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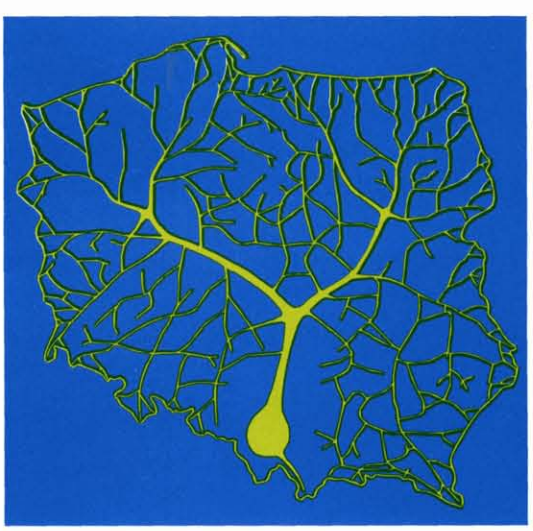
FIFTH INTERNATIONAL CONGRESS of the POLISH NEUROSCIENCE SOCIETY ABSTRACTS



Toruń, 6-9 September 2001

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Session 1 – Medical Symposium I

1.1 PLASTICITY OF THE BRAIN: AN INSPIRATION FOR SENSORY STIMULATION THERAPY

Liszcz Krzysztof
Therapeutic Institute of the Foundation "Give a chance", Toruń, Poland

Since forties of the 20th century knowledge of mechanisms of developmental plasticity has been used in treatment of children suffering from cerebral palsy. The Therapeutic Institute of the Foundation "Give a chance" has drawn its inspiration from those trials. For above 10 years we have been trying to develop therapeutic methods of functional improvement of the children. We focused on the improvement of their movements, vision, hearing, mechanical perception and spatial orientation. Our programs of rehabilitation concern both motor and sensory development of the children. We find extremely important to start sensory stimulation already during the first year of their life which makes the work relatively easy because of full plasticity of the nervous system. An example is so called photographic visual memory which allows early global teaching of visual reading. Visual stimuli are combined with acoustic ones. This gives the opportunity to improve sight fixation, sensory convergence, and stimulate hearing which is necessary for reading ability. This is of particular importance when cerebral palsy is accompanied by disordered articulation, the hypacusis and amblyopia.
The results of the therapy of 300 patients will be presented.

1.2 NEURODEVELOPMENTAL PROFILES – THE SOFTWARE FOR THERAPEUTIC PROGRAMS IN BRAIN INJURY

Marek Pietrzak
Wielkopolskie Centrum Neurologii Dzieci i Młodzieży Poznań

Nuerodevelopmental Profiles were originally developed for diagnostic purposes. They were tabular representation of the normal development of the central nervous system as it was manifested by the development of diverse CNS functions. By placing an individual child against such a table one could easily evaluate his neurological disability. Different Developmental Profiles vary in the composition of CNS functions considered crucial for describing the normality by their authors, as well as in the organization of the time frame. What they have in common is the appreciation of sequential development of abilities within each CNS function reflecting the hierarchic structure of the brain, with the achievement of any following ability depending on at least rudimental completion of preceding ones. This feature is the basis for the use of Developmental Profiles for constructing the programs of neurorehabilitation. One can find out which brain functions are affected by the injury and what is the level of their present development. One can also measure the rate of improvement with the therapy. The review of several different Neurodevelopmental Profiles will be presented and their applicability for the purpose of planning the programs of therapy for individual children will be dicussed.

1.3 NEUROBIOLOGY OF DRUG ADDICTION

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

Psychotropic substances having addictive properties have been known since the dawn of humanity. Originally their use had a definite survival value, but the recent changes in their use made them a threat for a modern society. Some of those substances, like alcohol and nicotine, are legally accepted, the other, like cocaine and morphine became illegal. Both legal and illegal drugs may induce addiction - an incurable mental disease, characterized by domination of drug seeking behavior and high incidence of relapse. The addictive substances overstimulate the brain reward system, which reinforces natural behaviors leading to reproduction or having survival value: sex, feeding, drinking, aggression, novelty seeking, accepting a risk. The main pleasure centers in the brain, detected as self-stimulation site, are mainly connected with the brain mesolimbic and mesocortical dopamine pathways. The dopamine neurons are activated by expectation of reward, while the pleasure of consumption of reward is mediated by opiate and gabergic systems. As the dopaminergic responses undergo sensitization, while tolerance develops to opiate and gabergic responses, the gap between the expected and really experienced pleasure widens and drives the victim of addiction to increase the demand for the substance of addiction. The mechanism of addiction is very similar among all mammals and thus animal studies shed some light on human addiction. There is both an environmental and genetic component in the preponderance to addiction. The modern pharmacotherapy offers some help for the addicts, and when combined with sociotherapy may met with a degree of success. Novel specific types of drugs are used against various types of addition: naltrexon and acamprozate are now promising agents in combating alcoholism

Session 2 – Medical Symposium II

2.1 DEMENTIA DISEASES, ALZCHEIMER TYPE.

Aleksander Araszkiewicz
Department of Psychiatry, University School of Medicine, Bydgoszcz, Poland.

Alzcheimer type of dementia (AD) is no homogenous disease. Current data show, that AD concerns heterogeneous subtypes of diseases.
In this talk new ethiological hypotheses of Alzcheimer diseases will be present. The most important to clinical practice appears clinical criteria useful for diagnosis of AD. Current diagnostic systems i.e. ICD-10 and NINCDS ADRDA, the most important clinical symptoms of AD and commorbidity with other mental disturbances, and also new standards of pharmacological treatment and therapeutic procedures will be present.

2.2 NEUROPSYCHOLOGICAL ASSESSMENT OF COGNITIVE DYSFUNCTION IN PATIENTS WITH EARLY PHASE OF DEMENTIA.

Alina Borkowska
Department of Psychiatry, University School of Medicine, Bydgoszcz, Poland.

Cognitive disturbances are core and enduring deficits in patients with dementia. These deficits show progression on most of cognitive areas such as memory, orientation, spatial and verbal abilities, planning, working memory and executive functions - related to level of brain impairment. Neuropsychological methods commonly used to assess level of cognitive deterioration as MMSE, ADAS, or 7 minut screen in early phase of diseases are not sensitive for detection of selective cognitive impairment. In our previous study concerning frontal lobe assessment in Alzheimer Disease patient, selected tests: TMT B and WCST – persewerative errors, some aspects of attention measued by CPT and eye movement disturbances (measured by infrared reflectometry) occurs as very sensitive to assess early cognitive and neurophysiological abnormalities in patients with high risk of dementia, when MMSE and ADAS performance was still good.
These neuropsychological tests are also very sensitive for assessment of cognitive disturbances during pharmacological treatment and effect of neuropsychological training. Three months of treatment with donepezil with neuropsychological training results in improvement on some neuropsychological tests, treatment with piracetam with neuropsychological training results with no significant improvement and no significant deterioration of performance on selected tests, however treatment with piracetam without cognitive training results in deterioration on all neuropsychological tests used.

Session 3 – Jerzy Konorski memorial lecture

3.1 CELLULAR SIGNAL-TRANSDUCTION CASCADES AS MOLECULAR AMPLIFICATION-ADAPTATION SYSTEMS

A pharmacologist's view on CNS plasticity and psychopathology

Fridolin Sulser, Vanderbilt University Medical Center
Nashville, TN 37232-2478

The term "CNS plasticity" implies that the CNS can adapt to conditions that threaten the physical and psychic/emotional well-being of the organism by altering programs of gene expression in specific neuronal and/or glial cell populations. Ultimately, changes in programs of gene expression determine the intensities of incoming signals, the sensitivities of neuronal systems to those signals and the nature, amplitude and duration of CNS responses. One of the earliest adaptive changes caused by psychotropic drugs is the antidepressant induced desensitization of the beta receptor coupled adenylate cyclase system. This deamplification is reflected down-stream from the second messengers at the level of nuclear CREB-P. Antidepressants also increase glucocorticoid receptor (GR) mRNA in the hippocampus, the density of GR's and, importantly, cause a translocation of the transcription factor (GR-H) from the cytoplasm to the nucleus. These molecular events are involved in achieving a stabilization of the signal integration, mechanism for adaptation. We have utilized the differential display technology in fibroblasts from control subjects and patients with a DSM-IV diagnosis of major depression. Among a number of differentially expressed genes is PTX3 which belongs to the subfamily of long pentraxins. PTX3 is not constitutively expressed in brain but is induced by interleukin-1. We hypothesize that PTX3 could be involved in the fine tuning of CNS plasticity. From a clinical-pharmacological perspective, this will enable us to formulate novel pharmacologic therapies designed to restore CNS plasticity that is lost in patients with major depression.

Session 4 – Plenary Lecture

4.1 DIABETES AND THE BRAIN

W.H. Gispen
Utrecht, Netherlands

Diabetes mellitus is associated with cognitive defects and an increased risk of dementia, particulary in the elderly. Hence, it is becoming increasingly clear that in addition to the well known diabetic peripheral and autonomic neuropathy the brain can be considered as end-organ of diabetic damage. In animal models of diabetes, impairments of spatial learning occur in association with distinct changes in hippocampal plasticity. At the molecular level these impairments seem to be associated with alterations in the NMDA-receptor subunit composition and altered signal transduction cascades. Collectively the human and animal literature suggests an accelerated ageing of the brain in diabetes, possibly implying an interaction between the two phenomena. Indeed in hipocampal CA1 cells of young diabetic rats the slow after hyperpolarization following a train of action potentials is increased compared to age-matched controls, generally accepted as a hall mark of neuronal ageing.

A number of processes that have been implicated in the pathogenesis of diabetic complications of the nervous system are also thought to relate to brain ageing. Another interesting lead is the potential role of insulin. Recent data indicate that insulin plays a modulatory role in synaptic transmission and directly affects learning and memory. Moreover, defects in insulin action, both in the periphery and the brain, have recently been suggested to contribute to the pathogenesis of sporadic Alzheimer's disease.

Session 5 – Parallel Symposium: Synaptic transmission

5.1 SYNAPTIC PLASTICITY AT THE MOSSY FIBRE-CA3 SYNAPSES IN THE DEVELOPING HIPPOCAMPUS

Enrico Cherubini

Neuroscience Programme, International School for Advanced Studies, Via Beirut 2-4, 34014 Trieste, Italy

A peculiar characteristic of the immature hippocampus is that mossy fibres (MF), the axons of *dentate gyrus* granule cells, have a postnatal development. They contact CA3 pyramidal cells by postnatal day 1 but they reach a full development only by the end of the second postnatal week. I will review recent work from our laboratory focussed on synaptic plasticity processes occurring at these connections during the first and second postnatal weeks.

At P1-P6, most of these synapses are silent: they do not respond at rest but they are functional at positive membrane potentials. They are silent because of a very low probability of glutamate release. Factors that enhance release probability such as paired-pulse stimulation, increasing the temperature or exposing the cells to cyclothiazide, are able to switch silent synapses into functional ones. During the second postnatal week, a high frequency stimulation to MF induces a long-term depression (LTD). This form of LTD is homosynaptic and independent of the activation of NMDA or mGluR but needs an increase of calcium in the postsynaptic cell for its induction. At the same synapse, another form of low-frequency LTD coexists. This form of LTD is NMDA independent but requires the activation of mGluR. In contrast with the high frequency LTD, its induction is presynaptic. The coexistence of two forms of LTD on the same synapse may expand the "mnemonic" capacity of the network during development.

5.2 WINDOW EFFECT OF TEMPERATURE ON CARBACHOL-INDUCED THETA-LIKE OSCILLATIONS IN HIPPOCAMPAL FORMATION SLICES.

J. Konopacki, T. Kowalczyk, H. Gołębiewski
Department of Neurobiology, University of Łódź, Łódź, Poland

The effect of different temperatures (18 - 42^{o}C) of artificial cerebrospinal fluid (ACSF) on carbachol (CCH)-induced field potentials were examined in the present study. Two hundred and thirty one experiments were performed on hippocampal formation slices maintained in the gas-liquid interface chamber. All slices were perfuzed with 50 μM CCH. Recording electrode was positioned in the region of CA3c pyramidal cells. The experiments gave two main findings. First, in a presence of continuous cholinergic stimulation the temperature of the bathing medium *per se* determined the rate of synchronization of the field potentials and pattern of EEG activity recorded. Second, within the temperature range from 33^{o}C to 37^{o}C the window effect of temperature on CCH-induced theta-like activity (TLA) was noted: at this temperature range all slices tested responded only with one pattern of EEG activity – TLA. The results are discussed in a light of temperature effect on hippocampal neuronal network.

5.3

MECHANISMS DETERMINING THE SHAPE AND PHARMACOLOGICAL MODULATION OF GABAERGIC SYNAPTIC CURRENTS

Jerzy W. Mozrzymas
Dept. Biophysics, Wrocław Medical University, Wrocław, Poland

GABAergic synaptic transmission is a key factor in integration of neuronal signalling. It is thus crucial to elucidate the mechanisms underlying the kinetics of GABAergic currents (IPSCs). It has been recently shown that synaptic GABA transient lasts for less than 1 ms, implying that activation of synaptic $GABA_AR$s occurs in the conditions of extreme non-equilibrium. The use of ultrafast application systems allowed to study the kinetics and pharmacology of receptors with time resolution approaching the time scale of synaptic agonist transient. Current responses to short agonist application closely mimicked the synaptic currents. The inhibitory effect of chlorpromazine (CPZ) on $GABA_A$ receptors, measured in conditions close to steady-state, was found to be one order of magnitude weaker than on synaptic currents. However, when the synaptic conditions were modelled by rapid GABA applications, the CPZ effect on IPSCs was well reproduced. Using this method, the mechanisms of action of several physiologically and therapeutically important compounds (e.g. Zn, pH, anaesthetics) has been described in terms of modulation of the $GABA_AR$ microscopic gating. It is concluded that the non-equilibrium conditions, dictated by synaptic agonist transient, play a crucial role in determining both the time course and susceptibility to pharmacological modulation of synaptic currents. The use of electrophysiological techniques together with rapid application systems offers a unique opportunity to determine kinetic and pharmacological mechanisms in the time scale of synaptic currents.

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5.4

WHY DOES THE CENTRAL NERVOUS SYSTEM NOT REGENERATE AFTER INJURY?

John Nicholls, Dept.Biophysics, SISSA, Trieste, Italy 34014

A problem for neuroscientists and clinicians is why the CNS does not regenerate after injury. Injured peripheral axons reform their connections, whereas injured spinal cord axons never regrow. Insights into mechanisms for repair after spinal cord injury have been obtained by recent experiments. One important finding is that injured neurons in adult mammalian brain can send out new fibers over a distance, if they are provided with appropriate tissue to grow along. Another key finding is that molecules exist in the brain that prevent the outgrowth of injured nerve cells. Strategies for promoting regeneration are to use bridges of tissue to provide conduits or to apply antibodies that neutralise effects of inhibitory molecules. An aim of our experiments is to define changes in development, as the spinal cord changes from being able to regenerate to the adult state of failure. Experiments show that after complete transection, spinal cords of new-born opossum pups regenerate when the animal is younger than about 12 days of age. After this the ability for repair becomes lost abruptly. We aim to determine which growth-promoting molecules decrease, and which inhibitory molecules increase during this critical period. Major hurdles must however be overcome before patients can be treated. Once a good understanding of mechanisms that promote and prevent regeneration is obtained, one will still have to devise safe treatments for patients with spinal cord injuries, as well as methods for applying them. Nevertheless the picture is not as discouraging as it was: one can think today of strategies for doing research on spinal cord injury so as to promote regeneration and restore function.

Session 6 – Parallel Symposium: Mechanism od emotions: neurobiological and psychological approach

6.1

PRECONSCIOUS DISCRIMINATION OF FACIAL EXPRESSION

6.2

Rafal Krzysztof Ohme, Maria Jarymowicz
Warsaw School of Advanced Social Psychology

Is preconscious processing complex enough to discriminate not only the valence but also the modality of an affective prime? We try to answer this question in series of experiments employing affective priming procedure. Subliminal affective primes (facial expressions of DISGUST, ANGER, FEAR were presented for 16 and 32ms. The primes preceded target stimuli (pictures of neutral faces and Japanese ideographs). Participants were to rate the target on different dimensions (neutral faces: „What emotion is she trying to hide?"; ambiguous ideographs: „Does it represent dangerous-not dangerous animal or edible-not edible substance). Some behavioral measures were applied as well. The results clearly show that preconscious processing is able to discriminate among negative facial expressions. Moreover the studies indicate that different subliminal exposure times may well represent different levels of preconscious processing, and thus provide new empirical arguments to the debate on the nature of consciousness.

6.3 **NEURAL MECHANISMS REGULATING RAGE AND AGGRESSION IN THE CAT: IMPLICATIONS FOR THE STUDY OF HUMAN AGGRESSION.**

Allan Siegel and Thomas R. Gregg

Departments of Neurosciences and Psychiatry, University of Medicine & Dentistry of New Jersey—NJ Medical School and Graduate School of Biomedical Sciences, Newark, NJ 07103 (USA)

It is well known that aggression and violence are presently major public health problems in most societies. It is our view that a rational basis for treatment of these disorders will involve the identification of the underlying neural circuitry and neurotransmitter receptors. Our studies have attempted to address this problem by utilizing electrical brain stimulation, anatomical – immunohistochemical techniques, and behavioral pharmacology to identify the neural systems regulating aggression and defensive rage in the cat. One group of studies has demonstrated that the key pathway mediating DR projects from the medial hypothalamus (MH) to the midbrain periaqueductal gray (PAG) and its functions are mediated by NMDA receptors in the PAG. A second group of studies has identified several key structures and pathways arising from the amygdala which powerfully modulate aggression and rage. These include: projections from the medial amygdala to MH whose excitatory functions are mediated by substance P (SP), and projections from the central amygdaloid nucleus to the PAG whose inhibitory functions are mediated by μ opioid receptors in the PAG. Other studies have shown that potent modulation of predatory attack and rage are mediated through local reciprocal GABAergic inhibitory pathways linking the lateral hypothalamus and MH. Our most recent studies have provided evidence that SP-NK_1 receptors in both MH and PAG significantly facilitate DR.

[Supported by NIH grant NS 07941-29].

6.4 GENETIC AND ENVIRONMENTAL DETERMINANTS OF EMOTIONS: DATA BASED ON TWIN STUDIES ON TEMPERAMENT

Jan Strelau,

Interdisciplinary Center for Behavior Genetic Research, University of Warsaw, Warszawa, Poland

By tradition temperament has been linked to emotions and emotional components of behavior. This traditio is present also in contemporary theories of temperament. Based on Warsaw-Bielefeld twin project a study was conducted aimed at assesing the contribution of genetic and environmental components to temperamental traits as represented by five different temperament theories. The psychometric measures based on five temperament questionnaires included a variety of triats directly reffering toemotions. Data were collected by means of two methods: self report and peer report (two observers rated each twin referring to questionnaire items converted into the third person). Altogether the study was conducted on over 3000 twins and over 6000 peers. The traits referring to emotions comprised: perservation, emotional reacivity, fear, distress, anger, mood quality, and neuroticism. Analysis of data based on path analysis and structural equation models has shown that it is mainly the genetic factor and non-shared environment that contribute essentially to the variance of emotional traits. The data give evidence for the universality of emotion-oriented temperament traits but at the same show some cross-cultured specificity.

6.5 ANXIETY AND NEUROPEPTIDES: WHAT HAVE WE LEARNED FROM CRF KNOCKOUT MICE?

Artur H. Swiergiel, Dept. Pharmacology, Louisiana State University Health Sciences Center, Shreveport, LA 71130-3932, USA

Corticotropin-releasing factor (CRF) plays a crucial role in hypothalamo-pituitary-adrenocortical (HPA) activity and in many physiological responses observed in stress. Substantial evidence suggests that CRF functions also as a neuropeptide and that it activates locus coeruleus (LC) noradrenergic neurons and norepinephrine (NE) metabolism in the LC terminal fields. Furthermore, CRF has been implicated in stress-related behavior, including anxiety and fear: antagonism of CRF receptors in locus coeruleus and central amygdala and peripheral administration of small-molecule CRF antagonists attenuate behavioral expression of fear. Recently, stress-induced behaviors were studied in mice lacking the CRF-gene (CRFko). CRFko mice displayed significantly impaired endocrine responses; on the other hand, several neurochemical and behavioral responses to stress were intact in CRFko. Thus, CRFko mice showed a clear dichotomy: the stress-related HPA responses were absent, but the stress-related neurochemical and behavioral responses thought to be mediated by brain CRF were unaffected. These findings suggest that when mice develop in the absence of CRF, other factor(s) assume the behavioral functions normally ascribed to brain CRF, but not activation of the HPA axis or the LC-NE system. Alternatively, the natural modulator of behavior may not be CRF, but other molecule(s) (e.g. urocortin) acting through the CRF receptors.

Session 7 – Parallel Symposium: Understanding of neuroprotection: molecular and cellular mechanisms

7.1 GLYCOGEN SYNTHASE KINASE-3β AS A TARGET FOR NEUROPROTECTIVE SIGNALING PATHWAYS.

M.Hetman, M.Chłystun, M.Higgins*, J.Cavanaugh*, Z.Xia*.
*International Institute of Molecular and Cellular Biology UNESCO/PAN, Warsaw, Poland, *University of Washington, Seattle, USA*

Glycogen synthase kinase-3β (GSK3β) activity is negatively regulated by several signal transduction cascades that protect neurons against apoptosis, including the phosphatidylinositol-3 kinase (PI-3K) and extracellular-signal regulated kinase 1/2 pathways (ERK1/2). This suggests the interesting possibility that activation of GSK3β may contribute to neuronal apoptosis. Consequently, we evaluated the role of GSK3β in apoptosis of cultured rat cortical neurons induced by trophic factor withdrawal or by PI-3K inhibition. Neurons were subjected to several apoptotic paradigms including serum deprivation, serum deprivation combined with exposure to N-methyl-D-aspartate (NMDA) receptor antagonists, or treatment with PI-3K inhibitors. These treatments all led to stimulation of GSK3β activity in cortical neurons. Inhibition of GSK3β reduced neuronal apoptosis, suggesting that GSK3β contributes to trophic factor withdrawal-induced death. Furthermore, overexpression of GSK3β in neurons increased apoptosis indicating that activation of this enzyme is sufficient to trigger programmed cell death. Activation of either ERK1/2 or PI-3K was able to decrease GSK3β activity and suppress apoptosis due to the activation of the kinase. We conclude that inhibition of GSK3β is one of the mechanisms by which PI-3K and/or ERK1/2 pathways protect neurons from programmed cell death.

7.2 **EFFECTS OF NMDA ANTAGONISM ON INDUCED ISCHEMIC TOLERANCE IN THE GERBIL HIPPOCAMPUS**

J. W. Łazarewicz[1], R. Gadamski[1] and W. Danysz[2]
[1]Medical Research Centre P.A.S., Warsaw, Poland and [2]Department of Pharmacology, Merz + Co., Frankfurt/Main, Germany

Preconditioning of the brain with sublethal ischemia protects against neuronal damage induced by the following lethal ischemic insult. Antagonists of NMDA receptors have been considered candidates for neuroprotectants in neurodegenerative disorders and brain ischemia. However, there are indications that NMDA antagonists may attenuate the induction of tolerance to ischemia. The aim of this study was to verify these suppositions. Ischemic tolerance was induced in Mongolian gerbils by 2-min. bilateral carotid occlusion 3 days before a 3-min lethal ischemia. MK-801 (3 mg/kg) and memantine (5 mg/kg) were injected i.p. 1 h before preconditioning ischemia, or memantine was administered s.c. 30 mg/kg/day by Alzet minipumps for 3 days, beginning 24 h before preconditioning. Rectal body temperature was measured during and after both ischemic insults, and hypothermia was prevented. The histology of the hippocampal CA1 was assessed after 14 days. The results (means±SEM) demonstrate that hippocampal CA1 was destroyed in 74.6±10.86 % (n=10) after 3-min ischemia without preconditioning, but only in 19.6±7.04 % (n=10) when preconditioning preceded lethal ischemia. Application of MK-801 or memantine before preconditioning did not influence the ischemic tolerance. Infusion of memantine for 3 days resulted in reduction of neuronal damage after 3-min ischemia to 49.1±9.26 % (n=10), while neurodegeneration after preconditioned ischemia combined with memantine infusion was reduced to 6.4±7.24 % (n=10). These results indicate that memantine at plasma concentration close to maximal therapeutically relevant, under normothermic conditions enhances ischemic tolerance in the Mongolian gerbils.

7.3 **REGULATION OF PATHOLOGICAL SIGNAL AFTER TRANSIENT BRAIN ISCHEMIA**

[1]Zabłocka B., [2]Domańska-Janik K.
[1]Mol. Biol. Lab. [2]Mol. Neuropath. Lab. Med.Res.Ctr. Warsaw,Poland

Various animal and *in vitro* models of injury provide an opportunity to study biochemical pathways leading to cell death or survival. Although, one of the well known function of MAPK signalling is regulation of gene expression in response to extracellular stimuli, several signals connected with MAPK pathways seem to be involved in formation of new complexes integrating in different cell compartments. Recent findings support the idea that mitochondrial permeability transition pore (mtPTP) and the integrity of outer membrane play a pivotal role for determining the fate of cells after different injury. The regulation of ischemic signal was examined in the model of delayed neurons death in ischemia vulnerable CA1 hippocampal region and changes in the balance between survival-promoting ERK and death-connected JNK kinases activity have been shown. In CA1 region this balance was shifted towards increased JNK activity and cells death. Concomitantly, changes in the amount of mitochondria coupled protein kinases were observed. The targets for these kinases are still unknown, but *in vitro* data suggest their action in regulation of mitochondrial membrane permeabilization and relation with members of Bcl2 group of apoptotic regulatory proteins. We have shown, that amount of phospho-Raf-1 decreases in total homogenate as well as in mitochondria fraction at 24 and 48 hrs postischemia. It corresponds with decreased phosphorylation of one of the Raf-1 target, proapoptotic BAD protein and the appearance of cytochrom c in the cytosol. The coexistence of mitochondria connected protein kinases, Bcl2 proteins with cyt c efflux will also be discussed in the context of neuroprotection by cyclosporin A and lack of its action in the longer postischemic time. Sponsored by SCSR 6P04A 01014 and Med. Res. Ctr.

Session 8 – Plenary Lecture

8.1 **SELECTIVE BRAIN COOLING: A MULTIPLE REGULATORY MECHANISM**

Michał Caputa
N. Copernicus Univ., Dept of Animal Physiology, Toruń, POLAND

The mechanism of defence against cerebral overheating, called selective brain cooling (SBC), allows the brain to remain cooler than the rest of the body. The aim of the present paper is to provide some new ideas to better understand this physiological phenomenon, with emphasis on how it works, how it is controlled and what are its functions. There are two distinct types of SBC: one, present in birds and some mammals, is based on precooling of the arterial blood destined for the brain, with cool venous blood returning from the nose and head skin, and the other, identified in some mammals (including humans), uses the venous blood to cool the brain directly. Both SBC systems use powerful effectors of evaporative heat loss. There is a common mechanism of nervous control of SBC intensity. A decrease in sympathetic activity results in simultaneous dilation of veins supplying the intracranial heat exchanges and constriction of veins supplying the heart. Therefore, SBC enhances during heat exposure, endurance exercise, relaxed wakefulness and NREM sleep, and vanishes in the cold and dufing emotional distress. SBC is a multifunctional effector mechanism: it protects the brain from heat damage; it intensifies in dehydrated medium-size mammals (at the expense of general body evaporative cooling), thereby saving water; it helps exercising animals prevent drop in arterial blood pressure, thereby delaying the onset of exhaustion; it is likely to thermally modulate central neurotransmission because SBC is abruptly halted at the start of alertness, which leads to instantaneous warming of the brain; it is used in diving animals to drop cerebral temperature much below its normal level, which expands diving capacity and protects the brain from asphyxic damage. Altogether, SBC is used to integrate both thermal and nonthermal regulatory functions.

Session 9 – Workshop: Methods of neuroinformatics

9.1 NEUROINFORMATICS: A NEW BRANCH OF NEUROSCIENCE (Report from OECD Science Forum)

Andrzej Wróbel
Nencki Institute of Experimental Biology, 02-093 Warsaw, Poland

Neuroinformatics combines neuroscience and informatics research to develop and apply the advanced tools and approaches that are essential for major advances in understanding the structure and function of the brain. Neuroinformatics research is uniquely placed at the intersections of medical and behavioral sciences, biology, physical and mathematical sciences, computer science, and engineering. There is a strong synergy in these interactions, including a positive feedback loop from the interactions between informatics and neuroscience. This synergy can lead to a rapid acceleration of scientific and technological progress, which in turn will have major medical, social, and economic impacts.

From January 1996 the OECD countries promote fostering neuroinformatics by eliminating the barriers that prevent cooperation and by providing incentives to potential participants. Strategic investment in Neuroinformatics research can have a strong leveraging role, accelerating progress in basic neuroscientific research and its translation to improved diagnosis and treatment in the clinical arena, in areas ranging from new pharmaco-therapeutic approaches to new prosthetic devices for restoring brain function. On a parallel front, Neuroinformatics can contribute to major technological advances in the areas of information and communication technology, robotics and intelligent machines, and the interface between machines and humans.

9.2 **NEURODYNAMICS OF BRAIN DAMAGES**

Zbigniew J. Kowalik
University of Duesseldorf, Germany

A set of data will be presented to show the advanteges of application of the Nonlinear Systems Theory methods in describing pathologic brain oscillatory processes. These methods can be applied in order to interpret the brain dynamics and/or to locate pathological areas from measured time series obtained in EEG/MEG experiments. Our examples include temporal lobe epilepsy, as well as cortical and subcortical strokes. The potential applications of our methods allow for estimation of predictability of seizures and their localizations.

9.3 MODELS OF TOPOGRAPHIC MAP'S FORMATION AND COMPARISON WITH EXPERIMENTAL DATA

Włodzisław Duch,
Department of Computer Methods, Nicholas Copernicus University, Grudziądzka 5, 87-100 Toruń, Poland,
http://www.phys.uni.torun.pl/~duch

Networks of simple neural elements with lateral and afferent connections trained with simple Hebbian algorithm self-organize creating receptive fields (RFs) and orientation maps. The primary visual cortex of mammals contains orientation and ocular dominance maps that form early during brain's development. Over 10 computational models have been proposed to explain the details of this process.

The availability of data for macaque striate cortex allows for detailed comparisons of properties of computational models with measured cortical map patterns. Accounting for both the local and the global relationships between orientation and ocular dominance map patterns is rather difficult. The overall map structure is explained by the band-pass-filtered white noise models and self-organizing maps while the field-analogy models and correlation based learning models focus on the singular points of orientation maps.

Model networks allow also to investigate effects of various lesions, such as retinal scotoma. Results match neurobiological data obtained on cats and psychophysical experiments with humans. Models of cortical reorganization after stroke and somatosensory cortex reorganization in case of phantom limbs may suggest some therapeutic actions.

MODELLING AS A RESEARCH TOOL. NEURAL NETWORKS GENERATING RHYTHMIC ACTIVITY 9.4

Stefan Kasicki
Dept. Neurophysiology, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Pasteur 3 Str., PL 02-093 Warsaw

How is the *in vivo* neurons' firing pattern generated? Modeling, based on computer simulations, has proved to be a very useful tool for investigations in various fields aiming to find the answer. At a high level it helps to understand how behavior results from the activity of neural networks (e.g. computational neuroethology). The more detailed level is based on the analysis of mechanisms responsible for activity of single neuron (like ion channels, membrane potential etc.). The high level modeling may take advantage of the results of realistic neuron's simulations, although in most cases it is not necessary. Among others, the mechanisms responsible for generation of rhythmic locomotor activity have been better understood due to simulations performed by various investigators. The amount of data gathered in electrophysiological experiments provides a possibility to feed models with various physiological parameters. The simulations help to realize how far the oscillations created by central pattern generators are based on a network phenomenon (connections, cell as a passive unit) or on cellular properties (e.g. voltage-gated currents). Experiments *in computo* use also realistic models to create new predictions that can be confirmed experimentally. This approach assumes that if a few simple guidelines are followed, one can build a realistic model, which integrates experimental data into a dynamic representation and enables prediction of new facts.

The work supported by a statutory grant from the Nencki Institute.

Session 10 - Workshop: Action of drugs of abuse on CNS

10.1 **DRUG ADDICTION: THE PATHOLOGY THAT RESULTS FROM DRIVE SATISFACTION DISORDERS.**

Wojciech Kostowski
Institute of Psychiatry and Neurology, Warszawa, Poland

The mechanism leading from an initial drug use to drug abuse and dependence remain unknown. Recept concepts emphasize the progressive dysregulation of brain reward system within the context of addiction cycle (Koob and LeMoal 2001). We suppose that drug addiction may involve a change in mechanism of satisfaction of drives and satiation state. The drive activity is characterized by general activation and tension while the drive satisfaction and satiety states are characterized by relaxation and relief. Consumatory act and satiety are, according to Konorski (19670, related to so called „antidrives". The drives and antidrives represent two sides of the motivational mechanisms controlling animal's behavior. When a particular drive is satisfied the operation of other drives become possible. We propose a model of motivational changes that occur during the development of addiction („antidrive" deficiency model of addiction) which focuses on the dysregulation of drive satisfaction and satiation. Thus, the deficiency of „antidrive" may cause at least two behavioral disorders. First, the appetitive (drug-seeking) activity persists and is continued, second, the operation of other drives is restrained thus forcing the organism to focus on current drug-related driver. We suppose that counteradaptative processes and drug-induced sensitization may dysregulate drive satisfaction („antidrive") mechanism.

THE NOCICEPTIN-ORPHANINFQ/ORL-1 RECEPTOR SYSTEM AS A TARGET FOR THE TREATMENT OF ALCOHOL ABUSE. 10.2

Massi M., Ciccocioppo R., *Panocka I.
*Dept. of Pharmacological Sciences Experimental Medicine, University of Camerino, 62032 Camerino (MC), Italy; *Dept. of Pharmacology and Toxicology, Military Institute of Hygiene and Epidemiology, 01-163 Warsaw, Kozielska 4, Poland.*

Studies of our group have shown that intracerebroventricular (ICV) treatment with the 17 aminoacid peptide nociceptin/orphaninFQ NC), the endogenous ligand of the opioid receptor-like 1 (ORL-1) receptor, reduces voluntary 10% ethanol intake in genetically selected Marchigian Sardinian alcohol-preferring (msP) rats (1). Studies aimed at the pharmacological characterisation of the receptor which mediates the effect have shown that the 13 N-terminal aminoacid sequence is crucial for activity and that the selective ORL-1 receptor antagonist [Nphe1]NC(1-13)NH2 is able to block the effect of NC on ethanol drinking. In place conditioning studies, NC abolishes the rewarding properties of ethanol in msP rats, as well as those of morphine in non-selected Wistar rats. In addition, NC reduces the oral self-administration of 10% ethanol under an FR1 schedule of reinforcement, both in msP and in non-selected Wistar rats. Lastly, NC abolishes reinstatement of alcohol-seeking behaviour following stress induced by electric foot-shock (2). Together, these findings suggest that the NC/ORL-1 system may represent an interesting target for novel pharmacological treatments of alcohol abuse.

References

1) Ciccocioppo et al., (1999) Psychopharmacology 141: 220-224.
2) Martin-Fardon et al., (2000) NeuroReport 11: 1939-1943.

10.3 **DOPAMINERGIC MECHANISMS IN DRUG ABUSE AND DEPENDENCE.**

I. Panocka, *Military Institute of Hygiene and Epidemiology Warsaw, Poland.*

Drugs of abuse belonging to different classes (psychostimulants, narcotic analgesics, nicotine, ethanol) increase extracellular dopamine (DA) in several areas of the brain. This activation shows a preference for the mesocorticolimbic DA system that is generally thought to be a critical component of the neural circuity mediating drug reward. Several lines of evidence indicate a dysfunction of DA system (mainly suppression of its activity) as a reason for various symptoms of drug withdrawal syndrome. Also, DA system is reported to be of critical importance for drug-induced behavioral activation and sensitization so that its drug-like response can be observed in individuals exposed either to drug itself or to environmental stimuli associated with previous drug administration (drug-related cues). This last phenomenon is thought to play an important role in drug craving and drug addiction relapse. In addition, inherent variants of genes coding for proteins involved in dopaminergic neurotransmission were shown to influence individual vulnerability to drug abuse. Taking into account afore-mentioned data, DA appears to be a neurotransmitter that is the most diffusely involved in the action of abused drugs. Thus, understanding of DA role in polydrug abuse as well as, for a given drug, in various steps of addiction history (abuse-withdrawal-relapse) may have a key role in searching for effective drug abuse and dependence counteraction. The present report summarizes current knowledge and perspectives in research on DA role in drug addiction.

Session 11 – Oral communications (part 1)

11.1 **BRAIN MICROVASCULAURE IN AGEING AND DEMENTIA.**

Bozena Berdel[1,5], Anne Koivisto[2], Hilka Soininen[1,2], Markku Laakso[3] Irina Alafuzoff[1,4]

[1]Department of Neuroscience and Neurology, [2]Department of Neurology, [3]Department of Internal Medicine [4]Department of Pathology, University of Kuopio; [5]Department of Anatomy and Neurobiology, Medical University of Gdansk.

Vascular dysfunction is considered to be the second most common cause of dementia in elderly population. The aim of this study is to investigate the influence of cerebro- and cardiovascular status and AD pathology on the small vessels in demented and non-demented aged subjects.

Material comprises 97 cases (32 males, 65 females); age at dead ranging from 73 to 88 years. Endothelial cells and β-amyloid loads were labeled using immunohistochemical stainings and quantified in frontal, parietal and temporal regions by means of morphometrical image analysis systems.

The significant spatial differences in the relative area covered by stained endothelial cells were found between the gray and white matter (gray > white) and between the different brain regions (temporal = frontal > parietal cortex). The endothelial cells area decreased with age in both demented and non-demented subjects and the decrease was more pronounced in the demented subjects. In patients with severe AD pathology relative area covered by endothelial cells was significantly higher than in patients without AD pathology. Our findings indicate that the small vessels in the brain are influenced by aging and AD pathology and might be of importance in provoking brain damage and subsequently clinical signs of dementia.

11.2 **THE AGE AND ITS INFLUENCE ON MALE AND FEMALE SLEEP**

W. Jernajczyk[1], L. Czerwosz[2], J. Antczak[1], K. Czasak[1]

[1]Institute of Psychiatry and Neurology, Warsaw, Poland
[2]Medical Research Centre, P.A.S, Warsaw, Poland

It is widely known that the biological value of human sleep after 40 years of life deteriorates.

A nocturnal polysomnography performed according to Rechtschaffen and Kales criteria was used to collect data of sleep patterns from 128 healthy volunteers of which 64 were men and 64 were woman, between the ages 20 and 80 years. This subjects were placed into six similar numbers group due to their age: 20 – 29, 30 – 39, 40 – 49, 50 – 59, 60 – 69, 70 – 80. Sleep patterns were statistically analysed to determine the sleep parameters on age and sex.

The obtained data were regarded as the norms of sleep pattern for healthy population between ages 20 and 80 years. Our data also confirm the deterioration of the biological value of sleep throughout the life. It is demonstrated by the prolongation of sleep latency, the increase of awakenings no and time during sleep as well as dramatic decrease in the amount of delta sleep. The data show the most pronounced deterioration of biological quality of the sleep is among males after their 40`s, however similar changes in sleep quality were found in 20 years older females. Even in the youngest group between 20`s and 40`s the quality of females sleep is better than of males one. In elderly group (60 – 80 y.) we found the longer total sleep time and shorter awakening time compared to American norms (Williams et al. 1974).

11.3 TAU GENE MUTATIONS IN FRONTOTEMPORAL DEMENTIA AND PARKINSONISM LINKED TO CHROMOSOME 17 AND THEIR ROLE IN NEURODEGENERATIVE PROCESS

Anna Kowalska [1, 2], Takeshi Tabira [2]
1/ Institute of Human Genetics, Polish Academy of Sciences, 32 Strzeszynska, 60-479 Poznan, Poland, e-mail: annkowal@rose.man.poznan.pl
2/ National Institute of Neuroscience, 187 Kodaira, Tokyo, Japan, e-mail: tabira@ncnp.go.jp

The identification of mutations in the tau gene in many families with autosomal dominant inherited frontotemporal dementia (FTD) and parkinsonism linked to chromosome 17 as well as a number of other mixed neurodegenerative disorders such as pallidopontonigral degeneration, familial progressive subcortical gliosis and familial multisystem tauopathy clearly indicates that the microtubule-associated tau protein plays a central role in the process of neurodegeneration. Tau protein promotes assembly of microtubules, and it is involved in neurite outgrowth, axonal development and maintenance, signal transduction pathways. Mutations in the tau gene cause disturbances, resulting in formation of filamentous inclusions and subsequent cell death. They are responsible for 25% of pedigrees with FTD. To date over 20 tau mutations have been identified in patients with different ethnic backgrounds.

We screened for tau mutations in a sample of 28 Polish and and Japanese patients with FTD. We found 2 missense mutations in exon 10: N279K and P301L and 1 novel intronic E10 +11 mutation. We discussed as well as molecular effects of the mutations and their neuropathological and clinical consequences.

11.4 EPILEPTOGENESIS RELEATED CHANGES IN GENE EXPRESSION REVEALED BY cDNA ARRAYS IN THE RAT MODEL OF TEMPORAL LOBE EPILEPSY.

Katarzyna Lukasiuk, Terhi Pirttilä, Asla Pitkänen. *A.I. Virtanen Institute for Molecular Sciences, University of Kuopio, 70 211 Kuopio, Finland.*

Epilepsy frequently develops as a result of brain insult and the epileptic process can be divided into three phases: 1) initial insult, 2) latency period (epileptogenesis) and 3) recurrent seizures (epilepsy). In the present study, we aimed at identification of genes that change their expression during the epileptogenesis. We used an amygdala stimulation model of temporal lobe epilepsy in which epilepsy is a consequence of 20-30 min stimulation of the lateral nucleus of the amygdala that is followed by self sustained status epilepticus (SSSE). Following stimulation rats were monitored with video-EEG until the end of experiment to detect the appearance of spontaneous seizures. Only the animals that had SSSE but did not experience spontaneous seizures were used for the experiment. Hippocampal RNA was isolated 14 days after induction of SSSE and was used for hybridization to cDNA arrays. Analysis of cDNA arrays revealed about two fold increase in expression of 118 genes, and decrease in expression of 50 genes. One of upregulated genes, cystatin C, was studied in details. Semiquantitative RT-PCR revealed 2.9 fold increase in cystatin C mRNA in the hippocampus. Increase in cystatin C immunoreactivity was observed at 4 d, 1 we and 2 we after stimulation, predominantly in microglia. We conclude that: 1) alterations in gene expression occur in the hippocampus during epileptogenesis before appearance of spontaneous seizures; 2) cystatin C has a novel, unknown function that could be related to recovery from SE induced damage.

11.5 The model of neurons growth with the branching probability depended of the neurons environment.

Artur Łuczak*, Jaap van Pelt**
*Jagiellonian University, Medical College, Biocybernetics Department, Kopernika 7, Krakow, Poland, e-mail: softromic@poczta.onet.pl; **Netherlands Institute for Brain Research, Meibergdreef 33, 1105 AZ Amsterdam, The Netherlands.

The geometrical properties of neurons are important for the way they function within neural circuits. The arborescent processes of neurons what are necessary for the transmission of the information are formed by branching and elongation of segments. In this work a model for the outgrowth of neurons is proposed. The presented model tries to investigate a relation between external and internal factors responsible for neurons shape. Assuming normal distributed concentration of substances stimulating branching we can explain observed phenomenon that during dendrite development after phase of elongation and branching, occurs a second phase with elongation only. The final outcome of this model is a 3D representation of a given type of dendrite. For the evaluation of the validity of this model we calculated different geometrical measures for the experimentally observed and model generated neurons, including topological aspects and fractal dimension. By optimizing the parameters of the model it was possible to reproduce very accurately experimentally observed distributions of dendritic segments calculated for four distinct groups of dendrites. The good fit of the model outcome values with experimental data shows that dendritic complexity can be described by a stochastic growth processes of branching and elongation, with environment depended branching probabilities.

11.6 ACTIVITIES OF ATP-ASES IN COURSE OF EXPERIMENTAL NEOPLASTIC DISEASE IN RATS AS IMPLICATION FOR PARANEOPLASTIC CEREBELLAR DEGENERATION.

S.Michalak, Z.Adamczewska-Goncerzewicz.
Department of Clinical Neurochemistry, Chair of Neurology, K. Marcinkowski University of Medical Sciences, Poznań, Poland.

Paraneoplastic cerebellar degeneration is found in association with neoplasms of lung, ovary, or breast. The relationship between the primary tumor and the resulting cerebellar dysfunction is not clearly understood. The immune response on one hand, and the metabolism disturbances on the other, are the proposed pathomechanisms. In this sudy we analyzed the activities of cerebellar ATP-ases, crucial enzymes involved in central nervous system metabolism.

Male, Buffalo rats, were sacrified 21days after intramuscular Morris hepatoma inoculation. The activities of Na^+/K^+, Ca^{2+} and Mg^{2+} - ATPases were analyzed basing on inorganic phosphorus release, and expresed as per miligram of protein.

