PAS, Lodz, Poland

PACAP – pituitary adenylate cyclase-activated polypeptide is a pleiotropic peptide exerting in vertebrates numerous actions related to neuromodulation, neurotrophism or neuroprotection. Its effects are mainly mediated through AC-coupled receptors named PAC1 and VPAC. Aim of work: (1) to evaluate the effect of hypoxia on PACAP-evoked receptor-mediated cAMP generation in glial and neuronal cells, and (2) to see whether PACAP has a neuroprotective activity. Methods: rat cell cultures maintained under normoxic or hypoxic conditions were used. cAMP generation was analyzed in [3H]adenine-pretreated cells. Protective activity of PACAP was tested in a model system utilizing astrocytes and adrenaline whose cAMP effect was inhibited by hypoxia. Results: in normoxia PACAP (15 min exposure) potently stimulated cAMP production in astrocytes and neurons, being distinctly more effective in glial cells. Acute cAMP effects of PACAP were significantly suppressed by hypoxia, while forskolin effects were unaffected. 24-h pretreatment of hypoxia-treated astrocytes with 1 nM PACAP had no effect on basal cAMP generation but completely restored the hypoxia-suppressed effect of adrenaline. Conclusion: PACAP is a powerful stimulator of cAMP generation in astrocytes and neurons, with glial cells likely being a primary target for PACAP actions. Antagonism by PACAP of hypoxia-evoked cell function confirms its neuroprotective potential. Supported by funds from Med Univ: 502-11-462, 503-1023-1.

P14.11 The endoplasmic reticulum/Golgi cross-talk in response to ischemia/reperfusion injury

Lehotsky J., Pavlikova M., Urban P., Kaplan P., Dobrota D.

Dept. of Medical Biochemistry, Jessenius Faculty of Medicine, Comenius University, Martin, Slovakia

Neural cells posses an ordered intracellular organelles with high Ca^{2+} concentrations, such as endoplasmic reticulum (ER) and Golgi apparatus (GA). On the tissue level, the complex responses have evolved with a great impact to cross-talk between ER/GA/mitochondria and finally to neural viability. Damage to the ER is involved in neuronal damage including ischemia/hypoxia. While the presence of GA secretory Ca^{2+}/Mn^{2+} ATPases(SPCA1) in the brain has already been shown, the cell-type specific expression pattern has not been established. Experiments show that SPCA1 is localized in neural cells to structures distinct from endoplasmic reticulum. In addition, in the rat forebrain ischemic model we examined the activation of genes involved in ER stress response at mRNA levels as well as SPCA1 gene from GA after 15 min ischemia and different times of reperfusion (1, 3 and 24 h) (IRI). The IRI induced both the changes in the early gene expression and the post-translational protein modification. The most striking differences was observed in mRNA and protein levels of ER resident Bip (Grp78). Measurement of SPCA1 mRNA after IRI clearly detected expression of the gene in injured area. In addition, mRNA expression pattern follows time dependent manner in reperfusion period. Since the pump plays major role in the refilling of Ca^{2+} stores, alterations between ER and Golgi derived intracellular calcium stores function might be an alternative pathway which control neuronal loss after injury. Supported by: VEGA 3380/06, COST B30, MVTS 39 and GRANT UK.

P14.12 1,2,3,4-tetrahydroisochinolines inhibit NMDA receptor-mediated excitotoxicity

Makarewicz D.[1], Kuszczyk M.[1], Zieminska E.[1], Salinska E.[1], Stafiej A.[1], Antkiewicz-Michaluk L.[2]

[1]Department of Neurochemistry, Medical Research Centre, PAS, Warsaw; [2]Department of Neurochemistry, Institute of Pharmacology, PAS, Krakow, Poland

Endogenous tetrahydroisochinolines, TIQ and 1MeTIQ are known for their mild neurotoxic or neuroprotective potential, respective-

ly. We reported that 1MeTIQ inhibits NMDA receptors and prevents glutamate excitotoxicity in cultured neurons. In this studies we examined effects of TIQ on NMDA receptor activation and NMDA and glutamate-induced excitotoxicity in the primary cultures of rat cerebellar granule cells. We evaluated neuroprotective potential of 1MeTIQ *in vivo* in the rat model of birth asphyxia. The results of our *in vitro* studies revealed that TIQ, acting with slightly lower potency than 1MeTIQ, inhibits the binding of [3H]MK-801 to rat brain membranes, reduces NMDA-evoked 45Ca uptake and glutamate-induced rises in the intracellular calcium levels evaluated with fluorimetric methods. Moreover TIQ and 1MeTIQ partially prevent NMDA-evoked neurotoxicity. *In vivo* studies demonstrated that 1MeTIQ applied specifically in a dose of 50 mg/kg, injected i.p. 3 times every 2 hours after the insult, significantly reduced brain damage in a model of perinatal hypoxia. However in these animals we did not observe typical symptoms of treatment with NMDA receptor antagonists like postischemic hypothermia or reduction of weight of the control hemisphere. Our results suggest that both, 1MeTIQ and TIQ inhibit NMDA receptor activity and reduce excitotoxicity in vitro, however it's mechanism is not clear.

P14.13 Time-dependent desensitisation of the adenosine A1-receptor during hypoxia

Rufke C., Nieber K.

Institut of Pharmacy, University of Leipzig, Germany

Because of the higher energy consumption under hypoxic conditions the release of adenosine, as the degradation product of ATP, is increased. It can modulate the neuronal function and is known to act neuroprotective mainly due to activation of adenosine A1 receptor (A1R), one of its four G-protein-coupled-receptor subtypes. Endogenously released adenosine as well as selective A1R agonists inhibit the postsynaptic potential in cortical neurones. A desensitisation of this receptor subtype yields to the loss of neuroprotection. Therefore, the time-dependent desensitisation of the A1R was investigated on CHO cells stably transfected with A1R by the measurement of intracellular markers using calcium imagin and ALPHA-Screen-technology. The activation of A1R by CPA, a selective receptor agonist, leeds to an increase of the intracellular calcium concentration, which decreases time dependently during persistent agonist exposure. Moreover, under hypoxic conditions the endogenous released adenosine inhibits the adenylate cyclase activity by an activation of the A1R. A desensitisation of this effect occurs after 6 and 12 hours of hypoxia exposure. Our results contribute to the understanding of the cellular mechanisms which underlie the time dependent A1R desensitisation.

P14.14 Inhibition of NMDA receptor by dantrolene: New putative mechanism of neuroprotection

Salinska E., Sobczuk A., Lazarewicz J.W.

Dept. of Neurochemistry, Medical Research Centre PAS, Warsaw, Poland

Excessive increase in $[Ca^{2+}]i$ in neurons evoked by ischemia or excitotoxic challenge may trigger neuronal injury and death. Increase in $[Ca^{2+}]i$ results from Ca^{2+} influx e.g. *via* NMDA receptors or its release from intracellular stores. Dantrolene, a drug useful in treatment of malignant hyperthermia, inhibits skeletal muscle isoform of RyR1. Dantrolene blocks also Ca^{2+} release from intracellular stores in neurons and may have neuroprotective effect. Previously we noticed neuroprotection by dantrolene in brain hypoxia/ischemia of 7-days-old rats, although in the developing rat brain only a negligible expression of these receptors was detected. We noticed also that dantrolene inhibits NMDA evoked 45Ca uptake in cultured neurons, which might suggest a direct interference of dantrolene with NMDA receptors. The aim of the present study was to recognize putative site of dantrolene action within the receptor complex. The results demonstrated that dantrolene in micromolar concentrations inhibits [3H]MK-801 binding to isolated brain membranes. Binding of [3H]glycine was also inhibited by dantrolene in a dose-dependent manner independently on the presence of NMDA. Strychnine (0.5 μM) only slightly reduced [3H]glycine binding, whereas application of both 0.5 μM strychnine and 100 μM dantrolene diminished the binding in 70%. Our results indicate that dantrolene inhibits activation of NMDA receptors by interfering with its glycine binding site. This mechanism may at least partially explaine neuroprotective effects of dantrolene.

