

POSTER SESSIONS

NEUROPHARMACOLOGY

P1.01 Effect of BU 224 on convulsive threshold in epilepsy model in miceAricioglu F.¹, Salanturoglu G.¹, Buldanlioglu U.¹, Hudson A.L.²¹Department of Pharmacology, Faculty of Pharmacy, University of Marmara, Istanbul, Turkey; ²Psychopharmacology Unit, School of Medical Sciences, University of Bristol, Bristol, UK

BU 224 is a new selective imidazoline (I) ligand which shows high affinity to I2 receptors. There is evidence for general I receptor ligands that they can change the convulsive threshold. The effect of I2 ligands is still unknown. The aim of the study was to investigate the effect of BU 224, a selective I2 ligand, on convulsive threshold by using maximal electroshock model in mice. Balb/c mice (25–30 g) were used. Convulsive current 50 value to produce seizures was found (46 mA). Saline or BU 224 was given intraperitoneally at doses of 2.5, 5 and 10 mg/kg 30 min before maximal electroshock. BU 224 increased convulsive threshold dose dependently. We conclude that I2 receptors may play a role in epileptic activity.

P1.02 Inhibitory effect of antipsychotic drugs on the human corticotropin-releasing-hormone gene promoter activity operate through a PI3-K/AKT mediated pathway

Basta-Kaim A., Budziszewska B., Jaworska-Feil L., Tetich M., Lason W.

Institute of Pharmacology, Polish Academy of Sciences, Krakow

Antipsychotic drugs can directly regulate some gene transcription, including genes involved in regulation of HPA axis, which activity is frequently disturbed in schizophrenic patients. However molecular mechanism of their action on the CRH gene activity has not been investigated so far. This study was undertaken to examine the influence of antipsychotic drugs on the CRH gene promoter activity in differentiated Neuro-2A cell cultures stably transfected with a human CRH gene promoter. It has been found that chlorpromazine, haloperidol, clozapine, thioridazine, promazine, risperidone and raclopride inhibited CRH-CAT activity. Sulpiride and remoxipride had no effect. The involvement of protein kinases in chlorpromazine and clozapine inhibitory action on CRH activity was also investigated. It was found that wortmannin, an inhibitor of PI3-K significantly attenuated inhibitory effect of chlorpromazine and clozapine on CRH gene promoter activity. In line with these results, a western blot study showed that these drugs increased phospho-Ser-473 Akt level, had no effect on total Akt and decreased GSK-3 β level. The obtained results indicate that inhibition of CRH gene promoter activity by some antipsychotic drugs may be a molecular mechanism responsible for their inhibitory action on HPA axis activity.

P1.03 Involvement of OCTN2 and B0,+ in the transport of carnitine through an *in vitro* model of the blood-brain barrier
Berezowski V.¹, Miecz D.², Marszalek M.², Broer A.³, Broer S.³, Cecchelli R.⁴, Nalecz K.A.²¹CELLIAL Technologies, Lens, France; ²Nencki Institute of Experimental Biology, Warsaw, Poland; ³Australian National University, Canberra, Australia; ⁴University of Artois, Lens, France

Carnitine is known to accumulate in brain, therefore transport of carnitine through the blood-brain barrier was studied in an *in vitro* system using bovine brain capillary endothelial cells (BBCEC) grown on filter inserts in a co-culture system with glial cells. Long-term exposure of BBCEC to carnitine resulted in a high accumulation of long-chain acyl carnitines, which decreased dramatically upon removal of carnitine. Kinetic analysis of carnitine accumulation indicated a possibility of functioning as more than one transporter. BBCEC were incubated in the presence of substrates and inhibitors of known carnitine transporters added from either apical or basolateral side. Inhibition by replacement of sodium and expression of OCTN2 (RT-PCR) were in agreement with Kido and coauthors (2001) on the functioning of OCTN2 in apical membrane. For the first time, functioning of OCTN2 was demonstrated in the basolateral membrane, as well as functioning in both membranes of a low affinity carnitine transporter B0,+ . Expression of B0,+ in BBCEC was confirmed by RT-PCR. These results suggest that OCTN2 and B0,+ could be involved in carnitine transport in both the apical and basolateral membrane.

P1.04 Calcium channel antagonists attenuate the expression of mecamlamine-precipitated nicotine withdrawal in mice
Biala G., Budzyska B.