The activity of Na^+/K^+-ATP-ase was increased in tumor bearing rats (264,3 ± 18,9 *vs* 71,6 ± 12,3 µmol P_i/min/mg protein in controls, $p<0.01$), as well as Ca^{2+}-ATP-ase (253,0 ± 30,2 *vs* 63,78 ± 16,4 µmol P_i/min/mg protein in controls, $p<0.01$). The decrease in activity of Mg^{2+} -ATPase was noticed in hepatoma rats (53,0 ± 16,0 *vs* 94,8 ± 32,5 µmol P_i/min/mg protein in controls, $p<0.05$). These changes were followed by decrease in conjugated dienes content in neoplastic rats (0.61 ± 0.23 *vs* 2.20 ± 0.66 µmol/ mg protein in controls, $p< 0.01$). The presented results indicate abnormal activities of ATP-ases as possible pathomechanisms involved in paraneoplastic cerebellar degeneration.

Session 12 – Poster Session: Neuroimmunology

12.1 **TIME-DEPENDENT CHANGES IN MICROGLIA REACTION IN THE COURSE OF EXPERIMENTAL INTRACEREBRAL HAEMATOMA IN THE RAT**

Przemysław Kowiański, Zbigniew Karwacki[1], Beata Ludkiewicz, Beata Domaradzka-Pytel, Sławomir Wójcik, Janusz Moryś, Olgierd Narkiewicz

Department of Anatomy and Neurobiology,
[1]Department of Anaesthesiology and Intensive Therapy,
Medical University of Gdańsk

In 15 adult rats an experimental intracerebral haematoma was produced by the injection of 100 µl of the arterial blood into the caudate nucleus during the period of 5 min. The animals were sacrificed after the survival period of 1, 3, 7, 14, 21 and 28 days. The immunohistochemical staining for microglial/macrophage lineage cells was performed with antibodies OX-42, OX-18, OX-6 and ED1. The reactive OX-6, OX-42, and ED-1-immunopositive microglia was observed since 24h after haematoma induction. The intensity of reaction increased gradually till the end of the second week of observation. OX-18-immunopositive cells were less numerous than the other studied populations and appeared later in the course of the haematoma. The reactive microglia/macrophages exhibited various morphological features – ameboid with thick processes, round and ramified cells with large soma were observed. Summarizing, microglia/macrophages cells exhibit time-dependent reaction both in morphology and intensity of staining, in the course of intracerebral haematoma, which reflects the reaction of the brain tissue to injuring agents. It could be useful in monitoring of the morphological changes observed in the brain during observation of the haematoma evolution and various drug interaction.

12.2 **REGION SPECIFIC DYNAMICS OF APOPTOSIS-LIKE NEURONAL DEATH IN THE RAT ISCHEMIC BRAIN IS CORRELATED WITH MICROGLIA ACTIVATION.**

O.Orzyłowska[1], M.Zaremba[1], S.Januszewski[2], B.Oderfeld-Nowak[1], M.Mossakowski[2]

(1) Nencki Institute of Experimental Biology, Warsaw; (2) Medical Research Center, Warsaw, Poland.

We examined the appearance of neurons with typical apoptotic features and changes in microglial cells, in various brain regions of rat at early (1, 6 and 24 hours) and late (3, 7 and 14 days) stages, after transient global ischemia evoked by cardiac arrest. We found a dissimilar time course of postischemic apoptosis in the neurons of the most vulnerable CA1 layer as opposed to neurons in the dentate gyrus (DG) (mainly in its crest), indusium griseum (IG), olfactory tubercle (Tu), reticular thalamic nucleus (Rt) and caudate putamen (CPu). However, in spite of differences in the time course, the sites of appearance of apoptotic-like cells correlated well with the especially strong microglial activation in the neighborhood. Dying CA1 cells appeared to be relatively silent, at least up to the 3rd day when single apoptotic-like cells were seen. Apoptosis was most visible there after 7 days, and after 14 days a loss of cells or cellular debris were observed. The time course of apoptosis in the remaining structures was characterized by very early onset. The number of apoptotic-like cells increased already from the 6th hour after recirculation. Between the 1st and 3rd day different stages of apoptosis were seen and after 7 days the apoptotic process seemed to be complete. No apoptotic-like cells or cellular debris were seen after 14 days. Our data point to differences in the timing of apoptotic-like cell death in brain regions of different vulnerability to ischemia and to its close association with microglia activation. The prolonged microglia activation seen after the completion of the apoptotic processes also indicates its other significance in the postischemic mechanisms.

12.3 **IMMUNOHISTOCHEMICAL INVESTIGETIONS OF PROTEIN S-100 IN NEURONS OF THE TRIGEMINAL GANGLION.**

Prośba-Mackiewicz M.* , Żółtowska A. **, Bąkowska A. **

* Department of Prosthetic Dentistry Medical University of Gdańsk, Poland

**Department of Immunopathology Medical University of Gdańsk, Poland

Protein S-100 occurs, among others, within the cells of central nervous system. It is considerd to be an indicator of the system defects. So far, however, the protein has not been found to occur in the neurons of the trigeminal ganglion.

The aim of the paper is to estimation the presence of protein S-100 in the neurons of the trigeminal ganglion in man.

The examinations were carried out on the trigeminal ganglion collected during autopsy. From the ganglia a part corresponding to ramifications II and III of the branch of nerve V there was isolated. Immunohistochemical examinations were performd using peroxidase method.

Positive immunohistochemical reactions of protein S-100 was observes in neuroplasm and/or in capsules of nerve cells of the trigeminal ganglia. There were neurons differend in size. Agglomerations of protein S-100 positive cells were observed.

Results of the investigations have confirmed the assumption that protein S-100 is found in the neurons of the trigeminal ganglion. The presence of that protein is very likely to be connected with dysfunctional changes or distrubsances of the peripherial part of that trigeminal neuron.

12.4 **INCREASED EXPRESSION OF BRAIN-DERIVED NEUROTROPHIC FACTOR AND ITS TRKB RECEPTOR IN THE RAT SPINAL CORD DURING EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS IS MAINLY CONNECTED WITH INFLAMMATORY CELLS**

Zaremba M.,(1), Aloe L.,(2), Tirassa P.,(2), Kwiatkowska-Patzer B.,(3), Lipkowski A.W.,(3), Petrykowski R.,(1) and Oderfeld-Nowak B.(1)

(1) Nencki Institute of Experimental Biology, Warsaw, (2) Institute of Neurobiology CNR, Rome, (3) Medical Research Center, Warsaw

While several recent findings convincingly demonstrate that the nerve growth factor (NGF) plays a role in experimental autoimmune encephalomyelitis (EAE), only very few link with this disorder the brain derived neurotrophic factor (BDNF). Using immunocytochemistry, we have examined the effect of EAE upon the expression of BDNF and its receptor, TrkB in the spinal cord of Lewis rats at acute (2 weeks postimmunization) and chronic (12 months postimmunization) phases of the disease. At the acute phase of EAE we have found that in addition to BDNF and TrkB immunoreactivity localized in motoneurons and in some glial cells, many small, round cells that were neither neurons, glia nor macrophages were strongly immunoreactive.These cells appeared to be the main source of the increased BDNF and TrkB expression. The identity of these cells remains presently unknown but their morphological appearance indicates that they may be lymphocytes or other cells of immune origin that invade the spinal cord during EAE. However, these immunoreactive cells were scattered throughout the whole spinal cord, particularly in its gray matter and their accumulation was not especially related to the perivascular infiltrate sites. Their number was at least twofold of that found in control tissue. In the chronic phase of EAE we observed a still increased, over its own age-matched control, number of small, round cells expressing the two immunoreactivities, particularly that of TrkB. These findings suggest that potentially damaging consequences of inflammation may be curbed by production of neurotrophic factors especially in the acute phase of the disease. In addition, TrkB upregulation in immune cells indicates mechanisms based on autocrine neurotrophin/receptor interactions.

12.5 **NEUROHORMONAL AND IMMUNOLOGICAL CHANGES INDUCED BY ACUTE PSYCHOLOGICAL STRESS IN PATIENTS WITH RHEUMATOID ARTHRITIS**

Żak W., Samborski W., Torliński L., Żaba R.
Department of Clinical Biochemistry and Chemistry
Department of Rheumatology and Clinical Immunology
Karol Marcinkowski University of Medical Sciences in Poznań

Although emotional distress often precedes the onset or exacerbation of rheumatoid arthritis (RA), very few data are available on patients' neurohormonal and immune system response to psychological stress under well-controlled, experimental conditions. With approval of the University Ethic Committee we used so-called confrontational role-play technique to induce stress in participants of our study. Each of 8 female volunteers (4 arthritics and 4 healthy age-matched controls) had to play the role of an employee called in for a talk with his employer (played by experimenter), who, displeased with the results of her work, was considering whether to dismiss her or not. Blood for evaluation of catecholamines concentration and lymphocytes number (total and % of: CD3, CD4, CD8, CD16, CD19, CD56) was twice sampled: at baseline (after resting for half an hour in sitting position and listening to a calm music) and after 15 minutes-lasting experiment, conducted every day in the same morning hours to control diurnal fluctuation. All the obtained results were subsequently analysed with respect to some psychological variables, which seemed to be essential in individual differences in stress response. Although the level of anxiety (Spielberger Inventory) and norepinephrin concentration increased in the whole group of participants, the epinephrin conc. and NK cells (CD16 and CD56) and lymphocytes B (CD19) number rose only in controls but fell in arthritics.

Session 13 – Poster Session: Brain aging

13.1 ALTERED CELLULAR DISTRIBUTION OF TAU1 PROTEIN COINCIDES WITH AXONAL TRANSPORT IMPAIRMENT IN AGED RATS.

Marta Baksalerska-Pazera and Grażyna Niewiadomska
Department of Neurophysiology, Nencki Institute, 3 Pasteur St., Warsaw

During aging, basal forebrain cholinergic neurons are prone to degeneration due to an impairment of either uptake or retrograde transport of nerve growth factor (NGF). Breakdown of the cytoskeleton may be involved in the axonal transport disturbances. The tau microtubule-associated protein may play an important role in this process. Tau exists in several isoforms and, in normal brain, is predominantly located in axons. In this study we evaluated the retrograde labelling of basal cholinergic neurons obtained after injection of fluorogold and nuclear yellow into multiple sites in neocortex of young adult (4-mo.-old) and aged (28-mo.-old) rats. After injection of fluorescent tracers the number of retrogradely labelled neurons in the horizontal diagonal band and basal nucleus was significantly lower in aged rats, by 38 and 45% respectively. In aged rats the numbers of neurons immunoreactive for ChAT and TrkA were also significantly lower, by 30-50%. The decline in the number of neurons retrogradely transporting tracers was greater than the decline in the number of immunoreactive neurons in aged rats. Our objective was also to characterize the neuronal distribution of Tau1 and Tau5 proteins in brain regions of young and aged rats. Immunohistochemical analysis was carried out using antibodies against tau isoforms. In young rats, the Tau1 immunostaining was predominantly localized in axons with minimal staining in neuronal perikarya. In aged animals, Tau1 staining was markedly redistributed to the cell bodies, however it was also present in axons. Contrastingly, immunostaining for another tau antibody, Tau5, was unchanged in young and aged rats and distributed both in perikaryon and neuropil. The findings demonstrate significantly reduced retrograde labelling of basal cholinergic neurons accompanied by altered cellular distribution of Tau1 in aged rats. These results suggest an impairment in axonal transport mechanisms which may contribute to the degenerative changes of basal cholinergic neurons observed in aging.

13.2 EFFECTS OF PRENATAL STRESS ON TONIC PAIN IN PREWEANLING RATS

I. P. Butkevich

Lab. of Ontogenesis of Nervous System, I. P. Pavlov Institute of Physiology, St. Petersburg, Russia, 199034

The present study was designed to determine the effects of prenatal stress (PS) on some indices of tonic pain induced by formalin injection. Pregnant rats (gestation days 16-21) were exposed daily to immobilization (1 h). The control rats were not put under the stress. Both pregnant rats and their offspring were in suitable conditions. Subcutaneous injection of formalin (2.5%, 10 µl) in the plantar surface of a hind paw induces in 25-day-old pups the nociceptive responses (flexing, shaking, licking) consisting of two phases lasting 3-5 min and 20-35 min, respectively. Between the phases an interphase interval (3-8 min) takes place during that the nociceptive responses are not shown. PS produced the following changes of nociceptive responses: the enhancement of flexing/shaking but not licking, the increase of the duration of the second (tonic) phase, the decrease of the interphase interval. In female, in contrast to males, nociceptive responses appeared during interphase interval. Data suggest that the interphase interval in formalin test probably is not a period of the rest as it was considered till now, but it is a period of active inhibition. This study shows in 25-day-old rats that PS alters the inhibitory processes involved in tonic pain and females are more vulnerable to PS in comparison with males.

13.3 RESPONSE OF THE SYMPATHO-ADRENAL SYSTEM ON TONIC PAIN IN PRENATAL STRESSED PREWEANLING RATS

V. A. Mikhailenko, I.P. Butkevich

Lab. of Ontogenesis of Nervous System, I. P. Pavlov Institute of Physiology, St. Petersburg, Russia, 199034

Effects of prenatal stress (immobilization in the last week of pregnancy) on the sympatho-adrenal response to formalin-induced pain (2.5%, 10 mμ, s.c. formalin injection into a hind paw) were studied in 25-day-old rats. In each rat total urinary adrenalin, norepinephrine and dopamine excretion during a 0-24 h period both before and after formalin-induced pain was investigated. Prenatally stressed rats showed a robust increase in flexing+shaking behaviors during the second phase of the formalin test. Formalin-induced pain evoked in all rats the increase of the urinary adrenalin and norepinephrine excretion. In prenatally stressed rats the increase of catecholamine excretion after nociceptive responses was pronounced to a lesser degree as compared to non stressed rats. Prenatally non stressed rats showed sex dimorphism in the urinary adrenalin and norepinephrine excretion whereas stressed rats only in norepinephrine one. The level of the catecholamine excretion was higher in males compared with females. Our data suggest that prenatally stressed 25-day-old rats show the enhancement of pain sensitivity and decrease of the reaction of the sympatho-adrenal system in response to tonic pain.

Session 14 - Poster Session: Neurodevelopment

14.1 **POSTNATAL CHANGES OF SYNAPTIC PROTEINS' IMMUNOREACTIVITY IN THE RAT HIPPOCAMPUS PROPER**

Joanna Biranowska, Bożena Berdel, Beata Ludkiewicz, Janusz Moryś

Department of Anatomy and Neurobiology, Medical University of Gdańsk.

Synaptic proteins are mediators of synapses' functions, including biogenesis, docking, fusion and recycling within nerve terminals. The electron microscopic analysis showed that the distribution of SY-staining fully matches the distribution of synapses, so it can be used as a synapses density maker. SNAP-25 plays a key role in membrane fusion events; SNAP-25, syntaxin and synaptobrevin form a protein complex that acts in the later steps of synaptic vesicle fusion with the plasma membrane and it enables neurotransmission release. GAP-43 plays a major role in regulated growth cone activity - during mammalian CNS development. A total number of 45 brains at various ages were examined. After perfusional fixation the brains were frozen and cut on the cryostat in coronal plane and stained either with cresyl violet or standard immunohistochemical method using SNAP-25, synaptophysin and GAP-43 antibodies. Synaptogenesis begins on the 4^{th} postnatal day when the expression of the all studied proteins increased. The strong immunoreactivity remains in most layers till P14. After 14^{th} postnatal day an amount of GAP-43, SNAP-25 and synaptophysin immunolabeled fibers and cells' bodies was gradually falling down and since 90^{th} postnatal day there were only a few stained terminals found. Our results indicate that immunohistochemical characteristic of synaptic proteins'reactivity in the hippocampus differs according to the layer and the developmental stages. The most intense synaptogenesis occur between 4^{th} and 10^{th} postnatal day and after that the synaptic terminals are established.

14.2 **BEHAVIORAL AND BIOCHEMICAL EFFECTS OF NEW CENTRAL DOPAMINE D3 (U-99194A) AND D4 (U-101958) RECEPTOR ANTAGONISTS IN RATS**

Ryszard Brus[1], Przemysław Nowak[1], Andrzej Sokoła[1], Richard M. Kostrzewa[2], Jashovam Shani[3]

[1]Department of Pharmacology, Medical University of Silesia, Jordana 38, PL-41-808 Zabrze, Poland, [2]Department of Pharmacology, Quillen College of Medicine, East Tennessee State University, Johnson City, TN 37614, USA, [3]Department of Pharmacology, The Hebrew University School of Pharmacy, Jerusalem 91120, Israel

Subtypes of the central dopamine receptors, namely D_3 and D_4 raised interest as potential targets for new groups of antipsychotic and neuroleptic drugs. The purpose of the present study was to examine behavioral and biochemical effects of the D_3 (U-99194A) and D_4 (U-101958), new dopamine receptor antagonists, in Wistar rats. Equimolar doses of both antagonists were administered, and the following behavioral parameters were recorded: yawning, locomotion, exploratory activity, stereotyped behavior and catalepsy. Also, the effect of the D_3 and D_4 antagonists on levels of biogenic amines and theirs metabolites (DA, DOPAC, HVA, 3-MT, 5-HT, 5-HIAA) in the striatum, and DA, DOPAC, HVA release in freely moving rats (by brain microdialysis), and DOPAC release (by in vivo differential pulse voltametry) in the striatum of anesthetized rats was determined. Release of biogenic amines in the striatum after dopamine receptors antagonists apply was compared with that of an equimolar dose of haloperidol, a D_2 receptor antagonist. U-99194A diminishes yawning behavior induced by 7-OH-DPAT, and modified its effect on locomotor and exploratory activities. U-101958 increased locomotor and exploratory activities after 7-OH-DPAT pretreatments. Neither antagonists modified apomorphine-induced stereotyped behavior, but intensified haloperidol-induced catalepsy. U-99194A increased levels of 5-HT and 5-HIAA in the striatum, while U-101958 increased DA and HVA levels in it. Neither antagonists affected release of biogenic amines in the striatum, as estimated by microdialysis or by in vivo voltametry, contrary to the effect of haloperidol in these amines.

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14.3 EARLY DEVELOPMENT OF THE CEPHALIC NEURAL CREST IN STAGED HUMAN EMBRYOS

Małgorzata Bruska, Witold Woźniak

Department of Anatomy, University School of Medical Sciences, Poznań

The neural crest is a transistory embryonic structure that detaches from the lateral folds of the neural plate. Neural crest cells migrate to diverse locations and they initiate first of all development of sensory and autonomic ganglia. The cephalic neural crest population has complex pattern and it contributes, together with placodes to cranial nerves ganglia. Present study was made in sectioned serially human embryos from the Collection of the Department of Anatomy. Age of embryos was determined according to international staging criteria and the age was between 30 and 32 days (developmental stages 12 and 13). In human embryos at stage 12 the neural crest extends at the level of rhombomeres 2, 4, 5, and 7. Also the mesencephalic, diencephalic, and optic neural crests are present. The crest cells contribute to trigeminal ganglion and facial-vestibulocochear ganglion. Neural crest for ganglia 9 to 11 form loose strands. In embryos at stage 13 the vomeronasal neural crest appears. Cells leave the nasal plate and form cellular buds. The trigeminal ganglion presents the division into two parts, and the superior and inferior ganglia of 9^{th} and 10^{th} nerve are visible. Also the differentiation within facial-vestibulocochlear ganglion is marked. Ganglia of the glossopharyngeal and vagus nerves show differences between superior and inferior components.

14.4 POSTNATAL MATURATION OF THE AMYGDALOCORTICAL PROJECTIONS - RETROGRADE TRANSPORT STUDY AND IMMUNOHISTOCHEMISTRY OF SYNAPTIC PROTEINS.

Jerzy Dziewiątkowski, Przemysław Kowiański, Joanna Biranowska, Janusz Moryś

Department of Anatomy and Neurobiology, Medical University of Gdańsk, Poland

The motor and somatosensory projection zones in the basolateral amygdala following the injection of the retrograde fluorescent tracer into the respective cortical fields were studied in species belonging to 6 groups of various ages (P7, P14, P21, P45, P90, P180, P-postnatal day). Additionally in 12 animals from four selected age groups the immunohistochemical study of microtubule associated protein and synaptic vesicle proteins in this complex was performed.

In all age groups for both motor and somatosensory cortex different projection zones was observed; the motor projection zone characterized also larger values of the numerical density as well as the total number of projecting neurons. There were observed the significant decrease of both parameters in the first three weeks of the postnatal life. Decreasing of the connections' intensity was correlated with significant changes in the amount and distribution of synaptic proteins. While in the youngest species besides of intensively stained neuropil there were found some immunolabeled cells, mature species characterized the presence only of the former. As a consequence, the significant decrease in the fraction of synaptic proteins during the first weeks of life was observed.

The coexistence of these two processes after the birth indicate that the final maturation of the amygdaloid connections takes place late in the postnatal period.

14.5 PATHOLOGICAL SPECTRUM OF DISCRETE GLIONEURONAL MALFORMATIVE LESIONS IN THE FETAL AND INFANTILE CEREBRAL CORTEX

Milena Laure-Kamionowska, Danuta Maślińska

Department of Developmental Neuropathology, Medical Research Centre, Polish Academy of Sciences, Warsaw

Microdysgenesis is a term describing microscopic cortical cytoarchitectural abnormalities. Histologically this change shows irregular glioneuronal tissue combination forming abnormal structure of cortex. The pathological features of this malformation are subtle and less well defined than other more distinctive cortical malformations. The clinical significance of these discrete glioneuronal malformations is controversial. Microscopic dysgenetic changes have been reported in the cases with intractable epilepsy but similar changes may be seen in neurologically normal adults. The purpose of our study is the investigation of microdysgenetic lesions in the developing nervous system with regard to normal neuronal migration, differentiation and maturation. The postmortem routine investigated fetal and infantile brains were analyzed histologically for the presence of discrete cortical malformations. The wide spectrum of cytoarchitectural glioneuronal malformations was found in investigated material. We have observed leptomeningeal glioneuronal heterotopias, subpial bands of heterotopic neurons, nests of ectopic neurons in the first cortical layer, neuronal and glial clusters, small foci with irregularity of laminar structure of the cortex. Microdysgenetic changes arouse from an insult occurring in the later stages of cortical development and influencing normal fate of neuroglial cells. Various types of focal morphological and cytoarchitectonical developmental abnormalities have been associated with behavioral and neuropsychological deficits in older infants.

14.6 THE MORPHOLOGY OF HIPPOCAMPAL FORMATION'S NEURON CELLS IN PIG IN ONTOGENESIS PROCESS

Iwona Łuszczewska-Sierkowska[1],Ryszard Eustachiewicz[1], Ryszard Maciejewski[2]

[1] Department of Animal Anatomy,Agricultural University,Lublin

[2] Department of Human Anatomy,Medical University,Lublin

The brain of domestic pig of both sex (taken during the following period of its life:a\from 6^{th} to 15^{th} week of intrauterine life, b \ newborn animals, c\ one-month and one-year animals) were used as the material for the examination.The brains were removed and processed conventionally by the microscope.The subject of this examination was hippocampal formation's stratum pyramidale and dental gyrus' stratum granulosum. Hippocampal formation's stratum pyramidale in 6^{th} and 7^{th} week of intrauterine life is made up of a big amount of thickly arranged neuroblasts.Their nuclei are in majority of chromatin.Celluar nuclei are surrounded by a very small amount of weakly coloured cytoplasm.In the subsequent weeks of intrauterine life , the differentiation concerning the shape,the size, the size of cellular nucleus and the amount of cytoplasm of homogenous cells takes place.From the 12^{th} week of intrauterine life hippocampal formation's stratum pyramidale doesn't undergo any important changes.One can say that the cells forming hippocampal formation's stratum pyramidale in pig are morophologically mature in newborn animals.The similar observations are true concerning the cells of dental gyrus' stratum granulosum.

14.7 **EFFECT OF THE CENTRAL DOPAMINE AND SEROTONIN RECEPTOR AGONISTS ON BIOGENIC AMINES RELEASE IN RATS' BRAIN WITH EXPERIMENTAL MODEL OF ATTENTION DEFICIT HYPERACTIVITY DISORDER (ADHD). IN VIVO BRAIN MICRODIALYSIS STUDY**

Przemysław Nowak[1], Ryszard Brus[1], Ryszard Szkilnik[1], Richard M. Kostrzewa[2]

[1]Department of Pharmacology, Medical University of Silesia, Jordana 38 St., 41-808 Zabrze, Poland, [2]Department of Pharmacology, Quillen College of Medicine, East Tennessee State University, Johnson City, TN 37614, USA

Attention deficit hyperactivity disorder (ADHD) is believed to be associated with an alteration of dopamine (DA) neurochemistry in the brain. This conventional view became solidified by the observation of ADHD in DA-lesioned animals, and effectiveness of dopaminomimetics, amphetamine (AMPH) and methylphenidate (MPH), in humans and in animal model of ADHD (1). AMPH and MPH release serotonin (5-HT) as well as DA in the brain. Previously we showed that AMPH and some central 5-HT receptor agonists attenuate hyperlocomotion in our animal model of ADHD (2).
Therefore in this study using of our rat's model of ADHD (1) we examined the effect of AMPH (central DA receptors agonist) and m-chlorphenylpiperazine (mCPP; 5-HT_2 central receptors agonist) on DA and its metabolite (DOPAC, HVA) release in the striatum of freely moving rats, using in vivo brain microdialysis technique. It was shown that both used agonists modified the DA and its metabolite release in different way in the brain of rats with experimental model of ADHD.

References:
1. Kostrzewa R.M. et al.: Brain Res. Bull. 1994, 34, 161-167.
2. Oświęcimska J. et al.: Pol. J. Pharmacol. 2000, 52, 152.

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14.8 **THE NEURONAL STRUCTURE OF THE MAMILLARY REGION IN POSTNATAL STAGE (P20) OF GUINEA PIG**

Anna Robak, Stanisław Szteyn

Department of Comparative Anatomy, University of Warmia and Mazury, 10-561 Olsztyn, Żołnierska 14, Pl.

This study is a one of a series dealing with the development of the mamillary region in guinea pig. On the basis of the Nissl stained sections as well as the Golgi impregnated preparations, the neuronal structure was examined and computerised reconstruction of selected neurons were made. The medial and lateral mamillary nuclei in 20 days old animals measure 1280 μm and 600 μm respectively. The supramamillary and paramamillary nuclei are not uniform, their cells are grouped or loosely arrayed around the mamillary nuclei. Mm cells are the most lightly stained neurons. The impregnated neurons were classified as aspiny: 1) piriform (11-22μm) with 1 thick dendritic trunk; 2) bipolar up to 40μm with fusiform perikarya and usually 2 thick dendritic trunks arising from two opposite poles; 3) multipolar: polygonal (16-40 μm) and smaller cap-like neurons. They have usually 3 - 4 (rarely 2 or 5) thick dendritic trunks. In general, all thick dendritic trunks measure about 5μm in diameter. An axon emerges from the cell body or from the dendritic trunk (especially in bipolar neurons) and can be followed on linear distance up to 35 μm. The small number of polygonal cells and bipolar with pen-shaped dendritic tree (at P20), differs this region from that examined in adult individuals. A few neurons, usually possessing small sizes (8-12 μm), have protoplasmic somatic processes, dendritic expansions as well as the growth cone on their axons. These cells are immature or in differentiating stage. It may be supposed that some discrete neural connections in mamillary region in guinea pig are formed postnatally (at P20).

14.9 **CHOLINERGIC INNERVATION AND CALRETININ-IMMUNOREACTIVE NEURONS IN THE HIPPOCAMPUS DURING POSTNATAL DEVELOPMENT OF THE RAT BRAIN**

Edyta Spodnik, Beata Ludkiewicz, Sławomir Wójcik, Beata Domaradzka-Pytel, Janusz Moryś

Department of Anatomy and Neurobiology, Medical University of Gdańsk

Learning and memory processes in the hippocampus are influenced by many neuromodulators. Among them acetylcholine is one of the most important. Calretinin-positive cells belong to nonprincipal neurons of hippocampus. In the present study we investigated correlation between the cholinergic innervation and neurons containing CR in the hippocampus during postnatal development.

Rat brains of various ages: P0, P4, P7, P14, P21, P30, P60 (P-postnatal day) were studied. Paraformaldehyde-fixed frozen brains were cut on the cryostat in coronal plane and stained immunohistochemically using the antibodies: anti-VAChT and anti-CR. In P0 VAChT-positive points were present in all hippocampal sectors, while CR-positive somata were present mainly in CA3 sector and in dentate gyrus (DG). Starting from P4 both VAChT-positive puncta and fibers were observed and they formed network thorough all hippocampal regions. During that time CR-positive somata appeared also in CA2 region. Since P14 they were present in all regions of hippocampus; also distinct CR-positive fibers appeared. CR-positive cells were mainly bipolar. Co-localization study showed that CR-positive cells formed sparse synaptic contacts with cholinergic terminals. According to our observation we conclude that the generation of synchronous rhythmic hippocampal activity connected with Ach fibers is rather not connected with the interactions of these fibers with subpopulation of CR-positive neurons.

14.10 **EFFECT OF COMBINED CADMIUM AND ETHANOL PRENATAL EXPOSURE ON BIOGENIC AMINES LEVEL AND RELEASE IN THE BRAIN AFTER HALOPERIDOL APPLY IN ADULT OFFSPRING RATS. IN VIVO MICRODIALYSIS STUDY**

Ryszard Szkilnik, Przemysław Nowak, Ryszard Brus, Łukasz Łabus

Department of Pharmacology, Medical University of Silesia, Jordana 38, Pl.-41-808 Zabrze, Poland

Ethanol (EtOH) abuse in pregnancy is known to produce serious damage to internal organs of the fetus, a condition in humans that is classified as „fetal alcohol syndrome". Cadmium (Cd), a heavy metal pollutant of the environment, represents another toxin that produces retarded fetal growth and teratogenic effects. The developing brains is particularly sensitive to both toxins, being affected morphologically and neurochemically. Previously we showed that both neurotoxins applied concomitanly during pregnancy modified reactivity of the central dopaminergic receptors and prevent Cd accumulation in the brain of offspring rats (1, 2). Pregnant Wistar rats consumed in their drinking water 50 ppm of Cd, with or without 10% v/v EtOH, throughout their entire pregnancies, and in adult male offspring the biogenic amines and theirs metabolites (NA, DA, DOPAC, HVA, 3-MT, 5-HT, 5-HIAA) were estimated in the striatum, cortex and hippocampus. Beside, in freely moving rats using brain microdialysis technique the release of DA and its metabolite (DOPAC, HVA) in the striatum was measured after haloperidol (1.0 mg/kg IP) apply. Our results demonstrate that EtOH and Cd exposure during pregnancy of rats separately or concomitanly modified level of biogenic amines mostly in the hippocampus of adult offspring, and their release in the striatum after haloperidol apply.

References:
1. Felińska W. et al.: Pol. J. Environm. Stud. 1995, 4, 31-36.
2. Brus R. et al.: Toxicol. Lett. 1995, 76, 57-82.

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14.11 **NEOCORTICAL AREAS AND THEIR CONNECTIONS IN THE OPOSSUM *MONODELPHIS DOMESTICA*.**

E. Wielkopolska, R. Djavadian, L. Krubitzer, K. Turlejski*
The Nencki Institute of Experimental Biology, Warsaw, Poland;
** University of California at Davis, USA*

The short-tail opossum is a new experimental animal, frequently used in the research on development and evolution of the brain. However, organization of its neocortical areas and their connections, have not been investigated in full yet. Our previous results suggested the existence of two secondary visual areas localized laterally and medially to the VI. Topography of visual areas in *Monodelphis* is similar to that in the closely related *Dydelphis*. In the present experiments we studied the cortical and thalamic connections of the somatosensory, auditory, visual and prefrontal areas. Four retrogradely or/and anterogradely transported dyes were injected into the presumed positions of the investigated areas of one hemisphere under xylazine/ketamine anesthesia. After 7 days of survival animals were killed with an overdose of Nembutal and perfused with 4% paraformaldehyde. Flattened cortex was cut tangentially to the pial surface while thalamus was cut coronally. Some sections were stained for myelin or Nissl. Injection sites and labelled neurons were plotted by a computerized system connected to a fluorescent microscope. We found evidence for a secondary visual area, the medial visual area, primary and secondary somatosensory area, one auditory area, the prefrontal cortex and an inhomogeneous belt of "association" cortex separating visual, auditory and somatosensory areas. We also found a new area, probably unique for the existence of *Monodelphis*, placed rostrally and laterally to SI. This area has connections typical for both somatosensory and prefrontal areas and it seems to be homologous to the orbito-frontal area.

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14.12 **PRIMORDIUM OF THE DENTATE GYRUS IN HUMAN EMBRYOS DURING 7 AND 8 WEEKS**

Witold Woźniak, Katarzyna Rapalska, Małgorzata Bruska

Department of Anatomy, University School of Medical Sciences, Poznań

The dentate gyrus is a part of the hippocampal formation and it is trilaminar structure that caps the distal tip of CA fields. The aim of the present study was to trace the early appearance of the gyrus in staged human embryos. All embryos were from the Collection of the Department of Anatomy in Poznań and their age was expressed according to developmental stages. Embryos were sectioned serially in three planes and in some of them graphic reconstructions were made. First signs of differentiation of the telecephalic wall is found in embryos at stage 16 (38 days). In these embryos the following regions may be distinguished: 1) the hippocampus, 2) the mesocortex, 3) the area dentata, 4) the area epithelialis, 5) the amygdaloid area, and 6) the olfactory area. The area dentata is the field between the hippocampus proper and area epithelialis which separates it from the lamina terminalis. The area dentata consists of ventricular layer and it is located medially to the hippocampus.The area dentata develops into dentate gyrus. In human embryos at stage 17 (41 days) and 18 (44 days) the area dentata is still undifferentiated and consists purely of the ventricular layer which is, however, slightly broader. During the last embryonic period (stages 21-23, 51-56 days) the area dentata occupies short part between already layered hippocampus and the epithelial layer. It consists of ventricular layer which is thicker than in previons stages.

14.13 **DEVELOPMENTAL CHANGES IN THE POSTNATAL PERIOD – STUDY OF CHOLINERGIC INNERVATION AND PARVALBUMIN-POSITIVE NEURONS IN THE RAT HIPPOCAMPUS**

Sławomir Wójcik, Beata Ludkiewicz, Edyta Spodnik, Beata Domaradzka-Pytel, Janusz Moryś

Department of Anatomy and Neurobiology, Medical University of Gdańsk

The hippocampus plays the important role in the control of cognitive-emotive behavior, especially in regard to learning and memory. The role of the cholinergic innervations seems to be essential for these functions. Nonprincipal neurons in the hippocampus are generally considered to be GABAergic and are divided into several subpopulations. Among them there are interneurons containing calcium-binding protein – parvalbumin (PV).The correlation between PV-positive neurons and cholinergic innervations was studied in the hippocampal formation during postnatal life. Rat brains of various ages: P0, P4, P7, P10, P14, P21, P30, P60 were studied. Frozen sections were stained using the antibodies against: vesicular acetylcholine transporter (VAChT) and PV. At P0 only VAChT-positive puncta were present. From P4 puncta and fibers were observed; on the end of first week they formed network in all hippocampal regions. The PV-reactive neurons were mainly located in the pyramidal and oriens layers of Ammon's horn and in the granular cell layer of the dentate gyrus (since P10). 3D reconstruction of double immunolabeled sections by using of confocal microscopy showed that relatively few PV-positive neurons possessed cholinergic endings.

It may indicate that cholinergic system is not the main factor that influences PV-positive neurons.

Session 15 - Poster Session: Biological aspects of major psychoses

15.2 DEPRESSION AND PAIN – WHAT WE CAN LEARN FROM ANIMAL STUDIES.

E. Kostarczyk
Department of Pathophysiology of Locomotor Organs.
University Medical School, Poznań, Poland

Clinical and epidemiological data indicate that depression and pain coexist. Depression is often accompanied with pain, and pain may lead to depression. Depression and pain are subjective human experience difficult to transpose for appropriate animal model. In this presentation we will explore some data from animal behavioral and electrophysiological experiments that might elucidate mechanisms that connect pain and depression. The hypothalamic lesions were shown to induce a variety of motor, alimentary, social and affective dysfunctions. These "depressive like" sympthomes were sensitive to the classical antidepressant treatment (imipramine). Although the distruction of hypothalamic area had high variability, the common damage included the medial forebrain bundle (MFB). The recent findings indicate that the spino-hypothalamic tract neurones (SHT) involved in pain processing enter the hypothalamus via MFB. From the studies of peripheral sensory neurones we know that axonal damage influence the function of the cell body.
We suggest that some sympthomes due to hypothalamic lesions, especially those that involve diffuse pain or discomfort may be generated by the milieu change (lesion) in the area of the passing SHT axons or by the degenerative process in the damaged SHT axon which affect the spinal neurons and also influence neuronal activity at the midbrain and brainstem level.

15.1 **DEPLETION OF NORADRENALINE COUNTERACTS THE INCREASE OF α_{1A}-ADRENOCEPTOR mRNA EXPRESSION INDUCED BY TREATMENT WITH ANTIDEPRESSANTS**

G. Kreiner, A. Zelek-Molik and I. Nalepa
Dept. of Biochemistry, Institute of Pharmacology, PAN, Krakow, Poland

Recently, we have found that chronic treatment with antidepressant agents, imipramine (IMI) and electroconvulsive shock (ECS), increased the mRNA level of α_{1A} subtype of α_1-adrenoceptor while no change in α_{1B} expression was observed. The aim of ε current study was to find out whether noradrenaline is involved in the above effect of antidepressants and how the central depletion of noradrenaline induced by noradrenergic neurotoxin, DSP-4, affects the expression of α_{1A}- and α_{1B}-adrenoceptors (α_{1A}-AR, α_{1B}-AR) mRNA in the prefrontal cortex of Wistar rats. Animals were pretreated with DSP4 (50 mg/kg, one dose, ip.) and then (after four days) IMI (10mg/kg ip, 2x daily) or ECS (130mA, 250ms, daily) were administered for 14 consecutive days. 24h after the last injection or ECS rats were decapitated and their brains were excised. Total RNA was isolated and analyzed using either *Northern blot* hybridization with specific cDNA probe for α_{1A}-AR or by competitive RT-PCR (α_{1B}-AR assessment). We have found that while the reduction of noradrenaline level induced by neurotoxic lesion did not influence the expression of either α_{1A}-AR or α_{1B}-AR, the IMI-induced increase in α_{1A}-AR mRNA was nullified and that induced by ECS was significantly diminished (by ~30%) in the cortex of animals depleted of this katecholamine. Our results show that the expression of gene coding for α_{1A}-AR is under positive control of noradrenaline which is involved in the action of IMI and ECS.

15.4 **NEUROLOGICAL MANIFESTATIONS AS THE RESULT OF NON-TYPICAL COURSE AND ENLARGED DIMENSIONS OF THE SUPERFICIAL VEIN OF THE TEMPORAL LOBE**

Maciejewski R., Sęk A. and Czerwińska E.*
Human Anatomy Department, Medical University of Lublin, Poland
** Neurological Outpatient Clinic, SPZOZ Staszów, Poland*

Neurological symptoms as the result of non-typical course of superficial cerebral veins are described in available literature very rarely. The case described below indicate that in some circumstances the compression symptoms derived from cerebral cortex may be incredible serious than their anatomical reasons.
Own observation: Young woman (20 years – case history 823/99), reported to the Neurological Clinic on October 1999 complaining of paroxysmal numbness of the left upper limb with irradiation to the left side of the face, to the left eye and to left half of the tongue. Paroxysms have increasing intensification. Duration in time from 2 to 5 minutes. The patient said, that in childhood she used to have paroxysmal itching of the left hand. She also said, that CT of the head made in 1994 after the car accident was without pathological changes. Neurological examination, x-ray of the skull and EEG test performed during first visit proved normal. From this time the natural case history was as follow: July 2000 – the Jackson type epilepsy combined with loss of sensation of the left half of the face. The neurological and ophthalmologic examination of the fundus of the eye proved normal. Skull x-ray –norm. October 13, 2000 – Disturbances of the vision in the left half of the field. EEG – norm. The MRI test showed the asymmetry in the course and dilated superficial vein between the basis of the right temporal lobe and the tentorium of the cerebellum. Fundus of the eye was normal, but in the field of vision the white and red colors were dominated. Then the patient was admitted to therapy in Neurology Department in Medical University of Lublin.

15.3 CHANGES IN GLUTAMATERGIC TRANSMISSION IN THE FRONTO-PARIETAL CORTEX AFTER CHRONIC TREATMENT WITH NEUROLEPTICS IN RATS.

Małgorzata Pietraszek, Maria Bijak, Krystyna Gołembiowska, Jadwiga Wardas, Stanisław Wolfarth, Krystyna Ossowska
Institute of Pharmacology, Polish Academy of Sciences, Kraków, Poland

Recently we have shown that chronic treatment with haloperidol and clozapine increased the number of cortical NMDA receptors labelled with competitive antagonist, [^{3}H]CGP 39653. Therefore, the purpose of this study was to examine the influence of haloperidol, typical neuroleptic and clozapine, atypical one, on glutamate-dependent neuronal activity and extracellular levels of excitatory amino acids in rat cerebral cortex.

Clozapine (30 mg/kg/day) or haloperidol (1 mg/kg/day) was administered to rats in drinking water for 6 weeks and was afterwards withdrawn for 4 days. Extracellular concentrations of basal and veratridine-evoked glutamate (Glu) and aspartate (Asp) in the fronto-parietal cortex were assessed using an in vivo microdialysis. Spontaneous neuronal discharges were recorded extracellulary in rat parietal cortex slices in a Mg^{2+}-free medium.

Haloperidol elevated basal, but not veratridine-stimulated, extracellular levels of Glu and Asp and increased the glutamate-dependent neuronal activity. In contrast, clozapine decreased both basal and veratridine-evoked extracellular levels of Glu and Asp in the fronto-parietal cortex, but had no effect on the activity of cortical neurones.

These results suggest that haloperidol and clozapine affect differently glutamatergic neurotransmission in the fronto-parietal cortex, which may reflect their diverse efficacy as antipsychotic agents.

15.5 **THE NUCLEUS OF THE LATERAL OLFACTORY TRACT:COMPARATIVE MORPHOMETRIC STUDY IN COMMON SHREW,RABBIT AND PIG.**

M. Równiak
Department of Comparative Anatomy, University of Warmia and Mazury in Olsztyn, Żołnierska 14, 10-561, Olsztyn, PL

All Nissl stained sections comprising nucleus of the lateral olfactory tract (NLOT) were scanned by the means of image analysis system. On the basis of such digital slices 3D reconstructions of examined structures were performed and their mean volumes calculated. Additionally, all analysed nuclei were characterised by the mean numerical density (the number of neurones in 1 mm^3 of brain tissue) and finally the average numbers of neurones in each of them. The neuronal populations of NLOT in all examined species were also analysed using image analysis system. Each neurone was characterised by a set of morphometric parameters: the cross-sectional area of soma, length, width, sum of length and width, shape factor and circumference.
The silver impregnated sections were analysed in the light microscope. The microscopic images of selected, impregnated cells were digitally recorded. From 50 to 100 such microphotographs were taken at the different focus layers of the section for each neurone. Computerised reconstructions of neurones were performed on the basis of these series.
The neuronal populations in all three layers of NLOT is composed of two sharply different subpopulations of neurones. The first one consists of principal spiny pyramidal and semi-pyramidal neurones. The second one is composed of small aspiny stellate-like cells with thin varicose dendrites and highly, arborised axons.

Session 16 - Poster Session: CNS plasticity

16.1 **THE INFLUENCE OF POTENTIATION AND OF THE TETANIC FUSION ON THE TIME COURSE OF FAST MOTOR UNITS TETANUS**

Edyta Bichler, Jan Celichowski & Kazimierz Grottel
Department of Neurobiology, University School of Physical Education, Poznań, Poland

The influence of potentiation and of stimulation frequency on the time course of the last contraction within an unfused tetanus was studied in fast fatigable and fast resistant (FF and FR) motor units in rat's medial gastrocnemius. Studied units were subjected to two series of trains of stimuli at increasing frequency. Within the first series the potentiation developed and the twitch tension before the second series amounted to 146% and 125% of the initial values for FF and FR units, respectively. Within the second series the twitch tension was stable. The contraction time and half-relaxation time were calculated for tetani fused to variable degree in the first and the second series of stimuli trains. In the first series, in tetani fused to a degree of 0.9 the half-relaxation prolonged by 110% and 60%, whereas the contraction time shortened by 48% and 33% as compared to a single twitch recorded before the first series for FF and FR units, respectively. In the second series, in similarly fused tetani, the half-relaxation prolonged only by 63% and 30%, whereas the contraction time shortened by 44% and 36% for FF and FR units, respectively. The present results showed that the potentiation influenced mainly the relaxation time and that this phenomenon was stronger in FF than in FR motor units. Moreover, the course of relaxation appeared to be more dependent on the fusion of tetanic contraction in FF than in FR units. Therefore, the summation of successive contractions in an unfused tetanus in FF motor units is more complex and dynamic process than in FR units.

16.2 **LONG TERM EFFECTS OF TEMPORALLY REDUCED ACTIVITY OF THE DEVELOPING NEUROMUSCULAR SYSTEM ON MUSCLE PROPERTIES**

A. Cabaj, K. Maleszak, U. Sławińska, L. Greensmith [1], G. Vrbová [2]
Nencki Institute of Experimental Biology and Institute of Biocybernetics and Biomedical Engineering, Warsaw, Poland [1] Institute of Neurology, London, UK (2) University College of London, UK

There is much evidence to show that activity of skeletal muscles and their motor nerves is important for the normal development and maintenance of the neuromuscular system. The possibility that the temporarily decreasing neuronal activity during early development by blocking NMDA receptors will influence muscle development was studied here. A non-competitive NMDA channel blocker, MK-801, was injected daily intraperitonealy to rat pups aged 0-12 days with a dose: P0 - 0.75mg/kg; P1 - 1mg/kg; P2 –1.5mg/kg; P3-P12 - 2mg/kg. At the same time the control pups were treated with saline. A single injection did not change a typical behaviour of neonatal rats (neither saline nor MK-801 solution). They remained quiet and did not move much around.