P14.15 Neuroprotective effects of methylnicotinamide in cultured cerebellar granule cells

Slomka M., Zieminska E., Salinska E., Stafiej A., Lazarewicz J.W.

Department of Neurochemistry, Medical Research Centre PAS, Warsaw, Poland

The aim of this study was to evaluate neurotoxic and/or neuroprotective effects of 1-methylnicotinamide in primary cultures of rat cerebellar granule cells. It has been suggested that MNA, may be neurotoxic due to mechanism resembling that of MPP+, while neuroprotective effects of its parent substance nicotinamide (NAM) has been demonstrated. Excitotoxicity was induced by 30 min incubation with 0.5 mM glutamate or 0.5 mM NMDA + 5 μM glycine. Neurotoxicity was evaluated 24 h later with propidium iodide staining. Our initial studies demonstrated very weak neurotoxic potential of both MNA and NAM, which in concentrations below 50 mM were devoid of neurotoxicity during 24 h cell exposure, whereas 50 μM MPP+ induced a considerable neurotoxicity. Subsequent experiments demonstrated that 25 mM NAM and MNA significantly reduced NMDA-evoked neurotoxicity. They also suppressed glutamate- or NMDA-induced $45Ca^{2+}$ uptake in CGC and glutamate-evoked increases in the intracellular Ca^{2+} level detected with fluorescent probe Calcium OrangeTM. These results primarily suggested that both NMA and MNA might directly interfere with activity of NMDA receptors. However, further experiments showed that MNA and NAM do not interfere with NMDA + glycine evoked [3H]MK-801 binding to rat brain membranes, pointing to other mechanism of neuroprotection. In conclusion, our results demonstrate that MNA is not neurotoxic, but instead induces neuroprotection in the excitotoxic insults comparable to NAM. The mechanism of this phenomenon remains unclear. Supported by grant PBZ-KBN-101/T09/2003/11.

P14.16 Neuroprotective effects of some neuropeptides, PACAP, VIP or NPY, in primary neuronal cultures
Smialowska M., Domin H., Kajta M.

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

Earlier data showed that modulatory neuropeptides, as pituitary adenylate cyclase activating polypeptide (PACAP), vasoactive intestinal peptide (VIP) and neuropeptide Y (NPY) may have neuroprotective activity in some *in vitro* and *in vivo* models. In the present study we investigated the neuroprotective effects of these peptides against kainic acid (KA) excitotoxicity in primary cultures of mouse cortical and hippocampal neurons. In order to evoke toxic effects, primary cultures were exposed to 150 μM KA for 24 h (hippocampus) or 48 h (cortex). PACAP or VIP at concentrations 10–15, 10–9 or 10–7 M and NPY or specific NPY receptor (Y1R, Y2R, Y5R) agonists (at concentrations 10–8, 10–7, 5–6 or 10–6 M), were applied 30 min before or 30 min after KA. Neurotoxicity was measured by lactate dehydrogenase (LDH) efflux from the damaged cells into the culture media. Our results showed a significant attenuation of KA-induced LDH release in cultures after the studied peptides application. The effect was observed after using all doses of PACAP or VIP and was stronger in the cortical cultures after PACAP and in hippocampal ones after VIP. NPY and specific Y2 or Y5 agonists were protective mainly at higher concentrations (5 mM or 10 mM) but no protection was found after the Y1R agonist. The obtained results indicate that PACAP, VIP, NPY and Y2R or Y5R agonists are neuroprotective against KA neurotoxicity given both 30 min before or 30 min after the KA. Moreover PACAP and VIP are neuroprotective at much lower concentrations than NPY. Supported by MNiI grant no. 2P05A 11428.

P14.17 ERK activation mediates astrocyte death and is a target for neuroprotectant FK506
Szydlowska K., Gozdz A., Kaminska B.

Nencki Institute of Experimental Biology PAS, Warsaw, Poland

Astrocytes modulate synaptic transmission, provide metabolic support for neurons and can modulate glutamate (GLU) homeostasis by its uptake, preventing GLU elevations, however, an excess of GLU under pathological conditions can induce astrocytes death by unknown mechanisms. Impairments of astrocyte function may play an important role in cerebral ischemia. Immunosuppressant FK506 is neuroprotective in animal models of brain and spinal cord injury, focal and global ischemia but molecular mechanisms underlying neuroprotection remain elusive. We have previously demonstrated that GLU (>50 mM) triggers an apoptotic death of cultured cortical astrocytes mediated by mitochondrial pathway. FK506, a calcineurin inhibitor and neuroprotective drug, inhibits efficiently glutamate-induced death of astrocytes in vitro and in ischemic brain (Szydlowska et al. 2006). Identifying mechanisms of astrocyte death, we found that a sustained activation of extracellular signal-regulated kinase (ERK) and JNK is involved in mitochondria-mediated cell death in glutamate-treated astrocytes. Furthermore, we found a rapid increase of phosphorylated ERK levels in the ischemic striatum and cortex, where a widespread astrocyte death has been detected. A single FK506 injection decreased ERK activation in the injured areas. Our findings suggest a possible involvement of ERK activation in glutamate-induced astrocyte death and potent inhibitory effect of FK506. We hypothesize that an attenuation of astrocyte death after ischemia may be a novel mechanism of its neuroprotective action.

P14.18 Alteration of UPR reaction after global forebrain ischemia/reperfusion
Urban P., Pavlikova M., Sivonova M., Dobrota D., Lehotsky J.

Department of Medical Biochemistry, Jessenius Faculty of Medicine, Comenius University, Martin, Slovakia

The functional significance and neuroprotective mechanism of simvastatin in ischemic brain injury is not yet solved. The purpose of study is to evaluate the effect of simvastatin on ischemic brain injury. A variety of endoplasmic reticulum (ER) stresses trigger the unfolded protein response (UPR). Sensors of stress are the ER membrane bound proteins IRE1 and ATF6. We were simultaneously examined the activation of ATF6, IRE1, Grp78 and Xbp-1 at mRNA and protein levels after 15 minutes 4-VO ischemia and different times of reperfusion (1, 3 and 24 h). The results showed that simvastatin increased Aft6 mRNA levels and caused significant difference at I stage between treated and untreated animals. Our results for Grp78 detect the highest mRNA level in 3 h of reperfusion in cortex. In addition to this we observed increased mRNA level after simvastatin treatment. Results also showed that statins kept increasing level of mRNA between R3 and R24 in treated animals. Protective effect of simvastatin is visible mainly in 24 h of reperfusion. Xbp1 mRNA level of untreated animals showed no significant shift between 1 and 24 h of reperfusion. It is possible that the Ire1 alteration reflects Ire1 activation, and the lack of detection of processed xbp-1 possibly indicates the inability to ligate the cleaved mRNA. These data indicates that statins, in addition to their cholesterol lowering effect, might exert a neuroprotective role in the attenuation of ER stress response after acute stroke/reperfusion.

P14.19 Mitochondrial apoptosis but not dysfunction is significantly affected by ischemic preconditioning
Racay P., Tatarkova Z., Drgova A., Kaplan P., Dobrota D.