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

The present study focused on the evaluation of nicotine abstinence syndrome in mice and on the influence of the L-type voltage-dependent calcium channel antagonists on the expression of the somatic signs of nicotine withdrawal. Our experimental protocol consisted of intermittent chronic administration of nicotine, 2.5 mg/kg, four times daily for 7 days. A single dose of the nicotinic receptor antagonist mecamlamine precipitated somatic withdrawal signs such as wet dog shakes, jumping, paw tremor, body tremor, chewing, ptosis and piloerection in nicotine-dependent mice. Additionally, 24 h after terminating nicotine treatment, we also observed additional nicotine abstinence measures, such as loss of body weight, decrease of spontaneous locomotor activity and anxiogenic responses in the elevated plus maze test. Interestingly, our data indicate that nimodipine, verapamil, flunarizine and diltiazem, injected before mecamlamine administration, dose-dependently attenuated the expression of nicotine withdrawal signs. These findings reveal the involvement of calcium-dependent mechanisms in the expression of mecamlamine-precipitated nicotine abstinence syndrome. Finally, we can suggest that the calcium channel antagonists offer an interesting approach for the pharmacotherapy of nicotine addiction.

P1.05 D1 receptor is involved in socio-sexual learningBialy M.¹, Kalata U.¹, Nikolaev A.¹, Golebiowska A.², Nikolaev E.²¹Department of Experimental and Clinical Physiology, Medical University of Warsaw, Poland; ²Nencki Institute of Experimental Biology, Warsaw, Poland

D1 receptor can be involved in incentive learning. During an acquisition of sexual experience male start to vocalize in 50-kHz band (PVs) before introduction of female and change copulatory performance (EL) (Behav Neurosci 114: 983). The D1 receptor agonist SKF-38393 has no effects on PVs and copulation in sexually experienced rats (Physiol Behav 75: 1). First, the role of s.c. injected a D1 receptor agonist SKF-38393, a selective D1 agonist SKF-82957, and a D1 antagonist SCH-23390 on the acquisition of sexual experience was investigated. The both agonists inhibit changes in PVs at all doses and at lower doses with weak facilitation effects on EL. The antagonist inhibits simultaneously changes in PVs and EL. Microinjection of D1 agonist SKF-82957 into nucleus accumbens (N. Acc.) shell inhibits changes in PVs but increase the number of males started to copulation. The results indicate role of D1 receptors in acquisition of socio-sexual experience and N.Acc. as a structure involved in these processes.

Medical University of Warsaw Grant (1M/W1/2005).

P1.06 Elevated expression of HSP72 mRNA in the prefrontal cortex of rats nonresponding to imipramine treatment in the chronic mild stress model of depressionBielawski A.¹, Papp M.², Nalepa I.¹¹Laboratory of Intracellular Signaling, Institute of Pharmacology, PAS, Krakow, Poland; ²Laboratory of Behavioral Pharmacology, Institute of Pharmacology, PAS, Krakow, Poland

The chronic mild stress (CMS) procedure induces depression-like symptoms in animals and is a useful tool to study the mechanisms of action of antidepressant drugs in animals. Heat shock proteins (HSPs) are induced not only by heat shock but also various other environmental stresses. The aim of the study was to investigate the expression of HSP72, HSP73, HSP86 and HSP84 mRNAs in the prefrontal cortex of rats subjected to the standard CMS procedure and then treated with an antidepressant drug, imipramine (IMI). Five groups of male Wistar rats were considered in the molecular study: sham-saline; stress-saline; sham-IMI; stress-IMI and IMI-non-responders (the group of stressed rats, which did not respond to IMI treatment). The expression of HSPs mRNAs was measured by quantitative real-time PCR method. We have found that though CMS alone did not affect the expression of any HSPs mRNAs, in IMI non-responding rats the level of HSP72 mRNA was increased (by 96%, $P < 0.04$) compared to IMI responders. Our results suggest the existence of permanent cellular stress reaction in the prefrontal cortex of rats non-responding to IMI treatment.

Supported by statutory funds of the Institute of Pharmacology

P1.07 The effects of antidepressant treatments on glutamatergic transmission in rat frontal cortexBobula B.¹, Hess G.^{1,2}¹Institute of Pharmacology, PAS, Krakow, Poland; ²Jagiellonian University, Krakow, Poland