Three months later the pattern and amount of EMG activity was studied. EMG activity of flexor muscle (extensor digitorum longus - EDL) and extensor muscle (soleus - Sol) was recorded during exploratory behaviour and locomotion. The analysis of EMG activity recorded in freely moving animals showed that the EMG activity during exploratory behaviour and the pattern of EMG burst activity of EDL and Sol muscles during locomotion were not different in MK-801 treated and untreated rats. The statistical analysis confirmed that the relationship between the EMG burst duration of Sol as well as EDL muscle and step cycle duration remained also unchanged. The final acute experiments demonstrated that the characteristics of single twitch of the Sol and EDL muscles also did not differ in both groups of animals. However, there was slightly bigger number of motor units in the Sol of the MK-801 treated rats when compare to that treated with saline (Student t-test $p<0.05$; 35±2.7 *vs.* 31±3.5 (mean±SD)). In EDL muscle this tendency was not confirmed by Student t-test ($p>0.05$; 42±2.5 *vs.* 41±2.2).

Thus, our results show that the treatment of neonatal animals with MK-801 has generally no permanent effect on the muscle properties and the development of locomotor activity.

16.3 **THE INFLUENCE OF POTENTIATION OF TENSION ON THE TENSION-TIME AREA IN FAST MOTOR UNIT UNFUSED TETANUS**

Jan Celichowski, Edyta Bichler & Kazimierz Grottel
Department of Neurobiology, University School of Physical Education, Poznań, Poland

The potentiation of tension can be observed in fast muscle fibers after their short-time activity and it appears as the increase in tension and the prolongation of twitch duration. The present study aimed at analyzing the influence of potentiation of tension on the unfused tetanic contraction. Fast motor units (fast fatigable – FF and fast resistant – FR) were studied in rat's medial gastrocnemius muscle. The tetanic contractions were evoked by trains of stimuli at increasing frequency. The tension-time area was analyzed for tetani fused to variable degree in both, non-potentiated and later, in potentiated tetani. It is known that the tension-time area is a parameter which enables the estimation of the effectiveness of muscle fibers contraction. The maximum tension-time area per one pulse is reached in the optimum contraction, i.e. realized at minimum metabolic costs. It was found that in potentiated tetani the maximum area per pulse occurred at lower frequency of stimulation (45.0 ± 5.8 Hz and 50.7 ± 11.7 Hz for FF and FR units, respectively) as compared to non-potentiated tetani (56.4 ± 11.2 Hz and 58.7 ± 11.8 Hz, respectively). Moreover, the direct values of tension-time area per pulse in potentiated tetani increased by 11.5 ± 0.25% and by 10.8 ± 0.48% for FF and FR motor units. It can be concluded that the potentiation is a phenomenon which enable performance of motor tasks at lower metabolic costs.

16.4 C-FOS PROTEIN EXPRESSION DURING CLASSICAL CONDITIONG INVOLVING STIUMULATION OF VIBRISSAE IN MICE.

Anita Cybulska-Klosowicz, Katarzyna Radwanska, Malgorzata Kossut
Nencki Institute, 3 Pasteur St., Warsaw, Poland

Tactile information acquired through the vibrissae is of high behavioural relevance for rodents. Sensory input, produced by deflection of vibrissae, is conveyed by the trigeminal system to the contralateral ventrobasal thalamus and from there to clusters of neurons in layer IV of the face region of the somatosensory cortex ("barrels").

It is known that body maps in the somatosensory cortex can be altered by training involving tactile stimulation. Functional representation of mystacial vibrissae in rats and mice was enlarged following pairing sensory stimulation with tail shock, and following a prolonged period of pairing sensory stimulation with alimentary award.

We examined c-fos expression in various brain structures after training using different reinforcement. c-fos protein is used to map functional activity of the brain and it is a transcription factor protein which changes in gene expression is believed to underlay neuronal plasticity.

Short-duration classical conditioning involving stimulation of a row of mystacial vibrissae in mice was followed with c-fos immunohistochemistry. Three conditioning sessions that paired stimulation of a row of whiskers with a tail shock or droplet of sweet water were applied. c-fos stained nuclei were counted in both hemispheres in the barrel cortex, thalamic reticular nucleus, ventral thalamic nucleus, perirhinal cortex, basolateral amygdaloid nucleus and posterior parietal cortex

16.5 **RAPID REPLENISHMENT OF SYNAPTIC ZINC IN THE MOUSE SOMATOSENSORY CORTEX AFTER LOCAL CHELATION**

A. Czupryn, J. Skangiel Kramska
Nencki Institute, 3 Pasteur St., 02-093 Warsaw, Poland

Synaptic zinc is sequestered in presynaptic vesicles of a class of glutamatergic neurons and it is an endogenous modulator in neuronal transmission. There is evidence that it is involved in experience-dependent synaptic plasticity. This pool of zinc can be selectively visualized using histochemical method by Danscher. Synaptic zinc is distributed unevenly in the somatosensory cortex of mice; layer IV presented the weakest staining (*J. Comp. Neurol. 1997* 386: 652-660). One way to examine the role of synaptic zinc *in vivo* is to chelate it. The aim of this study was to established the best conditions to deplete cortex of synaptic zinc and to investigate dynamic of chelation. We report now that implantation of a piece of spongostan containing 0,01 ml of 5 mM tetrakis-(2-pyridyl - methyl)ethylenediamine (TPEN), the high-specifity zinc chelator, resulted in a complete loss of zinc staining in the entire cortical depth under the implant. This effect could be firstly seen 30 minutes after implantation and remained up to 24 hours. A full restoration of zinc staining was observed after 48 hours. Furthermore, substantial increase of zinc staining intensity was found in cortical layer IV as compared to control untreated hemisphere and remained elevated after 7 days of survival. This observation indicates that compensatory effects take place as a result of transient disturbances of synaptic transmission involving zinc-containing circuitries in the cortex of mice. The established procedure of transient removal of synaptic zinc seemed to be useful to study the role of zinc in the neocortex.

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16.6 **LOCOMOTOR TRAINING IS AN EFFECTIVE TOOL TO STIMULATE BDNF AND NT-4 NEUROTROPHINS IN RAT SPINAL CORD.**

Dwornik A.*, Macias M., Sulejczak D., Wiater M., Skup M. and Czarkowska-Bauch J. *Department of Neurophysiology, Nencki Institute of Experimental Biology, Warsaw, Poland.*

Neurotrophins act not only as classical trophic molecules but also as potent modulators of neuronal activity and plasticity. Recent data indicate that exercise may induce BDNF mRNA expression in the adult rat brain. Our question was whether physical training might modulate neurotrophin pools in the spinal cord (SC) of adult Wistar rats. We studied distribution and intensity of BDNF and NT-4 neurotrophin staining following long-term, moderate locomotor training on a treadmill: 5d/wk, 1000 m/d at a speed of 20-25 cm/s. Untrained animals were used as controls. After 4 wks of training rats were perfused and the SCs were dissected out. Immunocytochemical labeling was performed [αBDNF and αNT-4 antibodies; Santa Cruz] on 40μm cryostat sections. To characterise cellular localisation of the label, double staining was performed [αMAP2-HM2 and αNeuN antibodies] on 14μm sections. ABC Vectastain or FITC/TRITC detection systems were used. In the control animals BDNF immunoreactivity (IR) was found in the neuropil of the spinal grey. NT-4 IR was found predominantly in the white matter fibres. Training led to change in BDNF IR distribution in the dorsal horn [DH] and to enhancement of BDNF IR in the ventral horn [VH]. VH BDNF labelling was particularly intense in a plexus of fibres and terminal swellings accompanying large neurones. Double labelling revealed that these fibres were mainly dendrites. After training NT-4 IR was significantly increased in the white matter fibres. The data show that our training regimen may increase BDNF/NT-4 availability in the SC, thus enhancing neurotrophin signalling. Supported by the SCSR 1305 and a grant for the Nencki Institute.

16.7 **Ketamine may enhance some cognitive processes in humans**

P. Jakubów, J.J. Braszko
Department of Clinical Pharmacology Unit, Medical Academy of Bialystok, Waszyngtona 15A, 15-274 Bialystok, Poland

Cognitive effects of anaesthetic drugs gain increasingly more attention. In this study we assessed influence of ketamine (2-d(1-2(o-chlorofenylo)2(methyl-amino-cykloheksanon) on several aspects of cognition in human subjects that underwent minor surgery. Twenty four patients (12 males and 12 females, age 35-58, mean 47.5 years) underwent following types of anaesthesia: regional block without intravenous drugs (n=7), regional block with analgosedation (fentanyl 100 μg + midazolam 1.5 mg, n=8) and regional block with analgosedation and low dose of ketamine (0.2 mg/kg, n=9). All subjects received similar treatment before and after the surgery.

On Day 5 before and Day 7 after the surgery all patients underwent the following cognitive tests: Rey Verbal Learning Test (RVLT), Wechsler Digits Recall Test (WDRT), Beck Depression Inventory, Hopkins System Checklist. Eleven drug-free healthy volunteers (controls) were evaluated with the same battery of psychological tests in parallel with the patients.

Better scores in RVLT and WDRT memory tests in patients receiving analgosedation with ketamine in comparison to controls and those receiving analgosedation alone were observed ($p<0.05$).

Conclusion:

Small doses of ketamine, enhancing analgosedation and used for short surgical procedures, appear to exert a beneficial influence on human memory processing.

16.8 **THE ROLE OF MELATONIN IN THE REGULATION OF LEARNING AND MEMORY PROCESSES IN THE RAT.**

Marlena Juszczak[1], Helmut Schwarzberg[2].
[1)]*Department of Pathophysiology, Medical University of Łódź, Poland;* [2)]*Institute of Neurophysiology, Otto-von-Guericke-University, Medical Faculty, Magdeburg, Germany.*

The aim of the experiment was to study the role of melatonin (MLT) as to the efficacy of vasopressin (AVP) in the regulation of learning and memory processes in the rat. For these studies, the active avoidance paradigm as well as the intracerebroventricular (icv) injection of AVP have been chosen.

Three months old male Wistar rats were housed under a 12/12 hr light-dark schedule (lights from 06:00 to 18:00). After icv implantation with stainless steel guide cannula, they were allowed to recover for 7 days before using for behavioural experiments. When finishing the first part of the experiment (i.e., 5 days of learning) animals were injected icv with AVP (10 ng AVP dissolved in 5 μl of an artificial cerebrospinal fluid [aCSF]) immediately after the end of last acquisition session. On the next day, the extinction trials started and run for 5 consecutive days. Injections of the vehicle (1% ethanol in 0.9% NaCl) or MLT solution (in a daily dose of 50 μg MLT per 100 g of b.w.) were given at the end of light phase (i.e., at about 17:00h), once daily during both acquisition and extinction sessions.

During the acquisition sessions MLT-injected rats learned to avoid shocks more effectively than rats injected with the vehicle. The AVP increased the number of avoidance responses during extinction trials, while MLT had similar effect on memory processes only at the first day of these sessions. However, MLT did not further modify the AVP-induced memory improvement in the rat.

The results of the present experiment show that MLT may influence both learning and memory processes in the rat.

Marlena Juszczak was supported by the DAAD fellowship.

16.9 EXPRESSION OF GluR1 mRNA IN THE BARREL CORTEX IS NOT ALTERED BY LEARNING-DEPENDENT PLASTICITY.

Monika Lech, Malgorzata Kossut
Nencki Institute, 3 Pasteur St., Warsaw, Poland

AMPA receptor is a very important component of excitatory transmission. It mediates fast excitatory potentials in responds to glutamate release. GluR1 is the most prominent subunit of AMPA receptor and it is crucial for receptor function. This subunit is widely distributed through the mammalian brain.

We have checked how the expression of GluR1 mRNA is changed after sensory conditioning in mice. This training changes representational maps in somatosensory barrel cortex. The training paradigm consisted of stroking the whiskers of row B on one side of the muzzle (conditioned stimulus, CS) followed by a single tail shock (unconditioned stimulus, UCS). Pairings were repeated four times/min for 10 min/d for 3 days. The mice were sacrificed one hour after the last treatment. A second group of mice (stimulated control) received only the CS delivered at the same schedule. The naive control group received no stimulation.

We used *in situ* hybridization method with ^{35}S labelled oligonucleotide as a probe for quantifying GluR1 mRNA level of expression. *In situ* hybridization revealed a laminar pattern of labelling with the greatest intensity in layer VI. Layers II/III and V were less intensively labelled. The weakest labelling was observed in layer IV, which additionally demonstrated clearly visible areas of extremely low signal. Those areas corresponded to barrel hollows.

We have not found any changes in expression of mRNA of this subunit in mice barrel field after conditioning training.

16.10 **NEURONS CONTAINING NITRIC OXIDE SYNTHASE AND NEUROPEPTIDE Y IN THE BASOLATERAL COMPLEX OF RAT AMYGDALA**

Beata Ludkiewicz, Joanna Biranowska, Sławomir Wójcik, Beata Domaradzka-Pytel, Janusz Moryś
Department of Anatomy and Neurobiology, Medical University of Gdańsk

Nitric oxide, the smallest neuroactive molecule is synthesized by the one of the largest enzymes - nitric oxide synthase (NOS). Besides of its role as neurotransmitter it may be also the important factor of excitotoxic processes that are thought to occur in neurodegenerative diseases affecting the amygdala (Alzheimer's disease or temporal lobe epilepsy). Neuropeptide Y (NPY) has been reported to modulation the activity of amygdaloid neurons in memory and anxiety. The present study utilized the immunohistochemistry to investigate the distribution and morphology of NOS- and NPY-containing cells in the basolateral complex of the rat amygdala. The material consisted of 5 adult rat brains. After perfusional fixation the brains were frozen, cut in coronal plane and stained using the antibodies: anti-NOS and anti-NPY. The lateral nucleus exhibited the highest density of NOS-positive neurons and fibers. Characteristically, numerous NOS-positive neurons were located along the ventral and medial border of the basolateral nucleus, but there were only few inside.

Their morphology indicated that they were non-pyramidal neurons. Similar pattern was observed in distribution of NPY-positive neurons; many of them were also non-pyramidal. The similarities in the distribution and their morphology suggest that NOS and NPY may coexist in the same neurons. These cells seem to be local circuit neurons of the rat basolateral complex.

16.11 **STIMULATION OF TRKB RECEPTOR PROTEIN EXPRESSION IN THE SPINAL CORD BY LOCOMOTOR TRAINING: CONTRIBUTION OF OLIGODENDROGLIA.**

Macias M*., Dwornik A., Sulejczak D., Wiater M., Czarkowska-Bauch J. and Skup M. *Department of Neurophysiology, Nencki Institute of Experimental Biology, Warsaw, Poland.*

Physicotherapy following spinal cord injury can bring motor improvement. Biochemical background of this effect is not fully understood, but recent studies point to neurotrophins as factors involved. Physical exercise was shown to upregulate brain expression of BDNF neurotrophin. Our recent study revealed that long-term locomotor training influences BDNF and NT-4 neurotrophins and their receptor TrkB also in the spinal cord (SC). Increase of TrkB is found in a population of small cells scattered in the spinal grey. Our aim was to further characterise the changes and to determine the phenotype of TrkB immunoreactive small cells. Male, Wistar rats walked on a treadmill 5d/wk, 1000m/d with a speed of 20-25 cm/s. After 4 wks of training rats were perfused, SC were dissected. To determine cellular localisation of the labelling, double staining was performed on 14 μm cryostat sections. ABC Vectastain with DAB, or FITC/TRITC/Texas Red detection systems were used. To visualise neurones or astrocytes, TrkB polyclonal antibodies (Abs) were combined with NeuN or GFAP Abs, respectively. NG2 and O4 Abs were used to identify oligodendroglial precursor cells and RIP and GalC - for mature oligodendrocytes. Microglia were stained with Bandeiraea Simplicifolia lectin. Our experiments reveal that TrkB is found both on mature oligodendrocytes and their precursors, indicating that the increase in number of TrkB expressing cells may be due to recruitment of newly generated cells. Increased TrkB receptor expression suggests that oligodendroglia may actively participate in training-driven responsiveness of spinal neuronal networks.

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16.12 **THE ROLE OF RE-ESTABLISHED SEROTONINERGIC INNERVATION IN IMPROVEMENT OF HINDLIMB LOCOMOTOR MOVEMENTS IN SPINAL ADULT RATS**

K. Maleszak*, H. Majczyński and U. Sławińska.
Nencki Institute of Experimental Biology, Warsaw, Poland
**Institute of Biocybernetics and Biomedical Engineering, Warsaw, Poland*

It was demonstrated recently that the grafts of embryonic raphe nuclei facilitate the improvement of hindlimb locomotor-like movement in adult rats after total spinal cord transection. The purpose of this study was to clarify whether this improvement was due to the re-established serotoninergic innervation. In 13 Wistar rats, 3 months old (both sex), the total spinal cord transection at the level T10 was performed. One month after spinalization the solid piece of embryonic tissue of raphe nuclei region was grafted into the spinal cord below the lesion in 7 animals. Five other rats, with their spinal cord transected, after a shame grafting operation, were left as spinal-control. Two weeks later the electrodes for EMG recordings were implanted in flexor and extensor muscles of the ankle joint. Two months after grafting the recovery of hindlimb locomotor function was tested.

Grafted rats, when put with their hindlimb on a treadmill, could be induced to walk with regular alternating hindlimb movements, with the plantar surface of their feet in contact with the ground during the stance phase, and ankle dorsi flexion during the swing phase of each step cycle. In the same situation the spinal-control rats were not able to initiate the dorsi flexion of the ankle joint to produce the swing phase of step cycle of locomotor-like movement induced by the tail pinching. Most of time the dorsal surface of the foot was dragged along the moving treadmill.

Following the pharmacological treatment with intraperitoneal injections of 5-HT_2 antagonist - cyproheptadine (1-2mg/kg) the locomotor-like hindlimb movement in grafted rats was impaired markedly for 2-3 hours. In spinal-control rats no such effect in hindlimb movement was obtained. The effect of cyproheptadine in grafted rats was reversed by intraperitoneal injections of 5-HT agonist - quipazine (0.5mg/kg).

Thus, our results demonstrate the crucial role of re-established serotoninergic innervation in graft-induced improvement in hindlimb locomotor abilities in spinal rats. (Supported by KBN grant 4.P05A.085.14)

16.13 **SYNAPSIN IA/B EXPRESSION IN MOUSE SOMATOSENSORY CORTEX.**

D. Nowicka, M. Liguz-Lecznar and J. Skangiel Kramska.
Nencki Institute of Experimental Biology, 3 Pasteur St., 02-093 Warsaw

The synapsins are a family of synaptic vesicle-associated phosphoproteins thought to be involved in the regulation of neurotransmitter release. There are three known genes coding for five isoforms: synapsin Ia, Ib, IIa, IIb and III. Synapsin I is broadly expressed in the brain, however, its expression is not uniform and it has been postulated that this differential distribution reflects differences in the functional properties of the synapses. In the present study we have used immunocytochemistry to examine the regional and cellular distribution of synapsin Ia/b in the mouse SI somatosensory cortex, especially in the barrel field, which is the cortical representation of face whiskers. To this purpose we have used polyclonal antibody supplied by Santa Cruz, recognizing both isoforms of synapsin I. The results show that there is a sharp regionalization of synapsin Ia/b expression in mouse neocortex. The synapsin Ia/b immunoreactivity is confined mainly to primary somatosensory areas. Neighboring regions of the cortex are practically devoid of immunopositive cells. These cells on coronal sections, were observed mainly in layers IV and VI of the barrel cortex. In the barrel field, we observed a distinct pool of neurons located in the barrel hollows, that were covered with synapsin Ia/b-immunoreactive product. Our results suggest that synapsin Ia/b expression in the neocortex is specific and may serve as a somatosensory cortex marker.

16.14 **EFFECT OF INJECTION OF PROCAINE INTO THE VENTRAL TEGMENTAL AREA ON HIPPOCAMPAL THETA RHYTHM IN THE RAT.**

J. Orzeł-Gryglewska, E. Jurkowlaniec, W. Trojniar, Department of Animal Physiology, University of Gdańsk, Poland.

Hippocampal theta rhythm is driven by activation of several sites in the lower brainstem, diencephalon and prosencephalon. In the present study we obtained evidence indicating a possible involvement of the midbrain ventral tegmental area (VTA) in the regulation of theta rhythm.

The experiment was done on urethane anaesthetized male Wistar rats implanted with bilateral hippocampal recording electrodes in the stratum moleculare of the dorsal blade of the dentate gyrus and an injection cannula unilaterally in VTA. Theta rhythm was evoked by tail-pinch before and after unilateral microinjection of procaine HCl (20% solution /0.5 μl) and control administrations of distilled water (drug solvent) into VTA. Stimulations were separated by 10 minutes intervals. The spectral analysis of hippocampal EEG (band 0-15 Hz) was performed off line by fast Fourier transformation (FFT) on three 5-s artifact-free epochs taken from 60 s sensory stimulation samples.

Procaine blocked sensory-elicited theta and caused a reduction of the mean FFT values for peak magnitude by about 60% and reduction of the mean FFT values for peak frequency by 1.3 Hz. The maximum decrease of the peak magnitude was 10 min. after drug administration. Despite unilateral injection this effect was observed bilaterally, but it was more pronounced on the ipsilateral side.

These results indicate that VTA belongs to the brainstem circuitry involved in the regulation of the hippocampal synchronous field activity.

16.15 THE EXPRESION OF TWO FORMS OF GLUTAMINIC ACID DECARBOXYLASE (GAD67 AND GAD 65) IMMUNOREACTIVE STRUCTURES IN BARREL CORTEX OF ADULT MICE AFTER LEARNING.

Ewa Siucinska
Nencki Institute, 3 Pasteur St. 02-093, Warsaw Poland.

We have previously reported that a aversive classical conditioning paradigm involving stimulation of facial vibrissae produced an expansion of the cortical representation of the "trained row", labeled with 2-deoxyglucose (2 DG), in layer IV of the barrel field. Functional reorganization of SmI cortex is accompanied by increased density of small GABAergic neurons. The inhibitory neurotransmitter GABA is synthesized by two isoforms of the enzyme glutaminic acid decarboxylase (GAD67 and GAD65). We asked if classical conditioning training involving facial vibrissa can selectively affect specific subcircuits within the GABAergic system of the barrel field. The present study has examined the pattern of GAD67 and GAD65 immunoreactive neurons and puncta, in the cortical representation of row B of facial vibrissae after short lasting of aversive training. The most notable observation was that average density of GAD67-IR neurons in the hollow of trained row B icreased by 53%, and density of GAD65-IR neurons did not change. An MCID Image Analysis System was used for the automated microscopic analysis. Coded sections were tiled by using 100x objective lens and immunoreactive terminals inside hollow were counted by unbiased 2D form of fractionator technique (Stuart, A.1984 *Basic Ideas of Sampling*. Griffin, London). Following learning GAD67-IR puncta in the hollow of "trained row" increased by 58%. The results suggest the GAD67 is involved in phenomenon learning dependent cortical plasticity.

16.16 LEARNING-INDUCED CHANGES IN αCAMKII AND PSD95 PROTEIN LEVELS IN CORTICAL SYNAPSES.

Anna Skibinska, Malgorzata Kossut
Nencki Institute, 3 Pasteur St., Warsaw, Poland

Proteins of the postsynaptic density are implicated in mechanisms of signal transduction and synaptic plasticity. The most prominent and the most extensively studied PSD protein is α subunit of CaMkII. Its involvement in induction phase of hippocampal LTP is a strongly supported idea. PSD95is a protein also prominent in PSD, and it is suggested as an anchoring and scaffolding protein for NMDA receptor and many signaling proteins. We examined involvement of PSD95 and αCaMkII in learning-induced expansion of representational maps in somatosensory cortex of adult mice. The barrel cortex of mice was examined following a 3 day long classical conditioning training, in which activation of facial vibrissae was linked to an aversive stimulus. In subcellular fraction enriched in postsynaptic densities from the barrel cortex, it was estimated by Western blotting that the level of PSD95 increased after the training by about 50%, while the level of CaMkII remained unchanged. It is suggested that the lack of changes in CaMkII expression may be due to the late phase of the plastic change at which the cortex was examined. On the other hand, this phase is associated with changes in PSD95, demonstrating its involvement in learning-induced plasticity of cerebral cortex.

16.17 **CHANGES IN GROUP I METABOTROPIC GLUTAMATE RECEPTORS IMMUNOREACTIVITY IN RAT HIPPOCAMPUS AFTER ELECTROCONVULSIVE SHOCK**

M. Śmiałowska, B. Kroczka, P. Brański, J. Wierońska, A.Pilc
Department of Neurobiology, Institute of Pharmacology, Polish Academy of Sciences, 12 Smętna Str., 31-343 Kraków, Poland.

Metabotropic glutamate receptors (mGluRs) of group I are generally postsynaptic excitatory receptors connected mostly with nerve cell bodies (mGluR1a) and dendritic tree (mGluR5a). The changes in the expression of their immunoreactivity (-IR) were studied in the hippocampus of the rat brain using immunohistochemical and western blot methods. It was found that electroconvulsive shock (ECS) (90mA, 0.5s) given chronically (21 days, every second day) induced a significant increase in the expression of mGluR1a and mGluR5a-IR in the CA hippocampal regions, especially in CA3, reaching 187% and 175% of the control level respectively. Some decrease in mGluR1a-IR were observed after single ECS.
The obtained results indicate the increase in mGluRs protein level in the studied structure, which may be a compensatory mechanism developing as a result of chronic ECS.

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16.18 SYNAPTIC TRANSMISSION IN MOUSE BARREL CORTEX SLICE PREPARATION

Joanna Urban[1], Malgorzata Kossut[1], Grzegorz Hess[2]
[1]Dept. of Neurophysiology, Nencki Institute of Experimental Biology, 3 Pasteur St., 02-093 Warsaw, Poland and [2]Institute of Zoology, Jagiellonian University, 6 Ingardena St., 30-060 Krakow, Poland

Vibrissal trigeminal pathway and representations of vibrissae in the primary somatosensory cortex (barrel cortex) are subjects of many investigations of plasticity. However, the details of the synaptic connection patterns within barrel field and the rules of their modifiability are not well understood. Vertical (intracolumnar) and horizontal (intralaminar) pathways of transmission of evoked field potentials were investigated in slices prepared from mouse barrel cortex. Slices were cut at the angle of 55 degrees to the midline, orthogonally to rows of barrels. Slices were next transferred to the interface chamber and incubated in the artificial cerebrospinal fluid. Barrels in layer IV could be visualized in the reflected light. Electrical stimuli applied to layer VI/V border evoked field potentials in layer IV and layer III of the same cortical column and in layers IV and III of adjacent columns. Preliminary data indicate that in intralaminar connections of layer V long-term depression (LTD) could be induced by 10 min of stimulation applied at 2 Hz. The same stimulation pattern was ineffective in LTD induction in intracolumnar, vertical pathways.

16.19 **PLASTICITY OF UTERUS-INNERVATING NEURONS OF INFERIOR MESENTERIC GANGLION AFTER OVARIOHISTERECTOMY IN THE PIG**

Wasowicz K., Podlasz P.
Dept. of Animal Anatomy, Faculty of Veterinary Medicine, University of Warmia and Mazury in Olsztyn, 10-957 Olsztyn, PL

Combined retrograde tracing and double-labelling immunofluorescence were used to investigate the changes in expression of biologically active substances in the neurons innervating the porcine uterus after ovariohisterectomy. The study was performed on 4 juvenile pigs (15 kg. of body weight) of the Large White Polish race. Retrograde fluorescent tracer Fast Blue (FB; total volume of 50 µl of 5% solution) was injected into the wall of the uterine cervix and uterine horn during laparatomy performed under pentobarbital anaesthesia. After a survival period of 3 weeks in 2 animals ovariohisterectomy was performed. After 7 days all animals were reanaesthetized and transcardially perfused with 4% buffered paraformaldehyde (pH 7.4). Collected inferior mesenteric ganglia (IMG) were cut into 10 µm-thick cryostat serial sections which were processed for double immunofluorescence using antisera against tyrosine hydroxylase (TH), galanin (GAL), substance P (SP), vasoactive intestinal polypeptide (VIP) and pituitary adenylate cyclase-activating peptide (PACAP). Retrograde tracing revealed many uterus-projecting neurons (UPN) that were localised mainly in the poles of left and right IMG. In control animals UPN contained TH, while being devoid of GAL, SP, VIP and PACAP. After ovariohisterectomy over 90% of UPN were devoid of TH, while expressing GAL. No expression of SP, VIP or PACAP was seen in UPN from ovariohisterectomized animals.

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16.20 **EFFECT OF NON-PEPTIDE SUBSTANCE P ANTAGONISTS ON NOCICEPTIVE TRIGEMINO-HYPOGLOSSAL REFLEX**

Zubrzycka M., Janecka A. Department of Physiology and Department of Chemistry, Medical University, 92-215 Łódź, Mazowiecka 6/8,

The aim of our study was to demonstrate the effect of perfusion of the cerebral ventricles with McIlwain-Rodnight's solution (control) and solutions containing SP and its non-peptide analogs RP-67580 and SR-48965 in 100 and 200 nmol/ml concentrations on evoked tongue jerks (ETJ) induced by tooth pulp stimulation. The rats were anesthetized with i.p. chloralose infusion at 150 mg/kg b.w dose. The recording of the amplitude of ETJ, whose sensory and motor centers are located near the aqueduct and the fundus of the IV ventricle, was the experimental method used to investigate the transmission of nociception within the brainstem. The reflex involves recording the movements of stretched tongue evoked by stimulating the sensory branch of the V nerve, i.e. tooth pulp. We observed that perfusion of the cerebral ventricles with 100 and 200 nmol/ml concentrations of SP caused a significant increase of mean ETJ amplitude by 70% and 87%, respectively, as compared with controls. The non-peptide analogs, RP-67580 and SR-48965 significantly blocked ETJ in a concentration-dependent way. RP-67580 perfused through the cerebral ventricles at 100 nmol/ml concentration blocked ETJ by 57%, and at 200 nmol/ml - by 80%, whereas SR-48965 at 100 nmol/ml concentration blocked ETJ by 48%, and at 200 nmol/ml - by 71% in comparison with controls. The above results suggest that SP exerts a dose-dependent effect on synaptic transmission in the investigated reflex arc and that the non-peptide analogs perfused through the ventricular system, competing for binding sites, effectively block the SP-ergic receptors inhibiting the trigemino-hypoglossal reflex.

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17.1 **OXIDATIVE AND HYDROLYTIC PROPERTIES OF BETA-AMYLOID**

Maria Brzyska, Andrzej Bacia, Danek Elbaum
Laboratory of Bio-Physical Methods, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland

β-amyloid protein is the major component of senile plaques found in the brains of Alzheimer's patients. Previously, a new biochemical property of amyloid, e.g. its ability to disrupt ester and peptide bonds, was described by us. In the present work we compare β-amyloid's ability to hydrolyse and oxidize model fluorescent derivatives of dichlorofluorescein (dichlorodihydrofluorescein [H_2DCF] or dichlorofluorescein diacetate [DCF-DA] respectively) to the same final product (dichlorofluorescein). Chemical modification studies revealed that hydrolytic properties are related to His, Ser and Asp/Glu triad, while residues of His, Tyr, Met are involved in oxidative activity of amyloid. Reduction of the hydrolysis product caused by inhibitors of serine esterases (PMSF and eserine) suggests that amyloid caused hydrolysis is serine sensitive. Antioxidants and metal chelators that reduced H_2DCF oxidation did not change or increased DCF-DA hyrolysis. Solvent isotope effects suggested involvement of hydrogen bonds in the hydrolysis reaction. Hydrolysis was inhibited by redox-active metal ions and practically oxygen independent while oxidation process was redox-active metal enhanced (Cu(II) and Fe(II) primarily), oxygen dependent. Product formation was significantly inhibited by catalase and superoxide dismutase as well as benzoquinone, a specific superoxide anion radical scavenger. Increase of fluorescence by oxidation was strongly inhibited by azide and histidine and enhanced in samples prepared with deuterated phosphate buffer, suggesting singlet oxygen intermediacy. These results indicate that hydrolytic and oxidative properties of amyloid are distinct features of this peptide.

17.2 **CORRELATION BETWEEN ACTIVITY OF METALLOPROTEINASES AND APOPTOSIS IN RAT PRIMARY CORTICAL CULTURE – THE EFFECT OF CsA AND FK-506**

P.Berdowska, M.Ziemka-Nałęcz, L.Bużańska, T.Zalewska
Mol. Neuropathol. Lab. Med. Res. Ctr., Warsaw, Poland

Matrix metalloproteinases (MMPs), can rapidly degrade critical protein components of extracellular matrix. In order to determine if activation of these enzymes is involved in the induction of neuronal cells death, we employed a paradigm of apoptosis induced in 7-day-old rat primary cortical culture by treatment with a known apoptosis-inducing factors: 500 µM glutamate, 25 µM hydrogen peroxide, 25 µM staurosporine or by trophic factors deprivation. The results showed that the apoptogens cause a widespread neuronal apoptosis. Concomitantly the activity of MMPs increased in all cases, while the dynamic and range of up-regulation demonstrates a certain degree of agent specificity. Next we examined the neuroprotective effect of two pharmacologically active immunophilin ligands- cyclosporin A (CsA) and FK-506. Neuronal cell death and MMPs activity were significantly attenuated by early CsA (0.5 µM) treatment (up to 2 hours after exposure to apoptosis-initiating factors), whereas FK-506 (0.5 µM) turned out to be ineffective. However, when apoptosis was induced by trophic factors withdrawal, FK 506 caused a reduction of MMPs activity.The findings indicate that there is a temporal correlation of MMPs activity and neuronal cells apoptosis, which tempts one to speculate that excessive degradation of extracellular matrix proteins contributes to neuronal cell death. The differences in the neuroprotective action of CsA and FK-506 in investigated models of apoptosis suggest engagement of different signaling pathways.

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17.3 **LIPID DIET REDUCES DELAYED, NEONATAL ASPHYXIA-INDUCED, COGNITIVE DEFICITS IN JUVENILE RATS - PRELIMINARY STUDY.**

Bohr I., Caputa M.

Department of Animal Physiology, N. Copernicus University, Toruń, Poland

Long term dietetic supplementation with essential fatty acids (EFA) ameliorates animal behaviour in cognitive tests. Moreover, there is a growing body of evidence suggesting neuroprotective effect of another lipid - cholesterol in various brain disorders such as brain stroke and Alzheimer's disease. Since neonatal anoxia leads to neurological abnormalities during entire life span, it was interesting to study effects of EFA and cholesterol on delayed postanoxic disturbances of cognitive functions in juvenile rats.

34 neonatal Wistar rats were divided into two groups: (1) subjected to extreme anoxia lasting 27 minutes, (2) subjected to control handling procedure (C-group). Anoxic group was divided further into two subgroups characterised by different diet regimens: commercial chow pellets as a standard diet for rats (S-subgroup) and diet enriched with 5% cholesterol and 5% EFA, so that total content of lipids in the diet was about 12% (L-subgroup). C- rats were fed the chow pellets only. Extreme anoxia was elicited by exposure of rat pups to pure nitrogen atmosphere for 27 min. C-rats were subjected to atmospheric air; the other procedures were the same as in rats subjected to anoxia. Spatial memory abilities were tested at the age of 45 days in a linear maze. The experiment was composed of 3 sessions repeated daily. Each session included two trials separated by 4-h resting period. Latencies of a reward finding were measured.

The latencies in successive trials shortened progressively in each experimental subgroup. In general, the improvement was the biggest in C-rats and the smallest in S-rats. Neither S-rats nor L-rats were able to perform equally well as C-rats. The performance of L-rats, however, was significantly better than that of S-rats.

17.4 **EFFECTS OF BODY TEMPERATURE AND CHELATION OF IRON DURING NEONATAL ANOXIA ON EMOTIONAL RESPONSES OF JUVENILE RATS**

M. Caputa, J. Rogalska, K. Wentowska, A. Nowakowska

N. Copernicus Univ., Dept of Animal Physiology, Toruń, POLAND

Our previous study has shown that critical neonatal anoxia in rats at body temperatures of 37°C and 39°C results in hyperferremia. The disturbance was absent at normal neonatal body temperature of 33°C. The surplus of iron is deposited in the brain and might be involved in delayed postanoxic neurotoxicity. One of the delayed postanoxic effects, frequently recorded in juvenile rats, is disturbed emotionality. Because long-term disturbances in daily rhythms of body temperature and motor activity following emotional stress can be used as an indicator of sensitivity to the stress we decided to study such disturbances following 5-min exposure to open-field stress in juvenile rats (aged 6-8 weeks), previously subjected to critical neonatal anoxia at 33, 37, and 39°C. The 39°C group was divided into two subgroups, one of them being injected with deferoxamine to prevent postanoxic hyperferremia. Body temperature and activity were recorded by means of radiotelemetry with abdominal transmitters in rats placed in their home cages. Open-field stress caused disturbances of daily rhythm of body temperature in groups of rats exposed to neonatal anoxia at 37 and 39°C. Rats subjected to neonatal anoxia at 39°C showed elevated body temperature at the end of the dark phase during 5 days following open-field stress. There was also the long-term increase in motor activity in rats subjected to neonatal anoxia at 37 and 39°C. Both reduced body temperature and chelation of iron protected juvenile rats from the emotional disturbances. In conclusion, postanoxic hyperferremia in newborn rats leads to delayed dysfunctions of the brain.

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17.5 **CYTOTOXIC EFFECT OF SODIUM GLUTAMATE ON THE STRUCTURES OF THE DORSAL ROOT GANGLIA OF THE RAT.**

R. Cybulska, J. Jaworska-Adamu, R. Maciejewski*

Department of Histology and Embryology, University of Agriculture in Lublin, Poland

**Department of Human Anatomy, Medical University in Lublin, Poland*

Based on the findings of other authors pointing to the fact that glutamate may be brought in to play a role in neurotransmission in the peripheral nervous system, we decided to investigate the effect of intraperitoneal application of sodium glutamate on the neurons and satellite glial cells of the dorsal root ganglia (L1-L4) of 3-months-old rats. Studies were carried out in 2 experimental groups. Group I rats were given once 1g/1kg body weight sodium glutamate. Group II rats were given similar doses for a period of five consecutive days. Control group were given saline solution.

Swelling of mitochondria and the loss of mitochondrial cristae together with concentrations of lysosomes in neuronal ganglia and satellite glial cells were observed in group I rats. More intense changes in the form of vacuolization of neurons and satellite glial cells and lysis of singular neurons were seen in group II rats.

Results obtained suggest that sodium glutamate crossed through fenestrated type blood vessels found in the dorsal root ganglia and caused a cytotoxic effect on neurons and glial cells.

17.6 **PROTEASOME HYPOFUNCTION INDUCES APOPTOSIS OF HIPPOCAMPAL GRANULE NEURONS IN CULTURE: POSSIBLE INVOLVEMENT OF NFκB TRANSCRIPTION FACTOR.**

Figiel I., Fiedorowicz A., Oderfeld-Nowak B., *Wilk S.

Nencki Institute of Experimental Biology, Warsaw, Poland;

*Mount Sinai School of Medicine, New York, USA.

Several recent studies have demonstrated that the ubiquitin/proteasome protein degradation pathway is linked to apoptotic cell death. However, the mechanism of this process varied in different cell types. We investigated the effect of a specific proteasome inhibitor, N-Benzyloxycarbonyl-Ile-Glu(O-*t*-butyl)-Ala-leucinal (PSI), on mixed neuronal/glial cultures derived from the rat hippocampal dentate gyrus. We found that PSI, depending on the concentration, evokes granule neuron apoptosis, as shown by morphological changes, as well as nuclear condensation and DNA fragmentation. In contrast, no degenerative changes were induced in glial cells. An alcohol analog of PSI did not evoke any sign of cell death. To further investigate the molecular correlation of granule neuron apoptosis, we analyzed NFκB transcription factor expression. Involvement of this transcription factor in neuronal apoptosis was reported previously. It was demonstrated that NFκB can function to promote either cell death or cell survival. As revealed by immunocytochemical staining with a p65-specific antibody, constitutive expression of this NFκB subunit found in control cultures decreased following treatment with PSI where neuronal apoptosis was observed. Our results suggest that a decrease of NFκB activation correlates with PSI-induced apoptosis of hippocampal granule neurons.

17.7 FORMS OF DYSGRAPHIA IN CHILDREN

Golubovic, S*., Jovanovic-Simic, N.*, & Markovic, M.**

*Faculty of Defectology, University of Belgrade
**Institute for Experimental Phonetics and Speech Pathology, Belgrade

The paper analyses both the frequency of the occurrence and the types of dysgraphia in children born in 1990 and in 1991. The number of 500 children attending the third and the fourth forms of the elementary school aged 9 and 10 of the normal hearing, seeing, and of the normal intelligence.
We analysed the dictation, rewriting, description of an experience, the graphomotoric string and the drawing of the man.
The analysis of the results has shown a high degree of frequency of the occurrence of dysgraphia in these children, as well as a prominent dysgraphic behaviour as compared with our earlier investigations (Golubovic, 1994). We have also established a more frequent occurrence of dyslectic forms of dysgraphia as compared to the graphomotoric forms of dysgraphia and the special dysgraphia, as well as the visual and auditory forms of it, which indicates a higher presence of certain
language deficiencies in these children, probably due to the events in Yugoslavia (the war in Bosnia and the bombardment of Yugoslavia in 1999).
Our investigation also points to the social relevance of the writing skill, which underlines the necessity of a compulsory pre-school diagnostics and the preparation including various forms of constructive games and graphomotoric tasks, but also establishing the existence of language deficiency in those children as well as the risk factors that cause the occurrence of dysgraphia..

17.8 **ESTIMATION OF POSTURAL STABILITY BY STATIC STABILOGRAPHY**

M. Kalejta[1], K. Flis[1], I. Lubiński[2]

[1] Institute of Physics, Nicholas Copernicus University,Toruń, Poland
[2] Rydygier Hospital, Toruń, Poland

Quantitative evaluation of human balance system is essential for diagnosis of balance disorders as well as for monitoring the patient's recovery. Several methods have been designed to asses the state of human balance via tests using a force platform that measures the trajectory of the centre of ground reaction forces (centre of pressure - COP). The static method involves registration of the trajectory of the COP during quiet standing.
This contribution presents a study of about one hundred persons that on the basis of classical neurological examination were divided into three groups: healthy, these with an organic pathology and subjects with a psychogenic pathology.
We have tested several measures of the COP trajectory that have been designed to evaluate the status of human balance system. Some parameters like: maximum and mean excursions in sagittal and lateral planes, mean radius, total length of trajectory were introduced in the past. Other, like: diffusion coefficient, scaling exponents and critical point coordinates have been introduced recently. We are also proposing a novel parameter, which reflects dynamic aspects of postural stability that could be derived from the COP trajectory taken under static conditions.

17.9 MPEP PROTECTS AGAINST METHAMPHETAMINE TOXICITY IN RATS.

J. Konieczny, K. Gołembiowska, K. Ossowska, S. Wolfarth
Institute of Pharmacology, Polish Academy of Science, Kraków, Poland

It has been suggested that drugs, which slow down the excessive dopamine (DA) and glutamate (GLU) release may have a potential for neuroprotection. Antagonists of group I of metabotropic glutamate receptors (mGluRs) have been shown to have neuroprotective properties in several models of neurotoxicity in animals. We have addressed this issue by using selective mGluR5 antagonist – 2-methyl-6-phenylethynylpyridine (MPEP), in rat model of neurotoxicity induced by methamphetamine (METH).

In our experiments, METH injected five times every two hours at a dose of 10 mg/kg sc decreased tissue content of striatal DA and its metabolites DOPAC and HVA in rats. MPEP (5 x 5mg/kg ip) given jointly with METH reversed effect of neurotoxin on DA and its metabolites level.

To investigate the influence of blockade of mGlu5 receptor subtype with MPEP on spontaneous and stimulated DA release in rat striatum we used *in vivo* microdialysis. MPEP (100-500 μM) perfused through microdialysis probe did not affect basal and stimulated with veratridine (100 μM) striatal DA release. However, MPEP given intraperitoneally (5 mg/kg) diminished basal extracellular DA level and inhibited veratridine-evoked DA release.

Results obtained in our study demonstrate that blockade of mGluR5 subtype with MPEP decreases DA release in rat striatum. The effect exerted by MPEP seem to be mediated by sites located outside the striatum and may result from relieving DA neurons of the facilitatory influence of GLU. Reversal of METH-induced DA depletion suggests a potential for neuroprotective activity of MPEP.

17.10 **WINDOW EFFECT OF TEMPERATURE ON CARBACHOL-INDUCED THETA-LIKE OSCILLATIONS IN HIPPOCAMPAL FORMATION SLICES**

Konopacki J., Kowalczyk T., Gołębiewski H.
Department of Neurobiology, University of Łódź, Łódź, Poland

The effect of different temperatures (18^0-42^0C) of artificial cerebrospinal fluid (ACSF) on carbachol (CCH)-induced field potentials were examined in the present study. Two hundred and thirty one experiments were performed on hippocampal formation slices maintained in the gas-liquid interface chamber. All slices were perfuzed with 50 μM CCH. Recording electrode was positioned in the region of CA3c pyramidal cells. The experiments gave two main findings. First, in a presence of continuous cholinergic stimulation the temperature of the bathing medium *per se* determined the rate of synchronization of the field potentials and pattern of EEG activity recorded. Second, within the temperature range from 33^0C to 37^0C the window effect of temperature on CCH-induced theta-like activity (TLA) was noted: at this temperature range all slices tested responded only with one pattern of EEG activity – TLA. The results are discussed in a light of temperature effect on hippocampal neuronal network.