Institute of Biochemistry, Jessenius Faculty of Medicine, Martin, Slovakia

Ischemia-induced mitochondrial dysfunction is considered to be an important event coupling cerebral blood flow arrest to neuronal cell death due to involvement of mitochondria in the process of apoptosis. Ischemic preconditioning (IPC) represents an important adaptation of CNS to sub-lethal ischemia, which results in increased tolerance of CNS to the lethal ischemia. In this study we have determined the effect of IPC on ischemia/reperfusion-induced inhibition of mitochondrial protein synthesis and activity of mitochondrial respiratory chain complexes I and IV in rat hippocampus. Global brain ischemia was induced by 15 minutes of 4-vessel occlusion. Rats were preconditioned by 5 minutes of sub-lethal ischemia and 2 days later, 15 minutes of lethal ischemia was induced. Our results showed that IPC affects dysfunction of mitochondria in two different ways. Ischemia-induced repression of mitochondrial translation was moderately attenuated by IPC. Slight protective effect of IPC was documented for complex IV, but not for complex I. With respect

to apoptosis, IPC abolished completely ischemia-induced translocation of p53 to mitochondria and led to significant inhibition of ischemia induced-activation of Caspase-9 activity. Our results indicate that although ischemia-induced mitochondrial dysfunction is not significantly affected by IPC, processes involved in mitochondrial apoptosis are almost completely abolished by IPC. Supported by grants VEGA 1/4255/07 and MVTS 39.

P14.20 Hypoxic preconditioning enhances glutamate receptor-mediated calcium transients in rat brain slices

Semenov D.[1], Belyakov A.[1], Samoilov M.[1], Lazarewicz J.[2]

[1]Pavlov Institute of Physiology, RAS, St Petersburg, Russia; [2]Medical Research Centre, PAS, Warsaw, Poland

Hypoxic preconditioning induces tolerance to subsequent severe hypoxia applied to the animals or their brain slices 24 hours after. In the present study we tested if changes in calcium transients mediated by glutamate receptors accompany development of brain tolerance induced by repetitive episodes of moderate hypobaric hypoxia. Glutamate receptor agonists (50 mM L-glutamate, 100 mM NMDA, 40 mM AMPA and 100 mM DHPG) were repeatedly bath-applied to slices of piriform cortex, obtained from rats 24 hours after their submission to the last trial of hypobaric preconditioning or to sham procedures. Responses of free and bound intracellular calcium (Ca-f and Ca-b, respectively) were detected fluorimetricaly. In the control slices NMDA or AMPA induced a transient rise in Ca-f, followed by sustained increase in Ca-b. Group I metabotropic glutamate receptor agonist with DHPG induced a modest transient drop in Ca-b and increase in Ca-f. The hypoxic preconditioning moderately enhanced calcium transients induced by NMDA and AMPA, whereas responses to DHPG were significantly potentiated. These results suggest that induced hypoxic tolerance is accompanied by moderate increase of intracellular Ca^{2+} level caused by sensitization of Ca^{2+} gating AMPA receptors and strengthening of NMDA receptor-mediated Ca^{2+} influx and especially of group I metabotropic glutamate receptor-mediated Ca^{2+} release.

P14.21 Antimyelin basic protein T cells stimulate hippocampal neurogenesis in trimethyltin intoxicated rats

Dzwonek K.[1], Kurkowska-Jastrzebska I.[2], Zaremba M.[1], Oderfeld-Nowak B.[1]

[1]Nencki Institute of Experimental Biology PAS; [2]Institute of Psychiatry and Neurology, Warsaw, Poland

Our recent studies demonstrated that administration of antimyelin basic protein (MBP) T cells improved survival of rat hippocampal pyramidal neurons in the model of trimethyltin (TMT)-evoked neurodegeneration (Kurkowska-Jastrzebska et al. 2007, Neuroreport 5:148). One of the possible mechanisms of beneficial effect of T cells could be inducing neuronal regeneration at the site of hippocampal injury to the extent that it is well known that neurogenesis occurs in the adult hippocampus and can be enhanced in various conditions. In the present study we have investigated, immunocytochemically, the expression of doublecortin, an immature neuronal marker and PCNA, a cell proliferation marker, in the rat hippocampus following TMT intoxication and administration of antiMBP T cells. We have found the enhancement of doublecortin expression in the subgranular layer evoked by administration of T cells, particularly evident on 21st day, the time of maximal neurodegeneration induced by TMT, accompanied by an enhancement of PCNA expression. Our results suggest enhancement of neurogenesis by administration of antiMBP T cells. It remains to be established whether newborn neuroprogenitors migrate from subgranular layer to the zone of maximal neurodegeneration (CA4 pyramidal cells) and differentiate there to mature neuronal phenotype.

PAIN

P15.01 Different effects of local and systemic administrations of morphine on G-protein gene expression

Askari N.[1], Mahboudi F.[2], Roohani A.H.[1], Yaraee R.[3], Kazemi B.[3], Maghsoudi N.[3]

[1]Faculty of Science-Tehran University; [2]Pasture Institute of Iran; [3]Neuroscience Research Center- Shaheed Beheshti University, Tehran, Iran

Opiates are considered as the most powerful analgesic in clinic. All three types of opioid receptors have been found on peripheral terminals of sensory neurons. Changes in the resting levels of G-protein subtypes could have an effect on intracellular signaling pathways. Our previous results have shown that acute systemic administration of analgesic doses of morphine could change the G-protein genes expression levels. The current study was designed to investigate whether the systemic and local administration of analgesic doses of morphine differently affect on the level of G-protein subunits gene expression in rat spinal. The gene expression of G-proteins was assessed by using real time PCR. According to the present data, a significant elevation in the mRNA levels of was observed in the group that received analgesic dose of morphine systemically while intraplantar treatment with analgesic dose of morphine had no effect on the gene expression. Systemic and local administration of analgesic doses of morphine in the presence of carrageenan induced different alterations in the levels of mRNA. Changes in gene expression following exposure to systemic administration of opiates are likely responsible for the alterations in responsiveness of opioid sensitive neurons.

P15.02 The role of interleukins 1-alpha and 1-beta in nociception in rat model of neuropathic pain

Mika J.[1], Korostynski M.[2], Kaminska D.[2], Osikowicz M.[1], Makuch W.[1], Przewlocka B.[1]

[1]Department of Pain Pharmacology; [2]Department of Molecular Neuropharmacology, Institute of Pharmacology PAS, Krakow, Poland

Recent research has shown that activated glia release various interleukins (IL), which can influence neuropathic pain. However, the role of IL 1-alpha and IL 1-beta in nociception is not well established. The goal of our study was to exam changes in transcript abundance for IL-1 alpha and beta genes in rat spinal cord and DRG (L5-L6) 7 days after chronic constriction injury (CCI). Moreover, the influence of intrathecal administration of IL 1-alpha, IL 1-beta and IL1 receptor antagonist on allo-

dynia and hyperalgesia was evaluated 7 days after CCI. The level of IL 1-alpha and 1-beta mRNA were quantified using qPCR. The expression of IL 1-alpha in ipsilateral DRG was strongly up-regulated 3 days after CCI. On 7th day the expression of IL 1-alpha was still increased, as well as expression of IL 1-beta. In the spinal cord transcription of the IL 1-beta gene, but not 1-alpha, was significantly increased on 7th day. The administration of IL 1-alpha and IL 1 receptor antagonist dose-dependently attenuated symptoms of neuropathic pain. Our results show that glia cells in spinal cord and DRG activated in response to nerve injury release IL 1-alpha as well as 1-beta cytokines. It seems that IL 1-alpha and 1-beta may play an important role in pain transmission and that their interactions are involved in the development of allodynia and hyperalgesia. Supported by IF PAN statutory funds and by a grant from MNiSzW 2P05A10528.