We studied adaptive modifications in glutamatergic synaptic transmission induced in rat frontal cortex by antidepressant therapies. Rats were treated with one of the three antidepressant drugs: imipramine, citalopram and tianeptine or with a non-pharmacological therapy, the electroconvulsive shock (ECS). Field potentials, evoked in *ex vivo* brain slices by stimulation of layer V, were recorded in layer II/III. Treatment with imipramine resulted in an average decrease of the field potential amplitude by approx. 10%, 35% and 34% for 7, 14 and 21 days of drug administration, respectively. Treatment with citalopram resulted in a decrease of the amplitude of field potentials by approx. 14%, 20% and 34% for 7, 14 and 21 days of drug administration, respectively. The amplitude ratio of pharmacologically-isolated NMDA to AMPA/kainate receptor mediated components of the field potentials was also altered. In contrast, repetitive ECS did not induce significant changes in the amplitude of field potentials. Current experiments are aimed at finding the effects of tianeptine treatment. These results indicate that pharmacological treatments, but not ECS, induce adaptive modifications of glutamatergic synaptic transmission in rat cortex, which depend on the duration of treatment. This effect is consistent with the "glutamatergic hypothesis" of the action of antidepressants.

P1.08 Antidepressant and anxiolytic-like effects of the antagonists of group I mGluRs after hypoxia

Car H., Nadlewska A., Wisniewska R.J.

Department of Pharmacology, Medical University, Bialystok, Poland

Potential antidepressant-like effects of ligands of metabotropic glutamate receptors (mGluRs) have been postulated. Hypoxia produced disturbances in excitatory transmission and impaired behavioral activity of rats. The present work assessed the effects of DHPG (0.01, 0.1, 1.0 nmol i.c.v.), the selective an agonist of group I mGluRs, AIDA (100 nmol i.c.v.) and LY367385 (5 nmol i.c.v.), the selective antagonists of mGluR1, mGluR 5 and mGluR1a, respectively, on activity of rats in Porsolt test and elevated plus maze after hypoxia. Neither a single doses of DHPG nor AIDA, or LY367385 influenced activity of rats in Porsolt test, and only, anxiolytic-like effect LY367385-induced was observed in the elevated plus maze. Short-term (4 min) episode of hypoxia (2% O₂, 98% N₂) produced depressant-like effect in forced swimming test and anxiogenic-like activity of rats. In rats after hypoxia we observed antidepressant and anxiolytic-like effects of the both antagonists of group I mGluRs, however, anxiolytic-like effects probably influence obtained antidepressant-like activity of rats. We suggest that single episode of hypoxia produced depression and anxiety, and that antagonists group I mGluRs have beneficial activity in those situation. Obtained results indicate also important role of mGluRs in hypoxia-induced disorders.

P1.09 Brain VMAT2 unchanged in portocavally shunted rats
Fogel W.A.¹, Michelsen K.², Lewinski A.¹, Panula P.², Maksymowicz M.³, Stasiak A.¹

¹Medical University of Lodz, Poland; ²Abo Akademi University, Finland; ³Medical Research Center, PAS, Warsaw, Poland

Rats with portocaval anastomosis (PCA), a model of hepatic encephalopathy (HE), have raised histamine (HA) levels in brain and stomach. HA transport into neurons and endocrine cell storage vesicles are mediated by vesicular monoamine transporter VMAT2. This study examined its function in PCA rats. Lewis rats with end-to-side PCA and sham-operated pairs were used 2–11 mo post surgery. Brain VMAT2 and HA localisation and density were analysed immunohistochemically, binding studies with 3H dihydrotetrahydrobenzazepine, a VMAT2 ligand. Reserpine an inhibitor of vesicular transport was given. Neuronal fibre HA immunofluorescence was greater in many areas of PCA brains but VMAT2 immunofluorescence did not differ in PCA vs. control. Occurrence and density of HA and VMAT2 immunoreactive neurons and nerve fibres and VMAT2 characteristics (Kd, Bmax) were similar in PCA and sham rats. All reserpine-treated rats (R, 5 mg/kg, i.p.) developed stomach ulcers; the number and severity were higher in PCA rats. Brain and stomach HA levels were less at 24 h and 120 h after R. Brain telemethylhistamine levels, increased by PCA, were not altered by R nor was its urinary output; suggesting a different R-insensitive storage compartment. Thus VMAT2 capacity is beyond the normal demands and may efficiently sequester even high histamine levels as found in HE.

P1.10 Effects of direct and indirect dopamine receptor agonists on the cannabinoid CB1 receptor agonist CP 55,940-induced discrimination

Frankowska M., Filip M., Przegalinski E.