17.11 PERIPHERAL NERVE EXTRACTS CHANGE THEIR NEUROPROTECTIVE ACTIVITY UPON CNS NEURITES AFTER NGF BLOCKADE

M. Larysz-Brysz[1], J. Lewin-Kowalik[1], D. Górka[1], K. Wolwender[2]
Department of Physiology[1], Neurology[2]; Medical University of Silesia, Medyków St., Katowice, Poland

Our earlier experiments dealing with the neurotrophic activity of predegenerated peripheral nerves showed that such nerves as well as their purified extracts exert neurotrophic effect on injured hippocampal neurites. This influence was strongest when 7-, 28- and 35-day-predegenerated nerves were used.

The aim of present work was to find, how big part of this activity belongs to one of the most potent neurotrophins - NGF. Therefore we blocked its activity using special antibodies against β-NGF.

Experiment was carried out on Wistar male adult rats. Extracts obtained from distal stumps of 7-, 28- and 35-days-predegenerated and non-predegenerated rat sciatic nerves were implanted into the hippocampus by means of autologous connective tissue chambers filled with fibrin as scaffold for outgrowing fibers. Half of animals received extracts mixed with anti-NGF antibody. Reference group was treated with NGF solution or fibrin only. Six weeks following surgery, FITC-HRP was injected to the free ends of the grafts. Next day whole grafted brains were dissected and histologically elaborated. Labeled hippocampal cells were counted near tip of the graft and subjected to statistical analysis.

We found that NGF blockade dramatically diminished neurotrophic power of peripheral nerve extracts, however it was still stronger than action of pure fibrin.

17.12 EFFECTS OF NMDA ANTAGONISM ON INDUCED ISCHEMIC TOLERANCE IN THE GERBIL HIPPOCAMPUS

J. W. Łazarewicz[1], R. Gadamski[1] and W. Danysz[2]
[1]Medical Research Centre P.A.S, Warsaw, Poland and [2]Department of Pharmacology, Merz + Co., Frankfurt/Main, Germany

Withdrawn

17.13 EPILEPTOGENESIS RELEATED CHANGES IN GENE EXPRESSION REVEALED BY cDNA ARRAYS IN THE RAT MODEL OF TEMPORAL LOBE EPILEPSY.

Katarzyna Lukasiuk, Terhi Pirttilä, Asla Pitkänen. *A.I. Virtanen Institute for Molecular Sciences, University of Kuopio, 70 211 Kuopio, Finland.*

Epilepsy frequently develops as a result of brain insult and the epileptic process can be divided into three phases: 1) initial insult, 2) latency period (epileptogenesis) and 3) recurrent seizures (epilepsy). In the present study, we aimed at identification of genes that change their expression during the epileptogenesis. We used an amygdala stimulation model of temporal lobe epilepsy in which epilepsy is a consequence of 20-30 min stimulation of the lateral nucleus of the amygdala that is followed by self sustained status epilepticus (SSSE). Following stimulation rats were monitored with video-EEG until the end of experiment to detect the appearance of spontaneous seizures. Only the animals that had SSSE but did not experience spontaneous seizures were used for the experiment. Hippocampal RNA was isolated 14 days after induction of SSSE and was used for hybridization to cDNA arrays. Analysis of cDNA arrays revealed about two fold increase in expression of 118 genes, and decrease in expression of 50 genes. One of upregulated genes, cystatin C, was studied in details. Semiquantitative RT-PCR revealed 2.9 fold increase in cystatin C mRNA in the hippocampus. Increase in cystatin C immunoreactivity was observed at 4 d, 1 we and 2 we after stimulation, predominantly in microglia. We conclude that: 1) alterations in gene expression occur in the hippocampus during epileptogenesis before appearance of spontaneous seizures; 2) cystatin C has a novel, unknown function that could be related to recovery from SE induced damage.

17.14 NORADRENALINE LEVEL CHANGES IN CEREBRAL CORTEX IN RATS TREATED BY TAUROCHOLATE SODIUM IN BILIARY-TYPE ACUTE PANCREATITIS

Maciejewski R.
Human Anatomy Department, Medical University of Lublin, Poland

Acute pancreatitis (AP) is a disease condition in which many changes in brain occurs. There are: oedema, petechia, fat embolism, degeneration changes turning into necrotic ones in ganglionic cells (from which Purkini's cells of the cerebellum and hypothalamus cells are the most susceptible). Among mechanisms which may take part in the process of encephalopathy coming into being in the pancreatitis course, the following should be also enumerated: hyperglycaemia, activity of inflammatory mediators and malabsorbtion from alimentary tract. Clinical signs of less or more intense encephalopathy are present at about 50% of patients admitted to hospital after 24 hours of AP.

The experiment was carried out on 100 male Wistar breed rats, divided into 3 groups: A-healthy (10), B-control (30) and experimental (C-60). Rats from B and C groups were anaesthetized by ketamine and AP was induced: in C group using Aho's method, but B-group rats were treated only by 0,9%NaCl. After 2,6,12,24 and 48 hours rats were anaesthetized again and brain was sampled. Hemisphere and commissural system was taken for chemical tests. Brodi's method modified by Chang was used. In healthy animals mean value of dopamine was 399,2 ± 31,1 μg/g and in next hours of the experiment in C group – 363,7 ± 30,2, 316,4 ± 27,7, 329,0 ± 18,6, 365,3 ± 37,0 and after 48 h – 403,4 ± 29,9 μg/g. Differences between levels of noradrenaline in A and B groups were less significant.

It was concluded that the lowest level of noradrenaline concentration in cerebral cortex occurred between 2nd and 12th hour of the experimental pancreatitis ($p<0.001$).

17.15 **NEUROPROTECTIVE POTENTIAL OF mGluR AGONIST ABHxD-I AND CALCIUM STABILISER DANTROLENE IN THE MODEL OF PERINATAL ASPHYXIA IN RATS**

D. Makarewicz[1], J. T. Wróblewski[2] and J. W. Łazarewicz[1]

[1]Medical Research Centre P.A.S., Warsaw, Poland and [2]Department of Pharmacology, Georgetown University, Washington, DC, USA

Putative mechanisms of neurodegeneration in perinatal asphyxia encompass excitotoxicity and neuronal calcium imbalance. The aim of the present study was to evaluate neuroprotective efficacy of ABHxD-I, a new mixed agonist of metabotropic glutamate receptors (mGluR) group I, II and III, and dantrolene, calcium stabiliser inhibiting ryanodine receptors, in the model of perinatal asphyxia in 7-day old rats. Hypoxia/ischemia (H/I) of immature rats was induced by unilateral carotid occlusion followed by 65 min exposure to hypoxia (7.3% O_2 in N_2). The drugs were administered 30 min after hypoxia, either intraperitonealy (ip) at doses of 10 and 20 mg/kg for dantrolene and 30 mg/kg for ABHxD-I, or intracerebro-ventricularly (icv), 0.17 μg of dantrolene or 7.5 of ABHxD-I into the ipsilateral hemisphere. The brain damage was evaluated two weeks after H/I as ipsilateral hemisphere weight deficit. The results demonstrate that dantrolene given ip but not icv, significantly attenuates brain injury induced by perinatal asphyxia, in 32.7 % and 25.8 % at doses of 10 and 20 mg/kg, respectively. ABHxD-I reduced brain damage in 55% and 37% when administered icv and ip, respectively. Both drugs given ip did not influence a rectal body temperature. ABHxD-I and dantrolene hardly penetrate the blood-brain barrier (BBB) of the adult animals. Yet, their neuroprotective efficacy after ip administration was most likely achieved here owing to immaturity and postischemic leakage of the BBB in PND7 rats. Thus, ABHxD-I and dantrolene are promising candidates for prototype drugs in treatment of perinatal asphyxia.

17.16 **MODULATION OF ARACHIDONIC ACID INCORPORATION INTO CULTURED SPINAL CORD NEURONS.**

Andrzej Małecki[1,3]; M. Chalimoniuk[2,3]; Henryk I. Trzeciak[1] and Michał Toborek[3]

[1]Department of Pharmacology, Silesian Medical University, Katowice, 40752, Poland; [2]Department of Cellular Signaling, Medical Research Center, Polish Academy of Science, Warsaw, Poland; [3]Department of Surgery, University of Kentucky, Lexington, USA

The massive release of free fatty acids occurs after primary spinal cord trauma and amplifies the initial injury. The most abundant released fatty acid is arachidonic acid (AA). Previously, we have shown that AA could exert several neurotoxic effects, such as induction of oxidative stress, an increase in intracellular calcium level, and compromised neuronal survival. In this study, we investigated the effect of different substances on AA neurotoxicity and on incorporation of AA into cellular lipids of cultured spinal cord neurons. Neurons were isolated from dissociated spinal cords of fetal mice (E-14) and maintained in Neurobasal medium enriched with N_2 supplement. Cells were exposed to 10 μM AA or to 10 μM AA containing 0,5 μCi ^{14}C-AA per dish (52,5 mCi/mmol; Amersham). Neurons viability was estimated by the rate of MTT conversion. Total cellular lipids were extracted and separated on silica gel TLC plates. Indomethacin, the inhibitor of cyclooxygenases, NDGA - the inhibitor of lipoxygenases, and methylprednisolone – a corticosteroid with antioxidant properties, did not protect spinal cord neurons against arachidonic acid neurotoxicity. However, indomethacin, NDGA and methylprednisolone changed the AA incorporation into different lipid pools. Specifically, these factors increased the level of free arachidonate in spinal cord neurons. We hypothesize that this effect may contribute to neurotoxic effects observed in spinal cord neurons exposed to AA in the presence of indomethacin, NDGA or methylprednisolone.

17.17 **REGENERATION OF SCIATIC NERVES OF ADULTS RATS INDUCED BY EXTRACTS FROM DISTAL STUMPS OF PERIPHERAL NERVES.**

W. Marcol[1], J. Lewin-Kowalik[1], M. Larysz-Brysz[1], E. Święch-Sabuda[1], D. Górka[1], B. Gołka[1], K. Wolwender[2], M. Pietrucha-Dutczak[1]

Department of Physiology[1], Neurology[2], Medical University of Silesia, Medyków Street, Katowice, Poland

Our previous papers had revealed that predegenerated peripheral nerve grafts, as well as their purified extracts (postmicrosomal fractions - PMFs) prevent both hippocampal and retinal ganglion cells from injury-induced death and facilitate the outgrowth of their neurites.

The purpose of the present paper was to examine whether PMF obtained from 7-day-predegenerated peripheral nerves would enhance the regeneration of the proximal stump of transected rat's sciatic nerve in the absence of its distal part. Experiments were carried out on adult male Wistar C rats. Animals were assigned into 4 equal groups. In all groups the sciatic nerve was totally transected and its distal fragment was removed. The proximal stump was introduced into the autologous connective tissue chamber filled with fibrin and 7D-PMF. Control animals were treated with PMF obtained from intact nerves, BDNF solution or fibrin only. The regeneration intensity was assessed by the number of DiI labelled motoneurons as well as the number of myelinated nerve fibres present in the central part of chambers.

Numbers of labelled cells and myelinated fibers were equal in groups treated with 7D-PMF as well as BDNF and it was significantly higher than in two other groups. This observation indicates that predegenerated nerves have relatively high neurotrophic capacity, which can overcome the absence of distal part of peripheral nerve.

17.18 **MATRIX METALLOPROTEINASES ACTIVITY IN DISTAL STUMPS OF RAT SCIATIC NERVES 1-7 DAYS FOLLOWING TRANSECTION**

M. Pietrucha-Dutczak[1], J. Lewin-Kowalik[1], D. Górka[1], I. Korczyńska[1], I. Matuszek[1], K. Wolwender[2], , M. Larysz-Brysz[1], Department of Physiology[1], Neurology[2], Medical University of Silesia, Medyków Street, Katowice, Poland

Matrix metalloproteinases (MMPs) are a class of structurally related enzymes that participate in the degradation of proteins that constitute the extracellular matrix pericellular of connective tissue and play an important role in both normal and pathological remodelling of different tissues. The MMP family consists of at least 18 members that have common propeptide and N-terminal catalytic domains.

Within this group, attention has been focused on the gelatinases (MMP-2 and MMP-9) with are thought to play a important role in tumour progression. The presence of MMPs in the CNS is well documented. MMP-2 and MMP-9 are localised in microglia and astrocytes. It is evident that MMPs have significant effects on the brain micro-environment and therefore may be essential in the regrowth processes.

Experiments were carried out on adult male Wistar rats. Sciatic nerves were totally transected and following 1, 2, 3, 4, 5, 6 and 7 days their distal stumps were homogenised and centrifuged. Presence of MMP-2 and MMP-9 in extract was determined by zymography with sodium dodecyl sulphate (SDS) polyacrylamide gel electrophoresis (PAGE). MMPs were detected in all extracts from predegenerated sciatic nerves but reached maximal level at the 4th, 5th and 7th day. Both gelatinases were absent or inactive in intact nerves.

17.19 **EFFECTS OF NEONATAL BODY TEMPERATURE AND CHELATION OF IRON ON DELAYED POSTANOXIC DISTURBANCES IN OPEN-FIELD BEHAVIOUR IN RATS**

J. Rogalska, M. Caputa, K. Wentowska, A. Nowakowska
N. Copernicus Univ., Dept of Animal Physiology, Toruń, POLAND

Neonatal anoxia leads to neurological dysfunctions during entire life span up to senescence. One of the putative factors involved is free iron, released from the red cells and ferritin, and deposited in the brain of asphyxiated newborns. The deposited iron catalyses delayed free radical damage to the brain. Because reduced body temperature (~33°C), typical of newborn rats, protects them from postanoxic acidosis and hyperferremia we decided to study effects of body temperature and chelation of iron, during a critical neonatal anoxia, on development of behavioural abnormalities in juvenile rats. Neonatal rats were exposed to the critical anoxia at body temperatures of 33, 37, and 39°C and the temperatures were kept unchanged during 2 hours postanoxia. The 39°C group was divided into two subgroups, one of them being injected with deferoxamine to prevent postanoxic hyperferremia. Both postanoxic and control temperature-matched rats were subjected to open-field stress, 5, 10, 15, 25, and 30 days postanoxia, and their behaviour was automatically recorded for 5 minutes. There were clear-cut differences in behavioural responses of juvenile rats to open-field stress between the experimental groups. Hyperactivity disorder, proportional to body temperature was recorded in rats from 37°C and 39°C groups. These behavioural disturbances were observed from 5th through 30th postanoxic day. Both reduced body temperature and chelation of iron protected juvenile rats from the behavioural disturbances. The present data should be taken into account in clinical studies of children suffering from the attention deficit-hyperactivity disorder (ADHD).

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17.20 **POST-ISCHEMIC CHANGES IN NMDA-EVOKED CICR IN SYNAPTONEUROSOMES OF THE RAT BRAIN CORTEX AND HIPPOCAMPUS**

Salińska E., Ziembowicz A., Łazarewicz JW.
Medical Research Centre, Pol. Acad. Sci., Warsaw, Poland

It has been recently suggested that dysfunction of endoplasmic reticulum (ER) may result in neurodegeneration in various pathological conditions including brain ischemia. There are indications that depletion of ER calcium pool may trigger these processes. It has been demonstrated, utilising different in vitro and in vivo models, that ischemia/reperfusion suppresses ER calcium pump (SERCA) and may affect ryanodine receptors (RyR). The aim of this study was to determine changes the NMDA-evoked Ca^{2+}-induced Ca^{2+} release (CICR) via RyR, detected as an increase in intracellular calcium concentration ($[Ca^{2+}]_i$) in synaptoneurosomes isolated from the rat brain cortex and hippocampus, one, two and three days after 10-min forebrain ischemia induced according to Pulsinelli. Isolated crude synaptoneurosomal fractions loaded with fura-2 were stimulated with 0.5 mM NMDA, and the effects of RyR blockers, 1 μM ryanodine or 0.5 μM dantrolene was detected. Our data demonstrated that NMDA-evoked increase in $[Ca^{2+}]_i$ in synaptoneurosomes isolated from the hippocampus on the first and second day after ischemia is attenuated in about 30%, and on the first day its ryanodine- and dantrolene-sensitive portion disappeared. On the third day after ischemia NMDA-induced increase in $[Ca^{2+}]_i$ was even potentiated, and its ryanodine- and dantrolene-sensitive increase in $[Ca^{2+}]_i$ partially recovered. In synaptoneurosomes isolated from the cortex NMDA-evoked increase in $[Ca^{2+}]_i$ was potentiated on the first day after ischemia and decreased on the second and third day, without significant changes in sensitivity to ryanodine and dantrolene. Thus, ischemia induces reversible inhibition of the NMDA-evoked CICR in the hippocampal neurones.

17.21 **TEMPORAL CHANGES IN Bcl-2 AND Bax PROTEINS IN RAT CEREBRAL CORTEX FOLLOWING DEVASCULARIZING LESION.**

Sulejczak D.* and Skup M. *Department of Neurophysiology, Nencki Institute of Experimental Biology, Warsaw, Poland.*

We have shown that cortical devascularization causes apoptotic cell death within infarcted cortical areas [Am. Soc. Neurosci. Abstr., 1999, 298.5]. Bcl-2 family of proteins is involved in the regulation of apoptosis. Ratio of Bcl-2 and Bax dimers was shown to have impact on this phenomenon. Here we investigate Bcl-2 and Bax protein postlesion responses to test whether these proteins contribute to apoptosis caused by devascularization. Study was performed on adult, Wistar rats. Animals were subjected to unilateral cortical lesion and allowed to survive for 0.5h, 1.5h, 3h, 6h, 1d, 3d or 7d. Naive and sham-operated rats served as controls. Rats were sacrificed by perfusion. Immunohistochemical study was carried out on 25μm free-floating or 14μm glass-mounted brain sections using αBcl-2 and αBax (1:400, Santa Cruz) antibodies (Abs). ABC Vectastain or FITC/TRITC detection systems were used. In control rats Bcl-2 and Bax immunoreactivity (IR) was found in neurones but not in astrocytes. Immediately [0,5h] after the lesion an increase of Bcl-2 IR and Bax IR was detected in multiple cell bodies within injured area. Enhancement of Bcl-2 IR was transient while that of Bax IR was sustained. Double labelling with Abs against markers: NeuN [neurones], GFAP[astroglia], Rip[oligodendroglia] and lectin[microglia] indicated that Bcl-2 and Bax IR upregulation occurred predominantly in neurones. Changes were found mainly in cells with condensed chromatin and in apoptotic bodies. As our correlative study revealed that the changes take place in areas where neurones are destined to die within following days (3-7d) we postulate that a decrease of Bcl-2/Bax protein ratio in time after the infarct may contribute to the fate of neurones. Supported by SCSR grants 1030 and 792 and a grant for the Nencki Institute.

17.22 **ASTROCYTIC RESPONSE IN CARDIAC ARREST-INDUCED GLOBAL CEREBRAL ISCHEMIA IN RAT.**

Grzegorz Sulkowski, Irena Bubko, Lidia Strużyńska, [a]Sławomir Januszewski, [b]Michał Walski, Urszula Rafałowska
Laboratory of Pathobiochemistry of the Central Nervous System, Department of Neurochemistry, [a]Laboratory of Experimental Nuclear Medicine and [b]Laboratory of Ultrastructure of the Cell, Medical Research Centre, Polish Academy of Sciences, 5 Pawińskiego St., 02-106 Warsaw, Poland.

The purpose of the present study was to investigated alterations in astrocytic cells after global cerebral ischemia resulting from cardiac arrest and in several intervals post resuscitation. Using electron microscopic, biochemical and immunochemical procedures, we examined the cellular fraction of astrocytic origin (glial plasmalemmal vesicles – GPV). A tendency towards an elevation in immunocontent of glial fibrillary acidic protein (GFAP) was noticed after 24 hrs post resuscitation whereas a significant increase was observed 7 days post ischemic event. The features of astrocytic stimulation were also observed in electron microscopy studies. An enhanced amount of gliofilaments was noticed in brain sections obtained from rats with 7 days of recovery.

At the same time, a gradual decrease of total glutathione level, depending on the duration of reperfusion, was observed in brain homogenates and in fractions of astroglial origin. The most considerable reduction was observed in brain homogenates in day 1 (52%) and day 7 (65%) of reperfusion so as in day 7 (47%) in the case of the GPV fraction. These results indicate the enhanced reactivity of astrocytic cells in ischemic conditions (resulting from cardiac arrest) concomitantly with a long lasting decrease of total glutathione.

17.23 GLUTATHIONE AND GLUTATHIONE-RELATED ENZYMES IN RAT BRAIN AFTER ACUTE LEAD EXPOSURE.

Lidia Strużyńska, Urszula Rafałowska
Laboratory of Pathobiochemistry of the Central Nervous System, Department of Neurochemistry, Medical Research Centre, Polish Academy of Sciences, Warsaw, Poland.

Numerous studies confirm the adverse effects of lead (Pb) and the functional abnormalities in brain as a results of its neurotoxic action, especially in young organisms. Glutathione functions in the protection of mammalian cells against oxidative damage and certain toxic compounds of endo- and exogenous origin. Evidence exists for two functionally distinct pools of GSH system – cytosolic and mitochondrial. It was of interest to study the effect of neurotoxic agent, lead, on the homeostasis of subcellular GSH systems. The purpose of the study was to determine the total glutathione level and the activity of two enzymes involved in its metabolism i.e. γ-glutamylcysteine synthetase (γ-GCS), a rate–limiting enzyme for GSH *de novo* synthesis and glutathione reductase (GR), involved in the regeneration of GSH from the oxidized form in the conditions of the acute lead toxicity. The adult rats from an experimental group were injected i.p. with 25 mg of lead acetate/kg b.w. for 3 days. Control rats obtained distilled water. Total glutathione level was elevated significantly when measured in the brain fraction highly enriched in mitochondria. The activities of enzymes both, γ-GCS and GR, were found to be increased in mitochondrial fraction by about 50% and 70%, respectively. The activities of enzymes measured in cytosolic fraction were only slightly elevated. The results indicating the active response of antioxidant GSH system in the mitochondrial compartment of the adult rat brain following Pb toxicity conditions.

17.24 **PROMOTION OF THE OPTIC NERVE REGENERATION AND SURVIVAL OF RETINAL GANGLION CELLS BY THE EXTRACTS FROM PERIPHERAL NERVES WHEN COMPARED TO BDNF**

Święch-Sabuda E., Gołka B., Lewin-Kowalik J., Larysz-Brysz M., Górka D., Marcol W.
Department of Physiology, Silesian Medical School, Medyków 18, 40-762 Katowice, Poland

Our previous studies revealed that peripheral nerve grafts facilitated neurite outgrowth as well as survival of retinal ganglion cells. The effect of 7-day-predegenerated (7PD) graft was stronger than non-predegenerated (NPD) one. The purpose of the present paper was to examine whether the extracts obtained from such peripheral nerves would exert similar effect. Experiments were carried out on adult male Wistar C rats. Animals were assigned into 4 equal groups. In all groups, fragment of optic nerve was excised and subsequently a connective tissue chamber was sutured into the site of excision. Chambers were filled with fibrin, mixed with: 7PD or NPD extract, BDNF or homogenizing buffer, respectively. Four weeks following surgery fluorescent dyes were applied: DiI into the end of implants and rhodamine B to the *corpus vitreum*. After 48 hours animals were perfused transcardially and the chambers and retinas were subjected to histological and immunohistochemical procedures. Labelled cells and growing fibres were examined using fluorescence microscope, photographed and counted. Myelinated fibres were also counted under light microscope. The results were subjected to statistical analysis.
Extracts from predegenerated nerves exert the strongest neurotrophic influence upon the injured retinal ganglion cells, comparable with BDNF effect.

17.25 **ALTERATION OF GABA TRANSPORT AND $GABA_B$ RECEPTOR BINDING IN RAT BRAIN CAUSED BY GLOBAL CEREBRAL ISCHEMIA.**

J. Waśkiewicz, G. Sulkowski, S. Januszewski*, U. Rafałowska
*Laboratory of Pathobiochemistry of Central Nervous System, Department of Neurochemistry and * Laboratory of Experimental Nuclear Medicine, Medical Research Centre Polish Academy of Sciences, Warsaw, Poland*

γ-aminobutyric acid (GABA) one of inhibitory neurotransmitters in the central nervous system play important role in activity of GABA-dependent Ca^{2+}, Na^+, Cl^- channels. One of the acknowledged targets of brain ischemia is synaptic transmission. This study was designed to determine the effects of global cerebral ischemia caused by cardiac arrest on GABA transport and $GABA_B$ receptor binding in different phases post resuscitation. The effects of 10 minutes global ischemia were measured immediately and after 1 h, 24 h and 7 days post resuscitation stages. The results of our studies have shown: the uptake and release of GABA in synaptosomes after 10 min global ischemia decreased by about 20 % compared to the control. This effect was enhanced to 30 % after 1 hour of recyrculation. The uptake and release of GABA normalized completely after 7 days post clinical death. Total ischemia and condition after recyrculation affected the $GABA_B$ receptor binding increasing its affinity (reduced K_D) and decreasing density of receptor (B_{max}) by about 15 % after 1 hour resuscitation and by about 30 % 24 hours after recyrculation. Kinetic parameters of $GABA_B$ receptor binding normalized after 7 days resuscitation. Our results show that global ischemia and recyrculation after cardiac arrest lead to disturbances in transport of GABA and its receptor binding. It may be reason of the instability in the central nervous system.

17.26 **ANALYSIS OF CHANGES IN THE LEVEL OF DOPAMINE IN RATS CEREBRAL CORTEX IN THE COURSE OF EXPERIMENTAL ACUTE PANCREATITIS**

Wieczorkiewicz A., Kopieniak M.*, Maciejewski R.
Human Anatomy Department, Medical University of Lublin, Poland
** Department of Anaesthesiology, Clinical Hospital No 4, Lublin*

In CNS dopaminergic neurons form three main tracts responsible for regulating motor processes, emotional and higher psychological activities and in regulating hormonal activities. Among mechanisms which may take part in the process of encephalopathy coming into being in the acute pancreatitis course, the hypo-, hyperglycaemia, activations of inflammatory mediators and malabsorbtion from alimentary tract should be counted. Clinical symptoms of encephalopathy (exitation, confusion, focal and general convulsions, stupor) are present at about half of the patients admitted to hospital with advanced stages of AP.

The experiment was carried out on 100 male Wistar breed rats, divided into 3 groups: A-healthy (10), B-control (30) and C-experimental (60). Rats from B and C groups were anaesthetized with ketamine. Acute pancreatitis was induced in C group using Aho's method, but B-group rats were treated only by 0,9%NaCl. After 2,6,12,24 and 48 hours rats were anaesthetized again and brain was sampled. Hemisphere and commissural system was taken for biochemical tests. Brodi's method modified by Chang was used and statistical analysis was carried out.

In healthy animals mean value of dopamine was 446,8 ± 64,7 μg/g and in next hours of the experiment in C group – 456,3 ± 34,1, 494,7 ± 15,6, 510,2 ± 32,8, 478,1 ± 33,5 and after 48 h – 491,5 ± 29,5 μg/g. Differences between levels of dopamine in A and B groups were less significant. We concluded that peak of dopamine concentration occurred between 6^{th} and 12^{th} hour of experiment ($p<0.05$).

17.27 **ROLE OF BCL-2 IN THE OPTIC NERVE REGENERATION INDUCED BY PERIPHERAL NERVE GRAFTS**

K. Wolwender[1], J. Lewin-Kowalik[2], M. Larysz-Brysz[2], Z. Fus[1]
Neurology Department[1], Physiology Department[2],Medical University of Silesia, Medyków Street, Katowice, Poland

Predegenerated peripheral nerve grafts are known to exert strong neurotrophic effect on injured central nervous system, but the mechanism of their activity remains to be elucidated. Our previous studies revealed that predegenerated peripheral nerve grafts prevent retinal ganglion cells (RGC) from axotomy-induced apoptosis and promote the outgrowth of their axons.

Here we report the examination of the possible role of anti-apoptotic gene bcl-2 in these processes. The experiments were carried out on bcl-2 deficient and wild-type mice, according to the Polish animal protection laws and the European Union directives. The predegenerated and non-predegenerated peripheral nerves were transplanted into the transected optic nerve of both types of mice. We have also studied the neurotrophic effect of bcl-2 deficient graft on wild-type optic nerve and the wild-type graft on bcl-2 deficient optic nerve. We assessed the number of surviving RGCs which had sprouted axons into the graft by means of fluorescent dyes: DiI and rhodamine-B. Non-predegenerated and predegenerated bcl-2 deficient grafts did not induce regeneration in the optic nerves. Contrary, predegenerated wild-type grafts promoted survival and outgrowth of RGCs axons in both types of mice.

Our results indicate that bcl-2 gene is essential for the neurotrophic activity of predegenerated peripheral nerve grafts. Moreover, the regenerative influence of such grafts seems to be independent from the bcl-2 expression in the injured central nervous system.

17.28 **EFFECT OF AMYLOID BETA PEPTIDES ON DNA DEGRADATION.**

Agata Zambrzycka, Grzegorz Czapski and Joanna B. Strosznajder
Dept. of Cellular Signalling, Medical Research Centre, Polish Academy of Sciences, 5 Pawińskiego St., 02-106 Warsaw e-mail: joannas@cmdik.pan.pl

There are suggestions that Aβ peptides are responsible for apoptotic cell death in Alzheimer's disease. The last data indicated that induction of apoptotic factor(s) locally in synapses might play important role in nuclear fragmentation. The aim of our study was to investigate the effect of Aβ peptides on induction of apoptotic cytosolic factor(s) in brain synaptosomes and to determine molecular processes involved in Aβ evoked DNA fragmentation. The studies were carried out using synaptosomal fraction from brain cortex. This fraction was incubated for 4 h with $A\beta_{25-35}$ at 25μM. Then synaptosomal cytosol was obtained and incubated with nuclear fraction for 30-120 min. DNA integrity was evaluated by agarose electrophoresis. Protein oxidation was measured using specific fluorescence probe and immunochemical detection. The free radicals' were determined by dichlorofluoresceine and thiobarbituric acid. The data indicated that cytosolic apoptotic and oxidative factor(s) are liberated during incubation of synaptosomes. These compounds are responsible for DNA degradation and dityrosine formation. Aβ peptides in early phase of incubation activate the synaptosomal cytosolic factor(s) that induced DNA fragmentation. However, Aβ itself incubated with nuclear fraction without cytosolic factor(s) induces DNA degradation probably by free radicals and alteration of Ca^{2+} concentration. The identification of synaptosomes apoptotic and oxidative factor(s) and the protective action of several antioxidants are under investigation.

17.29 **REGULATION OF CYTOKINE GENE EXPRESSION IN GLIAL CELLS AFTER IMMUNOSUPPRESSANTS TREATMENT.**

M. Zawadzka, B. Pyrzynska, B. Kaminska
Nencki Institute of Experimental Biology, Warsaw, Poland

Neurotrophins and cytokines, with both cytotoxic and neuroprotective activities, expressed in astrocytes during glia activation play a crucial role in the process of delayed neuronal death.

We demonstrated that the cyclosporin A (CsA) and tacrolimus (FK506), a widely used immunosuppressants, affect glial cells growth probably by inhibiting signalling pathways that regulate hypertrophic and/or proliferative responses.

The aim of this study was to determine the pattern of growth factors and cytokine expression and effect of immunosuppressants on glial cell function. FK506 inhibited proliferation of primary astrocytes and reactive astrocytes from striatal trauma and induced death accompanied by apoptotic changes in nuclear morphology and DNA fragmentation.

Using reverse transcription polymerase chain reaction (RT-PCR), we have studied the expression of mRNA coding for various growth factors and cytokines: bFGF, BDNF, CNTF, PDGF, LIF, Fas ligand, TGFβ, and TNFα in cultured reactive astrocytes, primary glial cultures and C6 glioma cells. Comparing the pattern of neurotrophins and cytokines expression in three cell types investigated, we found more similarities between C6 glioma cells and cultured primary astrocytes, particularly in level of growth factors' expression. It suggests that transformed glia cells retain most of the properties of the primary cells. Our RT-PCR findings show also similarity in pattern of cytokine expression in primary and reactive mature astrocytes.

Working hypothesis that immunosuppressant FK506 can modulate astroglial proliferation, hypertrophy and cytokine production is adressed *in vitro*. The influence of FK506 on cytokine expression pattern in primary astrocytes could be a model for investigation the role of FK506 as a modulator of reactive astrocyte responses after brain injury.

17.30 **MECHANISM OF AMMONIA-INDUCED TAURINE ACCUMULATION IN THE RAT STRIATUM IN VIVO: ROLE OF ACTIVATION OF GLUTAMATE RECEPTORS AND ION CHANNELS.**

M. Zielińska[1], W. Hilgier[1], H.D. Borkowska[1], S.S. Oja[2], P. Saransaari[2], J. Albrecht[1]
[1]Dept. of Neurotoxicology, Medical Research Centre, Pol. Acad. of Sci., Warsaw, Poland, [2]Tampere Brain Res. Center, University of Tampere, Tampere, Finland,

In vitro studies have shown that ammonia causes a massive release of endogenous taurine (Tau) from cultured CNS cells and brain slices (*cf. Zielińska et al., Neuroscience, 1999, 91, 631*). In this study, the effect of direct application of ammonia to the rat striatum *in vivo* on the extracellular accumulation of Tau was measured by means of the microdialysis technique. Infusion of 60 mM ammonium chloride („ammonia") to the microdialysis tube rendering the actual extracellular ammonia concentration of ~5 mM, increased the microdialysate Tau content by >2-fold. The stimulatory effect of ammonia was substantially attenuated upon addition to the microdialysis of NMDA or KA/AMPA receptor antagonists, dizocilpine and DNQX, respectively, whereas a noncompetitive Glu transport inhibitor-PDC potentiated the ammonia-induced, but not basal Tau accumulation. The effect of ammonia on the extracellular Tau content was not significantly affected by a $Na^+/K^+/Cl^-$ transport inhibitor, furosemide. An anion channel inhibitor, DIDS, increased basal Tau content in the microdialysates and only slightly supressed the stimulatory effect of ammonia. The results indicate that ammonia-induced accumulation of extracellular Tau is mainly due to its release from neurons and/or astrocytes, which is evoked by stimulation of NMDA and KA/AMPA glutamate receptors, with a relatively minor involvement of activation of osmosensitive ion channels.

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17.31 **RYANODINE RECEPTORS PARTICIPATE IN THE INDUCTION OF MITOCHONDRIAL PERMEABILITY TRANSITION AND EXCITOTOXIC DAMAGE OF CULTURED RAT CEREBELLAR GRANULE CELLS**

E.Ziemińska[1], W.Gordon-Krajcer[1], E.Matyja[2] and J.W. Łazarewicz[1]
Departments of Neurochemistry[1] and Neuropatology[2] Medical Research Centre P.A.S., Warsaw, Poland

Disturbances of intracellular Ca^{2+} homeostasis play a key role in the mechanisms of excitotoxic neuronal damage. Ca^{2+}-induced release of Ca^{2+} ions (CICR) from the intracellular stores in the endoplasmic reticulum (ER) via ryanodine receptors (RyR) participates in generation of pathological intracellular Ca^{2+} signal. On the other hand, mitochondrial Ca^{2+} overload may induce their permeability transition (MPT), swelling and release of proapoptotic cytochrome c. The aim of this study was to investigate relations between these two putative mechanisms of excitotoxic neurodegeneration, utilising as pharmacological tools RyR antagonist dantrolene (30 μM) and MPT blocker 0.5 μM cyclosporin A (CsA). Cultured rat cerebellar granule cells (CGC) were exposed for 30 min to 0.1 – 1 mM glutamate. Ultrastructural changes of mitochondria and cytochrome c release were tested immediately after incubation with glutamate, whereas neuronal death was evaluated 24 h later, based on LDH release. The results of this study demonstrate that dantrolene and CsA partially prevent glutamate evoked ultrastructural changes of mitochondria, cytochrome c release and CGC neurodegeneration, CsA being however more efficient than dantrolene. These results indicate that ryanodine receptors, mediating glutamate-evoked excessive CICR may participate in the induction of MPT, and this way may potentiate the excitotoxic neuronal damage.

This study was supported by the KBN Project 4.PO5A.031.17

Session 18 - Poster Session: Autonomic nervous system

18.1 **CHANGES OF ADRENAL MEDULLA CATECHOLAMINE (CA) CONTENT IN FASTED RATS**

B. Beszczyńska, A. Siejka, E. Wasilewska
Department of Animal Physiology, N. Copernicus University, Toruń, Poland

It is well known, that fasting significantly decreases sympathetic nerves activity and thereby, it is interesting, whether this decreasing is accompanied by changes in adrenal medulla activity, both in resting and stress conditions. Our experiments were carried out on albino Wistar rats divided into three groups: A – rats fed on standard laboratory chow, B – rats fasted 24h and C – rats fasted 72h. Each group was further divided into four subgroups: I – non-stressed control rats, II – rats immobilized at normal temperature (22°C), III – rats exposed to cold (-5°C) and IV – rats immobilized at cold. For the determination of adrenal CA content fluorimetric method was used. The statistical difference determined by Student's t-test was considered significant by $p<0.05$. Short fasting in non-stressed rats significantly ($p<0.02$) elevated adrenal noradrenaline (NA) level, but didn't change adrenaline (A) content. On the contrary, after prolonged fasting NA level remained unchanged, but A concentration was significantly ($p<0.01$) decreased. Stress exposure in normal fed rats significantly elevated adrenal NA content in all subgroups ($p<0.001$), but didn't influence A concentration. In short fasted animals we found significant decrease both of NA ($p<0.01$) and A ($p<0.001$) content only after immobilization at normal temperature, other kinds of stress didn't influence adrenal CA level. After prolonged fasting NA level was significantly diminished in rats immobilized at both normal ($p<0.01$) and low ($p<0.02$) temperature. Moreover, immobilization at cold significantly ($p<0.01$) decreased adrenal A level. These results suggest that short fasting can increase adrenal medulla activity, but such a reaction after prolonged fasting was not observed.

18.2 **IMMUNOHISTOCHEMICAL CHARACTERISTICS OF LEPTIN-RECEPTOR-CONTAINING NEURONS SUPPLYING THE SUBCUTANEOUS ADIPOSE TISSUE IN THE PIG**

K. Czaja[1], Z. Pidsudko[1], R.R. Kraeling[2], M. Łakomy[1]

[1]Department of Animal Anatomy, Warmia and Mazury University, Olsztyn, Poland

[2]Animal Physiology Research Unit, R.B. Russell Agricultural Research Center, Athens, USA

It is known that sympathetic neurons regulate the lipolytic activity of mammalian adipose tissue. Moreover, it have been found that leptin receptors (Ob-R) are expressed in sympathetic prevertebral ganglion neurons of mouse and rat. However there is no data dealing with the immunohistochemical characteristics of neurons projecting to the subcutaneous adipose tissue and containing Ob-R. The experiment was performed on 6 pigs (50kg b.w.). During operation the neuronal retrograde tracer Fast Blue (FB, 150μl) was injected into subcutaneous adipose tissue. After a survival period of 3 weeks sympathetic chain ganglia were removed from each animal and cut into 10 μm.-thick cryostat sections and next processed for double-labelling immunofluorescence with primary antisera against Ob-R, tyrosine hydroxylase (TH) and neuropeptide Y (NPY). Immunohistochemistry combined with tracing revealed that the vast majority of leptin-receptor-containing neurons supplying the subcutaneous adipose tissue in the pig were noradrenergic (TH-positive). Many $FB^+/Ob.\text{-}R^+$ neurons contained also NPY. These results raise the possibility that leptin may affect lipolytic activity by acting on leptin receptors located in sympathetic chain ganglia neurons.

Supported by grant ECCRA 2/99

18.3 **DISTRIBUTION AND CHEMICAL CODING OF NEURONS IN THE PREVERTEBRAL GANGLIA SUPPLYING THE URINARY BLADDER TRIGONE IN THE PIG**

Pidsudko Z., Kaleczyc J., Czaja K., Łakomy M. and Majewski M*

*Dept. of Animal Anatomy and *Dept. of Clinical. Physiology, Faculty of Veterinary Medicine, University of Warmia and Mazury in Olsztyn, 10-957 Olsztyn, PL*

Combined retrograde tracing and double-labelling immunofluorescence were used to investigate the distribution and chemical coding of neurons in prevertebral ganglia supplying the urinary bladder trigone (UBT) in the pig. The study was performed on 5 juvenile pigs (10 kg. of body weight) of the Large White Polish race. Retrograde fluorescent tracer Fast Blue (FB; total volume of 50 µl) was injected into the wall of both the left and the right side of the UBT during laparatomy performed under pentobarbital anesthesia. After a survival period of 3 weeks the animals were reanaesthetized and transcardially perfused with 4% buffered paraformaldehyde (pH 7.4). Collected prevertebral ganglia were cut into 10 µm-thick cryostat serial sections. Retrograde tracing revealed many urinary bladder trigone-projecting neurons (UBT-PN) that were localised mainly in the coeliac and superior mesenteric ganglion complex (C-SMG), as well as in the ovarian- (OG), aortico-renal- (ARG) and adrenal- (ADG) ganglion. Immunohistochemistry disclosed that the vast majority of UBT-PN neurons were noradrenergic (TH-positive). Many noradrenergic neurons contained NPY or, less frequently, SOM and/or GAL. This study has revealed a relatively large population of differently coded UBT-PN. As judged from their somatotopic and neurochemical organization these cells are probably involved in the complexity of the neural pathways.

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Session 19 – Plenary Lecture

19.1 **NEUROPLASTICITY AND CELLULAR RESILIENCE IN MOOD DISORDER**

H. Manji

Bethesda, USA

Mood diorders have traditionally been conceptualized as neurochemical disorders, but there is now evidence from a variety of sources demonstrating regional reductions in CNS volume, as well as reductions in the numbers and/or sizes of glia and neurons in discrete brain areas. It is thus noteworthy that lithium and valproate (VPA) have recently been demonstrated to robustly increase the expression of the cytoprotective protein bcl-2 in the CNS *in vivo*, and in cells of human neuronal origin. Consistent with these effects, lithium exerts marked neuroprotective effects in a variety of preclinical paradigms. VPA also robustly activates the ERK MAP kinase pathway, a signaling pathway utilized by *many endogenous neurotrophic factors*. Accompanying the activation of the ERK pathway, VPA also robustly promotes neurite growth. To determine if lithium also exerts neurotrophic effects *in the human brain in vivo*, brain tissue volumes have been examined using high resolution three dimensional MRI and validated quantitative brain tissue segmentation methodology. This study revealed an extraordinary finding that chronic lithium significantly increases *total gray matter content* in the human brain of patients with BD. Together with the recent morphometric studies demonstrating cell loss and atrophy in BD, these results suggest that a reconceptualization about the pathogenesis of BD may be warranted. Bipolar disorders may arise, at least in part, from impairments of neuroplasticity and cellular resilience. The future development of treatments which more directly target molecules involved in critical CNS plasticity and survival pathways thus hold promise as novel, improved long term treatments for this devastating illness.

Session 20 – Parallel Symposium: Plasticity of the cerebral cortex

20.1 MODULATION OF HUMAN CORTICAL EXCITABILITY BY PAIRED ASSOCIATIVE STIMULATION - A MODEL OF LONG-TERM POTENTIATION (LTP)?

J. Classen
Human Cortical Physiology Laboratory, Dep. of Neurology, University of Rostock, Germany

Low-frequency median nerve stimulation if paired with transcranial magnetic stimulation (TMS) over the cortical representation of the abductor pollicis brevis muscle (APB) leads to changes of excitability. The plasticity induced by this interventional paired associative stimulation (IPAS) protocol is located cortically, evolves rapidly, is persistent, and yet reversible. It is topographically specific, does not involve changes of cortical $GABA_A$-receptor dependent inhibition and depends on the activation of NMDA-receptors. Intracortical inhibition following afferent stimulation is temporarily disinhibited by the afferent pulse. This combination of features closely resembles features of associative LTP of cortical synapses as elucidated in animal experiments. A part of IPAS-induced increase of excitability may result from recruiting cortical neurones outside the original representational boundaries. IPAS-induced plasticity depends on the sustained attention of the subject. Preliminary results showed that cortical excitability may also be reduced by IPAS, by choosing appropriate interstimulus intervals suggesting long-term-depression-like phenomena. Excitability was also shown to be modifiable in the somatosensory cortex. Stimulation-induced changes of cortical excitability may serve as in-vivo models of cortical plasticity. IPAS may also be a tool for future therapeutic manipulation of cortical excitability. Supported by DFG grant Cl 95/3-1.

20.2 SYNAPTIC PLASTICITY IN RAT MOTOR CORTEX

Grzegorz Hess
Institute of Zoology, Jagiellonian University, Krakow, Poland

Activity-dependent modifications of synaptic efficacy are proposed to form mechanisms for experience-dependent regulation of adult cortical representations. Intrinsic horizontal pathways of the primary motor area (MI) are capable of both long-term potentiation (LTP) and long-term depression (LTD). LTP was induced in horizontal connections within layer II/III using the *in vitro* slice preparation by high-frequency stimulation when local synaptic inhibition was transiently suppressed. Alternatively, LTP could be evoked by conjoint activation of horizontally and vertically oriented inputs. Long-lasting increases of synaptic efficacy could also be induced in horizontal connections by transient exposure to increased $[Ca^{2+}]$ concentration or to a potassium channel blocker, tetraethylammonium. These data indicate that synaptic modification in motor cortex is regulated both by the arrangement of intrinsic circuitry and by the availability of mechanisms for modification at individual synapses. Superficial and deep cortical layers exhibit a significant potential for long-lasting potentiation of synaptic transmission within intralaminar connections.