P15.03 Behavioural visceral nociceptive response is associated with vagal nerves activity in rats

Nowak L.[1], Zurowski D.[1], Kaszuba-Zwoinska J.[1], Wordliczek J.[2], Thor P.J.[1]

[1]Department of Pathophysiology; [2]Department of Pain Treatment and Palliative Care, Collegium Medicum, Jagiellonian University, Krakow, Poland

Vagal nerves may participate in pain sensation. Vagal afferents activation seems to be related to cytokines release. Pentoxifiline (ptx) suppress mainly TNF-alpha production.The aim of this study was to evaluate behaviour changes and vagal activity in response to visceral nociceptive stimulus and PTX treatment. Rats were divided on 6 groups. In first group only peritonitis was induced, second group was pretreated with PTX (10 mg/kg i.p.) prior to peritonitis. Remaining groups were dedicated to vagal nerve recording: control (C), PTX, peritonitis (P), PTX + peritonitis (PTX+P). In all groups nodose ganglia of vagal nerves were taken for immunohistochemical studies. P induced nociceptive writhing response in rats. PTX inhibited pain-related behaviour. In C dominant frequency of the basal vagal afferents was 0.23 ± 0.08 Hz vs. 1.78 ± 0.34 Hz in P. In PTX + P frequency of vagal afferents discharge decreased to 0.56 ± 0.17 Hz, in PTX was similar to C. In P c-Fos expression [(17.8 ± 3.6 positive neurons /slide (pn/s)] confirmed high activity of vagal afferents vs. 11.8 ± 4.7 pn/s in PTX + P. Peritonitis increased TNF-alpha to 33.8 ± 8.6 pg/ml vs. 0.5 ± 4.7 pg/ml in C and returned near C in PTX+P. Vagal nerves play an important role in cytokine induced visceral sensation and seem to be, beside spinal afferents, parallel pathway of visceral nociception. In cytokine-dependent visceral vagal stimulation PTX use might offer new strategies of the treatment.

P15.04 The role of mGluR2/3 and mGluR7 agonists in neuropathic pain

Osikowicz M., Mika J., Przewlocka B.

Department of Pain Pharmacology, Institute of Pharmacology, PAS, Krakow, Poland

Within the last decade, the interest in the role of Group II and III mGluRs in nociceptive processing significantly increased. The aim of the present study was to exam the effects of mGluR2/3 and mGluR7 agonists (LY379268 and AMN082, respectively) on allodynia and hyperalgesia in mice model of neuropathic pain induced by sciatic nerve ligation (SNL). A single intraperitoneal (i.p.) administration of LY379268 (5–30 mg/kg) showed higher dose-dependent attenuation of allodynia (von Frey test) and hyperalgesia (cold plate test) 7 days after SNL than AMN082 (2–5 mg/kg). Chronic i.p. treatment (16 h and 1 h before ligation of the SNL and then twice daily for 7 days) of lower doses of LY 379268 (5 mg/kg) and AMN082 (3 mg/kg) significantly attenuated neuropathic pain symptoms in mice 3 and 7 days after SNL. LY 379268 induced higher antiallodynic and antihyperalgesic effects in comparison with AMN082 as observed 3 days after SNL. However, at day 7 LY 379268 showed lower analgesic effects when compared to AMN082. Moreover, repeated administration of LY 379268 (5 mg/kg; i.p.) but not AMN082, induced the development of weak tolerance to its analgesic effect measured 7 days after SNL by von Frey and cold plate tests. Summarizing, the above results demonstrate that the activation of receptors from Group II and III mGluRs evoked by single and chronic injections of its selective agonists may decrease the neuropathic pain-related behaviors in SNL mice. Supported by statutory funds (Ministry of Science and Higher Education) and by grant (MNiSzW 2P05A10528).

P15.05 Endovanilloids mediate neurotransmitter release in PAG-RVM descending pain pathways

Starowicz K.[1], Maione S.[2], Cristino C.[1], Palazzo E.[2], Marabese I.[2], Di Marzo V.[1]

[1]ERG, ICB CNR, Pozzuoli; [2]Department of Experimental Medicine, Second University of Naples, Italy

TRPV1 is expressed in the several brain areas, including the periaqueductal gray (PAG), yet data in favor of its brain function are missing. Here we examined in rats the effect of intra-VL-PAG injections of TRPV1 ligands on: the nocifensive response to heat in the plantar test and glutamate and GABA release in the RVM. The possible localization of TRPV1 in glutamatergic or GABAergic PAG and RVM neurons was studied. Capsaicin (CAP) injection into the VL-PAG increased thermal pain latency whereas the selective TRPV1 antagonist 5'-iodo-resiniferatoxin (I-RTX) evoked hyperalgesia. I-RTX (inactive dose) abolished CAP-mediated analgesia. Intra-VL PAG injection of CAP evoked glutamate release in RVM, whereas I-RTX significantly decreased it and at a dose inactive per se blocked the effect of CAP. As a secondary effect to glutamate discharge, CAP caused a faint stimulation of GABA release. TRPV1-immunoreacivity (ir) in the VL-PAG and RVM localized mostly to cell bodies. Both in VL-PAG and in the RVM, VGAT and VGLUT1 staining was found around TRPV1-ir cells, indicating glutamatergic and GABAergic input on TRPV1 neurons. Double immunofluorescence identified several TRPV1/VGLUT1 positive cells in the RVM. Our data indicate glutamatergic neurons of the VL-PAG respond to TRPV1 stimulation by releasing glutamate into RVM, thus activating other TRPV1-expressing glutamatergic neurons and produce analgesia. I-RTX significantly decreased glutamate levels indicating this pathway is tonically activated by endovanilloids.

P15.06 Gastric ulcers induce vagal afferents hypersensitivity and increase somatic sensitivity in rats

Zurowski D.[1], Nowak L.[1], Wordliczek J.[2], Thor P.J.[1]

[1]Department of Pathophysiology; [2]Department of Pain Treatment and Palliative Care Jagiellonian University, Krakow, Poland

Acetic acid-iduced gastric ulcer (GU) is a model of visceral pain in rats. We investigated whether visceral pain may trigger the changes in somatic nociception and gastric vagal afferent input may contribute to the altered somatic sensations associated with gastrointestinal disorders. For this aim somatic pain sensitivity and vagal afferent activity ware estimated in chronic GU and during GU healing. The study was carried out on rats divided into four group with experimental chronic GU and two groups of sham rats. Mechanical hypersensitivity using von Frey test was investigated on 3rd, 7th and 14th day after GU induction and compared to sham rats. In three groups, the nodose ganglia were removed respectively on 3rd, 7th, 14th day after gastric ulcer inducement and neuronal activation marker (c-Fos) in vagal primary afferent neurons was assesed and compared to control group. Experimental GU healed spontaneously within 2 weeks. Chronic gastric ulcers increased somatic pain sensitivity. Natural healing of gastric ulcers was accompanied by decreased mechanical sensitivity. Immunohistochemical studies showed significantly increased number of Fos-positive neurons in vagal nodose ganglia and it confirmed higher activity of vagal primary afferent neurons in experimental chronic GU and during GU healing. Obtained results suggest that gastric ulcer increased vagal afferents activity contribute in visceral nociception and visceral pain triggers somatic hypersensitivity.

STRESS AND MOOD DISORDERS

P16.01 Chronic stress and neurogenesis in structures of the limbic system in rat

Badowska-Szalewska E., Klejbor I., Cecot T., Sidor-Kaczmarek J., Lietzau G., Spodnik E., Morys J.