Institute of Pharmacology, PAS, Krakow, Poland

Of the two cannabinoid receptors, only CB1 receptors are expressed in the brain (Herkenham et al. 1991, *J Neurosci*). Recent data indicate an interaction between CB1 receptors and dopamine (DA) neurotransmission, especially on the level of DA D2 receptors. In fact, stimulation of CB1 receptors increases DA release (Romero et al. 1995, *Life Sci*), while stimulation of DA D2 receptors triggers the release of an endocannabinoid (Beltramo et al. 2000, *J Neurosci*). In our experiment male Wistar rats were trained to discriminate the CB1 receptor agonist CP 55,940 (0.1 mg/kg) from vehicle; moreover, we advanced the hypothesis that direct DA D2/3 receptor agonists (quinpirole and pramipexole) or indirect DA receptor agonists (amphetamine and cocaine) can alter CP 55,940 discrimination. In substitution studies, quinpirole (0.3–1 mg/kg), pramipexole (0.125–0.5 mg/kg), amphetamine (0.5–1 mg/kg) or cocaine (10–20 mg/kg) evoked only a 30% drug-lever responding. In combination studies, neither quinpirole (0.3 mg/kg) nor pramipexole (0.25 mg/kg) altered the dose-response curve of CP 55,940 (0.0125–0.1 mg/kg). The selective CB1 receptor antagonist rimonabant (0.2–6 mg/kg) antagonized CP 55,940 (0.1 mg/kg) discrimination. Our results indicate that – dependent on CB1 receptor stimulation – CP 55,940 discrimination is not altered by the enhanced DA D2 receptor function.

P1.11 Individual differences in sensitization effect of repeated amphetamine administration on NK-related cytotoxic activity
Glac W., Leszkowicz E., Tokarski J.

Department of Animal Physiology, University of Gdansk, Poland

In rats differing in locomotor reactivity to novelty (high responders, HRs, and low responders, LRs) the effects of repeated i.p. injections of amphetamine (AMPH) (7×1 mg/ml/kg, every other day) on natural killer cells cytotoxicity (NKCC) (51Cr-release assay), the number of LGLs (NK cells) (Timonen method) and plasma corticosterone level (CORT) (RIA) were evaluated. Animals exposed to seven injections of AMPH showed higher elevation of NKCC, LGL number as well as CORT level when compared to effects of a single injection (respectively on average from 122% above the baseline after acute AMPH to 174% above the baseline after repeated AMPH, from 35% to 55%, and from 432% to 537%). Repeated doses of AMPH resulted in stronger sensitizing changes in HR than LR rats (by 25% for NKCC, 18% for LGL and 375% for CORT). The surprising effect of the obtained data is that progressive NKCC enhancement (which determines defense against tumor and virus infections) may occur in response to repeated administration (addiction-simulating) of AMPH. Locomotor activity differentiates this effect: HR rats show higher sensitization to stimulatory effect of multiple doses of AMPH on NKCC.

P1.12 Effects of new, original serotonin (5-HT)_{2A} receptor antagonists on amphetamine discrimination in rats: A comparison between typical and atypical antipsychotics

Golda A.¹, Filip M.¹, Nowak E.¹, Byrtus H.², Obniska J.², Pawlowski M.²

¹Institute of Pharmacology, PAS, Krakow, Poland; ²Faculty of Pharmacy, Jagiellonian University, Krakow, Poland

Recent theories focus on the role of 5-HT_{2A} and 5-HT_{1A} receptors in controlling schizophrenic symptoms (Calsson et al. 1999, *Eur Arch Psychiatry Clin Neurosci*). In the search for new antipsychotic drugs, a group of new original derivatives of 5-spirohydantoin (I, II, III) and succinimide (IV) with antagonistic activity and high affinity for 5-HT_{2A} receptors were synthesized. We tried to determine whether those new drugs altered the discriminative effects of amphetamine, an indirect dopamine agonist which induces psychotic (positive) symptoms in humans, and compared their effects with those of classic (haloperidol or raclopride) and atypical (clozapine) antipsychotics. In rats trained to discriminate amphetamine (1 mg/kg) from saline, II weakly attenuated, while haloperidol and raclopride blocked the effects of amphetamine. Clozapine, the selective 5-HT_{1A} receptor antagonist WAY 100635 or the 5-HT_{2A} receptor antagonist SR 46349B were inactive in the amphetamine discrimination. These results indicate that selective 5-HT_{2A} and 5-HT_{1A} receptor antagonists are unable to antagonize the discriminative properties of amphetamine in rats, which reflect their lack of therapeutic efficacy to the positive symptoms of schizophrenia.

P1.13 Citalopram attenuates the enhancement of morphine dependence in rats subjected to chronic mild stress model of depression

Gruca P., Mrowiec S., Lason M., Litwa E., Papp M.