20.3 GLUTAMATERGIC AND GABA-ERGIC INVOLVEMENT IN LEARNING-DEPEDENT PLASTICITY OF SENSORY CORTEX.

M.Kossut
Dept. of Neurophysiology, Nencki Institute, Warsaw.

Neuronal properties of adult sensory cortices can undergo plastic changes as a result of sensory conditioning. We have described changes of cortical body maps occurring in the first somatosensory representation (the barrel field) in the cortex of rodents after classical conditioning involving stimulation of facial vibrissae. We have also found that this form of neuroplasticity depends on full activation of cortical NMDA receptors and that it is accompanied by a transient increase of binding to NMDA and AMPA receptors and by increased density of GABA-IR cells. The present study examined if mechanisms of plasticity of cortical body maps include changes of expression of mRNA for several elements of glutamatergic and GABA-ergic system. We used in situ hybridization with ^{35}S-labelled oligonucleotide antisense probes for NR1, NR2A, NR2B, GluR1 GluR2, GAD67 and GABARalpha1.GAD67 and GAD65 immunohistochemistry was also performed. The mice were trained in a classical conditioning task in which stimulation of a row of mystacial vibrissae on one side of the snout was paired with a tail shock. The effects were examined on brain sections after 3 daily training sessions. We found no changes in expression of NMDA receptor subunits and GluR1 mRNA, but GluR2 m RNA was significantly enhanced. Sensory training significantly increased the expresison of GAD67 mRNA and density of GAD67-IR neurons in the representation of the trained row of vibrissae. GABA receptor was also affected by the training–GABARalpha1 mRNA expression increased, although with longer latency than GAD67. In both major neurotransmission systems we found elments that were specifically affected by learning-dependent plastic modification of the cortex.

20.4 BRAIN PLASTICITY AFTER TRAUMATIC INJURY – A CASE STUDY

Joanna Seniów
The Institute of Psychiatry and Neurology, Warsaw

We would like to present the case of a young man suffering from traumatic brain injury. The patient survived a shot of the head. The bullet went horizontally through both hemispheres from the left to the right occipital lobe. The sequelae of such focal lesion were: severe associative visual agnosia, visuospatial and visuoconstructive disorders. These deficits dezorganized patient's cognitive functioning completely.

The patient was attending a neuropsychological rehabilitation programme at the Clinical Neuropsychological Unit of the Institute for over a year. Significant improvement of this cognitive functioning has been observed.

Session 21 – Parallel Symposium: Biological aspects of major psychoses

21.1 HUMAN EQUALITY AND DIVERSITY: MOLECULAR GENETIC INSIGHTS

RH Belmaker, MD
Div of Psychiatry, Ben Gurion Univ, Beersheva, Israel

The human genome contains over three billion base pairs on the verge of total sequencing. However, about one base pair in 1000 is polymorphic in normal human populations, forming the basis for the new "Human Genome Diversity Project".There are thus about 3 million single nucleotide polymophisms (SNP's). How different are these SNP's in different human populations and what can this tell us about human origins? Molecular genetic data do not fit the classical division of humanity into races based on skin color. Australian aborigines, for instance, are closely related genetically to Southeast Asian peoples from whence they derived about 40,000 years ago. American natives are closely related to Northeast Asians from whence they derived 15,00-30,00 years ago. However, human genetic variance is very small compared to that for most species. For instance, the amount of genetic variance in a small group of West African chimpanzees is several times greater than the total genetic variance in the population of six billion human beings. The most distantly related humans are closer genetically than chimpanzee brothers from the same troop and parents. This reflects the fact that our species is only about 100,000 years old. Thus the human species has diversity that can be tracked molecularly; population differences in diversity are small and human groups are amazingly equal in their genetic endowment. The implications of this for neuroscience research are discussed.

21.2 **NEONATAL HIPPOCAMPAL DAMAGE DISRUPTS PREFRONTAL CORTICAL FUNCTION IN THE RAT: IMPLICATIONS FOR SCHIZOPHRENIA**

Barbara K. Lipska
NIMH, CBDB, Bethesda, MD 20892, USA

We studied in the rat the effects of disrupting development of the hippocampus, a brain area consistently implicated in human schizophrenia. Excitotoxic lesions that involve regions of the hippocampus directly projecting to the prefrontal cortex, lead in early adulthood to the emergence of abnormalities in dopamine related behaviors, enhanced sensitivity to glutamate antagonists, deficits in sensorimotor gating and latent inhibition, impaired social behaviors and working memory problems. Emergence of the behavioral changes in adolescence is not related to the surge of gonadal hormones during puberty, suggesting that aberrant development of the prefrontal cortex in the context of early damage to the hippocampus may be a critical factor in the onset of the syndrome. Although the exact mechanisms of "dysconnection" and malfunction of the prefrontal cortex in the VH lesioned rats need to be elucidated, molecular and electrophysiological findings (e.g., reduced cortical levels of N-acetylaspartate, attenuated stress-induced cortical dopamine release, attenuated cortical expression of a membrane glutamate transporter EAAC1 and of a synthetic enzyme for GABA, glutamate decarboxylase-67 (GAD67), reduced BDNF expression, altered cortical expression of transcription factors, c-fos and ΔfosB, as well as altered firing pattern of cortical pyramidal neurons in response to VTA stimulation) suggest that aberrant cortical dopamine/glutamate/GABA interactions may underlie cortical dysfunction in the neonatally VH lesioned rats. Neonatal damage to the hippocampus of the rat appears to reproduce a broad spectrum of schizophrenia related phenomena.

21.3 CROSS-TALK BETWEEN α_1- AND β - ADRENOCEPTOR SYSTEMS: ROLE IN AFFECTIVE DISORDERS AND IN THE MECHANISM OF ANTIDEPRESSANT DRUGS ACTION

I. Nalepa
Department of Biochemistry, Institute of Pharmacology, Polish Academy of Sciences, Krakow, Poland

Pathologies of brain noradrenergic system may be involved in several psychiatric conditions, including affective disorders. This possibility is implicated by the results of postmortem studies that indicate the disturbances in the α-adrenoceptor (α-AR) and α_1-adrenoceptor (α_1-AR)-associated signaling cascades in bipolar subjects, and by the fact that chronic antidepressant treatment causes adaptive changes in noradrenergic system. Regarding the mechanisms of responses to chronic antidepressant drugs (AD), our previous studies done in the rat brain, showed the involvement of protein kinase C (PKC) in the mechanism of action of AD and electroconvulsive shock (ECS). Thus, antidepressant treatments inhibit the negative feedback between PKC and α_1-ARs and modulate the PKC-induced potentiation of responsiveness of β-AR. Those results made us to conclude that enhancement of noradrenergic transmission is the final effect of long-term administration of antidepressant agents. Recently we have found that AD and ECS increase the expression one of the α_1-AR subtypes, namely of α_{1A}, and this effect depends on noradrenaline level in the brain. As cyclic AMP (cAMP) was found to enhance the α_{1A}-AR gene expression, the function of α_{1A}-AR may be cross-regulated by receptors that stimulate cAMP formation. Thus, we propose that the antidepressant-induced increase in α_{1A}-AR mRNA expression might be related to the upregulation of the cAMP response-element binding protein (CREB) which was shown to be induced by AD and ECS. *Supported by PAN /NIH-97-312*

21.4 Serotonin 5-HT1A receptors as a potential target of novel antipsychotic drugs.

Krzysztof Wędzony and Marzena Maćkowiak, Institute of Pharmacology, PAN, 12 Smętna street, 31-343 Kraków.

In recent years there is increasing attention directed toward the role of the serotonergic neurotransmission in the neurochemistry of schizophrenia and the mechanism of action of antipsychotic drugs. Apart of 5HT2A serotonin receptors, which role is well documented in both aspects of schizophrenia research, there is growing evidence that 5HT1A receptors may be also responsible for certain cognitive deficits of that illness. For the first, in patients with schizophrenia, the majority of post-mortem studies have reviled increases in 5-HT1A receptor density in the prefrontal cortex and hippocampus. For the second, some clinically effective, or novel potential antipsychotic drugs have a considerable affinity toward 5HT1A receptors. Although the pathophysiological significance and therapeutic role of all above findings are unclear so far, data about location of 5HT1A receptors on pyramidal cells of prefrontal cortex, may reflect their potential role in restoration of abnormal glutamatergic tone observed in the course of schizophrenia. In the present lecture, evidences will be given that in pharmacological models of schizophrenia based on administrations of non-competitive antagonists of NMDA receptors, 5-HT1A receptors are regulated in the fashion similar to that observed in schizophrenic patients and that antagonist of 5-HT1A receptors WAY 100135 is capable of modifying the psychotomimentic effects of MK-801, a non-competitive antagonist of NMDA receptors. MK-801 is used here to model certain cognitive deficits of schizophrenia like impairment of sensorimotor gating and damage of working memory.

Session 22 - Parallel Symposium: Brain aging

22.1

AMYLOID β PEPTIDES INDUCE PHOSPHATIDYLCHOLINE HYDROLYSIS IN IMMORTALIZED RAT BRAIN ENDOTHELIAL CELLS AND BOVINE RETINA PERICYTES

Mario Alberghina
Department of Biochemistry, Faculty of Medicine, University of Catania, viale Andrea Doria 6, 95125 Catania, Italy
Email: malber@mbox.unict.it

We describe the inhibitory effect of Aβ 25-35 and bradykinin (BK) on phosphatidylcholine (PtdCho) metabolism in immortalized rat brain GP8.39 endothelial cells (EC) and the inhibitory effect of Aβ(1-42) and Aβ(25-35) on PtdCho metabolism in bovine retina capillary pericytes. In both cell cultures, peroxidation indices (MDA, CD and LDH release) significantly increased after Aβ (10-50 μM) treatment for 24 h. With 50 μM Aβ peptides, the [Me-^{3}H] choline incorporation into PtdCho strongly decreased while either ^{3}H-choline or ^{14}C-arachidonic acid release from prelabeled cells increased, indicating PtdCho hydrolysis. Reversed-sequence Aβ(35-25) peptide did not depress ^{3}H-choline incorporation nor stimulate PtdCho breakdown. BK strongly decreased [Me-^{3}H]choline incorporation into EC PtdCho, but did not stimulate significant ^{3}H-choline release. The action of 50 μM Aβ(25-35) on ^{3}H-choline release was not enhanced by 10 μM BK. With addition of Aβs at low concentrations (2-20 μM) to pericytes, marked ultrastructural changes, well connected to metabolic alterations. Cells treated with higher concentrations (50-200 μM) displayed characteristics of necrotic cell death. The data suggest that: a) Aβ(1-42) and Aβ(25-35) peptides may modulate phospholipid turnover in microvessel ECs and pericytes; b) Aβ and BK could not synergistically interact in vascular EC damage during processes involving amyloid accumulation and inflammatory response.

22.2

ALZHEIMER'S DISEASE – BRAIN AMYLOIDOSIS

Maria Barcikowska
Department of Neurodegenerative Diseases, Medical Research Centre, Warsaw, Pawinski 5

Alzheimer's disease can be defined as a syndrome that arises from chronic imbalance between beta-amyloid (Aβ) production and Aβ clearance. Genetic, epidemiologic studies have indicated that ~40% of the population variance in risk for Alzheimer's disease (AD) is attributable to genetic factors. Molecular genetic studies , focussing on pedigrees with autosomal dominant inheritance, have identified four genes which confer susceptibility to AD: presenilin 1 (PS 1), presenilin 2 (PS 2), β–amyloid precursor protein (βAPP) , and apolipoprotein E (apo E). Aβ is released from β APP by the sequential action of two protease, β–secretase and γ-secretase. Recently BACE 1 (a novel aspartic protease with all the known properties of the β–secretase) has been cloned . γ-secretase appears to be identical with the two presenilin proteins, PS 1 and PS 2 or/and play critical role in the γ-secretase processing of β APP. It is understandable that, β–secretase and γ-secretase are prime targets for therapeutic intervention.

One of the fundamental question in the development of AD concerns of molecular mechanism underlying aggregation of Aβ. Accumulating evidence indicates that processing of βAPP , including the generation of Aβ is modulatedby cellular cholesterol. Induction of apo E expression by cholesterol lowering drugs may be viewed as a potent therapeutic target for disease stabilization in Alzheimer's disease

22.3

THE ALZHEIMER'S AB PEPTIDE EVOKED OXIDATIVE AND METABOLIC STRESS IN RAT HIPPOCAMPAL AND CORTICAL NEURONS

Joanna B. Strosznajder
Dept. of Cellular Signalling, Medical Research Centre, Polish Academy of Sciences, 5 Pawińskiego St., 02-106 Warsaw e-mail: joannas@cmdik.pan.pl

Our previous data indicated that Aβ peptides (Aβ) are responsible for the alteration of phosphoinositides metabolism and calcium signaling. These changes might subsequently play important role in Aβ peptide generation from β amyloid precursor protein. The aim of this study was to determine the effect of Aβ on protein level and activity of different isoforms of phospholipase C (PLC) degradating phosphoinositides as well as to explain the mechanism of Aβ evoked inhibition of PLC. Moreover, the molecular events evoked by Aβ leading to DNA damage and apoptosis were investigated. Our new data indicate that Aβ enhances exclusively the protein level of PLCδ with no effect on PLCβ and γ. Concomitantly Aβ decreases Ca^{2+} dependent PLC activity probably by free radicals evoked alteration of Ca^{2+} binding site. Aggregated Aβ peptides $Aβ_{1-40}$ and its neurotoxic fragment $Aβ_{25-35}$ at 25μM concentration activate free radicals dependent lipid peroxidation processes, DNA damage and activation of DNA-bound, poli(ADP-ribose) polymerase (PARP) activity in hippocampus. The antagonist of NMDA receptor, MK-801 and N-nitro-L-arginine inhibitor of both constitutive isoforms of nitric oxide synthase decrease Aβ evoked stimulation of PARP by about 50%. These results indicate that Aβ through NO and other free radicals, induces DNA damage and PARP activation that may lead to NAD and ATP depletion and cell death.

22.4

DEATH IN NEURONAL CELLS EXPOSED TO Fe^{+2} AND β-AMYLOID(1-40) IS MEDIATED VIA A MAP KINASE NUCLEAR TRANSLOCATION

Ephraim Yavin
Department of Neurobiology, The Weizmann Institute, Rehovot, IL.

Neurodegeneration and cell death in Alzheimer disease (AD) may result from damaging reactive oxygen species, a fraction of which may arise from the interaction of β-amyloid (Aβ) protein with Fe^{2+}. The signaling cascades concordant with this possible scenario have been studied in primary rat neuronal cultures. Co-addition of A $β_{1-40}$ and Fe^{2+} (5 μM each) caused a rapid and sustained elevation of free radicals (FR) as detected by dichlorofluorescein staining and a 6 fold increase in lipid peroxides (LPO) as measured by thiobarbituric acid. Unlike $Aβ_{1-40}$, Fe^{2+} addition enhanced FR formation after 30 min, while a combination of both was effective after 5 min. Desferoxamine (DFX), at 25 μM blocked FR formation. Impaired mitochondria activity measured by MTT reduction assay, caspase 3 activation and TUNEL stain, all indicative of cell death were found. The extracellular signal-regulated kinases (ERK, 42 kDa and 44 kDa isoforms) were increased by 30 min after either $Aβ_{1-40}$ or Fe^{2+} addition. Activation of ERKs by $Aβ_{1-40}$ was associated with a rapid nuclear translocation which was blocked by the addition of Fe^{2+}. A combination of Fe^{2+}/$Aβ_{1-40}$ caused a delay of about 12 h in ERKs activation in contrast to a p38 kinase activation noted after 30 min. Addition of DFX prevented the above effects. The kinetics of ERK activation and nuclear translocation via $Aβ_{1-40}$ / Fe^{2+}-induced toxicity may constitute an important element in AD pathophysiology.

Session 23 - Parallel Symposium: Biological rhythms

23.1 THE POSSIBLE FUNCTION AND MECHANISM OF CIRCADIAN RHYTHMS IN THE FLY'S VISUAL SYSTEM

Elżbieta Pyza
Institute of Zoology, Jagiellonian University, 30-060 Kraków, Poland

In the visual system of the flies *Musca domestica, Drosophila melanogaster* and *Calliphora vicina*, L1 and L2, two classes of monopolar cell in the first optic neuropile, or lamina, show rhythmic changes in size and shape which are circadian. These first-order interneurons receive input at afferent tetrad synapses from photoreceptor terminals R1-R6 in the compound eye. In turn, L2's lamina axon forms feedback synapses back upon the R1-R6 terminals. The numbers of both classes of synaptic profile in *Musca* change during day and night. Tetrad profiles are more numerous in the day and seem to be regulated by direct exposure to light. The day/ night difference in profile number is blocked after injecting cytochalasin D, which disrupts actin microfilaments. For L2, the size of its lamina axon and the number of feedback synaptic profiles are both regulated by the circadian clock. Although neither mechanism nor function of these structural changes is known, larger sizes in L1 and L2 correlate with higher levels in locomotor activity. L1 and L2's rhythms disappear: a) when metabolism of the lamina's epithelial glial cells are disrupted by injected gliotoxins; b) by blocking a proton pump V-ATPase by injecting bafilomycin. Both treatments are effective only at night in preventing L1/L2's night-time shrinkage, suggesting involvement of both glia and ionic concentration changes. Night-time injection of colchicine produces similar results, suggesting that shrinkage also depends on the integrity of the microtubules. Daytime injections lack effect on the swelling in L1 and L2. Thus it appears that the cytoskeleton is involved in these changes, that actin microfilaments are involved in regulating tetrad number, whereas microtubules participate in regulating changes in cell size.

23.2 MOLECULAR GENETICS OF CIRCADIAN RHYTHMS IN FLIES, MICE AND HUMANS

Michael Rosbash, Patrick Emery, Carolyn Kotarski, Daniel Lee, Mike McDonald, Pipat Nawathean, Ying Peng, Jie Zhao. Howard Hughes
Medical Institute, Department of Biology, Brandeis University, Waltham, MA 02254 and NSF, Center for Biological Timing.

Genetic and molecular genetic analyses of the *Drosophila* circadian system originally identified the *period* protein as a clock molecule that contributes to circadian pacemaker function. The gene shows robust circadian rhythms of transcription, mRNA and protein expression, with strong evidence in favor of autoregulation. This raised the hypothesis that transcription and macromolecular metabolism, a transcription-translation feedback loop, is central to the mechanism(s) that generates circadian rhythms. This model is still viable, and transcriptional regulation plays a prominent role in contemporary circadian studies. Subsequent work identified at least seven new *Drosophila* clock genes: *tim, Clock, cycle, doubletime, cry, pdf*, and *vrille*. There is now a good picture of what these different gene products do and how they collaborate during a 24 hr cycle. Most if not all of these genes have mammalian orthologs, which function in a very similar manner. Therefore, the transcription-translation feedback loop hypothesis is also relevant to mammalian clocks. Moreover, a recent human sleep disorder family has been described, with a mutation in a human *period* gene protein. Despite this recent progress, there are a large number of questions that remain unanswered. Prominent among these are how 24 hr timing is achieved and why the apparent differences in *cryptochrome* (*cry*) function between flies and mammals? New features of these and other issues will be discussed, including a more global perspective on the circadian regulation of gene expression and some relevant aspects of neuronal function. An evolutionary perspective will also be discussed, in an attempt to understand the relationship between different circadian systems.

23.3 GENE EXPRESSION IN THE SCN REGION OF MICE

B. Rusak[1], J. Hogenesch[2], E. Marchant[1], Marleen de Groot[1], Donna Goguen[1], Yi Na Dong[1], Jennifer Villasenor[2] and S. Kay[2]
[1]Psychiatry and Psychology, Dalhousie University, Halifax, Nova Scotia, Canada; [2]Genomics Institute of the Novartis Research Foundation, San Diego, California, USA

Light is known to affect the expression of several immediate-early genes (IEGs), as well as identified clock genes in the mammalian suprachiasmatic nuclei (SCN). In addition, expression of several of these, and other, genes is known to oscillate spontaneously in SCN cells. In order to learn more about the range of genes that respond to light and circadian time with changes in expression, we used a high-density, oligonucleotide microarray screening method to assess expression of ~11,000 genes and expressed sequence tags in mice.

Punches (425 μm inner diameter) containing the bilateral SCN of C57Bl/6J mice were taken from the brains of mice exposed to light at one of three circadian phases or at the same time in total darkness. Levels of extracted mRNAs were compared between circadian phases and between light and no-light conditions using Affymetrix GeneChip microarrays. Several clusters of genes were identified as being regulated in parallel to each other. Among these were the familiar IEGs and some neuropeptides, which responded as had been reported previously. Novel findings included a number of structural genes, neuropeptide genes and others that were regulated by light exposure, varied with circadian phase, or both.

Subsequent confirmatory immunohistochemical studies revealed that some of these genes were expressed in SCN cells, while others were expressed in nearby structures, including the walls of the third ventricle. These results indicate novel mechanisms by which light may regulate brain function, including control of hormonal content of cerebrospinal fluid, which may have far-reaching effects on neural activity, behaviour and mood.

23.4 IS 5-METHOXYTRYPTOPHOL, LIKE MELATONIN, IMPLICATED IN THE INTEGRATION OF ENRIVONMENTAL INFORMATION : A COMPARATIVE APPROACH IN SPECIES LIVING IN DIFFERENT ENVIRONMENTS

Berthe Vivien-Roels
Neurobiologie des fonctions rythmiques et saisonnières, UMR CNRS-ULP 7518, Strasbourg, France

It is well-known that the pineal gland of vertebrates is implicated in a transduction of photoperiodic information via the daily pattern of melatonin (MEL) synthesis and secretion. In all species studied to date, MEL is synthesized during the night and the duration of the nocturnal MEL peak is proportional to the duration of the night. Accumulating evidence indicates that seasonal changes in the daily pattern of MEL synthesis are more complex than a simple change in the duration of the night peak, and that it might be affected by other environmental variables such as temperature, rainfall, humidity. Apart from MEL, other 5-methoxyindoles, especially 5-methoxytryptophol (5-ML), produced by the pineal gland can also exhibit a photoperiodic dependent synthesis. In species living in different environments, seasonal or experimental changes in environmental variables clearly affect the diurnal rhythms of MEL and 5-ML biosynthesis. Such changes may affect the duration, the amplitude or phase of the daily patterns of MEL or 5-ML. In some species (sheep, hamster), MEL and 5-ML rhythms are inversely correlated, in others (turtle, jerboa, arvicanthis), changes in environmental variables show a desynchronization between regulation of MEL and 5-ML, stressing the hypothesis that the vertebrate pineal gland could integrate several information about the state of environment via the daily pattern of different 5-methoxyindoles. Preliminary results obtained in snails support the hypothesis that 5-ML could be an informative molecule implicated in adaptative processes in invertebrates.

23.5 **REGULATION OF 5-METHOXYTRYPTOPHOL AND MELATONIN BIOSYNTHESIS IN AVIAN PINEAL GLAND**

Jolanta B. Zawilska

Department of Pharmacodynamics, Medical University of Łódź and Department of Biogenic Amines Polish Academy of Sciences, Łódź, Poland

Pineal glands of duck and hen rhythmically produces two 5-methoxyindole compounds, i.e. 5-methoxytryptophol (5-ML) and melatonin (MEL). These rhythms are circadian in nature and have opposite phases. 5-ML levels are low at night and high during the day, while MEL concentrations are high at night and low during the day. The MEL rhythm reflects oscillations in the activity of serotonin N-acetyltransferase (AA-NAT; a penultimate and key regulatory enzyme in MEL biosynthetic pathway). The activity of hydroxyindole-O-methyltransferase (HIOMT; an enzyme involved in the synthesis of both 5-ML and MEL) does not exhibit any significant rhythmic changes throughout the 24-h period. Acute exposure of birds to light at night significantly increased pineal 5-ML content, decreased AA-NAT activity and MEL concentrations, and did not affect HIOMT activity. 6 hr pulses of light applied during the early and late subjective night caused a delay and advance, respectively, in the phase of the circadian rhythms of 5-ML and MEL. Pharmacological modulation of pineal MEL synthesis produced opposite effects on 5-ML formation. Thus, inhibition of MEL biosynthesis by cycloheximide, revealed by a marked reduction in the nighttime AA-NAT activity and MEL concentrations, was accompanied by a significant increase in 5-ML content. In contrast, administration of aminophylline to light-exposed chicks significantly increased pineal AA-NAT activity and MEL levels and decreased 5-ML concentrations. It is suggested that in the duck and hen the pineal production of 5-ML and MEL is inversely correlated.

Session 24 – Plenary Lecture

24.1 **THE GENETIC BASIS OF NEURONAL PLASTICITY**

Colin Blakemore

University Laboratory of Physiology, Oxford OX1 3PT, UK

The cerebral cortex is organized into many functionally specialized areas, each with a distinctive arrangement of neuronal types, and of extrinsic and intrinsic connectivity. Not until after the innervation of the developing cortex by thalamic axons (postnatally in rodents) do these regional differences in cytoarchitectonics appear. The distribution of thalamic terminals, the effectiveness of the synapses that they form, the morphology and functional characteristics of their target cortical cells, and the local circuitry within the cortex all appear to be influenced by afferent *activity*. Recently Kind *et al.*[1] have described the transient expression in cortical neurons, of an intracellular organelle, the *botrysome*. Botrysomes are found only during early 'sensitive periods' when cortical neurons are known to be highly 'plastic'. They are characterized by a dense concentration of Phospholipase C-ß1, a phosphodiesterase that converts PIP2 to IP3 and DAG, which in turn stimulates production of the phosphorylating enzyme protein kinase C. PLC-ß1 in the botrysome may regulate protein transport, needed for dendritic modelling and synaptic modification. Measurement of IP3 production in synaptoneurosomes, in response to receptor agonists, suggests that during the crucial early postnatal period, PLC-ß1 is activated by G-proteins associated with metabotropic glutamate receptors and muscarinic acetylcholine receptors. Genetic analysis through gene knockout suggests that glutamate neurosecretion from thalamic fibres acting on mGluR5 receptors stimulates PLC-ß1, which regulates the selective growth of dendrites and hence the creation of local cytoarchitecture[2].

1. Kind, P *et al.* (1997) *J. Neurosci.* **17**: 1471-1480.
2. Hannan, AJ *et al.* (2001) *Nature Neurosci.* **4**, 282-288.

Session 25 – Workshop: Modern methods of neuroscience

25.1 **PROTECTIVE EFFECT OF GLIAL CELLS AGAINST LPS MEDIATED BLOOD-BRAIN BARRIER INJURY**

CECCHELLI R., L. DESCAMPS and G. TORPIER
Unité mixte Université d'Artois-Institut Pasteur de Lille, Faculté des Sciences de Lens, EA 2465, 62307 Lens, France.

Numerous infections of the central nervous system are characterized by altered blood-brain barrier functions leading to brain damage. To study the mechanisms leading to BBB disruption in such pathologies, we used an *in vitro* BBB model consisting of a coculture of brain capillary endothelial cells and glial cells. When endothelial cells from this coculture were submitted alone to lipopolysaccharides (LPS) added in the luminal compartment, a huge increase in the permeability of the monolayer was observed. As the microenvironment, i.e. the glial cells surrounding the brain capillaries, is of prime importance in specifying at least certain cellular properties, we investigated whether glial cells are able to modulate this endothelial cell response to LPS. When endothelial cells are incubated with LPS added luminally, but in the presence of glial cells, LPS surprisingly has no effect on the endothelial cell monolayer permeability, suggesting a protective effect of the glial cells on the LPS-mediated injury. This response depends on the route of administration of LPS, since incubations of endothelial cells and glial cells with LPS added in the abluminal compartment, result in a strong increase in the paracellular permeability, and this, independently of NO production by glial cells. As glial cells are a mixture of astrocytes, oligodendrocytes and microglial cells, further experiments performed with purified astrocytes revealed that microglial cells and/or oligodendrocytes are essential for the complete protection of the endothelial cell monolayer integrity in the case of a liminal activation of endothelial cells with LPS. All these results are direct evidence for a modulatory effect of glial cells on brain endothelial cell response in the pathogenesis of endotoxemia.

25.2 SIGNALING PATHWAYS AND APOPTOSIS – LESSON LEARNED FROM CULTURED CORTICAL NEURONS.

M.Hetman, M.Chłystun, J.Cavanaugh*, K.Kanning*, Z.Xia*.
*International Institute of Molecular and Cellular Biology UNESCO/PAN, Warsaw, Poland, *University of Washington, Seattle, USA*

Cultured rat cortical neurons are used as experimental system for various research applications. Here we present the usage of these cells to study the regulation of apoptosis by signaling kinases. Cultured cortical neurons were induced to die by either DNA damaging agents or by trophic deprivation. DNA damage mimics some aspects of neuronal death in pathologies. Trophic deprivation is a model of developmental apoptosis. Methods used to evaluate neuronal death included phase contrast microscopy, fluorescent microscopy with bisbenzimide staining, DNA fragmentation analysis and MTT viability assay. We found that BDNF can protect from both DNA-damage and trophic deprivation induced apoptosis. Combined application of biochemical, pharmacological and molecular methods resulted in the conclusions that protection from DNA-damage was mainly mediated by Extracellular signal-Regulated Kinase 1/2 whereas anti-apoptotic action against trophic withdrawal was due to activation of Phosphatidylinositol-3 Kinase.

25.3 VIRAL VECTORS AS A TOOL TO PROBE FOR A GENE FUNCTION IN THE NERVE CELLS

Jaworski J., Kaczmarek L.
Nencki Institute, Warsaw, Poland

Understanding of gene expression appears to be a prerequisite to gain insight into the molecular mechanisms of such phenomena like neuronal development, plasticity as well as programmed cell death. To approach these problems several tools allowing for gene transfer to neurons have recently been developed. In our studies we employed adenoviral vectors to investigate involvement of transcription factors in neuronal plasticity and neurodegeneration. Optimal conditions for adenoviral gene transfer into several types of neurons cultured in vitro, including dentate gyrus, cortical and SCG cells were initially established. Over 80% of neurons in all above types of cultures have been infected successfully infected by use of the optimized protocols. This allowed us to study role of overexpression of the ICER transcription factor (blocker of CRE-dependent transcription) in neuronal cell death. Infection of DG or cortical neurons with Ad-ICER resulted in robust (over 50% of neurons) apoptotic-like cell death. On the other hand, infection with the control virus encoding β-galactosidase did not cause neurodegeneration. Additionally, conditions for *in vivo* adenoviral gene transfer into hippocampus, amygdala, cortex and SCN were established. High level of transgene expression, lasting over 1 month, was obtained in dentate gyrus. However, it was accompanied by strong immune response (as indicated by infiltration of CD8- and CD4-positive cells). Approaches to overcome this problem, as well as newest advances in a gene transfer techniques into CNS will be also discussed.

Session 26 – Workshop: Natural neurotoxins: their targets, mode of action and structural-functional relationships

26.1

NEUROTOXIC ACTIVITY OF SNAKE VENOM PHOSPHOLIPASES A2

Grazyna Faure* & *Cassian Bon
Unité des Venins, Institut Pasteur, 25 rue du Dr. Roux, F-75724 Paris Cedex 15, France

Snake venoms have a large array of secreted phospholipases A_2 (PLA_2s) that catalyze the hydrolysis of the acyl-ester bond at the *sn*-2 position of phospholipids. In addition to their role in lipid degradation, snake venom PLA_2s may be cytotoxic, neurotoxic, myotoxic and may interfere with blood coagulation or inflammation processes. Neurotoxic PLA_2s, also known as β-neurotoxins, act on the neuromuscular junction, primarily at a presynaptic level inhibiting the release of the neurotransmitter acetylcholine (ACh), and cause death by respiratory failure. β-Neurotoxins, subdivided in three classes, are highly specific for various neuronal targets. Their molecular characterization and the elucidation of their mechanism of action on ACh release are underway.

We report here the solubilization and the identification of the 48-kDa high-affinity binding protein for crotoxin, a potent β-neurotoxin from *Crotalus durissus terrificus* venom. This crotoxin-acceptor from *Torpedo marmorata* electric organ was solubilized and purified to homogeneity by crotoxin-affinity chromatography. The presence of the acceptor in membrane detergent extract and in isolated fractions was detected by cross-linking experiments with radioiodinated crotoxin and by surface plasmon resonance (SPR) technology. SPR allowed also to determine kinetic parameters (association and dissociation rates and apparent affinity constant) for the (crotoxin/solubilized 48 kDa acceptor) interaction.

26.2

INTERACTIONS OF SCORPION NEUROTOXINS WITH SODIUM CHANNELS.

Marcel Pelhate [(1)], ***Maria Stankiewicz*** [(2)] & ***Wojciech Kadziela*** [(2)]
[(1)] *Lab. of Neurophysiology, Angers University, France.*
[(2)] *Inst. of Gen. & Molec. Biol., N. Copernicus Univ. Torun, Poland.*

Sea anemones, scorpions and spiders paralyze their crustacean, insect or vertebrate preys by injecting them toxins which alter differently sodium channel (Na_v) gating mechanisms. Na_v are voltage-sensitive transmembrane proteins responsible for the increase of sodium permeability that initiates action potentials. Na_v consist of three subunits, the most important, α = 260 kDa, delines the pore selectively permeable to Na^+ ions. Neuroactive agents bind to this α subunit at the level of six or more distinct receptor sites. Sea anemone, α-scorpion and some spider toxins bind to the site 3. Scorpion β-toxins bind to site 4. These toxins are openers of Na_v and the paralysis results from an extension or a suppression of action potentials. Structural differences among receptor sites 3 from mammals and insects have shown that α-scorpion toxins bind to homologous but not identical sites. Site-directed mutagenesis has been used to identify the molecular determinants involved in the functional effects of toxin binding to receptor site 3 . A large number of charged amino acids located on the outer surface of Na_v play an important role in the binding of neuroactive agents. On rat brain Na_v, E1613 (glutamic acid) is the major determinant for both α-scorpion and sea anemone toxins. D1612 (aspartic acid) in cardiac Na_v and D1428 in skeletal muscle play the same role. Biotechnology performances and a better knowledge of the subtle differences between insect and vertebrate Na_v receptor sites are promizing for agrochemical applications.

26.3

NEUROTOXINS: FROM SPECIFIC LIGANDS TO ACTIVE VENOMS AND APPLICATIONS.

Maria Stankiewicz [1], ***Marcel Pelhate*** [2], ***Ewa Kielbasiewicz*** [1] & ***Wojciech Kadziela*** [1].
[1] *Inst. of General & Molecular Biology, N.Copernicus Univ., Torun, Poland;* [2] *Lab. of Neurophysiology, Angers Univ., Angers, France.*

Animal venoms are rich sources of toxic peptides and proteins which have different, distinct, specific sites of action. Majority of them are neurotoxins affecting excitable membranes. They often show a special "strategy of function" depending on the animal group. Snake neurotoxin mode of action is pre- and post- synaptic, scorpion and spider neurotoxins modify mainly voltage-dependent K^+, Na^+, Ca^{2+} and Cl^- channels, *Conus* snail neurotoxins block nicotinic receptors, Na^+ and Ca^{2+} channels. Effect of all neurotoxin action mechanisms is generally the same in the poisoned animal: the transmission of information in the neuro-muscular system is disturbed and it causes, in very short time, a complete paralysis. Neurotoxins are used to catch a prey or to defend a single organism or a colony of social animals, but for human they may create significant troubles in public health. For science, they serve as excellent tools in fundamental research. They constitute specific and sometimes potent markers for many biological systems, e.g. molecular probe for the study of ionic channel function. Their application in diagnostic and therapeutic domains develops fast and they are considered as possible important factor in pest management in agriculture. Studies on neurotoxins progress, number of described molecules increases, wide applications develop, however it becomes more and more difficult to conserve a clear general view on these substances and now compendium works are necessary.

Session 27 - Oral Communications (part 2)

27.1 **Androgen Implants in Postero-Dorsal Medial Amygdala Briefly Maintain Noncontact Erections in Male Rats.**

Michał Biały[1,2] and Benjamin Sachs[2]
[1]*Dept. of Physiology, Medical University of Warsaw, Poland*
[2]*Dept. of Psychology, Univ. Of Connecticut, Storrs, CT, USA.*

Castration of male rats causes a rapid loss of noncontact erection (NCE; erection evoked by inaccessible estrous female). Such erections can be restored by systemic treatment with testosterone (T) or dihydrotestosterone (DHT) but not estradiol (E; Manzo et al., 1999). Several previous studies have shown that castration also results in a slower loss of copulatory behavior, an effect that can be blocked or reversed by systemic T and E, by forebrain implants of T and E, and by amygdala implants of DHT but not by systemic DHT treatment. Medial amygdala lesion completely destroy NCE with less effects on copulatory behavior (Kondo et al., 1997, Kondo & Sachs 1999). We examined whether androgen delivered to the medial amygdala (MeA) of sexually experienced rats would maintain NCE. Males from experimental groups received bilateral implants directed at the MeA of testosterone-filled (T-MeA, n=11), DHT-MeA, n=11, and E-MeA, n=10. Control animals had bilaterally implanted blank cannulae at the MeA and sc 2 cannulae with: T-sc, n=10, DHT-sc, n=6, E-sc, n=6. In additional control group males had cannulae bilaterally implanted at the caudate-putament (T-CP, n=10). During the two weeks after surgery, males were tested twice for NCE and 3-4 days after each NCE test, for copulation. Androgens implanted at the MeA were effective briefly (one week after castration) in maintain of NCE. In T-MeA 5 from 11 and in DHT-MeA 6 from 11 displayed NCE (2/11 and 3/11 at the second test respectively). No males with sc implanted androgens had NCE in both tests. The incidence of ejaculation was not significantly affected by steroid treatment or placement. Erections observed in NCE tests were not "spontaneous" but occurred in response to the estrous as another T-MeA group (n=8) was given two tests (balanced order) in which female were present or absent (5/ 8 males display NCE with female present vs. 0/8 with female absent). We can not excluded role of estrogens in maintain paradigm of NCE after castration as E-MeA (2 from 10) and E-sc (4 from 6) shown NCE in first and 5/10 and 2/6 in second test. Androgens implants were the most effective when the end of cannulae achieved posterior but not anterior part of dorsal medial amygdala. The results suggests that androgens at the postero-dorsal MeA are involved in NCE, a putative index of sexual arousability.

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27.2 **THE EFFECT OF OPIOIDS ON THE REGULATION OF TRANSCRIPTION AT THE CELLULAR LEVEL**

W.Bilecki, R. Przewłocki.
Institute of Pharmacology, 12 Smętna Street, 31-343 Kraków, Poland

Opiates, acting on opioid receptors (coupled to G_i/G_o classes of the G proteins) inhibit cyclic AMP (cAMP) formation, Ca^{2+} conductance and activate potassium conductance, leading to the suppression of neuronal excitability. However, opioids can also cause an increase in intracellular Ca^{2+} and activate mitogen-activated protein kinases (MAPK). Therefore it appears that opioids, at the cellular level, exert both inhibitory and stimulatory effects. The adaptations in cAMP and Ca^{2+} levels result in alterations in the activity of several transcription factors. Especially *Ca^{2+}/cAMP Responsive Element Binding Protein* (CREB) and *Activated Protein 1* (AP-1) can establish a direct link between opioid-regulated signal transduction pathways and the modulation of gene expression. It was found that acute administration of opioids increased CREB phosphorylation and binding to consensus CRE and AP-1 elements without affecting total CREB protein level. In contrast, prolonged opioid treatment normalized back to basal the levels of CRE and AP-1 DNA binding activity and slightly decreased the levels of phoshorylated CREB. Withdrawal from the drug elicited an increase in phosphorylated CREB levels and induced CRE and AP-1 DNA binding activity. Consequently, opioids regulated CRE- and AP-1-directed transcription of luciferase reporter gene as well as the expression of target genes (e.g. proenkephalin).

Our findings provide evidence that the regulation of gene expression may contribute to development of tolerance and addiction. Our results also highlight the role of transcription factors in the adaptations to opioids at the cellular level.

This study was supported by KBN grant P05A.107.20

27.3 INHIBITION OF THE INTERGENICULATE LEAFLET NEURONAL ACTIVITY BY SEROTONERGIC PROJECTION FROM THE DORSAL RAPHE NUCLEUS

Tomasz Błasiak, Marian H.Lewandowski
Jagiellonian University, Institute of Zoology
Ingardena 6, 30-060 Krakow, Poland.

The intergeniculate leaflet of the lateral geniculate nucleus (IGL) and the suprachiasmatic nucleus of the hypothalamus (SCN) are involved in generation and adjustment of mammalian circadian rhythms. Both structures receive strong serotonergic innervation, originating in the midbrain raphe nuclei [1]. Iontophoretic application of 5-HT receptor agonists, attenuate response of IGL neurons to photic stimulation [2]. Our previous study of IGL's electrophysiology revealed unique, isoperiodic oscillation of euronal firing rate in this structure [3]. The present study was undertaken to determine the influence of endogenous 5-HT on spontaneous oscillatory activity of IGL in vivo, by means of the electrical lesions or stimulation of the dorsal raphe nucleus (DRN). The DRN lesions always produced significant increase of IGL neuronal firing rate. In accordance with this, DRN stimulation resulted in decrease of IGL neuronal activity - effect lasting for several minutes. Concurrently, there was no change in the isoperiodic oscillation of the level of neuronal activity. These results suggest existence of inhibitory influence of DRN serotonergic projection on IGL activity. On the other hand, this data shows that serotonergic innervation of IGL neural network is not crucial for generation of isoperiodic oscillation within this component of circadian clock.

1.Meyer-Bernstein EL and Morin LP. *J Neurosci* 16(6):2097-2111 (1996).
2.Ying S-W, Zhang D-X and Rusak B. *Brain Res* 628:9-16 (1993).
3.Lewandowski MH, Błasiak T, Domosławski J and Wołkowska A. *NeuroReport* 11:317-321 (2000).

27.4 **AFFERENT INNERVATION OF THE VAS DEFERENS IN THE PIG**

Kaleczyc J., Pidsudko Z., Łakomy M.
Dept. of Animal Anatomy, Faculty of Veterinary Medicine, University of Warmia and Mazury, 10-957 Olsztyn, Poland

Combined retrograde tracing and double-labelling immunofluorescence was used to establish which spinal ganglia (SG) contribute to the innervation of the porcine vas deferens and to investigate whether these primary sensory neurons represent one or more neurochemically distinct populations. We also investigated nerve pathways from SG to the vas deferens with combined retrograde tracing and cutting the left hypogastric or pelvic nerve. The vast majority (90%) of the vas deferens-projecting neurons (VD-PN) were found mainly in the lumbar L2, L3 and sacral S2, S3 pairs of SG. However, approx. 80% of these nerve cells occurred in the ipsilateral (IL) ganglia. Immunohistochemistry revealed that most of VD-PN contained CGRP and/or SP. However, a distinct difference in the occurrence of these peptides was found between the neurons located within the lumbar and sacral ganglia. In the lumbar ganglia, virtually all the VD-PN (97%) contained CGRP and/or SP while in the sacral ganglia only 55% of the nerve cells expressed immunoreactivity to these peptides. Some of VD-PN expressed also GAL often in combination with SP and/or CGRP. Denervation experiments have revealed that the neurons located within both IL and contralateral (CL) lumbar SG project through the IL hypogastric nerve and those found within the sacral ILSG send their processes through the IL pelvic nerve. This study for the first time has revealed that primary sensory neurons projecting to the mammalian vas deferens comprise different regional-confined populations with respect to their neuropeptide content.

27.5 **ANALYSIS OF MULTIPLE STIMULUS PROPERTIES REVEALS SUBTLE DEFICITS IN AUDITORY RECOGNITION AFTER LESIONS TO ASSOCIATION AUDITORY CORTEX IN DOGS**

P. Kuśmierek, J. Sadowska, A. Łaszcz, D. M. Kowalska
Nencki Institute of Experim. Biology, 3, Pasteur St., 02-093 Warsaw

A method for detection of auditory perceptual deficits across many auditory variables has been developed. For each of 347 auditory stimuli, 59 parameters were derived. The data set was reduced by PCA to 9 factors: 1. main (middle) spectral band (0.4-1.2 kHz); 2. high band (3-11 kHz); 3. low band (<0.4 kHz); 4. proportion of silence; 5. spectrum skewness and kurtosis; 6. upper middle band (1.2-3 kHz); 7. top band (>11 kHz); 8. temporal variation of spectral shape; 9. temporal variation of intensity and spectral mean. Factors 4, 8, 9 were related to temporal structure of the sounds, whereas factors 1-3 and 5-7 tended to describe steady-state properties.

Performance on auditory Delayed-Matching-to-Sample task (which involves discrimination within many pairs of acoustic stimuli) was investigated. For each stimuli pair, difference and sum of the stimuli factor scores were calculated. In a big (n=18) group of intact dogs, difference in factor 4 scores was positively correlated with performance, however, this effect was not significant in a subset (n=4) of animals which were then subjected to bilateral lesions of association auditory cortex. After the surgery, performance level was positively correlated with difference of factors 1 and 5 scores, and with sum of factor 2 scores. These data suggest that processing of lower frequencies was impaired and that animals with lesions t the association auditory areas relied on high-frequency information. No specific impairment of temporal processing has been found.