Medical University of Gdansk, Poland

The limbic system is involved in response to stress stimulation. Incorporation of bromodeoxyuridine (BrdU) into DNA can serve as a marker of cells division associated with neurogenesis. In this study we investigated an influence of the chronic open field (OF) and forced swim (FS) stimulation on the level of cell proliferation in the hippocampus, amygdala and hypothalamus in the young and adult rats. 26 rats of ages P7 and P360 were exposed to chronic stress lasting 15 minutes daily for 21 days. BrdU was administered three times and brains were stained using immunohistochemical method. In the control groups BrdU-ir cells were observed in all examined structures, but their level was different. Moreover there were a large number of BrdU-immunoreactive cells in P7, whereas moderate in P360. In the rats exposed to chronic OF and FS the number of BrdU-ir cells in the investigated structures was considerably decreased in the young rats and slightly decreased in the adult ones in comparison to the controls. Little differences in the amount of BrdU-ir cells were observed after exposure to chronic stress both types. Our results indicate that newly proliferated cells are present in the investigated areas of the limbic system both in the young and the adult rats. Suppression of cell proliferation after chronic OF and FS is higher in the adult rats. Type of applied chronic stress does not have an influence on the neurogenesis level.

P16.02 Chronic mild stress affects mRNA expression of heat shock proteins in rat hippocampus

Bielawski A.[1], Papp M.[2], Nalepa I.[1]

[1]Dept. of Brain Biochemistry; [2]Lab. of Behavioral Pharmacology, Dept of Pharmacology, Inst. of Pharmacology PAS, Krakow, Poland

Heat shock proteins (HSPs) are induced by various sorts of environmental stressors and play a protective role against stress-induced damage of the cell. 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. The aim of the study was to assess the expression of HSP72, HSC73, HSP86 and HSP84 mRNAs in the hippocampus of rats subjected to the standard CMS procedure and then treated with antidepressant drug, imipramine (IMI). Five groups of male Wistar rats were considered in the molecular 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 HSPs mRNAs was measured by quantitative RT-PCR method. We found that CMS procedure increased the expression of HSP72 mRNA and HSP84 mRNA compared to sham-saline group (by 40% and 16%, respectively). In rats positively responding to IMI treatment in behavioral test, the stress-induced changes were abrogated and mRNA level was comparable to that of sham-saline group. Interestingly, in rats non-responding to IMI treatment, the expression of HSP72 mRNA remained increased in comparison to sham-saline and stress-IMI groups (by 59% and 57%, respectively). Our results suggest that CMS may induce the cellular stress reaction in rat hippocampus. The effect is reversed only in rats responding to IMI treatment. Supported by statutory funds of the Institute of Pharmacology, P.A.S.

P16.03 Age-related changes of BDNF and TrkB distribution under chronic stress exposure in rat

Cecot T.

Anatomy and Neurobiology, University of Gdansk, Poland

Chronic stress affects brain areas involved in learning and emotional responses. The brain-derived neurotrophic factor (BDNF), by acting through its receptor TrkB, is essential regulator of synaptic function, growth, and neuroprotective processes. BDNF activation is caused by both physical and psychological stress events. The present study examines the effect of chronic stress on BDNF and its receptor TrkB pattern in selected brain structures of the young (P7) and old (P360) rats. Twenty-six male Wistar rats were exposed to 15 min forced swimming daily during three weeks. Fluorescent immunohistochemistry was used to localize BDNF and TrkB in brain structures connected with stress response: paraventricular nucleus (PVN), supraoptic nucleus (SO), central and medial amygdaloid nuclei (CeA and MeA). Density of BDNF and TrkB positive cells in control animals is higher in P7 than P360. However, after chronic stress exposure an increase of these markers is considerably higher in the adult than in the young animals. In particular, we observed the increase of BDNF and TrkB in the magnocellular but not parvocellular part of PVN. In the Amygdala density of these markers was lower in MeA than in CeA. The results support the view that habituation to the stress conditions is easier in the early postnatal period.

P16.04 Effects of buspirone treatment of the neonatally stressed mice

Glowacz A., Djavadian R., Turlejski K.

Department of Molecular and Cellular Neurobiology, Nencki Institute of Experimental Biology PAS, Warsaw, Poland

Stress experienced during a critical period of ontogeny may influence animals' morphology, behavior and learning abilities. Efforts are being made to attenuate harmful effects resulting from such stress. We investigated the remote effects of separation stress and buspirone therapy in Swiss mice. The newborn pups were separated from their mothers for two hours daily from the first till the eighth postnatal day. One group of the stressed mice was injected with buspirone from the third till eighth postnatal day to attenuate negative effects of stress. Starting from the fifth week the animals were subjected to behavioral tests: open field, elevated plus maze and rotarod and tested in the Intellicage system. The buspirone-treated mice showed generally lower locomotor activity than the stressed mice. In the open field buspirone-treated animals spent more time in the central area than the stressed mice and left fewer fecal boli, which together may indicate reduced anxiety. However, they also spent less time in the open arms of the elevated plus maze and made fewer entries into its open arms, spending most time in the closed arms. In the consecutive rotarod sessions the buspirone treated mice improved their performance in the apparatus less than the stressed-only mice. Altogether these results show some effectiveness of buspirone treatment of the negative effects of neonatal stress.

P16.05 Analysis of the serotonergic system activity in various models of fear in the rats

Koprowska M., Krotewicz M., Strzelczuk M.

Laboratory of Neurophysiology, University of Lodz, Poland

The study presents a comparative analysis of the influence of different stressogenic stimuli on the serotonergic system activity in the brain structures forming the neural system, which detects threatening and stressogenic stimuli and organizes a response to them. The animals were subject to various behavioral tests, and next the serotonin turnover was examined and compared using the ratio of the metabolite concentration to monoamine (5-HIAA/5-HT). Stressogenic stimuli in the tests performed were: light of high volume (light dark transition test – LDT) or open field (open field test – OF, elevated plus maze test – EPM). HPLC-ED analysis showed an increased 5-HIAA/5-HT ratio in the frontal cortex and a decreased 5-HIAA/5-HT ratio in the pons after using the LDT-test. HPLC-ED analysis also showed a significant increase of 5-HIAA/5-HT ratio in the frontal cortex and in the hippocampus and a decrease of 5-HIAA/5-HT ratio in the pons after using OF-test. Concurrently, HPLC-ED analysis showed a significant increase of 5-HT turnover in the frontal cortex and hippocampus after using the EPM-test. These results indicate that different stressogenic stimuli caused an increase in the serotonergic activity in the emotional brain regions, especially in the frontal cortex and hippocampus.

P16.06 Strain-specific response to kappa opioid receptor ligands is modulated by social isolation stress

Kostrzewa E., Solecki W., Kubik K., Przewlocki R.

Institute of Pharmacology PAS; Institute of Applied Psychology, Jagiellonian University, Krakow, Poland

Various studies point to the involvement of kappa opioid system in stress-induced behavioural changes in rodents. In order to examine the modulating effect of kappa opioid receptor (KOR) ligands administration on stress response we conducted a series of tests on stress-naive and socially isolated animals measuring stress-induced immobility (Forced Swim Test) and natural reward sensitivity (sucrose oral self-administration) after systemic administration of selective KOR agonist (U50,488H; 5 mg/kg, i.p.) and antagonist (nor-BNI; 10 mg/kg, i.p.). Results show that the effect of KOR ligands' administration can be observed in DBA/2J (DBA) mice only after social isolation stress – the effect that was not seen in C57BL/6J (C57) mice. Social isolation changed natural reward sensitivity in DBA mice in a similar way as KOR blockade in stress-naive mice. What is more we observed time specific changes in natural reward sensitivity during isolation period with decrease in sucrose intake in C57 strain and increase in DBA mice. In conclusion, we hypothesise that social isolation stress causes similar changes in KOR system functioning in DBA mice as KOR blockade. Thus KOR system becomes responsive to social isolation and can modulate stress reactivity. Our results indicate that kappa opioid system sensitivity is increased after social isolation stress in strain-specific manner. Supported by EU grant LSHM-CT-2004-005166.