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

In chronic mild stress (CMS) procedure animals subjected to variety of mild stressors develop various abnormalities, which speak to the face and construct validity of this procedure as an animal simulation of depression and can be reversed by chronic administration of various antidepressants. Our previous studies showed that the CMS procedure provides an adequate basis for studying the relationship between depression and various aspects of drug addiction in animals. In the present studies we evaluated the effect of chronic citalopram on the development of morphine dependence in control and stressed rats. Citalopram attenuated the enhancement of both the motivational (naloxone-induced conditioned place aversion) and physical (abstinence syndrome precipitated by naloxone) aspects of morphine withdrawal observed in animals undergoing the CMS procedure. Citalopram also inhibited the aversive and physical symptoms of morphine withdrawal precipitated by presentation of conditioned stimuli (vanilla and tone associated with injections of naloxone), which were much more severe in animals subjected to the CMS procedure. These results confirm that the CMS model potentiates development of drug dependence, and suggest possible use of antidepressant drugs in the therapy of drug abuse and dependence in humans

P1.14 The influence of adenosine receptor agonists on the development of sensitization to diazepam withdrawal signs

Listos J.

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

Sensitization is thought as the mechanism associated with the process of the drug dependence. The most drugs of abuse have been shown to produce the sensitization with repeated exposure. The chronic treatment with benzodiazepines may lead to the state of dependence. In the present experiments the influence of adenosine receptor agonists on the development of sensitization to diazepam (DZ) withdrawal signs was evaluated in mice. DZ was administered at the single, daily dose of 15 mg/kg for 21 days (chronic treatment) or for 3 × 7-day periods, interspersed with two 72 h drug free periods (intermittent treatment). Forty-eight hours after the last injection of DZ the simultaneous administration of pentetrazole with flumazenil induced the withdrawal signs: clonic seizures, tonic convulsions and death episodes. We observed the marked intensification of withdrawal signs in intermittent DZ treated mice, in compare with mice chronically DZ treated. It confirmed that the sensitization of withdrawal signs has been developed. The administration of three injections with the selective adenosine A1 (CPA) or A2A (CGS 21680) receptor agonists or the nonselective adenosine A1/A2A receptor agonist (NECA), during 72 h drug free periods attenuated DZ withdrawal signs, indicating the involvement of adenosinergic system on the development of sensitization to DZ withdrawal signs.

P1.15 Effect of desipramine on depression- and anxiety-like behavior in mice selected for high and low stress-induced analgesia

Juszczak G.R., Sliwa A., Wolak P.M., Tymosiak-Zielinska A., Swiergiel A.H.

Dept. of Animal Behavior, Inst. of Genetics and Animal Breeding, PAS, Jastrzebiec, Poland

Numerous attempts have been made to create animal models of genetic predisposition to depression. Mice selected for high (HA) and low (LA) stress-induced analgesia were subjected to tail suspension (TST) and open field (OFT) tests. TST is widely used to screen for antidepressants and OFT provides measures of motor activity and anxiety-like behavior. Mice were injected with antidepressant desipramine (DMI). HA mice display increased depressive-like behavior in TST and are sensitive to DMI. LA mice display decreased depressive-like behavior and are insensitive to DMI. Differences in duration of immobility are independent of motor activity because both lines display similar locomotor activity in the OF. HA mice are more anxious than LA mice as determined by the latency of first entrance to the open field center an display also some peculiarities in locomotion. DMI does not affect behavior in open field, thus conferring its specificity to depression-but not anxiety-like behavior in both HA and LA lines. Supported by KBN Grant 3PO4C 0582 and the EU Framework 6 NEWMOOD Integrated Project to AHS.

P1.16 Effect of chronic mild stress (CMS) on behavior of mice selected for high and low stress-induced analgesia

Juszczak G.R., Sliwa A., Wolak P.M., Tymosiak-Zielinska A., Swiergiel A.H.