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27.6 **GLUTAMATE RECEPTORS MEDIATING COCKROACH JUVENILE HORMONE BIOSYNTHESIS**

Maciej A Pszczolkowski[*,#], Hsin-Ping Liu [#], Shu-Jane Lin [#], Wei-Shiang Lee [#], and Ann-Shyn Chiang [#]*

**Department of Entomology, Washington State University, Pullman, WA 99164, USA, [#]Department of Life Science, National Tsing Hua University, Hsinchu 300, Taiwan R.O.C.*

We show that JH syntesis by corpus allatum (CA) of a cockroach, *Diploptera punctata*, is modulated by calcium influx to CA cells by ionotropic glutamate receptors. An elevation of cytosol calcium concentration was observed when CA cells were exposed to L-glutamate or NMDA (N-methyl-*D*-aspartate). Both L-glutamate- and NMDA- stimulated rates of JH biosynthesis in a dose-dependent manner. Changes in cytosolic calcium concentration caused by L-glutamate or NMDA were abolished at presence of NMDA receptor antagonists, Mg2+ or (5R,10S)-(+)-5-Methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine, MK-801. Similarly, *in vitro* JH synthesis was significantly suppressed by addition of Mg2+ or MK-801 to incubation media. Furthermore, the CA's were most sensitive to L-glutamate or NMDA on the day when JH synthesis peaks in *D. punctata*. We postulate that in *D. punctata* JH biosynthesis is regulated via functional NMDA-subtype glutamate receptors. Our additional preliminary results suggest that JH synthesis in this species may be also regulated by another ionotropic glutamate receptor, sensitive to kainate, but not to (RS)-α-amino-3-hydroxy-5-methyl-4-isoxazolepropanoic acid (AMPA), function of which may be impaired by (6-Cyano-7-nitroquinoxaline-2,3-dione), CNQX.

Session 28 – Posters Session: Biological membranes

28.1 **HISTAMINE-EVOKED STIMULATION OF CYCLIC AMP FORMATION IN THE CHICK HYPOTHALAMUS: INTERACTION WITH VIP AND PACAP**

Małgorzata Bereziński[1], Jolanta B. Zawilska[1,2], Agata Woldan-Tambor[1,2], Katarzyna Kuba[1] and Jerzy Z. Nowak[1]
[1]Department of Biogenic Amines Polish Academy of Sciences, Łódź, and [2]Department of Pharmacodynamics, Medical University of Łódź, Łódź, Poland

Histamine (HA; 1-1000 μM) potently and concentration-dependently stimulated cyclic AMP (cAMP) production in[^{3}H]prelabeled slices of chick hypothalamus, with an EC_{50} values of 6.5 μM. The effect of HA was mimicked by agonists of HA receptors: HA > 4-methylHA (H_2) ≥ N_α,N_α-dimethylHA (H_3 >> H_2=H_1) > 2-methylHA (H_1) >> amthamine (H_2) >> dimaprit (H_2), *tele*-methylHA. The HA-evoked increase in cAMP production in the chick hypothalamus was antagonized by selective H_2-receptor blockers (aminopotentidine >> cimetidine > ranitidine >> zolantidine), and not significantly affected by mepyramine and thioperamide, selective H_1- and H_3-receptor blockers, respectively. Aminopotentidine inhibited the HA action in a noncompetitive manner. Vasoactive intestinal peptide (VIP; 0.3-3 μM) weakly, whereas pituitary adenylate cyclase activating polypeptide ($PACAP_{38}$; 1-30 nM) potently stimulated cAMP synthesis, giving maximal increases of 150% and 300% above control, respectively. HA (1μM) potentiated the action of VIP and did not modify effects produced by $PACAP_{38}$. It is concluded that the HA receptor in the hypothalamus of chick represents an avian specific H_2-like HA receptor, whose pharmacological profile is distinct from that described for the mammalian H_2-HA receptor. In the chick hypothalamus a hyperadditive synergistic interaction between HA and VIP results in large increases in cAMP production.

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28.2 **ADAPTATION OF C6 GLIOMA CELLS TO THE ABSENCE OF GLUTAMINE IN THE CULTURE MEDIUM: CHANGES IN CELL PHENOTYPE AND GLUTAMINE TRANSPORT CHARACTERISTICS.**

M.Dolińska, M. Zielińska, A.Dybel, B.Zabłocka[1], L. Bużańska[2], W. Hilgier, J.Albrecht
Dept.of Neurotoxicology, [1]Lab.of Mol. Biology, [2]Dept.of Neurochem., Lab.of Mol. Pathology, Medical Res.Centre, Pol. Acad. of Sci., Warsaw, Poland

Glutamine (Gln) is one of the key metabolites in the CNS and as such is a routine supplement of CNS cell culture media. C6 cells normally grown in a Gln-rich medium adapt to a Gln-deprived medium without pronounced phenotypic changes. The present study compared the enzymes of Gln metabolism and characteristics of cell membrane transport of Gln in cells grown in the presence or absence of Gln. Growth in Gln-deprived medium was associated with activation of glutamine synthetase, whose activity was barely detectable in cells grown in the presence of Gln. The activity of the Gln-catabolizing enzyme, phosphate-activated glutaminase was not affected by medium change. In cells grown in the presence of Gln, ~80% of the uptake was Na^+ dependent, mediated by (a) system(s) strongly inhibited by ASC substrates (Thr, Ser, Cys) but relatively insensitive to the model system A substrate, methyl-amino-isobutyric acid (MeAiB). The uptake also showed strong pH sensitivity and partial tolerance to substitution of Na^+ by Li^+, resembling both system ASCT2 and ATA1 variant of system A. The absence of Gln in the medium did not induce MeAiB-sensitive uptake typical of the classical system A, but rendered the uptake via the „ASC+A" system more active than in cells grown in a Gln-rich medium. Both ATA1 and ASCT2 mRNAs are expressed in cells grown in either medium. Supported by SCSR grant no. 4 P05A 060 18.

28.3 **THE STUDY OF CALMODULIN ACTION ON CORTICAL PLASMA MEMBRANE Ca^{2+}-ATPASE IN DIFFERENT PHOSPHORYLATION STATES.**

Gromadzinska E, Lachowicz L, Zylinska L
Department of Biochemistry, Medical University, Lodz, Poland,

The plasma membrane Ca^{2+}-ATPase in neuronal tissue plays an important role in fine tuning of the intracellular Ca^{2+} concentration. Here we analyzed the relationship between separate modes of cortical Ca^{2+}-ATPase regulation i.e., reversible phosphorylation processes mediated by protein kinases A and C, protein phosphatases PP1 and PP2A, and stimulation by calmodulin (CaM). The activity of PKA or PKC-phosphorylated Ca^{2+}-ATPase rose by the further addition of CaM. In both cases the fluorescence study has shown the increase in formation of Ca^{2+}-ATPase/CaM complex, and for PKA-mediated phosphorylation it was correlated with a higher affinity of calcium pump for calmodulin. The incubation of Ca^{2+}-ATPase with CaM prior to protein kinases action revealed that CaM presence counteracts the stimulatory effect of PKA and PKC. Under the *in vitro* assay the Ca^{2+}-ATPase was a substrate for PP1 and PP2A. Protein phosphatases decreased both, the basal activity of Ca^{2+}-ATPase and its affinity for CaM. Fluorescence analysis confirmed the lowered ability of dephosphorylated Ca^{2+}-ATPase for calmodulin binding. These results may suggest that interaction of CaM with calcium pump and its stimulatory action could be a partly separate phenomenon that is dependent on the phosphorylation state of Ca^{2+}-ATPase.

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28.4 **MODIFICATION OF INSECT DUM NEURON BIOELECTRICAL ACTIVITY BY DELTAMETHRIN**

E. Kiełbasiewicz,[1] M. Stankiewicz,[1] B. Grajpel[2] & W. Kądziela[1]
[1]Department of Biophysics, Nicolaus Copernicus University Toruń.
[2]Department of Animal Physiology, Nicolaus Copernicus University Toruń.

Although pyrethriod action on different nerve membranes has been investigated, deltamethrin effects on DUM neurons is still unproved. The neurosecretory DUM neurons from cockroach CNS are known for their endogenous pacemaker activity. Several ionic conductances are involved in this activity.Voltage-gated sodium channels (Na_v) are responsible for action potential (AP) fast depolarizing phase. The type II pyrethroid, deltamethrin (1, 10, 100 μM), has been used to study its action on DUM cells. Experiments were performed on desheated cockroach last abdominal ganglions by microelectrode *in situ* recording of the DUM cell activity .

Obtained results gave three types of effects: 1. A relatively fast decrease of AP amplitude simultaneous to resting potential changes: small (2-4 mV) hyperpolarization immediately after application and then progressive neuron membrane depolarization. 2. Evident AP amplitude oscillations 10-15 min after application. 3. Spontaneous activity was present during strong artificial hyperpolarizations (e.g. to -100 mV) while in control conditions APs completely disappeared when RP was about -60mV.

Results confirm that deltamethrin modifies Na_v: resting depolarization and AP decrease may be explained by slowing of activation-deactivation mechanism. Activity at very negative potentials and AP amplitude oscillations can be attributed to shift of activation towards more negative potentials and to deltamethrin-induced subconductance states. However effects on other cooperative ionic currents (e.g. Cl^-, Ca^{2+} and background Na^+ channels) cannot be excluded. To confirm that, further investigations are needed.

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28.5 **LEM-TRP-1 NEW ANALOGUES AND THEIR MYOTROPIC EFFECTS IN COCKROACH *Leucophaea maderae***

Danuta Lombarska-Śliwińska[1], Dick R. NÄSSEL[2], Danuta KONOPIŃSKA[1]

Faculty of Chemistry, University of Wrocław, 50-383 Wrocław, Poland[1], Department of Zoology, Stockholm University, S-10691 Stockholm, Sweden[2]

The subject of our investigation on structure/activity relationship in insect are tachykinin Lem-TRP-1 (Ala-Pro-Ser-Gly-Phe-Leu-Gly-Val-Arg-NH_2) and its five analogues modified in position 2 of the peptide chain.The synthesis of Lem-TRP-1 analogues modified in position 2 of the peptide chain, such as: Ala-X- Ser-Gly-Phe-Leu-Gly-Val-Arg-NH_2, where X = Hyp(**1**), Acp(**2**), Val(**3**), Ach(**4**), and Sar (**5**) was performed. Biological activity of these neuropeptides was estimated *in vitro* on the hindgut of cockroach *Leucophaea maderae* at the 10^{-9} to 10^{-7} M range of concentrations. Effects were compared with native Lem-TRP-1 activities. Among the analogues tested, peptide 1 retained a myotropic activity similar to the native peptide, whereas peptide **2** showed a higher maximum response than Lem-TRP-1, [Val^2]-Lem-TRP-1 (**3**) preserved over 15% of the native peptide activity. Peptides **4** and **5** were almost inactive.

28.6 **GABA BINDING SITES ON $GABA_A$ RECEPTOR SHOW POSITIVE COOPERATIVITY**

Jerzy W. Mozrzymas[1], Andrea Barberis[1,2], Katarzyna Mercik[1,3], Ewa D. Żarnowska[1]
[1] Department of Biophysics, Wrocław Medical University, Wrocław, Poland [2] International School for Advanced Studies (SISSA), Trieste, Italy [3] Institute of Physics, Technical University of Wrocław, Wrocław, Poland

In most kinetic studies it is assumed that $GABA_A$ receptor, prior to opening, binds two GABA molecules to two identical and independent binding sites. The goal of the present study was to verify to what extent this assumption is correct for $GABA_A$ receptors in cultured rat hippocampal neurons. For this purpose, current responses to ultrarapid (ca. 50 μs solution exchange time) applications of GABA were recorded in the outside-out configuration of the patch-clamp technique. The experiments were performed on cultured rat hippocampal neurons. GABA concentrations varied from 10 μM to 10 mM and dose-dependencies of both current amplitudes and the 10-90% current rise times were constructed. The quantitative analysis, aiming to determine the microscopic gating of $GABA_A$ receptors, was based on the model assuming one open state, one desensitized state and a postulated binding reaction. In the frame of this model, we tested the binding schemes with different number of binding sites that were either cooperative or non-cooperative. We found that the best reproduction of the experimental data was obtained with the model postulating three positively cooperative binding sites.

We conclude that the number of agonist molecules, required to activate the ionotropic $GABA_A$ receptor may be larger than two and that the binding sites show a positive cooperativity.

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28.7 **CHANGES IN EXTRACELLULAR pH AFFECT $GABA_A$ RECEPTOR MICROSCOPIC GATING**

Jerzy W. Mozrzymas[1], Ewa D. Żarnowska[1] Katarzyna Mercik[1,3]
[1]*Department of Biophysics, Wrocław Medical University, Wrocław, Poland,* [2]*Institute of Physics, Technical University of Wrocław, Wrocław, Poland*

It is known that local changes in pH may occur due to metabolic processes and membrane transport phenomena. In particular, transport of HCO_3^- anions through $GABA_AR$s is supposed to strongly modulate pH around these receptors. It has been reported that variations in pH affect the GABA-evoked currents but a limited resolution of recordings did not allow to describe these effects in terms of changes in $GABA_AR$ microscopic gating. In the present study we examined the effect of pH (in the range 5 - 9) on current responses to ultrarapid (ca. 50 μs solution exchange) GABA applications. Experiments were performed in the outside-out mode of the patch-clamp technique on cultured rat hippocampal neurons. For responses to low [GABA] (100-300 μM), acidic pH slowed down, while basic pH accelerated the rising phase of current responses. Since at low [GABA], binding process is rate limiting, these results indicate that increasing pH accelerates association of GABA to its binding site. Paired pulses experiments demonstrated that the recovery of response to the second GABA application was slowed down by increasing pH, suggesting a decrease in the dissociation rate constant for GABA. In addition, increase in pH accelerated the desensitization onset and slowed down the deactivation process. We conclude that pH modulates $GABA_A$ receptor by modifying its microscopic gating.

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28.8 **THE PHOSPHORYLATION OF BRAIN SYNAPTOSOMAL MEMBRANE PROTEINS BY PKC CAN BE MODULATED BY 17βESTRADIOL IN VITRO.**

E.Rębas, L.Lachowicz, A.Lachowicz
Dept.Biochemistry, Institute of Physiology & Biochemistry Medical University of Lodz, Poland

The reversible phosphorylation of proteins by kinases and phosphatases plays important role in control of many cellular processes. The activity of protein kinases could be modulated by various agents including steroid hormones. One of them is 17βestradiol a neuroactive steroid which can cross the blood-brain barier and modify neuronal functions. The aim of our work was to examine the influence of estradiol on phosphorylation of synapsins, the synaptosomal membrane proteins which are substrates for PKC in *in vitro* experiments. We used 1mM and 0.1mM solution of estradiol water-soluble (SIGMA) and the time of preincubation was 5 to 10 minutes. Control samples did not contain steroid. We assumed that degree of ^{32}P incorporation into proteins of control group amounted to 100%. We conclude that estradiol decreased the phosphorylation of synapsins by PKC in rat cerebral cortex and hippocampus to 50-70% of the control values and this effect was dependent on estradiol concentration.

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28.9 **THE MECHANISM OF INSECTICIDE-INDUCED CHANGES IN THERMOREGULATION OF INSECTS.**

E.Tęgowska, B. Grajpel, M. Stankiewicz[1], M. Wojciechowski, B. Piechowicz, R.Olszak[2],
Dept. Animal Physiology, Dept. Biophysics[1], Institute General and Molecular Biology, N. Copernicus University, Toruń; Institute of Pomiculture and Floriculture[2], Skierniewice.

Neurotoxic effect of pyrethroids is diminished by high ambient temperature, simultaneously after pyrethroid exposure insects prefer higher ambient temperature. Symptoms of neurotoxicity of pyrethroids have been attributed to an induced modification of the gating kinetics of fast sodium channels (opening) in the nerve membrane. Indoxycarb blocks sodium channels. A set-point theory was based on the balance of the concentrations of sodium and calcium ions in the thermoregulatory center. We studied changes in the survival, thermoregulation (both preferred and body tempe-perature), metabolic rate, and in activity, caused by of indoxycarb and pyrethroid in locusts (*Locusta migratoria).* Because locusts exist in solitary and gregarious forms, experiments were performed both on individual insects and on clusters. The survival of animals exposed to pyrethroid was higher in the warm environment (at ambient temperature selected by animals under the exposure), while after indoxycarb intoxication, animals exposed both to warmer, and to cooler (the later corresponds to selected temperature of the intoxicated animals) environment - showed a reduced survival. Both forms insects displayed: an increase of body and preferred ambient temperature after pyrethroid, and a decrease after indoxycarb. In conclusion: patterns of thermoregulatory responses to intoxication were correlated with a specific modification of the gating kinetics of sodium channels: i.e. opening was connected with elevated body temperature, and closing with lowered body temperature.

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28.10 **CYTOARCHITECTURE AND CHEMOARCHITECTURE OF THE ENTORHINAL AND PERIRHINAL CORTICES IN THE DOG**

Woźnicka, A., Kosmal, A. and Malinowska, M.
Laboratory of Neuroanatomy, Department of Neurophysiology, Nencki Institute of Experimental Biology, Warsaw, Poland.

Differentiation of Nissl, Timm, cytochrome oxidase and acetylocholinesterase staining allow the determination of the cortical architecture, and areas of the entorhinal and perirhinal cortices. The entorhinal cortex, which occupies the posterior extent of the piriform lobe, is characterised by a bilaminar and six-layered cell arrangement. Between the external (layers I - III) and internal (layers V and VI) laminas, the lamina dissecans (layer IV) can be distinguished as a cell-poor zone. Using cytoarchitectonic criteria, the cortex can be subdivided into four areas (DLEA, VLEA, VMEA and MEA). The perirhinal cortex extends into the fundus of the posterior rhinal sulcus (area 35), and into the lateral bank of this sulcus (area 36). Cytoarchitectonic division of the ento- and perirhinal cortex into areas coincide well with the chemoarchitecture, and is most clearly visible in Timm stain. The method visualizes zinc-containing axonal terminals forming a characteristic pattern related to the laminar structure of the cortex. In the entorhinal cortex, the most dense staining predominantly overlaps the external lamina. Within the VLEA and MEA, an additional pattern of wide patches can be observed. In contrast to this, in the perirhinal cortex, two distinct dark bands are seen that extend into the laterally adjacent temporal neocortex. The internal band mainly overlaps layer V of areas 35 and 36, which in area 35 is thinner than in area 36. A thick external band overlaps cortical layers II and III of the entire perirhinal cortex.

28.11 **THE OPIOID-REGULATED ACTIVITY OF MITOGEN ACTIVATED PROTEIN KINASES AT THE CELLULAR LEVEL**

G. Zapart, W. Bilecki, R. Przewłocki.
Institute of Pharmacology, 12 Smętna Street, 31-343 Kraków, Poland

The inhibition of neuronal excitability via the regulation of cAMP levels or Ca^{2+} conductance represents the traditional mechanism of action of opioids. However, opioids can also activate mitogen-activated protein kinases (MAPK). The opioid-induced increase of MAPK activity was shown to initiate μ-opioid receptor desensitization. The activated MAPK can also translocate to the nucleus where it can activate or induce transcription factors thereby leading to the expression of several genes. The present study was undertaken to evaluate the direct effect of opioids on MAPK activity in Neuro2a MOR1 cells expressing μ-opioid receptors. We report here that opioids, via μ-opioid receptors exert a stimulatory effect on MAPK phosphorylation. In contrast, prolonged opioid treatment decreased the levels of phoshorylated MAPK. Moreover, withdrawal from the drug enhanced the opioid-induced inhibition of MAPK phosphorylation. We have also found that the opioid-regulated MAPK activity opposed the adaptation in the well-known cAMP system. The stimulatory effects of opioids on MAPK activity had similar pharmacological profiles and concentration-response relationships to those reported for the inhibitory effects of opioids on cAMP pathway.

Our results provide evidence that, at the cellular level, cAMP and MAPK cascade can serve opposing functions and may contribute to the development of tolerance and addiction

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28.12 **PROTEIN KINASES ACTIVITIES IN ASPHYXIATED MEMBRANES.**

Zylinska L[1], Sobolewska B[2], Gulczynska E[2]
[1]Department of Biochemistry, Medical University, Lodz, Poland,
[2]Research Institute of Polish Mother Center, Lodz, Poland,

Perinatal asphyxia represent a major cause of acute brain impairment and mortality in neonatals. The therapies able to prevent or reduce post-asphyxial damages in newborns involve the characterization of biochemical processes underlying asphyxia. In our study we compared the activities of endogenous protein kinases in erythrocyte membranes of healthy and asphyxiated infants. We have detected the increased activities of serine/threonine protein kinase A and tyrosine protein kinases in asphyxiated erythrocyte ghosts (150% and 180%, respectively), whereas under the same conditions the activity of protein kinase C was reduced by 20%. In asphyxiated membranes the amounts of phosphothreonine and phosphotyrosine were significantly higher as compared to healthy newborns, but phosphoserine comprised only 50% of detected in control samples. Another membrane protein - anion-exchange protein, band 3, which is generally recognized as the major substrate for protein tyrosine kinases, was present in asphyxiated membranes at the lower level than that of healthy one. Our results indicate that under asphyxia the changes in protein kinases activities, the phosphorylation state of membrane proteins and anion exchanger protein content could underlie the disturbance in membrane function.

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28.13 **CHLORPROMAZINE EFFECTS ON NMDA AND NON-NMDA GLUTAMATE RECEPTORS IN CULTURED RAT HIPPOCAMPAL NEURONS**

Ewa D. Żarnowska, Jerzy W. Mozrzymas
Department of Biophysics, Wrocław Medical University, Wrocław, Poland

The effect of chlorpromazine (CPZ) on ionotropic glutamate receptors was investigated using the patch-clamp technique in cultured rat hippocampal neurons. The current responses were recorded in the whole-cell configuration of the patch-clamp technique and the agonist (glutamate or NMDA) was applied using a multibarrel perfusion system (solution exchange 10-30 ms). The non-NMDA receptors were found to be insensitive to CPZ. The currents evoked by NMDA (30-100 μM) showed a wide range of variability of both the rate and the extent of desensitization. The responses characterised by fast (τ_{des} < 300ms, for 100 μM NMDA) and profound desensitization were strongly inhibited by 30 μM CPZ (up to 40% of control amplitude) while the CPZ (100 μM) effect on slowly desensitizing currents (τ_{des} > 300ms) was weak (decreased to about 94% of control value). The extent of desenstitization was found to be increased by CPZ (for 100μM NMDA steady-state to peak was 0.37 ± 0.04 and in 100 μM CPZ 0.18 ± 0.03). The time course of deactivation phase (after agonist removal) was slowed down by 30 μM CPZ (in control τ = 158 ± 14 ms and in presence of CPZ τ = 215 ± 44 ms). Since synaptic currents represent mainly the deactivation process, this finding seems particularly important. We conclude that CPZ inhibits the NMDA receptors and propose that the sensibility to CPZ may depend both on the subunit composition and on the state of receptor modulation by intracellular factors.

Supported by KBN funds for Wrocław Medical University.

Session 29 – Poster Session: Behaviour

29.1 SIMPLE MODEL OF LAMPREY NEURONAL NETWORK REALIZED IN MATLAB/SIMULINK ENVIRONMENT

Małgorzata Baszczak, Stefan Kasicki
Dept. Neurophysiology, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Pasteura 3, PL 02-093 Warsaw

We built in MATLAB/SIMULINK environment a realistic neuron model based on physiological data obtained from lamprey, which is one of the simplest vertebrates. The neuron model consists of one compartment with voltage-gated Na^+, K^+, Ca^+, $K_{Ca}{}^+$ channels. Na^+ and K^+ channels were modeled using H-H model, Ca^+ and Ca^+-dependent K^+ channels were modeled using Ekeberg's model and synaptic currents (NMDA, AMPA, $GABA_a$, $GABA_b$, $GABA_c$) were modeled using Destexhe's model. Parameters of the cell models were adjusted to match their activity as close as possible to the biological counterparts. We built a one segmental network, in which the rhythmic activity can be produced in an analogous manner like in large segmental network. The oscillator network was made of motoneurons, contralateral inhibitory interneurons, excitatory interneurons and lateral inhibitory interneurons. Except for their different synaptic effects and connectivity patterns, the main distinguishing feature was the cell size. Each neuron represented a population of functionally similar neurons in the real lamprey. This model can simulate the experimental results obtained from the lamprey.

The model of single neuron with the synaptic interactions has proven to be useful in the analysis of the segmental network for locomotion in the lamprey. The model is sufficiently general to be also useful for realistic simulation of other neural systems and will be used for building more complicated networks in future.

Supported by a statutory grant from the Nencki Institute.

29.2 INFLUENCE OF D-1 RECEPTOR AGONIST SKF 38393 ON POSTCOPULATORY DEPARTURE IN MALE RATS

Józef Beck[1], Michał Biały[1], Wojciech Kostowski[2,3]

[1]Department of Physiology and [3]Department of Pharmacology, Medical University of Warsaw, 26/28 Krakowskie Przedmieście, 00-927 Warsaw, Poland, [2] Department of Pharmacology and Physiology of the Nervous System, Institute of Psychiatry and Neurology, 1/9 Sobieskiego, 02-957 Warsaw, Poland

Eight male rats were tested in apparatus in which the goal compartment of runway was connected with start compartment by one-way door. In such apparatus after mount bout (the cluster of one or more copulatory events) male spontaneously left goal compartment containing tethered estrous female and new run starts. This phenomenon seems to be a result of competition between incentive value of stimulus female and runway. SKF 38393 in the doses 1-5 mg/kg s.c. significantly prolonged the time spent by male in the goal compartment while run latency and run duration remained unaffected.

In the separate experiment 9 male rats were tested in situation when the free access to female was allowed. In such situation SKF 38393 in the doses 1-2.5 mg did not influenced the ejaculation latency (the time between first intromission and ejaculation). Hence the prolongation by SKF 38393 the time spent by male rat in goal compartment cannot be a result of prolongation of ejaculation latency. It is suggested that this phenomenon is a result of interaction between primary and secondary rewards with dopamine-mediated signal at D1 receptor, produced by SKF 38393 .

29.3 THALAMO-CORTICAL CORRELATION OF BETA ACTIVITY IN DIFFERENT STATES OF ATTENTION

Marek Bekisz and Andrzej Wróbel
Nencki Institute of Experimental Biology, Warsaw, Poland

We have previously shown that local field potentials recorded in the lateral geniculate nucleus (LGN) and primary visual cortex (VCx) of cat contain significantly more beta (16-24 Hz) activity when the animal attends to visual than to auditory stimuli. In the present study we calculated crosscorrelation between envelopes of thalamic and cortical beta (16-24 Hz) activity in different attentional states. Except most peripheral thalamic recording (30° above the area centralis, a.c.) the thalamo-cortical beta envelope crosscorrelation functions showed clear central peak with maximum located within 25 ms time window around the centre of the crosscorrelograms. For all LGN (or perigeniculate nucleus) / VCx recording pairs which corresponded to the central representation of the visual field (5° from a.c.) correlation values obtained for visual trials were significantly higher than those calculated for the auditory task (n=11). The visual and auditory correlation values were, however, not different when one (thalamic or cortical) recording site of a pair was located 10° or farther above/below the representation of the a.c. (n=12). Some observations showed that intrathalamic correlation values were significantly different for visual and auditory attentional states: (i) visual correlation was stronger than auditory for central LGN / PGN recording pair, (ii) visual correlation was lower for central / peripheral LGN pair. Taken together our findings showed increased correlation of beta activity within cortico-thalamic neuronal loop participating in central vision during visual attention. We suggest that such correlated activity play an important role in coordinating different visual structures during attentional task.

29.4 ACOUSTIC STARTLE RESPONSE IN OPOSSUMS *MONODELPHIS DOMESTICA* AND WISTAR RATS

Janusz W. Blaszczyk and Krzysztof Turlejski
Nencki Institute of Experimental Biology, Warsaw. Poland.

Twenty three gray opossums from the breeding colony of the Nencki Institute of Experimental Biology and the same size group of Wistar albino rats were used in the experiment. The opossums were 3-5 months old and their mean body weigh was 77.9 $\pm$ 17 g. Their acoustic startle reactions (ASR) were compared with responses of young Wistar albino rats (mean body weight 98 $\pm$ 18g). The ASR testing was performed in a ventilated, double-walled sound-attenuating chamber (Coulbourn Instruments). The animals were placed on force platforms that recorded the vertical reaction force of the animal's startle response. A sequence of the acoustic stimuli (10-ms in duration white noise pulses at an intensity of 120 dB SPL) separated by a pseudo-random interstimuli interval, was presented to them. Startle parameters (the latency-to-peak and the peak amplitude) normalized to a body weight were computed online.

No significant sex differences in the normalized ASR amplitude were found within each group. Mean of normalized ASR amplitude was 0.29 $\pm$ 0.14 in the opossum group. The probability of the non-freezing response (exceeding 0.3) was only 23.7 %. In the same test the rats responded with significantly higher amplitudes ($t=-8.5$, $p<0.0001$). Their mean normalized amplitude of ASR was 0.81 $\pm$ 0.23 and the probability of nonfreezing response exceeded 69%. Thus it can be concluded that a tendency for freezing in response to an unexpected stimulus is a dominant future of opossum's behavior.

29.5 **PRINCIPAL COMPONENTS ANALYSIS (PCA) AS A METHOD FOR ASSESSING BEHAVIORAL DEFICITS IN OLD RATS**

Paweł Boguszewski, Jolanta Zagrodzka
Nencki Institute of Experimental Biology, 3 Pasteur St., 02-093 Warsaw, Poland

The aim of our study was to differentiate motivational factors influencing spontaneous behavior of young and old rats in open field test (OF) and Elevated Plus Maze (EPM) in order to exploit further the question of age-related changes in emotionality. Animals of the same strain bred in the same conditions formed two experimental groups – young adults (n=20) tested at the age of 4 months and old rats tested at the age of 24 months. Video based tracking system EthoVision was used for automated acquisition of OF and EPM data. Then the data were analyzed separately for young and old animals by Principal Components Analysis (PCA) with varimax rotation which ensures that the extracted factors are independent of one another and therefore reflect separate motivational processes. Our experiment demonstrated that in OF the behavior of rats is driven primarily by locomotor activity, however anxiety factor accounted for 31% of the variance in old rats while in young ones only for 21% of the variance. In EPM the behavior of old rats was characterized mainly by anxiety (factor 1 accounting for 52% of the variance), while in young animals factor 1 represented locomotor activity. PCA proved to be a very useful tool for the interpretation of tests' results. It allowed to move beyond the description of behavior to the definition of the different factors and underlying motivation to which they refer.

29.6 **HINDLIMB MOTOR DEFICITS AFTER PARTIAL SPINAL LESION IN RATS**

B. Chojnicka, T. Górska, W. Zmysłowski[1)]
Nencki Institute of Experimental Biology and [1)]Institute of Biocybernetics and Biomedical Engineering, Polish Academy of Sciences, Warsaw, Poland

Hindlimb motor deficits were analysed in rats subjected to two kinds of spinal lesions performed at the low thoracic level: I) a lesion of the dorsal columns or dorsal quadrants and II) a much larger lesion sparing solely the ventral funiculi or part of them. To evaluate the hindlimb motor deficits a battery of behavioural and some neurological tests were used. In animals from group I and II, the ability to support the body weight on the hindlimbs, and hence quadrupedal walking, returned spontaneously after 1 and 2-3 weeks, respectively. This was correlated with the reappearance of some prioprioceptive reflexes (response to strong displacement of the feet and limbs), and backward hindlimb placing to stimulation of the rear part of the body. The hindlimb locomotor functions successively improved and reached a relatively stable level after 3-4 (group I) and 6-7 (group II) weeks. However, up to the end of postoperative period (3-5 months) the animals from group II showed clear deficits such as walking on a broader base of support (footprint analysis) and missing the rungs with the hindlimbs when walking on a horizontal ladder. Group I animals showed negligible or less marked deficits. In both groups the tactile and tactile + prioprioceptive placing to stimulation of the feet were permanently absent. On the other hand, the noticeptive flexion reflexes were present from the first postoperative week. These results show that in the rat ventral funiculi or even part of them are sufficient to to permit recovery of the hindlimb locomotor activity, although the distal movements remain impaired.

29.7 **LOCOMOTOR CHANGES AFTER PARTIAL SPINAL LESIONS IN RATS**

B. Chojnicka, T. Górska, W. Zmysłowski[1)]
Nencki Institute of Experimental Biology and [1)]Institute of Biocybernetics and Biomedical Engineering, Polish Academy of Sciences, Warsaw, Poland

In rats with different partial spinal lesions, performed at the low thoracic level, varying from destruction of the dorsal columns to large lesions sparing only the ventral funiculi or part of them, various indices of overground locomotion were analyzed using the contact electrode method and/or emg recording of movements. Compared to the preoperative period the following changes were found: i) a decrease in the spontaneous speed of locomotion; ii) an increase in the step cycle duration, which was related to changes in the locomotor speed; iii) an increase in the stance phase duration particularly in the hindlimbs, which could not be accounted for by an increase in the step cycle duration. This increase was best evaluated by changes in the duty factor i.e. the ratio of the stance phase duration to the step cycle duration; iv) a decrease in the electrical activity of soleus muscles best estimated by the emg duty factor i.e. the ratio of the integral of rectified emg to the emg duration multiplied by the maximal emg amplitude. The magnitude of all these changes depended, in general, on the extent of the spinal lesion and the postoperative survival time. In all cases, the most sensitive indices of hindlimb impairment appeared to be the duty factor calculated from the stance to step duration and the emg duty factor. E.g. in rats with small and large lesions the decrease in the emg duty factors shortly after surgery could attain 20 and 60% of the preoperative values, respectively, the extent and speed of recovery being far greater in the former group. These results show that adequate methods of analysis can disclose gait deficits not previously recognised after partial spinal lesions in rats.

29.8 TWO ANTIDEPRESSANT DRUGS, TIANEPTINE AND FLUOXETINE, INFLUENCE THE RAT CENTRAL DOPAMINERGIC RECEPTORS-AUTORADIOGRAPHY AND *IN SITU* HYBRIDIZATION STUDY.

Daniel Dlaboga, Zofia Rogóż, Jerzy Maj, Marta Dziedzicka-Wasylewska
Departament of Pharmacology, Institute of Pharmacology, Polish Academy of Sciences, Smętna 12, PL. 31-343 Kraków, Poland

In the present study the effects of two antidepressant drugs (ADs) with opposite pharmacological profile; tianeptine (TIA 5 and 10 mg/kg *po*) a serotonin reuptake enhancer, and fluoxetine (FLU 10 mg/kg *po*), a serotonin reuptake inhibitor, on dopaminergic receptors were compared after single and repeated administration (twice daily for 14 days) in the rat brain. We have previously reported that TIA and FLU, administered repeatedly, potentiate behavioural effects evoked by dopamine receptor stimulants. The aim of present study was to establish whether TIA and FLU administered repeatedly induce changes at the level of dopamine receptors similar to those produced by tricyclic ADs.

This study used *in situ* hybridization to examine the effect of TIA and FLU on the levels of mRNA encoding dopamine D_1 and D_2 receptors and receptor autoradiography, using [^{3}H]raclopride and [^{3}H]spiperone (D_2/D_3 antagonists), [^{3}H]quinpirole (D_2/D_3 agonist), [^{3}H]7-OH-DPAT (D_3 agonist) and [^{3}H]SCH 23390 (D_1 antagonist). Dopamine receptors were studied in the nucleus accumbens (shell and core), caudate putamen and islands of Calleja.

The obtained results show that TIA and FLU administerd repeatedly decreased the level of D_1 mRNA in the nucleus accumbens shell but not in the other brain regions. The same effect was observed for D_2 mRNA expression in the nucleus accumbens shell and core (except for TIA 10 mg/kg). FLU also decreased the level of D_2 mRNA in the caudate putamen. Autoradiographic analysis showed that TIA and FLU after repeated administration increased [^{3}H]quinpirole binding in the nucleus acccumbens core and caudate putamen. In the islands of Calleja TIA and FLU decreased [^{3}H]quinpirole and [^{3}H]7-OH-DPAT binding. Binding of [^{3}H]SCH 23390 was also decreased in the nucleus accumbens following FLU (but not TIA) administration. [^{3}H]spiperone binding was not changed. In contrast, [^{3}H]raclopride binding was increased following TIA 5 and FLU 10 in the nucleus accumbens core and caudate putamen.

Relevance of the obtained results to the effects of other ADs will be discussed.

29.9 **SPATIALLY STRUCTURED SYNCHRONIZED INTRACORTICAL ACTIVITY IS AVERAGED IN EEG SCULL RECORDINGS**

A. Ghazaryan, M. Bekisz, M. Wypych and A. Wróbel
Nencki Institute of Experimental Biology, 02-093 Warsaw, Poland

Our previous experiments revealed that attentive state of the visual cortex is characterized by an elevated amplitude within beta band (16-24 Hz) of fast Fourier transform functions (FFTs) calculated from signals recorded by means of electrodes implanted at the depth of layer IV in different regions of the visual cortex (VCx) (Bekisz and Wróbel, 1993). We have further found (Krakowska et al., 1995) that correlation coefficients (CC) of the frequency filtered beta signals calculated between pairs of simultaneous LFPs were in most cases lower than 0.75 in not aroused cortex and decreased further during visually attentive situation. In opposite, when CC exceeded 0.75 (a few remaining cases) it grew further in aroused VCx proving that attention-related activation may be organized in a specific functional pattern. Present experiment carried on attention-related changes in human EEG showed that the amplitude of the beta-frequency spectrum registered from occipital electrodes decreased with increased visual attention. We therefore hypothesized that EEG electrodes tended to average EEG signals from a large cortical area of the occipital cortex resulting in increased FFT amplitude during synchronized beta activity and decreased EEG power during periods of spatially structured activity. In the next experiment we recorded in parallel from EEG-type electrodes placed on the cat's skull and from many intracortical chronic microelectrodes. As expected, the highly structured activity within VCx was accompanied by decreased beta activity within EEG signal averaged from the cat's skull.

29.10 **THE POST-MORTEM CONCENTRATION OF GLUTAMATE IN THE CEREBELLUM OF RAT AS AN EXPONENT OF SHORT AVERSIVE SENSORY STIMULATION PRECEDING DEATH**

Gos T, Hauser R, Krzyżanowski M

Institute of Forensic Medicine,
Medical University of Gdańsk, Poland

The problem of post-mortem assessment of short central fear reaction preceding death has been obscure till now. The preliminary results obtained by authors have encouraged them to further research. Time of aversive sensory stimulation in the study present was considerably decreased to 15 s. The different modalities of sensory stimulation were applied. The dominant role of the mechanical stimulus was suggested in observed reaction as measured by glutamate concentration increase in the whole cerebellum homogenate. By using presented experimental paradigm a possible application of the biochemical assessment of human brain tissue would be developed in forensic pathology in future. Such an evaluation of biochemical „frozen frames" of neurotransmission could help in the reconstruction of events just before sudden and violent death.

29.11 **REGIONAL DISTRIBUTION OF GLUTAMATE IN THE CENTRAL NERVOUS SYSTEM OF RAT TERMINATED BY CARBON DIOXIDE EUTHANASIA**

Gos T, Hauser R, Krzyżanowski M

Institute of Forensic Medicine
Medical University of Gdańsk, Poland

The CO_2 euthanasia is an established method of termination of small laboratory animals. It was also applied by authors in the previous neurobiological research based on the post-mortem glutamate concentration assay in the structures of rat brain. The presented investigations were aimed to optimize the termination procedure based on CO_2 saturation rate of the inhaled air. The two rates of CO_2 flow were applied and the higher one significantly augmented the glutamate level in the the hippocampus and cerebellum. A possible relation of the observed phenomenon to the central fear reaction was discussed. The potential value of the lower CO_2 flow in the euthanasia procedure was pointed out.

29.12 **HEREDITY OF RESISTANCE TO PESTICIDES IN COCKROACHES**

Barbara Grajpel[1], Ewa Kiełbasiewicz[2], Eugenia Tęgowska[1] Maria Stankiewicz[2]
[1]Department of Animal Physiology, [2]Department of Biophysics, N. Copernicus University, Toruń,

It has been known that ion channels of cell membrane are the major target sites of action of several insecticides. Ion channels are ubiquitous and play crucial role in generation of resting and action potentials as well as in transmitter release. Therefore, dysfunction of ion channels results in changes of organism's functions at both cellular and behavioural levels. The aim of the present study was to compare the effect of a pyrethroid (Bulldock EC-preparation contains 2.5% beta-cyflutrin as bioactive substance) on thermal behaviour and muscle resting potential in two succesive generations of cockroaches. Changes of selected ambient temperature were monitored during 24 h using a temperature gradient system. Thermal behaviour was automatically recorded by means of a photocell detector. The resting potential was recorded from dorso-ventral muscles *in situ* using the conventional microelectrode technique. After application of pyrethroid cockroaches of the first generation preferred much higher ambient temperature (by about 10°C) and their muscle resting potential was significantly lower (by about 9mV) in comparison with untreated control group. These changes are probably due to disturbances of the function of sodium channels induced by synthetic pyrethroides. Surprisingly, the parameters of resting potential and thermal behavior in insects of the second generation were similar to the respective parameters found in untreated insects. These results suggest that insects can gain hereditary resistance or tolerance to pyrethroid.

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29.13 BEHAVIOURAL AND PHYSIOLOGICAL MECHANISMS OF DEFENSE AGAINST HYPOXIA IN COCKROACHES

Barbara Grajpel, Przemysław Grodzicki, Michał Caputa
Department of Animal Physiology, N. Copernicus University, Toruń

Anapyrexia, i.e. defended drop in body temperature, is a general, protective response exhibited by both invertebrates and vertebrates. The anapyrexic response improves the animal's ability to survive or resist the environmental insult, such as hypoxia. In poikilotherms anapyrexia manifests itself as a reduction in selected ambient temperature. The aim of the present study was to examine the thermal behaviour of cockroaches under both hypoxic and anoxic conditions. The effect of behavioral changes of body temperature on muscle resting potential was also studied. Changes of selected ambient temperature under control, hypoxic (10% O_2) and anoxic conditions were monitored during 1 hour using a temperature gradient system. Thermal behavior was automatically recorded using a photocell detector. A part of insects was exposed to hypoxia at ambient temperature fixed at a level typical of normoxic insects. In each experimental group the resting potential was measured. The measurements were performed *in situ* on dorso-ventral muscles. The conventional microelectrode technique was used. Under both hypoxic and anoxic conditions insects preferred significantly lower ambient temperature (by 8°C and 11°C, respectively) than during control normoxia. The resting potential measured immediately after one hour of hypoxic treatment was significantly reduced in insects prevented from selecting ambient temperature. However, if insects could select low ambient temperature their resting potential was hardly influenced by hypoxia. In conclusion, behavioural anapyrexia protects insect's muscles from hypoxic damage.

29.14 EFFECTS OF LOW DOSE D1 AND D2 DOPAMINE RECEPTOR BLOCKERS ON SPATIAL MEMORY IN RATS

Ewa Jakubowska-Doğru and Başak Alsaç
Dept. Of Biology, Middle-East Technical University, Ankara, Turkey

The present study examined the effects of preferential D1 and D2 dopamine (DA) receptor blockers, SCH 23390 (i.p. 0.015 mg/kg) and raclopride (i.p. 0.125 mg/kg), on spatial memory in rats. Reference memory was assessed by rats' performance in partially baited, 12-arm radial maze, while working memory was tested in the delayed nonmatching-to-position task (DNMP), carried out with three different delays: 1, 5, and 10 min. Additionally, the interaction of DA receptor antagonists with non-specific DA agonist, apomorphine hydrochloride (i.p. 5.0, 2.5, and 1.25 mg/kg) was investigated. Neither D1 nor D2 DA receptor antagonist was effective in significantly altering radial maze choice accuracy in the course of task acquisition. In DNMP task, raclopride administration had no effect on the rat's performance. In contrast, SCH 22390 caused significant deterioration in the task solving but only under longer delays of 5 and 10 min. These results confirmed involvement of DA D1 but not DA D2 receptor in the spatial working memory. Drug combinations remained with no effect on the rat's performance in the radial maze, however, apomorphine administration compensated for the adverse effect of SCH 23390 in the DNMP task. Interestingly, two higher doses of apomorphine produced pronounced oral stereotypy, and in few cases circling behavior, but only when apomorphine was combined with raclopride. Conversely, joint administration of SCH 23390 with apomorphine caused profound slowing of animal responding.

29.15 NEGATIVE VS POSITIVE IMPLICIT PRIMES EXPOSED IN LVF, CVF, RVF AND EXPLICIT EVALUATIONS

Maria Jarymowicz, Dorota Karwowska, Dorota Kobylińska, Katarzyna Piotrowska
Faculty of Psychology, University of Warsaw

Research question. The research in affective priming paradigm (Murphy & Zajonc, 1993; Ohme & Jarymowicz, 1999) show that the implicit affect (elicited by suboptimal affective priming) influences the conscious judgments about the neutral target stimuli. We decided to check what is the influence of affect on conscious judgments, when the affective stimuli is addressed to the left cerebral hemisphere, or to the right one, or to both of them. Hypothesis 1 predicted that suboptimal affective stimuli exposed in right visual field (RVF) will cause more positive evaluations of primed neutral stimuli than affective stimuli exposed in central or left visual field (CVF or LVF). Hypothesis 2 predicted that the effect mentioned in Hypothesis 1 will be stronger under the influence of negative primes exposed in LVF, but positive primes exposed in RVF. **Method**. Four experiments in affective priming paradigm were conducted. Neutral target stimuli (Japanese ideographs) were suboptimally primed by photographs of faces expressing joy or disgust. In Experiment 1 the exposure duration of primes was 4ms, in Experiments 2-4: 16 ms. Primes were exposed either in LVF or in RVF or CVF. Targets stimuli were always exposed in CVF. Subjects were requested "to say how negative/positive is the character trait that is represented by a given ideograph. **Results**. The hypotheses were no confirmed. In four experiments there were no differences between the evaluations of ideographs primed by either positive or negative stimuli exposed in LVF or RVF. The influence of VALENCE OF PRIMING was only detected when the priming stimuli were exposed in CVF.