P16.07 Neurobiological changes in the frontal cortex in rats differing in the strength of a fear reaction

Lehner M.[1], Taracha E.[1], Skorzewska A.[1], Turzynska D.[1], Sobolewska A.[1], Wislowska-Stanek A.[2], Plaznik A.[1,2]

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

After 10 days of acclimatization, the animals were subjected to the conditioned fear test, S group, while the control group C was placed in the conditioning box only. Next, S animals were divided into three experimental groups: the low sensitivity animals (LS, time of freezing behavior below 205 s), the intermediate sensitivity animals (MS, time of freezing behavior between 205–236 s) and the high sensitivity group (HS, with time of freezing behavior above 236 s). This criterion was established according to the mean time of freezing reaction in fear-conditioned animals (group S: 220.6 s ± 15.54, mean ± SEM). The most interesting results of the present research showed that the group of LS rats had a higher c-Fos activity in the prefrontal cortex, PCX, stronger 5-HT and CRF complexes in the PCX in comparison to HS group. This group also vocalized more during test session, and had higher serum levels of corticosterone, examined 10 min after testing session. An increase in serum corticosterone can be considered another coping mechanism, which, along with serotonin-related activation of PCX, could help to better prepare the organism to handle a threatening stimulus. In conclusion, the type and strength of a fear reaction to conditioned affective stimuli are related to the activity of brain structures controlling anxiety, e.g. PCX.

P16.08 Does Ras in neurons promote antidepressant activity?
Leske O., Heumann R.

Molecular Neurobiochemistry, Ruhr-University Bochum, Germany

Stress is believed to be an important factor in the pathogenesis of depression resulting in reduced hippocampal neurogenesis, whereas treatments for depression enhance neurogenesis. In contrast, the elimination of neurogenesis by irradiation does not produce the symptoms of depression. Thus, the aim of this study was to elucidate the possible contribution of neurogenesis in depression by using a synRas transgenic mouse model expressing constitutively activated Ha-Ras in differentiated neurons (Heumann et al. 2000, J Cell Biol 151: 1537–1548). In these synRas mice the volume of neuronal cell somata, axons and dendrites was increased and the number of synaptic structures was 2-fold elevated. However, in the dentate gyrus of the hippocampus there was a dramatic reduction in progenitor cell production (Manns and Heumann 2002, Soc Neurosci, abstr No 618.3). Applying the forced swim test we observed an antidepressant-like effect in the synRas mice as compared to their wildtype littermates suggesting that changes in neuronal Ras activity may play a role. Chronic fluoxetine treatment decreases the immobility values in the forced swim test and increases the proliferation of hippocampal progenitors in both, wildtype and synRas mice. However, chronic citalopram treatment stimulated hippocampal progenitor proliferation only in the synRas mice but not in wildtype mice. Neither wildtype nor synRas mice responded to chronic citalopram treatment in the forced swim test. Our data suggest that changes in hippocampal neurogenesis do not strictly correlate with the behavioral response in animal models of depression.

P16.09 Stress-induced changes of interleukin-1beta (IL-1beta) within the limbic system in rat
Lietzau G., Sidor-Kaczmarek J., Badowska-Szalewska E., Klejbor I., Domaradzka-Pytel B., Cecot T., Morys J.

Medical University of Gdansk, Poland

IL-1beta is the proinflammatory cytokine highly produced by CNS glia under conditions of disease, damage and stress. It is reported that cytokines are released not only in response to the traumatic stress, but also immobilization or psychophysical stress. Many studies suggest that acute stress increases the level of cytokines, but the reports on the chronic stress are contradictory. The aim of this study was to compare the influence of different chronic stress stimulations on IL-1beta level in relation to age. We applied psychological (open field) and psychophysical (forced swimming) chronic stress to investigate IL-1beta changes in hypothalamus, hippocampus and amygdala in the young (P7) and adult (P360) male Wistar rats. Activation of the limbic system under the chronic stress was estimated by c-Fos expression level. IL-1beta immunoreactivity was detected by immunohistochemical staining and semi-quantitative protein expression was estimated with chemiluminescent immunoblotting. We found no differences in c-Fos level in the limbic system in the young rats exposed to both types of chronic stress and the control groups. The adult rats, however, presented an increased c-Fos expression under both chronic stress conditions. Our results indicate the decreased expression of IL-1beta under the chronic stress exposures in both age groups compared to the controls. The highest expression of IL-1beta we observed in the hippocampus of P7 and P360, however, the protein level was lower in the adult rats.

P16.10 Divergent behavioral and molecular response to novelty in RHA/verh and RLA/verh rats
Meyza K., Nikolaev E., Olszewski M., Zagrodzka J.

Nencki Institute of Experimental Biology PAS, Warsaw, Poland

The investigation of individual differences in emotional reactivity requires use of well-established animal models of diverse emotionality. Psychogenetically selected Roman Low Avoidance (RLA/verh) rats, contrary to their Roman High Avoidance (RHA/verh) counterparts, demonstrate high rate of emotional activation in response to stress (as measured with several behavioral and biochemical parameters). This provides a unique opportunity of elucidating the neuronal background of their diverse emotional behavior. In our experiments the two sublines differed with anxiety-related and exploratory behavior in novel environment (open field with illuminated center, elevated plus maze and hole board tests, quantified by EthoVision, Noldus), especially during first three minutes of the tests. They also reacted with diverse neuronal activation in key structures of the fear-anxiety circuit to this behavioral challenge. We observed subline-specific differences in the expression of c-Fos protein in the basolateral, central, medial and cortical nuclei of amygdala and CA1 field of hippocampus. At the same time no such difference was seen in structures directly responding to stress, such as the paraventricular or dorsomedial nuclei of the hypothalamus. This result might indicate that the two sublines of Roman/verh rats differ in their reaction to novelty, due to differences only in activation of structures involved in higher processing of emotional cues and not the HPA axis.

P16.11 The effects of exercise and restraint on the NK cytotoxixity in rats of different motor activity
Orlikowska A.[1], Wierzba T.[2], Glac W.[1], Badtke P.[1], Tokarski J.[1]

[1]Department of Animal Physiology, University of Gdansk; [2]Department of Physiology Medical University of Gdansk, Poland

The effects of treadmill exercise (45 min, 25 m/s) and restraint stress (plastic tubes, 45 min) on lytic activity (51Cr-release assay), number of large granular lymphocytes (LGL-NK) (Timonen method) and plasma corticosterone level (CORT) (RIA) were evaluated in motor high responders (HR) and low responders (LR) to novelty locomotor response in new enviroment. The exercise increased natural killer cell cytotoxicity (NKCC) in the peripheral blood (HR: +128 delta%; LR: +68 delta%) and decreased NKCC in the spleen (HR: –22 delta%; LR: –29 delta%). The most intense decrease in the number of LGL in both the blood and spleen was in the HRs (–22 delta% and –17 delta%) then LRs (–16 delta% and –14 delta%). After the restraint stress NKCC increased in the peripheral blood (LR: +140 delta%; HR: +50 delta%) while in the spleen remained unchanged. The number of LGL increased in the blood (HR: +23.5 delta% and LR: +61 delta%) and was unchanged in the spleen. The most intense increase in CORT was observed in HR rats both after the exercise (+1 200 delta% in comparison to +875 delta% in LRs) and after the restraint stress (+1 000 delta% in comparison to +775 delta% in LRs). It appeared that both the exercise and the restraint stress evoked an increase in natural killer cell cytotoxicity. The exercise stress resulted in a greater rise in natural killer cell cytotoxicity in the HRs whereas restraint stress had grater effect on the LRs.