Dept. of Animal Behavior, Inst. of Genetics and Animal Breeding, PAS, Jastrzebiec, Poland

Chronic mild stress (CSM) is model of depression developed for rats and mice. It has been reported that mild, unpredictable and long lasting different stressors produce in animals neuroendocrine and behavioral responses resembling those observed in human depression. Male mice belonging to lines selected for high (HA line) and low (LA line) swim stress-induced analgesia were subjected to CMS for at least 6 weeks. sucrose solutions intake, nociception and behavior in tail suspension (TST) and forced swim (FST) tests were then studied. CMS reduced 5% though not 3% sucrose intake but no significant differences between the lines were observed. There were no obvious effects of CMS on performance of LA mice in TST and only small effects were observed in HA mice. No differences between the control and CMS groups were observed in FST. CMS significantly shortened latency of a paw withdrawal on a hot plate in HA mice but not in LA mice suggesting that CMS produced hyperalgesia in depression-prone HA mice. It is concluded that CMS induces in mice behavioral alterations comparable to some depressive symptoms in humans, the effects being dependent on genotype. Supported by KBN Grant 3PO4C 0582 and the EU Framework 6 NEWMOOD Integrated Project to AHS.

P1.17 Effect of MK-801 and lipopolysaccharide on acoustic startle reflex in mice selected for high and low stress-induced analgesia

Juszczak G.¹, Blaszczyk J.W.², Sliwa A.¹, Wolak P.M.¹, Swiergiel A.H.¹

¹Dept. of Animal Behavior, Inst. of Genetics and Animal Breeding, PAS, Jastrzebiec, Poland; ²Nencki Institute of Experimental Biology, PAS, Warsaw, Poland

Acoustic startle reflex (ASR) is a protective response that is enhanced in threatening situations or following an aversive event. It is known that peripheral injections of lipopolysaccharide (LPS) produce changes in stress hormones secretion and sickness behavior. Since LPS affects the neural circuitry involved in emotional states, we have put forward a hypothesis that LPS influences the magnitude of ASR. Male mice selected for high (HA line) and low (LA line) swim stress-induced analgesia (SSIA) were used. Before measuring ASR, mice were injected with LPS, MK-801 (NMDA receptors antagonist) or saline. Pronounced differences between HA and LA mice, with HA mice displaying considerably higher basal ASR magnitudes than LA mice, were observed. The results suggest that the two lines of mice differ in the emotional reactivity. MK-801 augments ASR in LA but not in HA mice suggesting that NMDA receptors are involved in mediating ASR in LA line. However, LPS does not affect ASR and does not impair responses to salient stimuli.

Supported by KBN Grant 3PO4C 0582 and the EU Framework 6 NEWMOOD Integrated Project to AHS

P1.18 Effect of the biogenic amines octopamine, tyramine and dopamine on behaviour of ants reunited after a period of social isolation

Korczynska J., Szczuka A., Kieruzel M., Majczynski H., Khvorostova N., Godzinska E.J.

Nencki Institute of Experimental Biology, PAS, Warsaw, Poland

We tested the hypothesis proposing that the same basic neurochemical system is involved in two reward-related phenomena: isolation-induced trophallaxis in ants, known to be reduced by octopamine (OA) treatment, and sensitization to cocaine in *Drosophila* fruit flies, known to involve both tyramine (TA) and dopamine (DA) systems. To that purpose, we investigated the effects of abdominal injections of OA, TA and DA on the behaviour of minor nurse workers of the ant *Camponotus herculeanus* during the nestmate reunion tests carried out after 5 or 20 days of social isolation. OA influenced significantly all forms of social interactions between the tested ants, suppressing trophallaxis and antennal contacts with the nestmate, but stimulating allogrooming. Both OA and TA influenced significantly some forms of self-focused behaviour and some forms of activity directed to the elements of physical environment of the ants. The effects of OA and TA were never antagonistic. TA alone influenced significantly further two behaviour patterns of unclear function, and DA had no significant effect on any feature of behaviour displayed by the tested ants. Our data imply that isolation-induced trophallaxis in ants and sensitization to cocaine in *Drosophila* flies are mediated by different neurochemical mechanisms.

P1.19 Age-related effects of midbrain 5-HT1A receptors stimulation in rats

Krotewicz M., Koprowska M., Strzelczuk M.

Laboratory of Neurophysiology, University of Lodz, Poland

The effects of 5-HT1A receptor agonist 8-hydroxy-dipropylaminotetralin (8-OHDPAT) injection into the dorsal raphe nucleus (DRN) on behavioral indices measured in the light-dark transitions test (LDT) were examined in young – 3-month-old, mature – 12-month old, and aged – 24-month-old Wistar rats. The most indicative markers used for the assessment of anxiety-like behavior were: the number of returns (RET) from the dark to the illuminated compartment of experimental chamber, the number of head dipping (HDD) from the dark to the illuminated compartment, and the time of motionless behavior (TML) in the illuminated compartment. Administration of 8-OHDPAT (300 ng) into the DRN evoked diversified behavioral effects depending of the rat age. The largest differences in the behavioral parameters occurred in young rats and demonstrated itself in a significant increase in RET and HDD with TML decreasing at the same time as compared to the same 3-month old saline-injected rats. In aged rats 8-OHDPAT administration exerted only a significant increase in HDD and decrease in TML as compared to the same 24-month old saline-injected rats. We observed weakest effects in mature rats showing only a tendency of RET and HDD growth with a TML decreasing tendency at the same time. The observed differences revealed the age-dependent effects of 8-OHDPAT action in midbrain of rats.