29.16 DANGER AND SAFETY SIGNALS DIFFERENTIATE HIPPOCAMPAL THETA ACTIVITY IN CLASSICAL CONDITIONING IN RATS

Piotr Jeleń, Urszula Sławińska, Stefan Kasicki
Dept. Neurophysiology, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Pasteur 3 Street, PL 02-093 Warsaw

The effect of fear and relief from fear on hippocampal deep EEG activity was studied in partially restrained adult male rats implanted with chronic electrodes. The emotional state of animals was influenced using Pavlovian aversive conditioning procedure, during which the EEG was recorded. In excitatory trials a 5s signal of danger (DS, light) preceded tail-shock and was used to evoke the state of conditioned fear. In inhibitory trials a signal of safety (SS, tone) overlapped the last 3s of DS. It predicted an omission of the expected aversive event and served to inhibit the conditioned fear and to evoke the state of relief. In order to compare the EEG activity during pre-DS, DS and DS/SS periods we analyzed the power spectra of EEG signal in 0-50 Hz frequency band. During the 5s pre-DS period the delta band (0-2 Hz) dominated, but the theta rhythm was also present. In reaction to DS exposure the theta peak amplitude increased and the delta maximum was lowered. Presentation of SS resulted in increase of frequency and amplitude of the theta rhythm. Moreover, there was an additional maximum in the range of 13-15 Hz, which was absent in the DS situation. Our results clearly demonstrated differentiation of hippocampal EEG activity in opposite emotional states.

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29.17 THE PERCEPTION OF TEMPORAL ORDER FOR AUDITORY AND VISUAL STIMULI

Kanabus, M., Szeląg, E., Rojek, E., Weker, M.M.
Department of Neurophysiology, Nencki Institute of Experimental Biology, Warsaw, Poland

A number of psychological studies has suggested that the temporal order (TO) of two short auditory stimuli can be identified if their onsets are separated by a temporal gap of at least 20-40 ms. That can be explained by oscillations with frequencies in the range of about 30 Hz which create temporal system states.

The aim of the present study was to investigate whether the similar mechanism controls identification of the order for auditory and visual stimuli. We tested 12 right-handed students aged 20-25 years. The task was to identify the TO of two 300 and 3000 Hz tones of 15 ms duration exposed binaurally with interstimulus intervals (ISI) of 5, 10, 20, 40, 80, 150, 300 and 500 ms or of two diodes (red and green) presented at the same location. Both the stimulus duration and ISIs in the visual task were exactly the same as those applied in the auditory task. The responses were given by pressing the two buttons in order of stimulus presentation. The data showed no difference in the general level of performance between the two modalities and the criteria of 75% correct were reached at a 40 ms ISI or longer. For shorter ISIs percent of correct was systematically lower for visual than for auditory modality, especially, at a 5 ms ISI where this difference proved significant.

The results suggest that although the TO can be correctly identified for ISI of about 40 ms independently of the modality, for shorter ISI it is more difficult to identify TO of visual than auditory stimuli probably because of the different kind of transmission at the level of receptive cells for vision and audition.

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29.18 THE EFFECT OF SELECTED TOXICANTS ON CERTAIN ASPECTS OF BEHAVIOUR AND MUSCLE RESTING POTENTIAL IN COLORADO POTATO BEETLE

Katkowska, M.J.
Department of Animal Physiology, N. Copernicus University, Toruń, Poland

The aim of this study was to determine the susceptibility of Colorado potato beetles to sodium azide and sodium m-arsenite, and a combination of KCN with one of them, both when the insects were injected either with one of these compounds or with their combination (control insects were injected with saline) and when they were fed on potato leaves sprayed either with one of toxicants or with above mentioned combination two of them. The feeding and general activity of insects as well as their mortality were assessed. In addition, the muscle resting potential (RP) was recorded both in insects fed on contaminated leaves and in those kept on normal diet, whose muscles had been treated directly after their exposure with tested agents introduced into physiological saline, to compare the effect of toxicants acting upon insects different way.

The effect of toxicants on muscle RP was not marked in beetles. The only exception were insects fed on arsenite+KCN-treated leaves.

Insects injected with saline or with arsenite showed at once usual activity and the most often started feeding after 18 or 3-4h, respectively. The amount of food eaten by treated insects was up to 48h about the same as that eaten by control insects, decreasing with time. 33.3% insects survived for 5 weeks. Most of beetles injected with azide seemed to be paralysed just after the injection (up to 20-30 min), then they recovered showing the activity similar to that of control ones, their feeding activity was even higher up to 72h. The mortality was 66.6% after 6 days. Insects injected with KCN+azide showed hyperexcitability for 15-25 min but soon started to behave as control ones, only eating more for 3 weeks. 91.66% beetles survived for 5 weeks. Insects injected with KCN+arsenite, first looked excited but soon (in 30 min) started to behave as control ones but during 4h their activity decreased (no food ingestion) and none of them survived 24h. Insects fed on contaminated leaves, in general, ate less food than the control ones (or avoided eating intoxicated leaves, e.g. azide-treated).

29.19 **IMPLICIT AFFECTIVE PRIMES EXPOSED TO RVF, CVF, LVF AND EXPLICIT EVALUATION**

Dorota Kobylińska, Dorota Karwowska, Katarzyna Piotrowska
Faculty of Psychology, University of Warsaw

Research question. The research in affective priming paradigm (Murphy & Zajonc, 1993; Ohme & Jarymowicz, 1999) show that the implicit affect (elicited by suboptimal affective priming) influences the conscious judgments about the neutral target stimuli. We decided to check whether the influence of affect on conscious judgments is different when the affective stimuli is addressed to the left cerebral hemisphere, or to the right one, or to both of them. The hypothesis predicted that suboptimal affective stimuli exposed in right visual field will cause more positive evaluations of primed neutral target stimuli than affective stimuli exposed in central or left visual field. **Method**. Four experiments in affective priming paradigm were conducted. Neutral target stimuli (Japanese ideographs "representing different character traits") were suboptimally primed by photographs of faces expressing joy or disgust. In Experiment 1 the exposure duration of primes was 4 ms, in Experiments 2-4 the duration was 16 ms. Primes were exposed either in LVF or RVF or CVF accordingly. Target stimuli were always exposed in CVF. Subjects were requested "to say how negative/positive is the character trait that is represented by a given ideograph". **Results**. The data didn't confirm the hypothesis. In all of four experiments the main effect of VISUAL FIELD was detected. The data show that evaluations of ideographs are significantly more positive, when they are primed by suboptimal affective stimuli exposed in either LVF or RVF, than when they are primed by stimuli exposed in CVF.

29.20 **THYROLIBERIN DIALYSED INTO THE HIPPOCAMPUS IMPROVES MEMORY PROCESSES IN RABBIT**

P.Kolodziejski, B.Naziemblo, M.Orlowska-Majdak, W.Z.Traczyk
Department of Experimental and Clinical Physiology, Institute of Physiology and Biochemistry, Medical University of Lodz, Poland

Thyroliberin (TRH) and its receptors are widely distributed in the central nervous system, where it may act as neuromodulator (Winokur and Utiger 1974). It reversed experimentally evoked amnesia in mice (Yamazaki et al. 1983, 1985). Moreover TRH enhanced long-term potentiation in guinea pig hippocampal slices (Ishikawa et al. 1991). Hippocampus is thought to be the site of TRH action on memory processes. Eyelid conditioning has been used to study influence of TRH on learning processes in rabbit. Rabbits underwent 5 days of acquisition and then 5 days of extinction training. Eyeblink responses were detected by photoelectric transducer and recorded. Then each animal was stereotaxically implanted with microdialysis probe into the hippocampus. Acquisition training was perform simultaneously with 0.9% NaCl dialysis, and extinction training with TRH dialysis of the hippocampus. Four concentrations of TRH were successively applied: 0.05; 0.5; 5.0 and 50.0 μg/ml with the rate of 1 μl/min. Percentage of conditioned responses observed during acquisition training after TRH treatment, achieved greater values, as compared with control (0.9% NaCl). Data obtained during extinction with simultaneous TRH treatment had the greater values than the control either, especially from the 3rd to the 5th day of training. The effect was highly significant and dose dependent.

It is concluded, that TRH dialysed into the hippocampus restrains process of forgetting of the learned task and improves process of succeeding learning.

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29.21 **BIOLOGICAL METHODS OF FIGHTING AGAINST PESTS BASED ON THEIR SENSORY ABILITIES**

Korpała Małgorzata, Tyczyński Marek
Dept. of Animal Physiology, N. Copernicus Univ., Toruń, Poland

Injurious insects are important factors threatening various cultures. Some of them attack plants and food stores in all developmental stages. Other pests exist in the museum relics, library collections and archives, where they cause losses. Therefore, pesticides are used to regulate the density of pest's population. But some pesticides such as organophosphate and carbamates are very toxic both for invertebrates and vertebrates. Considerably less toxic are pyrethroids but they also attack useful insects. Pyrethroids modify sodium channels and by this way they influence cell membrane excitability which is the main cause of insects death. Chemical methods of fighting against pests are very often dangerous. So new safe methods based on reception of sensory stimuli, i.e. chemo- and photoreception, are indispensable. Chemoreception plays a great role in the life of insects. This method is based on various kinds of traps or scaring away (repellents). In the present study we used an olfactometrical method. The insect's reaction to light in phototaxic behaviour is body orientation to the light. Reactions depend on the light intensity, wavelength and the rate of transformation of the photopigments of the insects eye and the developmental stage. Colour vision or wavelength discrimination means the ability to distinguish between spectral lights of different wavelengths independently of their intensity. The light of specific wavelengths, which is absorbed in the rhodopsin, gives the spectral eye sensitivity. Insect's eye responses are different in various species, but for many of them the most attractive is UV, the blue-green light and sometimes the yellow, too. The beetle *Stegobium* was tested in a photometr consisting of 1 m long tunnels with different colour lights at their ends. Beetles could choose the most attractive colour and approach to it. They usually selected the blue light.

29.22 **INFLUENCE OF VENTRAL SUBICULUM LESION ON INFORMATION FLOW IN LIMBIC-MOTOR CIRCUITRY ASSESSED BY EEG STUDY IN FREELY MOVING RAT**

Anna Korzeniewska*, Iwona Wiśniewska, Stefan Kasicki
Neurophysiology Dept., Nencki Institute of Experimental Biology, Polish Academy of Sciences, Pasteur 3 Str., Warsaw 02-093, Poland

To study an involvement of structures engaged in limbic-motor integration processes we investigated the information flow in the circuit of the basolateral amygdala (BLA), ventral subiculum (VSB), n. accumbens (ACC) and subpallidal area (SPL). The involvement of VSB in this circuitry was investigated by analysis of the EEG activity recorded from above structures during various behavioral states in normal rats and after VSB lesion. All EEG signals were taken into account simultaneously and the directions, intensities and spectral characteristics of the information flow within the studied circuit were calculated for normal and lesion animals by use of a newly created function, combining the directed transfer function (DTF) and partial coherences. Comparison of flows during situations with various motivational/emotional aspects showed that lesion of VSB caused significant changes in the information flow patterns among preserved structures in respect to normal rats. The differences in flows accompanying exploration and regular locomotion appeared for frequencies lower than 8 Hz or higher than 60 Hz. The flows in situation with stressful stimulus or without clear emotional component became similar. During locomotion performed in difficult task, lesion of VSB changed information flows between structures, which have been left intact, especially in frequency ranges of 8-10 Hz, 21-24 Hz and 33-42 Hz. Summarizing, although the investigated behavior of lesion animals did not visible change, the information flow patterns among remained structures were significantly different.

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29.23 **Brain monoamines and GABA alterations evoked by the light-dark and shelter-seeking tests in rats.**

Koprowska, M., Krotewicz, M., Romaniuk, A., Strzelczuk, M. and Wieczorek, M.

Dept. of Neurophysiol., Univ. of Lodz, 66 Rewolucji 1905 r st, 90 – 222 Lodz. E-mail: marek@taxus.biol.uni.lodz.pl

Alterations of monoamines (NA, DA, 5-HT) their metabolites (MHPG, DOPAC, HVA, 5-HIAA) and GABA in the hypothalamus, midbrain central gray matter, amygdala and hippocampus were measured in rats exanimate in the light-dark (LDC test) and in the shelter-seeking test (SS test). In the rats examined in the LDC test HPLC analysis demonstrated a significant increase of NA, MHPG, 5-HT and 5-HIAA concentrations in all investigated brain regions, and DOPAC and HVA levels in the hypothalamus and amygdala. In the rats examinated in the SS test a significant increase of MHPG level occurred in the midbrain central gray matter, amygdala and hippocampus. Simultaneously, in the hypothalamus, amygdala and hippocampus a significant decrease of GABA level occurred in SS tests. The results indicate that neurochemical basis for anxiety drive is above all a coincidental increase of NA and 5-HT metabolism, as well as GABA level reduction in the key "emotional" brain regions, i.e. hypothalamus, midbrain central gray matter, amygdala and hippocampus. The reasons for some different neurochemical effects observed after the LDC test and SS test are discussed.

29.24 **Effects of 8-OH-DPAT administration on the social behavior and regional brain monoamine distribution in the submissive cats**

Krotewicz M., Koprowska M., Strzelczuk M., Romaniuk A. and Wieczorek M.

Department of. Neurophysiology, University of Łódź 66 Rewolucji 1905 r., 90-222 Łódź, Poland

The neurochemical data obtained in our previous studies showed that a submissive position in the social hierarchy was mainly caused of a very close interaction between the noradrenergic and GABA-ergic systems in central regulation of anxiety. In the present study behavioral parameters as well as regional brain concentration of monoamines (NA, DA and 5-HT), their metabolites (MHPG, DOPAC, HVA and 5-HIAA) and GABA in the hypothalamus, amygdala, hippocampus, midbrain central gray matter and frontal cortex were measured after administration of 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT) to submissive animals. Administration of 8-OH-DPAT reversed of hierarchy and increased fight against dominant cats for getting a good position to attack a mouse in submissive animals. Simultaneously, neurochemical data showed a significant reduction of NA concentration in the anterior hypothalamus. Additionally, a significant increase in GABA content occurred in the hippocampus and amygdala. These results confirm the control role of NA and GABA in the formation of social hierarchy.

29.25 **PROJECTION FROM THE AMYGDALOID COMPLEX TO THE CLAUSTRUM IN RAT**

Katarzyna Majak, Maria Pikkarainen, Samuli Kemppainen, Esa Jolkkonen, Asla Pitkänen

Epilepsy Research Laboratory, A.I.Virtanen Institute for Molecular Sciences, University of Kuopio, Finland

Recent studies have implicated that the claustrum is one of the relay stations that spreads epileptiform activity from the temporal lobe, including the amygdala, to the other brain areas. To investigate the projections we iontophoresed Phaseolus vulgaris-leucoagglutinin into various amygdaloid nuclei in 101 rats and detected immunolabeled terminals using immunohistochemistry.

The magnocelullar division of basal nucleus projected moderately to the dorsal aspect of the rostral two-thirds of the claustrum. The parvicelullar and the intermediate divisions provided a light projection to the same zone. The parvicelullar division of accessory basal nucleus and the lateral division of amygdalohippocampal area projected lightly to the most caudal aspect of the claustrum. Only single fibres with varicosities were emanated to the caudal two-thirds of the claustrum from different divisions of the lateral nucleus. The anterior cortical nucleus, the periamygdaloid cortex, the posterior cortical nucleus and the central nucleus of the amygdala did not project to the claustrum.

The amygdaloid complex sends sparse projections to the claustrum which mainly originate in the deep amygdaloid nuclei, particularly, the basal nucleus. These projections target the portion of the claustrum that has bilateral, reciprocal connections with the motor and somatosensory cortices. These data provide an anatomic background for the observations suggesting that the claustrum is a candidate brain area via which the seizures of amygdaloid origin may become secondarily generalized.

29.26 REBOXETINE ADMINISTERED REPEATEDLY INFLUENCES THE CENTRAL DOPAMINERGIC SYSTEM IN THE RAT

Wojciech Margas, Zofia Rogóż, Jerzy Maj, Marta Dziedzicka-Wasylewska
Department of Pharmacology, Institute of Pharmacology, Polish Academy of Sciences, Smętna 12, PL. 31-343 Kraków, Poland

Reboxetine (REB) is a new potent antidepressant drug (AD), selective noradrenaline reuptake inhibitor with no affinity for serotonin or dopamine transporters. In contrast to typical tricyclic ADs, REB shows no affinity for various central neurotransmitter receptors nor does it inhibit the rat brain monoaminooxidase A or B. The clinical efficacy of REB is comparable to that of typical tricyclics (like desipramine or amitriptyline).

Our earlier studies showed that ADs administered repatedly, but not in a single dose, potentiated behavioral effects (locomotor hyperactivity) evoked by dopamine stimulants, such as D-amphetamine, quinpirole or 7-OH-DPAT. Those findings indicate that ADs given repeatedly activate the dopaminergic system. Further support to this concept comes from the biochemical studies, which show that repeated administration of ADs increases the binding of ligands specific for dopamine D_2 and D_3 receptors in various regions of the rat brain.

The present study was aimed at determining whether REB evokes, when given repeatedly, the changes similar to those induced by tricyclic drugs. To this end, we administered REB (10 or 30 mg/kg *po*) acutely or repeatedly, i.e. twice daily for 14 days) and studied the behavioral response of rats to the agonists of dopamine D_2 and D_3 receptors. The obtained results showed that REB administered repeatedly (30 but not 10 mg/kg) increased the rat locomotor hyperactivity induced by D-amphatamine or 7-OH-DPAT (but not by quinpirole), measured at 24 h after the last dose.

In biochemical experiments we used the autoradiography of dopamine receptors with the radioligands of various specifity: [^{3}H]raclopride (D_2/D_3 receptor antagonist), [^{3}H]quinpirole (D_2/D_3 receptor agonist) and [^{3}H]7-OH-DPAT (D_3 receptor agonist), as well as *in situ* hybridization to measure the level of mRNA coding for dopamine D_1 and D_2 receptors in different regions of the rat brain. Biochemical data indicate that neither acute nor repeated treatment with REB (10 or 30 mg/kg) induced any statistically significant alterations in the binding or expression of dopamine receptors in the rat brain.

The behavioral and biochemical effects of REB will be discussed in comparison to the effects induced by typical tricyclic ADs.

29.27 **PROJECTIONS OF NEURONES LOCATED IN SACRAL SPINAL CORD SEGMENTS TO THE CEREBELLUM AND THE INFERIOR OLIVE IN THE CAT**

Włodzimierz Mrówczyński, Piotr Krutki

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

Dual projections of sacral neurones (S1/S2 segments) of the spinal cord ascending to the cerebellum and the inferior olive were electrophysiologically investigated in 3 adult cats under α-chloralose anaesthesia. Antidromic action potentials were recorded extracellularly from 19 cells following stimulation of their axons in both the contralateral restiform body (coRB) and contralateral dorsal accesory olivary nucleus (coDAO). Two groups of investigated cells were distinquished in the grey matter of S1/S2 segments: one distributed in the medial part of Rexed's lamina VII (n=6), the other located in lamina VIII (n=13). Axons of all neurones identified ran contralaterally in lateral funiculi of the spinal cord. Two patterns of axonal projections of sacral neurones have been demonstrated in this study. 14 neurones were found to ascend dually to coRB and coDAO, while 5 cells projected to coDAO only. Axonal conduction velocities of neurones investigated were comprised in the range 32 - 55 m/s. The presented results lead to the conclusion that dual ascending projections enable transmission of sensory information from the hind limb to the cerebellum through two parallel tracts - the one direct and the other indirect - with a relay in the DAO.

29.28 **EFFECT OF LY 367385 ON BEHAVIORAL ACTIVITY IN RATS.**

Agnieszka Nadlewska, Halina Car, Konstanty Wiśniewski

Department of Pharmacology, Medical Academy of Białystok, Mickiewicza 2c, PL 15-222 Białystok, Poland

(+)-2-Methyl-4-carboxyphenylglycine (LY 367385) is a potent and selective antagonist of 1a metabotropic glutamate receptor (mGlu1a), linked to phosphoinositol phosphate hydrolysis.

LY 367385 induces long term changes in the expression or function of mGlu receptors, it is neuroprotective and may have analgesic properties.

In this study we estimated the influence of the blockade of mGlu1a on behavioral activity in male Wistar rats, using tests: the open field test, passive avoidance response and elevated "plus" maze.

LY 367385 was administered intracerebroventricularly (icv) in a dose of 100 nmol 30 min before all tests.

In the open field test LY 367385 significantly increased the number of crossings and rearings. It significantly improved consolidation, but it impaired retrieval in the passive avoidance situation.

LY 367385 significantly shortened time spent in closed arms and prolonged time spent in open arms; it increased number of entries into closed and open arms also in elevated "plus" maze.

These results indicated that the antagonist of mGlu1a - LY 367385 (100 nmol icv) enhanced locomotor activity, improved consolidation, but impaired retrieval processes and had anxiolytic activity.

29.29 CHANGES INDUCED BY CHRONIC STRESS IN RATS – THE INFLUENCE OF MAO-A INHIBITORS

Nowakowska E., Chodera A., Kus K.
Department of Pharmacology Karol Marcinkowski University of Medical Sciences, 10 Fredry, 61-701 Poznań, Poland

Studies on animals have shown that chronic stress is able to evoke behavioural changes such as locomotor activity deficit, decreased sleep, reduced food and water consumption and impaired memory. Chronic stress produces changes in concentrations of neurotransmitters, mainly in the hippocampus. The hippocampus is a vulnerable brain structure that is involved in learning and memory functions. In this study we investigated the effects of chronic stress procedure and moclobemide in rats, and the influence of chronic stress on the levels of monoamines (NE, DA,5-HT) in rats hippocampus (as well as their metabolites). It was found out that chronic 16-day stress caused worsening of memory: the well trained rats after stress procedure lost their ability to find food quickly. Because of many errors on the way, the time these animals needed was 5-times longer than that of the control group. One-off as well as prolonged (21 days) treatment with moclobemide (10 mg/kg/day) counteracted the deficit of memory induced by chronic stress. In stressed animals we observed increase of DA, decrease of DOPAC, 5-HT and 5-HIAA and decrease of NE levels. Moclobemide modulated the changes in the levels of neurotransmitters in the hippocampus, decreasing their turnover. The results demonstrate that moclobemide improves memory impaired by stress. They suggest also that moclobemide has a modulatory effect on stress induced neurotransmitter changes which may be of importance in the protective effect of the drug with regard to memory impairment.

ECS CHANGE BEHAVIORAL EFFECT BICUCULLINE. 29.30

Oksztel Robert, Car Halina, Wiśniewski Konstanty.
Department of Pharmacology, Medical Academy, Mickiewicza 2c, PL15-222 Białystok, Poland.

Bicuculline – selective antagonist modulation of GABA-A receptor activity causes changes in behavioural activity. Electric convulsions induced in the experiment (ECS) interfere in the GABA system and the functions of GABA receptor. The use of GABA receptor antagonist (bicuculline) in Wistar rats was to estimate its influence on locomotor activity in the open field test as well as the processes of consolidation and retrieval, evaluated in the test of passive conditioned reflexes. Activity against anxiety in elevated "plus" maze test in physiological state and after amnesia induced with single nervous shock (ECS) were also estimated. Bicuculline improves the process of passive conditioned reflex retrieval, both in physiological state and amnesia. It weakens the process of consolidation. The process of retrieval is inhibited after ECS. Animals after ECS present increased motility, inhibited by bicuculline in the state of amnesia. Bicuculline prolongs the time of sojourn in open arms and shortens in closed (anxiolitic activity). Bicuculline after ECS lowers the time and quantity of the entrance in closed and open arms. ECS levels the effect of bicuculline and diminishes the number of entrances in closed arms. Maintenance of profitable effects of bicuculline on retrieval process despite induced amnesia will be essential regarding clinical implication enabling practical use.

29.31 EFFECTS OF VASOPRESSIN (AVP) HIPPOCAMPAL MICRODIALYSIS ON EYELID CONDITIONING IN RABBIT

M.Orlowska-Majdak, P.Kolodziejski, W.Z.Traczyk
Department of Experimental and Clinical Physiology, Institute of Physiology and Biochemistry, Medical University of Lodz, Poland

The physiological involvement of AVP in learning and memory is up to now a controversial issue. The rabbit's classically conditioned eyelid response is one of the model systems for studying associative learning. In white male rabbits microdialysis probe (CMA/Microdialysis) was implanted into the hippocampus (Traczyk et al.1977) and AVP was perfused through the probe with the rate of 1 µl/min. in concentration of 0.05, 0.5, 5.0 and 50.0 µg/ml during the 5 successive days of conditioned reflex extinction. Conditioned responses - eyelid closures were monitored by the photoelectric transducer acting in close infrared and registered during acquisition and extinction procedure (M.Orlowska-Majdak et al. in press).Two-way ANOVA followed by the LSD test was used to compare percentage of responses in the control (0.9% NaCl as dialysis medium) and in AVP groups of variables, separately for acquisition and extinction procedure. The course of the both processes during 5 successive days of training was also analysed. It was noticed that AVP dialysed into the hippocampus in concentrations of 0.05 and 0.5 µg/ml significantly restrained extinction in rabbits, especially during the 3rd – 5th day of training. The tendency to diminishing the level of acquisition was also observed following AVP dialysis in concentrations of 0.05 and 0.5 µg AVP/ml.

It is postulated that hippocampal AVP microdialysis restrains process of forgetting of the learned task and diminishes the level of succeeding learning.

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BEHAVIOURAL EFFECTS OF A PYRETHROID CYPERMETHRIN IN FROGS (*Rana temporaria*). 29.32

Przybylska Ewa, Tyczyński Marek
Dept. of Animal Physiology, N. Copernicus Univ., Toruń, Poland

Pest insects are factors threatening various cultures. Radical restriction of their numbers by insecticides is commonly accepted. Recently very often pyrethroids are applied. The mechanism of their action depends on blockade of sodium channels in membranes of nervous cells in open position. Such a disturbance leads to a general stroke finishing with death. Experiments were carried out on 12 frogs exposed to following conditions: control; toxication; detoxication I (D1) 2 days after the toxication; detoxication II (D2) 5 days after D1 and detoxication III (D3) 5 days after D2. The data were recorded and analysed by computer method. The pyrethroid cypermethrin was given in a form of a drop on the skin at 0.07 µg dose dissolved in a 8.6 µl of solution (i.e. 2.37 ng per 1 g of body weight). Effects of the pesticide were studied using recording of thermal preference of the frogs in a temperature gradient system with a series of thermoelements, connected in pairs to infrared sensors and motor activity of the animals was detected using a ultrasonic system. Breathing activity was recorded using a pressure converter. Every frog was examined in 5 successive 50-min seesions in the thermal gradient and then breathing frequency was recorded during 25 minutes. Obtained data can be classified into various patterns of responses to the pesticide. In the majority of individuals toxication caused an increase of preferred ambient temperature. There was also a moderate increase of locomotor activity in some individuals. Some frogs, however, selected reduced ambient temperature. In 4 of 12 frogs, however, the pesticide didn't affect the above mentioned behavioural responses. In spite of such a small dose of the poison (nonlethal for cockroaches) there were behavioural symptoms of poisoning in frogs. The process of detoxication was visible.

29.33 EFFECTS OF GLUTAMATE RECEPTOR AGONISTS AND ANTAGONISTS ON FEEDING IN ECONOMICALLY IMPORTANT INSECT PEST

Maciej A. Pszczolkowski, Luis F. Matos, Sandye M. Bushman and John J. Brown

Department of Entomology, Washington State University, Pullman, WA 99164, USA

We investigated feeding behavior in neonates of important apple pest, the codling moth, *Cydia pomonella*. Our research identified monosodium glutamate as a substance increasing feeding in dose-dependent manner. Antagonists of vertebrate ionotropic glutamate receptors, significantly inhibited feeding as measured in short- term (3h) and long-term (24h) assays. A broad- spectrum antagonist of metabotropic glutamate receptors, ((S)-α-Methyl-4-carboxyphenglycine), (S)-MCPG, also inhibited feeding in long-term assays, however, in short-term tests inhibitory action of (S)-MCPG was not observed.

We hypothesize that glutamate receptors play a role in regulation of feeding behavior in codling moth larvae. The localization of these receptors is unknown yet, however our preliminary data on effects of N-methyl-*D*-aspartate , NMDA, (5R,10S)-(+)-5-Methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine, MK-801, and $CaCl_2$ on feeding, suggest that NMDA receptor is involved in regulation of feeding in the codling moth.

29.34 EFFECT OF COMBINED TREATMENT WITH IMIPRAMINE AND AMANTADINE ON THE PHARMACOKINETIC AND ENDOCRINE PARAMETERS OF RATS SUBJECTED TO THE FORCED SWIMMING TEST.

Rogóż Z, Budziszewska B, Daniel WA, Kot M, Wójcikowski J, Maj J.
Institute of Pharmacology, Polish Academy of Sciences , Smętna 12, PL 31-343 Kraków, Poland.

The problem of therapy-resistant depressive patients has been studied for a long time, but with no significant success. The present study was aimed at determining the effect of imipramine (IMI) given alone or in combination with amantadine (AMA), a non-competitive NMDA receptor antagonist, on the immobility time in the forced swimming test (FST) in rats and on FST-induced changes in pharmacokinetic and endocrine parameters in the rats Experiments were carried out on male Wistar rats . All the drugs examined were administered three times (24, 5 and 1 h before the test). The obtained results showed that IMI (10, but not 5 mg/kg) and AMA (20, but not 10 mg/kg) – each of them given alone – had an antidepressant-like activity in the FST in rats. Joint administration of IMI and AMA induced a more potent antidepressant-like effects in the FST than did treatment with either of those drugs alone. A synergistic effect was also observed when IMI or AMA was used at a dose which was ineffective when either of those drugs was given alone. Combined treatment with IMI (5 and 10 mg/kg) and AMA (20 mg/kg) did not change the level of IMI and its metabolite-desipramine, in the rat plasma and brain, measured 1 h after FST exposure. However, the level of corticosterone in plasma, measured immediately (but not 1h) after the FST, was decreased.

The obtained results indicate that joint administration of IMI and AMA induced antidepressant-like effect in the FST procedure. This effect did not result from a pharmacokinetic interaction. These findings may be of particular importance in the case of drug-resistant patients.

29.35 THE EFFECT OF CONGENITAL DEAFNESS ON THE MAXIMUM SPEED OF FINGER TAPPING TASK

Rojek, E., Szeląg, E., Kowalska, J.
Nencki Institute of Experimental Biology, Dept. of Neurophysiology, Warsaw, Poland

A number of experimental data suggests the left hemisphere involvement not only in language functions, but also in motor control of simple repetitive movements, like Finger Tapping Task (FTT). Moreover, there exists evidence that these two functions are rooted in similar temporal constraints.

In order to look deeper into these relationships we examined the temporal aspects of the maximum speed of FTT, performed with the left and right hand, in eight right handed normal hearing and eight congenitally deaf boys, aged between 16-18 years. The onset of speech therapy in the deaf subjects was relatively late, they displayed disturbed articulation and communicated using sign language.

The results showed that hearing boys tapped significantly faster with the right hand (controlled by the left hemisphere) than with the left hand (right hemisphere). In contrast, this difference was not observed in the deaf group. We concluded that although the left hemisphere is involved in temporal control of movement, a long linguistic deprivation may influence this relationship.

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29.36 Interactions between melanocortins and opioids. *In vivo* and *in vitro* studies.

Katarzyna Starowicz[a], Barbara Przewłocka[b], Ryszard Przewłocki[b]
[a]*International Institute of Molecular and Cell Biology UNESCO/PAN, Warsaw*
[b]*Institue of Pharmacology, Polish Academy of Sciences, Cracow*

Melanocortins and opioids both are located in a spinal structures and might interact in nociception. We compared the effects of the opioids: morphine, endomorphin-1 (EM-1) and DAMGO, with those of the melanocortins: MT II (receptor agonist) and SHU 9119 (SHU, receptor antagonist) after their i.th. injection in a rat model of neuropathic pain (sciatic nerve ligation). Antinociceptive effects were measured using a tactile (von Frey) and cold allodynia tests. EM-1 (2.5-10μg) and DAMGO (0.1-0.5μg) increased the mechanical and the cold allodynia effects in rats with sciatic nerve ligation, whereas morphine (10-30μg) was effective at higher doses only. The anty-allodynic effect of SHU was caused by considerably lower concentrations (0.15-1.5μg) when compare with μ-opioid agonists. In contrast, MT II administration (0.03- 0.5μg) enhanced allodynia. The present results show a potent analgesic action of SHU at a spinal cord level, which suggests a possible use of the melanocortin receptor antagonist in pain therapy.

In further study we compared the MC4R level in the L4-L6 parts of the spinal cord in animals with ligated or crushed sciatic nerve *versus* sham-operated or intact ones using a RT-PCR method. Post-*in vivo* studies showed up-regulation of the MC4R after chronic constriction injury.

In conclusion, our study postulates that the melanocortin system can modulate the nociceptive transmission and can be involved in the development and/or perception of neuropathic pain.

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29.37 **EARLY DEPRIVATION OF A VARIETY OF FOOD TASTES CAN REVEAL AN INBORN FOOD PREFERENCE IN CATS**

Maciej Stasiak
Department of Neurophysiology, Nencki Institute of Experimental Biology, 3 Pasteur St., 02-093 Warsaw, Poland

Fourteen cats from the Nencki Institute colony were used. Four cats were fed with a variety of foods during the first six months of their lives. Once w week, cats were fed with Whiskas (Whiskas was a standardized, nutritionally complete canned cat food produced by Master Foods) with beef (WB) or Whiskas with tuna (WT). Both kinds of Whiskas – WB and WT - were eaten willingly. After six months, these cats were fed with only one kind of food: WB (two cats) or WT (two cats). The cats were then trained in alimentary instrumental conditioning. After criterion performance was achieved they were retrained with alternated food. The results indicated that both WB and WT had a similar reward value. Other ten cats were reared on a variety of foods during their infancy as well as adulthood. The results of a preference test indicated no preference to WB or WT in these adult cats. The data indicate that there is no difference in attractiveness (palatability) between WB or WT in cats non-deprived of a variety of food tastes. In contrast, it is known that WB and WT have a different reward value for cats continously fed with only one specific food (WB or WT) during early life, i.e. in cats deprived of a variety of food tastes; the WT reward is more effective than WB reward [Stasiak 1997, Acta Neurobiol. Exp. 57 (suppl.): 35].

One can conclude that, if the taste environment is heterogeneous in early life – in other words - rich in many tastes of food, the natural or potential, i.e. inborn, attractiveness (palatability) of the WT is overshadowed or masked. In contrast, if the taste environment is impoverished, the potential palatability of the WT is revealed.

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29.38 **Effects of 8-OHDPAT and UH-301 administrations into the dorsal raphe nucleus on fear behavior and regional brain monoamies distribution in rats.**

Strzelczuk, M., Koprowska, M., Krotewicz, M., Romaniuk, A. and Wieczorek, M.

Dept. of Neurophysiol., Univ. of Łódź, 66 Rewolucji 1905 r St, 90 – 222 Łódź, Poland E-mail: marek@taxus.biol.uni.lodz.pl

The effects of 8-OHDPAT (300 ng) and UH-301 (300 ng) administrations into the dorsal raphe nucleus on fear behavior in modified version of light-dark transitions test and regional monoamines (NA, DA, 5-HT) and their metabolites (MHPG, DOPAC, 5-HIAA) in the hypothalamus, midbrain central gray matter, amygdala, hippocampus, pons and cortex were examined. The results indicate that administration as 8-OHDPAT as well UH-301 produced the same behavioral changes suggesting the anxiolytic actions of both substances. In the case of 8-OHDPAT decrease of 5-HT activity was connected with the increase of the activity of DA. On the other hand in the case of UH-301, relation between these monoamines were not as clear as in the case of 8-OHDPAT, and concern only the NA – 5-HT.

The results show, that activation of 5-HT_{1A} presynaptic receptors caused the anxiolytic like behavior of the rat connected with infringement of dynamic balance between the 5-HT and catecholamines.

29.39 **DOES THE PRETRAINING PROCEDURE OR THE CS SALIENCY INFLUENCE INTERTRIAL RESPONDING IN THE TWO-WAY AVOIDANCE CONDITIONING IN RATS?**

Krystyna Świrszcz, Grażyna Walasek, Kazimierz Zieliński and Tomasz Werka
Nencki Institute of Experimental Biology, Pasteur St. 3, 02-093 Warsaw, Poland

It was shown that low salient visual CS resulted in clear enhancement of intertrial responding (ITR) combined with relatively slow avoidance acquisition. A positive correlation between the number of ITRs and speed of avoidance acquisition was also found. According to hypothesis of Zieliński (1993) a residual fear caused by insufficient discrimination of sporadic CS of low saliency and context stimuli, is the main source of ITRs during shuttle-box training. However, this hypothesis was based on results obtained in experimental procedures, which did not employ any pretraining, except slight habituation of stimuli presented during the avoidance training. Thus, in 18 male Möll-Wistar rats the effect of pavlovian preconditioning on ITR rate and avoidance performance was studied. The same auditory CS was used in both pavlovian and instrumental training. The classical pretraining caused no changes of avoidance rate. However, the frequency of ITRs was decreased, in spite of clear symptoms of fear caused by context stimuli. We conclude that the pavlovian pretraining inhibits ITRs during the shuttle-box training, and it seems to exert more significant effect than the saliency of CS. Probably, the avoidance performance to visual CS became irrelative to the ITR rate evoked by a residual fear, after classical preconditioning. However, this hypothesis needs verification.

Ref: Zieliński K. (1993). Intertrial responses in defensive instrumental learning. Acta Neurobiol. Exp. 53: 215-229.

29.40 **INFLUENCE OF A PYRETHROID DELTAMETHRIN ON THE CHOSEN BEHAVIOURAL PARAMETERS IN *Hylobius abietis* L.**

Tyczyński Marek, Korpała Małgorzata, Przybylska Ewa, Wilczyńska Małgorzata, Chomaciuk Jolanta.
Dept. of Animal Physiology, N. Copernicus Univ., Toruń, Poland

Hylobius abietis (the large pine weevil) belongs to most important primary pine pests, especially in young pines (1 – 3 years old) and very often they kill the little pines. Fight against them is difficult, therefore insecticides are used to reduce the density of their population. Pesticides from the pyrethroid group are widely used. They modify the sodium channels and in this way they influence the organism's excitability. The experiments were carried out on 14 individuals of *Hylobius abietis*. They were treated with a pesticide Deltamethrin. It was given in a form of a drop on the upper surface of abdomen at a dose of 1.96 μg dissolved in 17,5 μl of solution.The insects were placed individually in a thermal gradient system during five successive 50-min sessions, as follows: control, one minute after toxication with deltamethrin and during three stages of detoxication (D1 – 2 days; D2 – 7 days; D3 – 12 days after the exposure to the pesticide, respectively. Motor activity of the animals during the sessions was detected using an ultrasonic system. All the data were recorded and analysed by computer. In spite of such a small dose 4 individuals were killed – one at the stage of toxication and three at the stage D1. In remaining ten insects pesticide caused an increase of preferred ambient temperature and a slight increase of locomotor activity. In one case, the increase of motor activity was considerable at the stage of toxication. The process of detoxication in successive detoxication periods was visible with a lapse of time (D1 – D3) (i.e. slow return to the control temperature and activity was observed).

29.41 **ONE-TRIAL FORWARD FEAR CONDITINING AS REFLECTED IN CONDITIONED SUPPRESSION OF BAR PRESS RESPONDING IN RATS.**

Grażyna Walasek, Małgorzata Węsierska and Kazimierz Zieliński.
Department of Neurophysiology, Nencki Institute of Experimental Biology, 3 Pasteur St., 02-093 Warsaw, Poland

Previous researches have shown using off-baseline technique of conditioned suppression of licking, that this conditioned reaction produced by the one-trial simultaneous and backward procedures reflected a genuine associative process (Mahoney and Ayres 1976). In the present one-trial forward fear conditioning was studied. During the experiment fourteen hooded rats were housed individually in the Skinner operant chambers. In this way, the rats perfectly habituated all elements of the experimental context. Then rats were divided into experimental and control groups. Both groups of rats were trained to press the bar for food reinforcement and then, experimental group was exposed to three minute long conditioned trial (80 dB white noise) that was terminated during the last second with nociceptive unconditioned stimulus (2 mA shock of 1 sec duration). The control group received presentation of white noise alone. Thereafter, suppressive effect of defensive CS-US pairing was extinguished during subsequent sessions. The present results show that in the experimental group only one pairing of the CS and nociceptive US in forward procedure would be effective in acquisition of excitatory fear conditioning manifested in suppression of bar press responding, resistant to the extinction during five subsequent sessions, compared with control animals.

*) *Mahoney W.J., Ayres J.J.B. 1976 One-trial simultaneous and backward fear conditioning as reflected in conditioned suppression of licking in rats. Animal Learning Behav. 4: 357-362.*

29.42 **GENERALIZATION OF THE CONDITIONED ENHANCEMENT OF THE BAR PRESS RESPONDING IN RATS.**

Grażyna Walasek, Janina Kilijanek and Kazimierz Zieliński
Department of Neurophysiology, Nencki Institute of Experimental Biology, 3 Pasteur St., 02-093 Warsaw, Poland

Twenty four male hooded rats divided into three groups were trained in eight Skinner operant chambers in the bar press responding for food, reinforced according to 2.5 min VI schedule. On this alimentary background, the sporadic stimulus: one minute tone of 3000 Hz intensity, which signalled continuous food reinforcement (CRF) was introduced. In the alimentary group only the food motivation was employed. In two other groups the conditioned enhancement trials were contrasted with the defensive trials: the darkness one minute stimulus which terminated during last second with shock of 2 mA intensity. Then five frequencies of tones were introduced in the generalization test. All generalization gradients were flat across of the tone frequency dimension independently on preceded experience.

The long lasting test of stimulus generalization in the form of chronic extinction of the conditioned enhancement of alimentary motivated bar press behaviour, and resulted in easier transformation of the conditioned suppression into the conditioned enhancement.

29.43 **TRANSFER OF THE TWO-WAY AVOIDANCE IN RATS TRAINED IN THE PROCEDURE OF SECOND-ORDER CONDITIONING**

Tomasz Werka and Grażyna Walasek
Nencki Institute of Experimental Biology, Pasteura St. 3, 02-093 Warsaw, Poland

In pavlovian first-order fear conditioning a conditioned defensive reaction (CR) is evoked by a conditioned stimulus (CS1), which has acquired motivational value by virtue of being paired with a foot-shock (US). The second-order procedure consists in elicitation of CR by another CS2, which precedes CS1 used as US. Thus, fear and reaction evoked by US are conditioned to CS1 and then transferred to CS2. In the instrumental avoidance learning procedure fear is a classically conditioned component, which provides the motivation for instrumental responding. On the other hand, the avoidance reaction is able to modify the emotional state caused by CS1. The aim of our research, which was carried on five male Möll-Wistar rats, was to examine whether the transfer of fear motivation to CS2 goes together with an analogous transfer of the two-way avoidance responding. An auditory stimulus (white noise, 70 dB) was used as CS1, and the visual one (darkness) as CS2. Both stimuli were habituated before training. The second-order conditioning implies no presentation of punishment during test trials. To estimate a relative strength and stability of the instrumental responding during the second-order conditioning, the extinction of previously acquired avoidance response to CS1 was studied in another five animals. The results showed some transfer of the two-way avoidance to the second-order stimulus. In contrast to extinction training, instrumental responding evoked by CS1 during the second-order training was fast and very stable.

29.44 **SPONTANEOUS BEHAVIOR OF THE GRAY SHORT-TAILED OPOSSUMS (*Monodelphis Domestica*) IN THE OPEN FIELD (OF) AND IN THE ELEVATED PLUS-MAZE TEST (EPM) IN COMPARISON WITH THE LONG-EVANS RATS.**

Węsierska M., Walasek G., Olkowicz S., Turlejski K.
Department of Neurophysiology, Nencki Institute of Experimental Biology, 3 Pasteur St., 02-093 Warsaw, Poland.

We compared the spontaneous behavior of both sexes of the laboratory opossums and the Long-Evans rats in the OF and EPM. In the OF the animals were observed during four 10 min daily sessions. During the last two sessions they were exposed to a new object (NO). We counted the numbers of crossings in the peripheral (P), internal (In) and central (C) part of the OF. In the EPM animals received six 5 min daily sessions. Entries into the open (OA) and enclosed (EA) arms, number of rearing and grooming, time of grooming, time in C and defecation scores in both test were calculated. Opossums displayed high activity in the OF. During the first exposition they made more crossings within P and more rearings, then they gradually increased their activity in the In and C. Rats showed stable level of activity in each sessions, higher in P than in In and C. Opossums displayed higher numbers of contacts with the NO. In the EPM both species spent most time in EA, but opossums showed higher number of entries into OA and stayed longer there. From the third session opossums reduced their entries into OA and spent more time in C. In both tests rats defecated more. In conclusion, both species showed anxiety evoked by open space, but opossums habituated faster and spent more time in C. In the rats thigmotaxic behavior dominated, whereas opossums displayed strong exploratory drive to the whole space. This curiosity-driven behavior of opossums is probably a consequence of their species-specific adaptation to predatory, solitary and nomadic life.