P16.12 Corticosterone-induced increase in the responsiveness of 5-HT7 receptor is reversed by imipramine

Pitra P.[1], Hess G.[1,2]

[1]Institute of Pharmacology PAS; [2]Jagiellonian University, Krakow, Poland

One of the symptoms that are seen in humans with depression is hypercortysolemia which can be mimicked by repetitive administration of corticosterone to rats. It has been speculated that 5-HT7 receptors are affected by antidepressant drugs. In the present study we evaluated whether imipramine treatment of rats receiving corticosterone will result in a reversal of corticosterone-induced changes in 5-HT7 receptor-mediated effects in *ex vivo* hippocampal slices. Rats were injected with corticosterone for 7 or 21 days. The third group of rats received corticosterone for 21 days and since the eighth day of this treatment, animals additionally received imipramine for 14 days. Spontaneous epileptiform bursts were recorded from the CA3 area. 5-HT7 receptor-mediated increase in bursting frequency was induced by bath application of 5-carboxamidotryptamine (5-CT) in the presence of N-[2-[4-(2-methoxyphenyl)-1piperazinyl]ethyl]-N-2-pyridinylcyclohexanecarboxamide (WAY 100635), an antagonist of 5-HT1A receptor. Application of 100 nM 5-CT induced increase in bursting frequency, which was significantly enhanced in slices prepared from animals treated repeatedly with corticosterone for 7 and 21 days compared to slices prepared from control rats. This enhancement was reversed by imipramine administrated concurrently with corticosterone. It indicates that imipramine administrated concurrently with corticosterone normalizes the corticosterone-increased responsiveness of 5-HT7 receptors.

P16.13 The anxiolytic-like effects of acute administration of corticosterone

Skorzewska A.[1], Bidzinski A.[1], Lehner M.[1], Turzynska D.[1], Szyndler J.[2], Maciejak P.[1,2], Plaznik A.[1,2]

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

Rats were injected with corticosterone at the doses of 5 or 20 mg/kg or vehicle. Behavioral effects in the conditioned freezing test (CFT) were compared to changes in corticosterone concentration and expression of c-Fos and CRF in brain structures. Acute pretreatment with corticosterone 90 min before training session attenuated their emotional behavior in the CFT, and increased plasma corticosterone concentration, examined 24 h later. This effect of corticosterone was accompanied by selective enhancement of stress-induced c-Fos expression in the parvocellular and magnocellular neurons of the paraventricular hypothalamic nucleus (pPVN, mPVN), medial amygdala nucleus, MeA and cingulate cortex, area 1, Cg1. Furthermore, acute glucocorticoids administration induced CRF expression in the pPVN, mPVN, MeA, cingulate cortex, area 2, Cg2 and secondary motor cortex, M2. Additionally, a single injection of corticosterone decreased CRF expression in the central nucleus of the amygdala, CeA. The present results indicate the anxiolytic-like effects of acute corticosterone administration. It is suggested, that the enhanced activity of Cg, MeA, pPVN and mPVN, with ensuing stimulation of the HPA axis and increased serum glucocorticoid concentration, might serve to better prepare the organism to survive in a threatening situation.

P16.14 Effects of repeated brief restraint stress on LTP in the dentate gyrus of mice

Spyrka J.[1,2], Hess G.[1,2]

[1]Institute of Zoology, Jagiellonian University; [2]Institute of Pharmacology PAS, Krakow, Poland

Chronic stress influences the structure and function of the hippocampal formation, a brain part crucial for learning and memory. In has repeatedly been shown that chronic stress reduces the potential for hippocampal long-term potentiation (LTP), however, a brief exposure to swim stress enhances LTP in rat dentate gyrus. The present study investigated whether repeated brief restraint stress affects LTP in mice dentate gyrus. C57BL/6 male mice were subjected to the restraint lasting 10 minutes for 1, 3, 7, 14 and 21 days. *Ex vivo* hippocampal slices were prepared 24 h after last restraint. Field excitatory postsynaptic potentials (fEPSP) were evoked in the molecular layer of the dentate gyrus 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). LTP recordings lasted for 4 hours. Preliminary data suggest that restraint stress enhances LTP induced in slices prepared from stressed animals. This finding might help understand the mechanisms involved in different stress models.

P16.15 Chronic imipramine decreases glutamatergic transmission in rat frontal cortex

Bobula B.[1], Tokarski K.[1], Wabno J.[1,2], Hess G.[1,2]

[1]Institute of Pharmacology PAS; [2]Jagiellonian University, Krakow, Poland

Previous work demonstrated that treatment with antidepressants may decrease glutamatergic transmission in the frontal cortex but the mechanism of this effect remains obscure. In the present study imipramine, a tricyclic antidepressant, was administered to rats (10 mg/kg) twice daily, for 14 days. *Ex vivo* frontal cortical slices were prepared 2 days after last drug administration. Spontaneous and stimulation-evoked EPSCs were recorded. There were no differences in basic membrane properties between layer II/III pyramidal neurons in slices prepared from imipramine-treated rats and in controls. A lack of changes in the mean frequency of spontaneous EPSC after blockade of Na^+ channels suggests that most of recorded spontaneous EPSCs corresponded to miniature EPSCs. In slices prepared from imipramine-treated animals the mean frequency of sEPSCs was two times lower in comparison to that in cells from control animals. The mean amplitude of sEPSCs remained unchanged. Comparison of the initial slope of AMPA/kainate and NMDA receptor-mediated, evoked EPSCs showed that the ratio of the latter to the former was significantly lower in neurons obtained from imipramine-treated rats. The decrease in the mean frequency but not in the mean amplitude of sEPSCs is consistent with the decrease of glutamate release from afferent boutons. The decrease in the ratio of NMDA to AMPA/kainate eEPSCs, suggests changes in postsynaptic AMPA and/or NMDA receptor function.

P16.16 Up-regulation of GABAergic system in suicide victims with mood disorders

Gos T.[1], Bielau H.[2], Dobrowolny H.[2], Gunther K.[2], Mawrin Ch.[3], Truebner K.[4], Bernstein H.G.[2], Bogerts B.[2]

[1]Institute of Forensic Medicine, Medical University of Gdansk, Poland; [2]Department of Psychiatry; [3]Institute of Neuropathology, Otto-von-Guericke-University, Magdeburg; [4]Institute of Legal Medicine, University of Essen, Germany

Alterations of GABAergic neurotransmission are assumed to play a crucial role in the pathophysiology of mood disorders. Glutamic acid decarboxylase (GAD) is the key enzyme of GABA synthesis. Immunohistochemical staining of GAD 65/67 was performed in dorsolateral prefrontal cortex (DLC), orbitofrontal cortex,anterior cingulate cortex,entorhinal cortex (EC), hippocampal formation,mediodorsal and laterodorsal thalamus with consecutive determination of GAD-immunoreactive (-ir) neuropil density. The study was performed on paraffin-embedded brains from 21 depressive patients (14 of whom had committed suicide) and 18 matched controls. The data were tested statistically using Kruskal-Wallis (KW) and *post-hoc* Mann-Whitney tests. KW-test revealed significant differences in GAD 65/67-ir neuropil density between suicides, non-suicides and controls bilaterally in EC, dentate gyrus and CA1 field of hippocampus. As shown by post-hoc tests, the increased density of GAD-ir neuropil existed in these regions in suicides only. In this subgroup of patients, the negative correlations were found between the density of GAD-ir neuropil and mean doses of psychotropic medication. The current data point to the up-regulation of the GABA-ergic system in suicidal patients with mood disorders, specific for the declarative memory-system structures.