P1.20 Immunomodulatory effect of antidepressant drugs in animal models of depression

Kubera M., Basta-Kaim A., Budziszewska B., Jaworska-Feil L., Roman A., Leskiewicz M., Tetich M., Korzeniak B., Lason W.

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

Recently it has been suggested that activation of pro-inflammatory response is involved in the pathophysiology of depression. Immunomodulatory effect of several types of antidepressants were tested in new model of depression induced by repeated intermittent lipopolysaccharide (LPS) injection (C57BL/6 mice) and Porsolt test (Wistar rats or wild-type and noradrenaline-transporter knockout C57BL/6J mice). Antidepressogenic effect of these drugs were associated with decrease of proliferative activity of lymphocytes in response to mitogens and production of superoxide anions by macrophages, switch from Th1 type cytokines to Th2 type cytokines and reduction of serum corticosterone level. These results suggest that therapeutic activity of antidepressant drugs is connected with their immunosuppressive effect on some aspects of cell-mediated immunity.

P1.21 Behavioral effects of repeated treatment with amitriptyline and citalopram in the olfactory bulbectomy model of depression in C57BL/6J mice

Legutko B., Dudys D., Ciszek M., Wieronska J., Palucha A., Branski P., Pilc A.

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

Olfactory bulbectomy (OB) in rats is one of the currently used models of depression. The OB induced deficits in rat behaviors are similar to human depression symptoms and can be reversed by long-term (but not acute) administration of effective antidepressant drugs (ADs). The objective of the present study was to investigate the effects of treatment with ADs on the behavioral changes produced by the removal of olfactory bulbs in strain C57BL/6J mice. The behavioral effects of OB and subsequent ADs treatment in mice were compared in the locomotor activity and the passive avoidance tests, two most adequate behavioral paradigms employed in the OB model of depression. Repeated administration (twice daily for 14 days) of amitriptyline (10 mg/kg) or citalopram (10 mg/kg), attenuated the hyperactivity of OB mice. In the passive-avoidance paradigm, chronic amitriptyline treatment caused non-significant attenuation of the effects of OB; however, in the case of amitriptyline a tendency to counteract the effect of bulbectomy was observed. Our preliminary results suggest that the OB procedure in C57BL/6J mice may be a useful model for studying the mechanism of ADs in knockout and transgenic mice of the same strain.

Supported by the KBN, Poland (Grant No K058/P05/2003) and BMBF, Germany.

P1.22 Inhibitory effect of neurosteroids on glucocorticoid receptor-mediated gene transcription: An involvement of protein kinases and interaction with psychotropic drugs

Leskiewicz M., Basta-Kaim A., Budziszewska B., Tetich M., Otczyk M., Lason W.

Institute of Pharmacology, Krakow, Poland

Some neurosteroids attenuate the stress-activated HPA axis activity. However, intracellular mechanism of their interaction with glucocorticoids has not been elucidated. To this end we evaluated effects of some neurosteroids on functional activity of glucocorticoid receptor (GR) *in vitro*. Combined treatment with antipsychotic drugs and involvement of some protein kinases in allopregnanolone effect on GR function was also studied. We found that allopregnanolone and to a lesser extent its both isomers in concentration dependent manner inhibited the GR-mediated gene transcription, whereas dehydroepiandrosterone sulfate (DHEAS) was inactive. Allopregnanolone and chlorpromazine or clozapine showed additive inhibitory effect on the GR function. It was also found that allopregnanolone inhibited PKC activity and decreased the amount of active phosphorylated form of ERK-MAPK in LMCAT cells, but had no effect on PKA activity. These data indicate that allopregnanolone and its isomers like antidepressants and antipsychotic drugs may attenuate some glucocorticoid effects *via* inhibition of GR-mediated gene transcription. The inhibitory effect of allopregnanolone on the corticosterone induced gene transcription in LMCAT cells depended on inhibition of PKC and ERK-MAPK pathways.