29.45 **Effects of 8-OHDPAT administration into the dorsal raphe nucleus and dorsal hippocampus on fear behavior and regional brain monoamines distribution in rats**

Wieczorek, M., Romaniuk, A., Koprowska, M., Krotewicz, M. and Strzelczuk, M.

Dept. of Neurophysiol., Univ. of Łódź, 66 Rewolucji 1905 r. St., 90-222 Łódź, E-mail: marek@taxus.biol.uni.lodz.pl

The effects of 8-OHDPAT administration into the dorsal raphe nucleus (DRN) (200 ng) or bilaterally (100 ng per site) into the dorsal hippocampus (HIP) on fear behavior in modified version of the light-dark transitions test and regional brain monoamines (NA, DA, 5-HT) and their metabolites (MHPG, DOPAC, 5-HIAA) in the hypothalamus, midbrain central gray matter, amygdala, HIP and pons were examined. The results indicate that changes in intensification of fear response are not so closely dependent on changes in the 5-HT system activity but they are regulated by complex interactions between the 5-HT – NA – DA systems. The results indicate that the neurochemical base of anxiolytic effects evoked by 8-OHDPAT administration into the DRN is the decreased 5-HT system activity accompanied by an increased DA system activity, with no change in the NA system activity. On the other hand, a neurochemical base of anxiogenic effects by 8-OHDPAT administration into the HIP is the heightened activity of catecholaminergic systems, especially noradrenergic, in all the brain structures of the emotional defensive circuit.

In summary, the results show that the activation of pre- and post-synaptic 5-HT_{1A} receptors resulted in the opposite behavioral effects, anxiolytic and anxiogenic, respectively, which have a different neurochemical base in the structures of the brain constituting the emotional-defensive system.

DOPAMINERGIC PROJECTION TO THE NUCLEUS ACCUMBENS MEDIATES THE FACILITATORY EFFECT OF CCK-8US AND CAERULEIN ON MEMORY IN RATS 29.46

M. M. Winnicka[1,2], P. Kosiorek[1], R. Ł. Zbucki[1], K. Wiśniewski[2]

[1]Department of General and Experimental Pathology, [2]Department of Pharmacology, Medical Academy of Białystok, Mickiewicza 2c, 15-222 Białystok, Poland

The involvement of dopaminergic projection to the anterior and to the posterior part of nucleus accumbens in the facilitatory effect of cholecystokinin-unsulfated octapeptide (CCK-8US) and caerulein (CER) on memory motivated affectively was investigated in male rats. CCK-8US and CER were given subcutaneously at the doses of 10 μg/kg and 0.5 μg/kg, respectively, immediately after a single learning trial in a passive avoidance situation, after bilateral 6-OHDA lesions (desipramine pre-treatment; 25 mg/kg) to the anterior, or to the posterior part of nucleus accumbens. Bilateral 6-OHDA lesions to the anterior part of nucleus accumbens totally abolished, while to the posterior part of this structure significantly attenuated the facilitatory effect of CCK-8US and CER on retention of passive avoidance behaviour evaluated 24 h after the learning trial. Neither, destruction of dopaminergic endings in both parts of the nucleus accumbens, nor application of CCK-8US and CER changed the spontaneous psychomotor activity of rats estimated in an "open field" test, as well as rats' behaviour in elevated plus maze. These results may indicate that dopaminergic projection to the anterior part of the nucleus accumbens is mainly responsible for the facilitatory effect of tested peptides on memory for affect.

Session30 - Poster Session: Biological rhythms and oscillations

30.1 **THE ROLE OF RETINAL D1-DOPAMINERGIC SIGNAL IN THE REGULATION OF MELATONIN BIOSYNTHESIS IN THE CHICK PINEAL GLAND**

Małgorzata Berezińska, Jolanta B. Zawilska, Jerzy Z. Nowak

Department of Biogenic Amines PAS, Łódź, Poland

The vertebrate pineal gland produces melatonin in a light-dependent diurnal rhythm. Rhythmic melatonin formation is regulated mainly by changes in the activity of serotonin N-acetyltransferase (AA-NAT). AA-NAT activity and melatonin content of the pineal gland are low during the light phase and high during the dark phase of a light-dark illumination cycle. The nighttime melatonin production is suppressed by light. The avian pineal contains functional photoreceptors, so light can affect melatonin formation in this organ without participation of the visual system. In addition to that, some extra-pineal factors, including retinal dopamine (DA), are postulated to be capable of modulating melatonin production by the pineal gland. In this work we studied effects of activation of retinal DA-ergic system on the nocturnal AA-NAT activity of the chick pineal gland.

Intravitreal administration to chicks (into both eyes) of DA, apomorphine (D1- and D2-DA receptor agonists) and SKF 38393 (a D1-agonist), but not quinpirole (a D2-agonist), decreased the nighttime AA-NAT activity of the pineal gland in a dose-dependent manner. The action of apomorphine and SKF 38393 was blocked by SCH 23390 (a D1-antagonist), but not affected by sulpiride (a D2-antagonist). Moreover, SCH 23390 antagonized the suppressive effect of light on the pineal AA-NAT activity.

These results indicate that the retinal D1-dopaminergic signal may be an important factor in the regulation of melatonin biosynthesis in the directly photosensitive chick pineal gland.

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GENERATION AND APOPTOSIS OF THE BRAIN CELLS IN THE LIFE CYCLE OF SHREWS (INSECTIVORA). 30.2

K. Bialoskorska[1], J. R. E. Taylor[2], R. Djavadian[1], K. Turlejski[1].

1) Nencki Institute of Experimental Biology, Warsaw, Poland; 2) Institute of Biology, The University of Bialystok, Bialystok, Poland

Soricid shrews of the temperate zone undergo seasonal changes in the weight of their body and brain. They are born in early summer, mature after wintering, breed and then die in the next autumn. They decrease their size during autumn and grow again in the spring. Dehnel (1949) found that the capacity of their brain case decreases and the volume of their brain is reduced by 20-30%. In the spring the brain regains about half of the loss of weight. We investigated the death and generation of new neurons in the brains of *Sorex araneus* and *Sorex minutus*. Using TUNEL method we found, that the number of labeled nuclei was low in all seasons. Therefore decrease of the size of the brain is not caused by the cell death. In order to measure the rate of generation of new cells in the brain, shrews were injected with bromodeoxyuridine (BrdU). For about one month after weaning production of neurons migrating to the dentate gyrus and olfactory bulb was profuse and some BrdU labeled nuclei were also found in the neocortex. Later, numbers of labeled nuclei in the dentate gyrus sharply decreased, to stop in the autumn and never recover again. Cells migrating towards the olfactory bulb were produced in high numbers throughout summer. This generation decreased in the autumn and in the winter it dropped to almost null. Generation started again in the spring and continued at a moderate rate throughout summer, to decrease again at the end of life. Therefore increase of the brain size in the spring does not depend on production of new neurons. Relevance of these findings for the biology and evolution of shrews and for understanding of regulation of proliferation of the stem cells in the brain will be discussed.

30.3 IN VITRO NEURONAL ACTIVITY OF THE RAT INTERGENICULATE LEAFLET

Anna Błasiak, Tomasz Błasiak, Marian H.Lewandowski
Jagiellonian University, Institute of Zoology
Ingardena 6, 30-060 Krakow, Poland

The mammalian circadian timing system contains a distinct set of neural structures that generate and regulate circadian rhythms. The suprachiasmatic nucleus (SCN) functions as the dominant pacemaker of this system which receive direct input from the retina. The intergeniculate leaflet (IGL) of the thalamus is a second an important component responsible for the adjustment of the mammalian circadian rhythms to non-photic clues. In our recent paper we described at the first time ultradian rhythmic oscillation of IGL neurons in vivo [1].

To goal of this study was to determine whether the same activity of IGL neurons persist also in vitro. To test this question we recorded a multi-unit neuronal activity (MUA) from IGL slices preparation. The IGL neurons exhibited spontaneous activity in the presence of elevated concentration of a potassium ions (8mM) in the incubation medium. The most important feature of IGL neuronal activity was its oscillatory nature, recorded for the first time *in vitro*. The observed oscillation had similar period (T≈120s) to this revealed during *in vivo* experiments.

These results suggest that IGL neurons are capable to generate the isoperiodic phasic discharge (T≈120s) in vivo and in vitro. However, at the moment we have not enough date to discuss the cellular mechanism of this oscillation.

1. Lewandowski MH, Błasiak T, Domosławski J and Wołkowska A. *NeuroReport* 11:317-321 (2000).
2. Miller JD and Fuller CA. Am J Physiol 263:R51-R58 (1992).

30.4 **THE EFFECT OF POSTERIOR HYPOTHALAMIC INJECTION OF CHOLINERGIC AGENTS ON HIPPOCAMPAL FORMATION THETA IN THE CAT**

Bocian R., Kowalczyk T., Eckersdorf B., Gołębiewski H., Konopacki J.
Department of Neurobiology, University of Łódź, Łódź, Poland

Recent electrophysiological observations suggest that, in addition to the medial septal area (MS), several different structures are involved in generation and modulation of hippocampal formation theta rhythm. The number of recent investigations showed that reticular influences were transmitted to the limbic cortex *via* the posterior hypothalamus area (PH).

The aim of these studies was to analyse the pharmacological profile of PH in the production of the hippocampal theta in the cat. The following cholinergic agents were administrated into the posterior hypothalamus area: hexamethonium (nicotinic cholinergic antagonist), atropine (muscarinic cholinergic antagonist), gallamine (M_2 muscarinic antagonist) and pirenzepine (M_1 muscarinic antagonist). Finally, the effect of posterior hypothalamic injection of cholinergic agonist – carbachol on the hippocampal slow activity was also examined.

Our results showed that the posterior area of the hypothalamus is actively involved in the mechanisms responsible for the production of theta oscillations in the freely moving cat. Involvement of the posterior hypothalamus M_1 cholinergic receptors in generation of hippocampal field activity in this species is discussed.

30.5 **NEUROKININ A AND THE OXYTOCIN RESPONSE TO MELATONIN: in vitro studies**

Ewa Bojanowska, Bożena Stempniak and Marlena Juszczak
Department of Pathophysiology, Medical University of Łódź, Poland

Tachykinins are postulated to play a role of as regulators of the posterior pituitary endocrine function. The aim of the present investigations was to study the role of neurokinin A (NKA), a member of a family of tachykinins, in the regulation of basal and K^+-evoked oxytocin (OT) secretion as a response to melatonin.

Male Wistar rats served as donors of the hypothalamo-neurohypophysial (HN) explants which were incubated *in vitro* in Krebs-Ringer fluid (KRF) enriched with NKA (neuropeptide which acts preferentially on NK-2 receptors) at the concentrations of 10^{-7}, 10^{-8}, 10^{-9}, 10^{-10} or 10^{-11} M/L. The HN explants were incubated successively in: 1 - normal KRF (B1); 2 - modified KRF containing the excess (56 mM) of K^+ (S1); 3 - the incubation fluid as B1 alone or with NKA in the respective concentration (B2); 4 - the KRF as S1 alone or with NKA in the same concentrations (S2). Next, the B2 and S2 fluids were additionally enriched with melatonin at the concentration of 10^{-9} M/L. After 20 minutes of incubation, each medium was collected and frozen before estimation of OT by the radioimmunoassay.

In agreement with previous *in vitro* studies high concentration of K^+ stimulated the release of OT from the isolated HN explants. Under basal conditions, the OT release was increased by NKA when used at the concentration of 10^{-9} and 10^{-7} M/L; melatonin significantly inhibited this effect of NKA. K^+-evoked release of OT was not further modified by either NKA or melatonin.

The present results show that tachykinins may be involved in the pineal-neurohypophysial interactions. However, more studies are necessary to estimate the respective mechanisms.

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30.6 **INTRASEPTAL MICROINJECTION OF CHOLINERGIC ANTAGONIST: THE EFFECT ON CARBACHOL-INDUCED HIPPOCAMPAL THETA IN THE FREELY MOVING CAT**

Gołębiewski H., Eckersdorf B., Bocian R., Kowalczyk T., Konopacki J.
Department of Neurobiology, University of Łódź, Łódź, Poland

Hippocampal formation (HPC) theta field activity depends on the integrity of cholinergic input from the medial septal/vertical limb of diagonal band of Broca (MS/vDBB). It has been histochemically demonstrated that approximately 50% of the fibers forming septo-hippocampal projection are cholinergic. Cholinergic nature of HPC theta was well documented both *in vivo* and *in vitro*.

In the present study intrahippocampal microinjections of carbachol-induced well-synchronized long lasting episodes of theta activity in freely moving cat. This effect was observed at least for 30 min. Then the influence of intraseptal microinjections of atropine sulphate on HPC carbachol-induced theta was studied. No changes in carbachol-induced HPC theta was observed during the first 30 to 40 min postatropine. The blocking effect of medial septal area on HPC theta field activity evoked by local cholinergic stimulation is discussed.

30.7 TACHYKININS AND THE RESPONSE OF VASOPRESSIN TO MELATONIN: in vitro studies

Marlena Juszczak, Ewa Bojanowska and Bożena Stempniak
Department of Pathophysiology, Medical University of Łódź, Poland

The role of tachykinins as regulators of the posterior pituitary endocrine function is postulated recently. The aim of the present investigations was to study the role of neurokinin A (NKA), a member of a family of tachykinins, in the regulation of basal and K^+-evoked vasopressin (AVP) secretion as a response to melatonin.

Male Wistar rats served as donors of the hypothalamo-neurohypophysial (HN) explants which were incubated *in vitro* in Krebs-Ringer fluid (KRF) enriched with NKA (neuropeptide which acts preferentially on NK-2 receptors) at the concentrations of 10^{-7}, 10^{-8}, 10^{-9}, 10^{-10} or 10^{-11} M/L. The HN explants were incubated successively in: 1 - normal KRF (B1); 2 - modified KRF containing the excess (56 mM) of K^+ (S1); 3 - the incubation fluid as B1 alone or with NKA in the respective concentration (B2); 4 - the KRF as S1 alone or with NKA in the same concentrations (S2). Next, the B2 and S2 fluids were additionally enriched with melatonin (MLT) at the concentration of 10^{-9} M/L. After 20 minutes of incubation, each medium was collected and frozen before estimation of AVP by the radioimmunoassay.

In agreement with previous *in vitro* studies high K^+ concentration stimulated the AVP release from the isolated HN explants. Under basal conditions, the AVP release was increased by NKA only when used at the concentration of 10^{-7} M/L and MLT significantly inhibited this effect of NKA. K^+-evoked release of AVP was not further modified by either NKA or MLT.

The present results show that tachykinins may be involved in the pineal-neurohypophysial interactions. However, more studies are necessary to estimate the respective mechanisms.

Supported by the Medical University of Łódź (grant No. 502-11-632).

30.8 24-HOUR TRNDS IN BRAIN HEMISPHERIES EFFICIENCY IN PROCESSING OF VERBAL AND PICTORIAL STIMULI

Iskra-Golec I.
Jagiellonian University, 31-004 Krakow, Poland

The aim of this study (a part of a larger experiment) was to find out the 24-hour pattern of brain hemispheres efficiency in processing of verbal and pictorial stimuli at semantic and physical features of the stimuli levels. The data from three 24-hour constant routine studies of the experiment were taken to the analysis. The materials were parallel sets of words and pictures exposed laterally on the screen by purposely-designed computer program. The subjects were to press one of two buttons reacting to picture or word (stimuli physical traits level of processing) or answering the question concerning the meaning of stimuli (semantic analysis level). Performance was measured every 3 hours starting from 06.30. The subjects were 8 right-handed students aged 21-25 years. Four factorial ANOVA was performed on the data. The factors were level of processing (LP), visual field (VF), stimulus (S), and measurement time (MT). There were different temporal patterns of speed of processing of words and pictures exposed in the right and in the left visual field (VF*S*MT: F=3.363, p=.005) and at the semantic and physical traits levels of processing (LP*VF*S*MT: F=3.195, p=.051). It seems that at 13.00- 16.00 there was a kind of specialisation in stimuli processing in both hemispheres. The left processed the most effectively words at the semantic level when the right one pictures at both levels, and words at the level of physical traits. At 16.00-19.00 there appeared that the right hemisphere is more efficient in processing both stimuli than the left. At 04.00 am the left hemispheres seemed to be more effective in semantic processing of words and pictures and the right in processing of pictures at the stimuli physical traits level. At the other times of the day left hemisphere seems to be more effective than the right.

30.9 CURRENT-SOURCE DENSITY ANALYSIS OF THETA-LIKE ACTIVITY (TLA) RECORDED IN THE HIPPOCAMPAL FORMATION SLICES.

Kowalczyk T., Gołębiewski H., Eckersdorf B., Bocian R., Konopacki J.
Department of Neurobiology, University of Łódź, Łódź, Poland.

The earlier study demonstrated that a number of properties of the *in vivo* recorded theta rhythm could be successfully replicated *in vitro*. Specifically, it was found that the pharmacological profile of carbachol (CCH)-induced TLA, its frequency and amplitude, postnatal development, and loci of the amplitude maxima closely resembles properties of theta rhythm described *in vivo*.

In the present study we investigated the laminar distribution of cholinergic-induced theta-like activity recorded *in vitro* and we focused on its current-source density analysis. Depth profile of TLA was constructed by vertical tracking the roving electrode in 100 μm steps through the CA1, DG, and CA3c regions of the hippocampal formation slice preparation. The reference electrode was positioned in the stratum oriens of CA1 pyramidal cells. One-dimensional current-source density analysis was calculated from the laminar profile of averaged theta-like activity waves.

Location of sources and sinks of current of carbachol-induced theta like activity as well as its similarities to *in vivo* recorded theta are discussed.

30.10 RESPIRATORY MODULATION AND THE RATE OF RESTING DISCHARGE IN RENAL SYMPATHETIC NEURONS

K. Lupa, R. Tarnecki, M. Pencuła, A.Niechaj
Department of Human Physiology, Medical University School, Lublin and Nencki Institute of Experimental Biology, Warsaw

The coupling between medullary respiratory and cardiovascular neurons underlies respiratory modulation (RM) of the resting sympathetic discharge. In the present experiments we tried to assess the relationship between the size of the RM and the rate of spontaneous discharge in the renal sympathetic neurons while altering the respiratory drive by positive pressure ventilation. In vagus-intact rabbits anaesthetized with urethane + chloralose the resting discharge was recorded in single renal neurons. RM was determined from time-histograms triggered by changes in tracheal pressure and expressed as difference between the peak and minimal activity in percentage of peak activity. In spontaneously breathing animals the mean rate of discharge was 2.7 ± 0.3 spikes/s (x ± S.E.; n = 30) and the RM amounted to 67.2 ± 3.1 %. There was statistically significant correlation between the rate of discharge and the RM (r = -0.432; P = 0.02). Then the animals were paralyzed and artificially ventilated at a rate close to that occurring during spontaneous breathing. Now the rate of discharge decreased to 1.9 ± 0.2 spikes/s and RM augmented to 72.9 ± 4.1 %. Both changes were significant as compared to data in spontaneously breathing rabbits (P < 0.001 and P = 0,047, respectively; t-test). The relationship between the rate od discharge and RM was no longer significant (r = -0.128; P > 0.05). These findings suggest that tonic inhibition of the rate of resting discharge by positive pressure ventilation prevents significant correlation between the rate od discharge and RM in vasomotor renal neurons.

30.11 **SEASONAL CHANGES IN THERMAL BEHAVIOUR OF THE HELIX POMATIA SNAIL**

Nowakowska A., Caputa M., Rogalska J., Wentowska K.
N. Copernicus Univ., Dept of Animal Physiology, Toruń, POLAND

Before going into winter torpor Helix pomatia snails form the operculum to close their shell. Are they able to arrange winter torpor in advance? Recording seasonal changes in their thermal behaviour should allow to answer this question because mammalian hibernators, placed in a thermal gradient, actively select a cold place before going into hibernation. The snails were collected from their natural habitat as soon as they appeared after winter torpor and their thermal preference was immediately recorded during a period of 48h. The same recordings were repeated in mid summer and in November. The autumn session was continued for 3 weeks. Between the sessions the snails were kept outdoors. Directly after spontaneous arousal from winter torpor snails showed circadian changes in thermal preference (acrophase occurred during the day). Both in summer and autumn, however, they selected uniformly warm environment over 24-h period. Mean selected temperature of the first 24-h period of the spring (22.6±0.8°C) was significantly reduced ($p<0.01$) comparing with the remaining data (25.9±0.02). Starting from the second 24-h period of the spring there were no seasonal differences in selected temperatures. There was a progressive increase in amplitude of irregular changes of selected temperature during the prolonged autumn recording, but its mean value remained unchanged throughout the recording period. Latency of arousal from outdoors winter torpor in snails placed in the thermal gradient increased exponentially at lower temperatures. A threshold temperature to elicit arousal was ~8°C. Within 2 days each aroused snail selected a warm ambient temperature of ~25°C. In conclusion, both winter torpor and arousal from the torpor in Helix pomatia snails are passive responses to environmental thermal changes.

Michał Caputa

IS THE CHICK PINEAL GLAND DIRECTLY SENSITIVE TO THE UV-A LIGHT? 30.12

Jolanta Rosiak, Jolanta B. Zawilska
Department of Biogenic Amines, Polish Academy of Sciences, Łódź, Poland

Pineal glands of various vertebrates synthesize melatonin (MEL) in a daily/circadian rhythm. Rhythmic changes in MEL production are mainly driven by changes in the activity of serotonin N-acetyltransferase (AA-NAT, a penultimate and key regulatory enzyme in MEL synthesis). Light is a predominant environmental factor controlling, in an inhibitory manner, MEL-generating system. It has been demonstrated that, in contrast to mammals, the pineal gland of birds, including chicken, is directly photosensitive to visible light. Recently we have found that near-ultraviolet radiation (UV-A; λ_{max} = 365 nm) suppresses AA-NAT activity and MEL content of the chicken pineal gland. The aim of this study was twofold: to examine whether the pineal gland of chick is directly sensitive to UV-A radiation, and, additionally, to analyse a possible role of proteosomal proteolysis in the process of the UV-A-induced suppression of AA-NAT activity. In *in vivo* experiments the eyes of chicks were tightly covered by black, nontransparent tape and the animals were exposed to UV-A in the middle of the night. In *in vitro* experiments cultured pineal glands were exposed to UV-A light. Exposure of chicks and cultured pineal glands to UV-A radiation significantly decreased the nighttime pineal AA-NAT activity. The magnitude of the observed changes was dependent on a duration of the pulse. MG-132 (30 μM), a proteasome inhibitor, abolished the suppressive effect of UV-A light on pineal AA-NAT activity. These findings indicate that (1) chicken pineal gland is directly sensitive to UV-A radiation, and (2) UV-A-induced decrease in AA-NAT activity of the chicken pineal gland involves proteosomal proteolysis

Session 31 – Plenary Lecture

31.1

CYTOKINES IN BRAIN ISCHEMIA

Mirosław Mossakowski
Kraków, Poland

Not Received

Session 32 – Parallel Symposium: Neuroimmunology

32.1 **CYTOKINES AND SICKNESS BEHAVIOUR: LESSONS FROM CYTOKINE GENE-DELETED (KNOCK OUT) MICE**

Wiesław Kozak
Med Coll Georgia, Dept Physiol, Augusta, USA

Sickness behaviour (SB) is a brain-mediated response to infection and acute inflammation manifested, among others, by fever and anapyrexia, sleepiness and lethargy, reduced appetite and cachexia. SB is considered adaptive and critical to survival and recuperation. Based on studies using peripheral and central injection of cytokines, principally IL-1, IL-6 and TNFα, it has been concluded that these cytokines are indispensable for SB. It has been postulated that physiologic in vivo effects of these cytokines are characterized by a redundancy, that their biologic actions overlap, and that they act in a cascade fashion. Therefore, one may hypothesize that deletion of a cytokine gene in a mouse embryo, resulting in the absence of an individual cytokine during the mouse life span, may not have a dramatic effect on SB. To test this hypothesis, we were using IL-1β, IL-6, and TNFα gene deficient adult mice treated with various inflammation-inducing agents. Responses to systemic inflammation induced by bacterial endotoxin were slightly reduced or unaffected in these mice. Responses to localized muscle tissue injury induced by turpentine oil, or lung inflammation provoked by influenza virus were significantly attenuated or abolished in the absence of either IL-1β or IL-6. In contrast, the absence of TNFα had no effect on SB of mice in response to localized inflammation. Lack of TNFα, on the other hand, revealed a complex role this cytokine is playing in controlling the sickness responses upon systemic inflammation. In conclusion, the redundancy among cytokines in induction of the SB is more likely to occur upon systemic inflammation, whereas responses to a local inflammation appear to be mediated by an individual cytokine.

Session 33 - Parallel Symposium: Development of brain cortex

33.1 **COMMITMENT OF STEM-LIKE CELLS INTO NEURAL FATE BY EPIGENETIC AND GENETIC STIMULATION *IN VITRO***

Bużańska Leonora.
Mol. Neuropath. Lab., Med. Res. Ctr. Warsaw, Poland

Human neural stem cells are of a high interest because of their possible therapeutic properties due to their ability to integrate into a brain tissue after transplantation. However the repairing process requires, depending on a type of damage, a sufficient source of either immature neural progenitors or already committed into a certain neural cell line. Thus it is important to work on mechanisms underlying neural stem cells differentiation and to evaluate methods enabling directional steering of their fate *in vitro*. Epigenetic stimulation by growth factor dependent instructive mechanisms and genetic, selective stimulation by ectopic administration of transcription factor genes were employed to induce this differentiation. The regulation of the differentiation of human neural stem-like cells of DEV line into neurons by the cascade of HLH transcription factors (*Mash-1 Neurogenin-1, -2, -3,* and *NeuroD*) and into glial phenotypes by the *Gcm* "master gene" will be presented. The ability of tissue-specific stem cells to give rise to not related developmentally, differentiated cell types was recently demonstrated for the neural stem cells and bon marrow stromal cells isolated from adult animals and humans. Here the evidence will be presented that the cells bearing characteristic of neural stem cells (nestin-positive and clonogenic) can be obtained from the subpopulation of human cord blood cells and further stimulated to differentiate into neuronal, astrocytic and oligodendroglial phenotype *in vitro*, by means of epigenetic, stimulation. This was achieved by co-culturing of cord blood derived nestin positive cells with rodent cortical cells *in vitro* or by growing them in defined growth-factors containing media.

33.2 **Development of corticotectal projection in the monocularly enucleated opossum**

Djavadian R.L., Bialoskorska K., Turlejski K.
Department of Neurophysiology, Nencki Institute of Experimental Biology, 3 Pasteur St., 02-093 Warsaw, Poland

Plasticity of the developing corticotectal projection was studied in the short-tailed opossum (*Monodelphis domestica*). Neonatal pups were monocularly enucleated and in some of them serotoninergic fibers were lesioned with s.c. injection of 5,7-dihydroxytryptamine. Pups survived for three months and then the retrogradely transported fluorescent dyes (Diamidino Yellow and Fast Blue) were injected into the superior colliculi of the enucleated and control animals. The fluorescent dyes labeled neurons of the cortical layer 5 of three visual areas: the striate area (V1), the laterally placed peristriate area (V2) and the medial visual area (MV). Numbers of the labeled neurons in these areas on the side ipsilateral to the remaining eye did not differ from control, while on the contralateral side they were much lower. The largest reduction (65%) was observed in the striate area. In the V2 and MV numbers of the labeled neurons were reduced by about 40%.
Effects of the serotoninergic lesion were visible in the brains for about three weeks. After that period serotoninergic axons were fully regenerated. Therefore, at least during the phase of axons' elongation, the level of serotonin was decreased. In the group that was enucleated and 5-HT-lesioned the density of the labeled neurons was comparable to that in the enucleated group. This form of plastic changes was found for the first time and is not explained by the present theories on the mechanisms of plasticity. Temporary serotoninergic depletion did not permanently influence the course of development of the corticocollicular projection and neither its plastic changes induced by the monocular enucleation.

33.3 **THE EFFECT OF INTERRUPTING THE BIRTH OF LAYER 4 ON SUBSEQUENT DEVELOPMENT OF CEREBRAL CORTEX.**

Sharon L. Juliano
USUHS, Bethesda, MD 20814, USA

For normal development of cerebral cortex, a precise sequence of events must occur. We developed an epigenetic model that delivers an antimitotic (MAM) during corticogenesis of the ferret, resulting in severe diminution of layer 4 in somatosensory cortex (SCX). This model allows us to determine: (i) the influence of layer 4 on further development of sensory cortex, including growth and termination of thalamic projections, (ii) if layer 4 is essential to normal function of sensory neocortex, and (iii) if disruption of layer 4 leads to breakdown of information transfer. After MAM treatment, projections from the thalamus were aberrant and terminated in all remaining cortical layers, rather than focussing in central layers. Although several aspects of cortical responses were normal in these animals, more detailed analysis revealed that many functional properties were disrupted, including current source density (CSD) profiles and ability to entrain intermittent stimuli. The responses suggested that flow of information was disrupted by an imbalance of the normal distribution of excitatory and inhibitory elements. This idea was reinforced by finding an abnormal distribution of GABAergic cells and receptors in the SCX of MAM treated animals. Further studies using in vitro recordings of normal or MAM-treated cortical slices, or organotypic cocultures of thalamus and cortex, indicated that treatment with GABA antagonists results in CSD profiles and thalamic terminations in normal slices that mimic those observed in untreated MAM treated slices. This supports the notion that interruption of layer 4 development impairs the flow of information by altering the intracortical balance of excitation and inhibition.

33.4 **DEVELOPMENT AND EVOLUTION OF THALAMOCORTICAL INTERACTIONS**

Zoltán Molnár, Lucy Jones, Juan Small, Guillermina Lòpez Bendito
Department of Human Anatomy and Genetics, University of Oxford, South Parks Road, Oxford, United Kingdom

The sequence of events in the development of thalamocortical projection is similar in all mammals. After descending through the ventral thalamus they advance in the internal capsule amongst cells already innervated by dorsal thalamic projections, then reach the cerebral cortex by associating with the subplate cells and their corticofugal projections. They usually pause in the internal capsule and the subplate layer. Interactions of thalamocortical projections with the early generated, largely transient cells of the subplate, marginal zone, internal capsule and ventral thalamus play a crucial role in their deployment and establishment of a functional cortical architecture. Selective fasciculation, contact guidance, release of neurotrophic factors and early neuronal activity all are important factors in this process. We tested their importance in the *reeler* and other strains of mice (*Tbr1* K.O., *Emx2* K.O., *Pax6* K.O., *SNAP-25* K.O.). Behaviour of the thalamocortical and corticothalamic projections at the cortico-striatal junction is particularly puzzling. Evolutionary origin of these transient cell groups and their early development are not fully understood yet. Comparing expression of early genes and cell migration patterns with the developmental steps forming early connections in the forebrain of various vertebrates, we begin to understand the cellular and molecular interactions employed in the development of thalamocortical projection and evolutionary origins of mammalian cerebral cortex.

Supported by Swiss National Science Foundation, EU, HFSP, McDonnell-Pew Centre for Cognitive Neursocience, Oxford.

33.5 **POSTNATAL MATURATION OF THE CLAUSTRO-CORTICAL PROJECTIONS INTO THE MOTOR AND SOMATOSENSORY AREAS IN THE RAT**

Janusz Moryś, Przemysław Kowiański, Joanna Biranowska, Sławomir Wójcik, Jerzy Dziewiątkowski
Department of Anatomy and Neurobiology, Medical University of Gdańsk, Poland

The retrograde axonal transport and immunocytochemical methods were used to study the development of claustrocortical projections. 30 rats were divided into 6 groups of various postnatal ages (P0, P7, P14, P21, P60, P90). The occurrence of four selected neuroactive peptides was studied: neuropeptide Y (NPY), somatostatin (SOM), vasoactive intestinal peptide (VIP) and nitric oxide synthase (NOS).

The cortical projections were detected in all age groups. The morphology as well as the distribution of projection zones within the claustrum was differentiated. The intensity of the claustrocortical projections decreased significantly during the postnatal period. Only NOS was detected in projecting and intrinsic neurons. The remaining substances were present exclusively in the interneurons. The quantitative differences among immunoreactive interneurons were detected. The claustrocortical connections, although well established in the postnatal life, undergo significant changes in the first three postnatal months.

The decrease of the total number and numerical density of projecting neurons, as well as the changes in the numerical density of interneurons, may reflect the process of adjustment of the claustral function in the matured brain.

Session 34 - Parallel Symposium: Neurodegeneration

34.1 TETRAHYDROISOQUINOLINES AS ENDOGENOUS NEUROTOXINS AND NEUROPROTECTANTS.

Lucyna Antkiewicz-Michaluk and Jerzy Vetulani Institute of Pharmacology Polish Academy of Science, Kraków, Poland

Several of 1,2,3,4-tetrahydroisoquinoline derivatives (TIQs) present in the brain are endogenous. In the present study we investigated the biochemical effects of potentially neuroprotective 1MeTIQ, and neurotoxic 1BnTIQ administered in a single dose or chronically on two pathways of dopamine catabolism: oxidative MAO-dependent pathway and O-methylation COMT-dependent pathway. The subjects were male Wistar rats treated with 1MeTIQ (50 and 100 mg/kg ip) or 1BnTIQ (25 and 50 mg/kg ip) once or daily for consecutive 17 days. The substantia nigra (SN), striatum, and nucleus accumbens were dissected immediately after decapitation, and obtained tissue was frozen on solid CO_2 till used for biochemical assay. Dopamine (DA) and its metabolites, homovanillic acid (HVA), 3,4-dihydroxyphenylacetic acid (DOPAC) and 3-methoxytyramine (3MT), were assayed by means of high-performance liquid chromatography (HPLC) with electrochemical detection. The rate of DA catabolism in the striatum along the N-oxidative and O-methylation pathways was assessed by calculation of the ratio of appropriate metabolites to DA concentration. The results have shown that 1MeTIQ and 1BnTIQ produced different effects on DA catabolism. 1MeTIQ did not change the rate of total DA catabolism, it strongly inhibited the oxidative MAO-dependent catabolic pathway and significantly activated the COMT-dependent O-methylation. In contrast, 1BnTIQ produced the significant increase of the rate of DA metabolism with strong activation of the oxidative MAO-dependent catabolic pathway. The results may explain the biochemical basis of neuroprotective and neurotoxic properties of endogenous tetrahydroisoquinolines.

34.2 ANTI-INFLAMMATORY TREATMENT DIMINISH NIGROSTRIATAL DEGENERATION IN MICE MODEL OF PARKINSON'S DISEASE

A. Członkowska

2nd Department of Neurology, Institute of Psychiatry and Neurology, Department of Pharmacology, Medical Academy, Warsaw

It is emphasised that the inflammatory reaction may contribute to the neuronal impairment in various degenerative processes in the CNS. The mice model of Parkinson's disease is caused by toxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropiridine (MPTP). MPTP damages the nigrostriatal system and produces the inflammatory reaction consisting of glial activation lymphocytic infitration and the increase of various proteins. In this study we investigated an effect of different anti-inflammatory agents on the nigrostriatal degeneration. We used dexamethason (dxm), propentofylline (ppf) and indomethacine (ind). Animals (C57Bl mice, 8-10 months old) were injected ip with tested agents before and after MPTP intoxication. The protective effect was observed using dxm 1mg/kg and ind 1mg/kg before MPTP intoxication and dxm 1mg/kg after MPTP. Both agents diminished a dopamine content depletion in striatum and a decrease in the number of dopaminergic cells in the pars compacta of the SN. Ppf had no effect on nigrostriatal degeneration. Dxm inhibited also lymphocytic infiltration and diminished microglial reaction. Ind diminished lymphocytic infiltration but not microglial reaction. In conclusion, we showed the protective properties of anti-inflammatory agents as dxm and ind in MPTP model of Parkinson's disease. This gives a hope that anti-inflammatory treatment may be effective in other neurodegenerative disorders as Alzheimer's and Parkinson's diseases.

34.3 **ENDOGENOUS TOXIC EVENTS LEADING TO NEURODEGENERATION: THE CASE OF PARKINSONS'S DISEASE**

Peter Riederer

Clinical Neurochemistry and NPF Center of Excellence Research Laboratories at the Clinic and Policlinic for Psychiatry and Psychotherapy of the University of Wurzburg, Dept. Psychiatry, Wurzburg, Germany

The patogenesis of Parkinson's disease (PD) is still unknown. Nevertheless there are several hypotheses which per se or by interactive mechanisms give evidence or the onset and progression of a neurotoxic cascade of events leading to cell death of dopaminergic neurons. Such endogenous toxic events include oxidative stress, inflammatory and acute phase mechanisms, excitotoxicity, mitochondrial disfunction, lack of neurotrophic support, metabolic disturbance leading to protein aggregation and building of Lewy bodies, genetic abberration in dopaminergic neurons proned to synthesise neuromelanin, toxicity derived from dopamine, its metabolism and neuromelanin, developmental disturbances leading to malfunction and disconnectivity of dopaminergic fibre systems and last but not least genetic events of primary and secondary (i.e. interactive with the above mentioned disfunctions) importance. Except for a few families all our current knowledge points on the view, that sporadic PD with its spectrum variation of symptomatology is based onthe interaction of genetic, developmental, metabolic and environmental disturbances. Although „neurodegeneration" is thought mostly to be connected with loss of neurons and accompanying gliosis this is true only in part and mainly focused onthe degeneration of the substantia nigra pars compacta. However, „neurodegeneration" without gliosis either through apoptotic other as yet unknown progressive endogenous toxic processes also seem to be of great importance. Knowledge about these various mechanisms leading to cell death are important for the development of neuroprotective drugs.

34.4 **cDNA MICROARRAY FOR STUDYING MODELS OF NEURODEGENERATIVE AND NEUROPSYCHIATRIC DISEASES AND RESPONSE TO NEUROPROTECTIVE AND ANTIDEPRESSANT DRUGS**

Moussa B.H. Youdim, Edna Grunblatt, Gila Maor, Yona Royak, Noam Derigus, Silvia Mandel and Marta Weinstock* *Eve Topf and National Parkinson Fouindation (US) Centers of Excellence for Neurodgenerative Diseases, Technion-Faculty of Medicine and Department of Pharmacology, Haifa, and *Hebrew University, Departement of Pharmacology, Jerusalem*

Parkinson's Disease (PD) AND Alzheimer's disease are aging disorders associated with progressive degeneration of dopamine and cholinergic neurons respectivley and both diseases have comorbidity with depressive illness. Although the etiology of neurodegeneration are not known, biochemical evidence support the notion for pivotal roles of iron, oxidative stress and inflammatory processes in cascade of events leading to neurodegeneration. The Parkinson inducing neurotoxin MPTP (N-methyl-4-phenyl-1,2,3,6- tetrahydropyrdine) which is used in animal models to initiate dopaminergic neurodgeneration brings about similar biochemical changes. Neurodegeneration is a complex cascade of events many of which have not been identified. We have investigated the alteration in gene expression in brains from PD and MPTP-treated mice using the human and mice cDNA expression array membranes where thousands of genes can be analyzed at once, followed by quantitative RT-PCR and in-situ hybridization methods for confirmation. Chronic MPTP treatment in mice induced alterations of some 51 different genes involved in iron metabolism, oxidative stress, inflammatory processes, neurotorphic factors, glutamate, nitric oxides synthase, heat shock proteins and cell cycle and a number of other unknown genes. Pretreatment of mice with neuroprotective antiParkinson drugs eg. Rasagiline, R-apomorphine and EGCG prevented the increase or decrease of most, but not all these genes and induced neuroprotection of dopamine neurons. These results indicated the neuroprotective activity of specific genes. We have also employed this technique to study brain gene expressions as a means to arrive at a possible unity mechanism for the action of different classes of antidperessants (lithium, MAO inhibitors, tricyclic antidepressants, SSRI) on chronic (3 weeks) treatments in rat. We believe that this technique will enhance the studies on mechanism of action of neuropsychiatric drugs. The expression pattern of genes can provide "indirect" information about function and dysfunction. It will also give an over view of known and unknown mechanisms, for example in the process of neurodegeneration, neuroprotection or neuropsychiatric abonormanlity. It will provide new directions mechanism of action and development of drugs, which are not possible with the present conventional biochemical techniques.

Session 2 – Medical Symposium II

2.3 **SURGICAL TREATMENT OF MOVEMENT DISORDERS**

Marek Harat
Clinic of Neurosurgery and Head Surgery, 10 Military Clinical Hospital, Bydgoszcz

Progress of techniques of the central nervous system imaging and development of operative instruments and computer software allowed on return of stereotactic methods in the treatment of the extrapiramidal disorders in the second half of the nineties. Macrostimulation and examination of the cell potentials increase safety and effects of surgical treatment. Besides lesion procedures, frequently performed, stimulators can be implanted. The most frequent entity is Parkinson Disease which occur in 80000 people in Poland and most of them may have quality of life improved. Other entities are: essential tremor, posttraumatic and poststroke tremor, chronic pain syndromes, dystonies. Since 1999 in our clinical department over 200 functional stereotactic procedures in these entieties have been performed. Techniques, effects of treatment and complications are presented.

2.4 **VASCULAR DEMENTIA – CURENT CONCEPTS ON DIAGNOSIS AND THERAPY**

Zdzisław *Maciejek*
Dept of Neurology, Military Clinical Hospital, ul. Powstańców Warszawy 5, 85-681 Bydgoszcz

The increase of elderly population leads to increased number of cases of dementia – also mixed: vascular and Alzheimer type. Cortical and subcortical infarcts may be the cause of vascular dementia. Clinical criteria for vascular dementia (ICD-10, DSM-IV, NINDS-AIREN) are: impairment of memory and cognitive functions, neurological symptoms and signs of focal cerebral lesion, confirmed by brain CT and MRI, the beginning of dementia within 3 months after stroke. The other symptoms of vascular dementia are: changes of personality and mood, disturbances of equilibrium and gait, drop attaks, urine inconfinend, pseudo-bulbar syndrome. Symptoms that make the diagnosis of vascular dementia imposible are as follows: early onset of memory and cognitive function deficiency (aphasia, apraxia, agnosia), lack of focal neurological signs and "coresponding" focal lesions in brain CT-scans and MRI. Disturbances of consciousness exclude the diagnosis of vascular dementia. Complex clinical, psychological, laboratory and genetic examinations are necessary for proper diagnosis of vascular dementia.

The main aim of the treatment is to delay the occurrence of first symptoms of the disease, to slow its course and to minimalize the degree of clinical symptoms. The therapeutic methodes are based on the normalisation of neurotransmition, improvement of cerebral metabolism, stabilisation of cerebral membranes, antyinflamatory treatment and the inhibition of excitatory aminoacids.

Session 7 - Parallel Symposium: Understanding of neuroprotection: molecular and cellular mechanisms

7.4 MITOCHONDRIAL INVOLVEMENT IN ISCHEMIC BRAIN DAMAGE

Tadeusz Wieloch[1], T. Melcher[2], M. Gonzalez-Zulueta[2], [1]Wallenberg Neuroscience Center, Lund University, BMC A13, S-22184 Lund, Sweden; [2]AGY Therapeutics Inc., South San Francisco, CA 94080.

Mitochondria as been implicated in ischemic brain injury. We analyzed the temporal changes in gene expression in the rat brain in an in vivo model of ischemic tolerance induced by a brief 3 min ischemic period of global ischemia. By using differential cloning techniques and cDNA array analyses we found increased expression of mitochondrial uncoupling protein-2 (UCP-2), a cation carrier present in the inner mitochondrial membrane. Using *in situ* hybridization, increased expression of UCP-2 was found at 48 h of reperfusion following 3 min ischemia, while no increase was seen after 10 min ischemia which causes massive cell death in the CA1 region. Transgenic mice overexpressing UCP2/3 (tgUCP2/3) were subjected to focal ischemia, and displayed a dramatic reduction in infarct volume compared to wildtype controls. Furthermore, flowcytometric analysis of isolated brain tgUCP2/3 mitochondria showed that UCP2 attenuated mitochondrial production of reactive oxygen species. We conclude that UCP-2 can act as an inducible neuroprotective factor in the brain, possibly by inhibiting mitochondria-induced cell death.

Session 28 – Poster Session: Biological membranes

28.14 GABA INDUCED PRESYNAPTIC INHIBITION OF SPIDER CUTICULAR MECHANOSENSORY NEURONS

Panek, I and Torkkeli, PH

Dept. Physiology and Biophysics, Dalhousie Univ., Halifax, Canada.

Presynaptic inhibition of mechanosensory afferents is an ubiquitous phenomenon throughout the animal kingdom. This inhibition involves a depolarization of the afferent terminals called primary afferent depolarization (PAD) that leads to blockade of action potentials (AP) or reduction of their amplitude, and consequently to a reduction in the effectiveness of synaptic transmission to postsynaptic neurons. Several mechanisms have been suggested to be responsible for PAD, the most widely accepted model assumes that the depolarization is induced by efflux of Cl^- from GABA-gated Cl^- channels which transiently drives the membrane potential toward the Cl^- equilibrium potential.

We recorded the electrical responses of VS-3 neurons, innervating the lyriform slit sense organ the spider, *Cupiennius salei*, to bath application of GABA. GABA application induced an increase in membrane conductance and blocked AP propagation by membrane depolarization. The amplitude of depolarization was dependent on holding voltage and reversed at about -35 mV, more than 20 mV positive to the normal resting membrane potential of these neurons.We are currently testing the known agonists and antagonists of different types of GABA receptors to identify the types receptors located in these neurons. While presynaptic inhibition of sites close to output synapses can selectively block transmission at specific axons, inhibition of peripheral regions could be more significant because its effect is directed at all sites of neurotransmission. More subtle control than total inhibition can be achieved by peripherally located synapses, such as adjusting neuronal sensitivity to stimulus frequencies as an adaptation to the animal's behavioral or physiological state.

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