P16.17 Effects of cytokines in the striatum on motivated behaviour in rats

Karrenbauer B.D.[1], Ho Y.J.[2], Ludwig V.[1], Schwarting R.K.W.[1], Pawlak C.R.[3]

[1]Physiological Psychology, Philipps-University of Marburg; [2]School of Psychology, Chung-Shan University, Tai-Chung City, Taiwan, ROC; [3]Psychopharmacology, Central Institute of Mental Health, Mannheim, Germany

There is evidence that immune messengers like cytokines can modulate motivated behaviour, and are involved in psychiatric conditions like anxiety, and depression. Previously, we showed that cytokine expression in specific brain tissues correlated with anxiety-like behaviour (open arm time) in the elevated plus-maze in rats. These relationships indicated that cytokines in the brain can be related to avoidance behaviour, and that this relationship is site- (striatum, frontal cortex), and cytokine-specific (interleukin-2 (IL-2) mRNA). Subsequently, we tested rats after a single striatal IL-2 injection followed by an elevated plus-maze, or open field, test acutely and 24 h later. Overall, rats with IL-2 showed dose-dependent effects for anxiety-related behaviour compared to saline-treated rats, showing a trend for anxiolytic-like behaviour with the highest dose (25 ng), and an anxiogenic-like effect with the lowest dose tested (0.1 ng). In addition, dose-dependent suppression of rearing activity was shown for IL-2 doses (0.1; 1 ng). However, locomotion did not differ between groups so that general activity cannot account for these differences. In the ongoing study we tested for proactive drug mechanisms, that is, rats were tested in an open field 24 and 48 h after striatal IL-2 injection. These results will then be presented.

P16.18 Possible participation of the brain dopaminergic system in the control of the animals anxiety behavior

Strzelczuk M., Krotewicz M., Koprowska M.

Department of Neurophysiology, University of Lodz, Poland.

It is difficult to understand anxiety states and research is particularly needed into its neurochemical grounds. The dynamics of metabolic processes and multiple wide interactions between the neurotransmission systems are most significant. In the present study we analyze changes in the DA and its metabolite DOPAC concentrations, which occur in the brain structures forming the emotional-defensive system, i.e. hypothalamus, midbrain grey matter, amygdala and frontal cortex after exposition of rats on LDT-test. The light-dark transition test is using to study a natural tendency of the rats avoiding bright places and trying to hide in the darkness. The HPLC-ED analysis showed an elevated DA and DOPAC concentrations in the amygdala and increased DOPAC/DA ratio in the cortex. An increasing activity of the DA system might as well be one of major elements responsible for a fear drive. Key words: fear, light-dark transitions test, DA, DOPAC, HPLC, rat.

P16.19 Comparison of activity of the noradrenergic system in rats exposed to different ethological tests

Krotewicz M., Strzelczuk M., Koprowska M.

Laboratory of Neurophysiology, University of Lodz, Poland

The ethological tests are widely used to study behavior of rodents for exploration and emotionality. Moreover, the ethological tests are useful in investigating multiple behavioral responses in the same animal studied in a battery of ethological tests. Here, we asked whether different ethological tests, namely, the elevated plus-maze, the open field and author's LDT-test, are related to similar activity of noradrenergic system in rats. To analyze noradrenergic system activity, we examined regional brain concentration of noradrenaline (NA), its metabolite 3-methoxy-4-hydroxyphenylglycol (MHPG) and MHPG/NA ratio. The high performance liquid chromatography with electrochemical detection were used to neurochemical analyses. The neurochemical data were compared with biochemical indices received in naive rats. The obtained data showed differences in neurochemical indices measured in animals previously exposed to the various ethological tests. It indicates that activity of the central noradrenergic system is dependent on the used ethological test.

Abstracts missing

E1 New transgenic mouse lines for temporally-controlled targeted somatic mutagenesis in astrocytes

Slezak M.[1], Metzger D.[2], Chambon P.[2], Pfrieger F.W.[1]

[1]Dept. Neurotransmission, Inst. Cell. Integrative Neurosci., Strasbourg, France; [2]Inst. Genetics Mol. Cell. Biol., Mouse Clinical Inst., Illkirch, France

The functions of astrocytes, in particular their interactions with synaptic connections, are mainly derived from *in vitro* studies, but their relevance *in vivo* remains elusive, mainly due to a lack of animal models. We decided to establish transgenic mice that enable temporally controlled targeted mutagenesis in defined subpopulations of astrocytes based on the Cre-ERT2 system (Metzger and Chambon 2001). To drive expression of Cre-ERT2 in astrocytes, we generated lines carrying Cre-ERT2 under the control of large genomic DNA fragments of astrocyte-specific promoters (GLAST, Connexin-30, Aquaporin-4, ApoE) contained in bacterial artificial chromosomes (BACs). A first characterization of our transgenic lines in combination with reporter mice revealed that (a) Cre expression patterns across different organs and brain regions matched the activity pattern of the driving promoter, (b) transgenic lines derived from a given construct differed in the level of Cre-mediated recombination but not in its regional distribution and (c) Cre activity was strictly tamoxifen-dependent. Out of the four promoters chosen, Glast and Cx30 induced strong Cre expression in brain cells with non-overlapping regional distribution, whereas ApoE-CreERT2 and Aqp4-Cre-ERT2 lines showed low levels of Cre activity in the CNS. In GLAST-CreERT2 lines, Cre-mediated recombination occurred in cerebellar Bergmann glia, retinal Mueller cells, as well as in the olfactory bulb, cortex, hippocampal dentate gyrus and subventricular zone. In contrast, in Cx30-CreERT2 lines the highest level of recombination occurs in midbrain, thalamus, hypothalamus and brain stem. The initial characterization indicates that the new transgenic mouse models will help to determine the relevance of astrocytes during brain development and in the adult. Supported by DFG SPP1172, CNRS.

E2 Ischemia effects ECM-FAK signaling pathway in gerbil hippocampus

Ziemka-Nałęcz M., Zalewska T.

NeuroRepair Dept., Medical Research Institute, Warsaw, Poland

Cell attachment with ECM proteins generates intracellular signals which lead to the specific tyrosine phosphorylation cascade of limited number of protein substrates, and these participate in the regulation of cytoskeletal organization and gene expression. This pathway involves FAK a non-receptor tyrosine kinase, a key component responsible for the flow of information from the ECM to the cell interior. Phosphorylated FAK may interact directly with other kinases, adaptor molecules and cytoskeletal proteins, perhaps providing a pathway by which ECM may regulate cell viability. Therefore it remains conceivable, that excessive matrix protein degradation in pathological conditions may lead to the disruption or even loss of the cell-ECM interaction and can further affect the downstream signaling pathways. This prompted us to verify if ischemia induced degradation of ECM proteins is temporally coincident with the modulation of intracellular pathway to which it is connected. We sought to clarify this point by measuring the activity of metalloproteinases – MMP-2 and MMP-9 as well as the activity of intracellular protein – FAK. We found that short-time forebrain ischemia leads to the activation of MMPs in CA1 region of the hippocampus at 48 and 72 h of reperfusion. At the same time a significant decrease of total FAK protein as well as its activation/phosphorylation, and the reduced amount of FAK/Src complex was observed. It may be concluded that ischemia-induced changes in ECM-FAK signaling pathway may contribute to delayed neuronal degeneration. Sponsored by MES grant 2P05A 09928.

INDEX OF AUTHORS