P1.23 The influence of kindling and hippocampal group I mGluR's on fear conditioning

Maciejak P.¹, Lehner M.¹, Szyndler J.², Taracha E.¹, Skorzewska A.¹, Bidzinski A.¹, Plaznik A.^{1,2}

¹Department of Neurochemistry, Institute of Psychiatry and Neurology, Warsaw, Poland; ²Department of Pharmacology, Medical University, Warsaw, Poland

The effects of post-training (a conditioned fear test), intra-hippocampal injections of group I mGluR ligands were examined in chemically kindled rats (a model of temporal lobe epilepsy). It was found that in control rats intra-hippocampally given AIDA (a mGluR1 antagonist) increased, while DHPG (a mGluR1 and 5 agonist) decreased conditioned freezing reaction on retention test. In kindled group, freezing reaction was attenuated by AIDA, CHPG (a mGluR5 agonist), and (MPEP) (a mGluR5 antagonist). Microdialysis (hippocampus) showed that baseline concentration of GABA was lower, whereas that of and glutamate (Glu), aspartate and Glu/GABA ratio were higher, in kindled animals. Factor analysis revealed opposite patterns of changes in Glu/GABA ratio in kindled and control animals. In kindled rats post-conditioning administration of AIDA increased c-Fos in CA3 layer of the hippocampus and basolateral amygdala. Classification Tree analysis showed Glu/GABA ratio and c-FOS induction in basolateral amygdala to have similar prediction accuracy in discriminating between kindled and control animals. It is suggested that enhanced neuronal activity evoked by kindling is the result of an imbalance in activity of central GABAergic and glutamatergic systems.

P1.24 Herbal drugs used to prevent neurodegenerative diseases – risk of interactions

Ozarowski M., Mrozikiewicz P.M.

Research Institute of Medicinal Plants, Poznan, Poland

Neuronal cell degeneration and death are critical components of CNS diseases (i.e., Alzheimer's disease, Parkinson's disease). Several active compounds of herbal medicines shows similar pharmacological mechanisms as synthetic drugs, i.e., inhibition of the neurotransmitter degradation (ACh), binding to receptors (GABA, NMDA) and influencing CNS microcirculation. Herbs could supplement conventional CNS pharmacotherapy, however several reviews on herb-drug interactions (positive or negative) have been published recently. We have summarized this possible pharmacodynamic interactions and tried meta-analysis for this interaction. The similarity of CNS effects of herbs and synthetic drugs can be affirmed, thus the interactions between herbs and synthetic drugs are possible. This interaction in CNS could result in stronger neuroprotective and antiexcitotoxic effects in neurodegenerative cascades. *Huperzia serrata* (huperizin A), *Galantus nivalis* (galantamine), *Withania somnifera* (withaferin) slightly decrease acetylcholinesterase (AChE) activity. Ginkgolides (*Ginkgo biloba*) and ginsenosides (*Panax ginseng*) can interact with the cholinergic system. *Hypericum perforatum*, *Passiflora incarnata* showed possible nootropic action. In conclusion, possible pharmacodynamic interaction between herbal and synthetic drugs in CNS could result in stronger synergistic effect in therapy.

P1.25 Nicotine potentiates the effect of imipramine related to its antidepressant-like action through activation of noradrenergic system

Michaluk J., Romanska I., Krawczyk M., Kos T., Antkiewicz-Michaluk L.

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

Many lines of evidence demonstrated that nicotine potentiates the antidepressant-like actions of classic and atypical antidepressant drugs. The present experiments were carried out on male Wistar rats to investigate the effects of chronic treatment with imipramine with or without nicotine on a behavioral (locomotor activity) and biochemical (metabolism of monoamine) response produced by apomorphine challenge (0.15 mg/kg s.c.). The content of dopamine (DA) noradrenaline (NA), serotonin (5-HT) and their metabolites was assayed by HPLC. The results have shown, that chronic administration of imipramine caused hyper-responsiveness to apomorphine and this behavioral effect was significantly potentiated by co-administration with nicotine. Biochemical results have shown that apomorphine produced in saline treated rats strong inhibition of DA metabolism in STR and N. Acc., and significant inhibition of NA metabolism in BrSt. Co-administration of nicotine with imipramine not only antagonized the inhibitory effect of apomorphine on noradrenaline metabolism, but significantly increased it. In conclusion, co-administration of nicotine with imipramine leads to the activation of noradrenergic neurons during dopaminergic stimulation by apomorphine, as the result of adaptation changes within central nervous system.

P1.26 Some aspects of (-)-2'-thionicotine behavioral activity in rats-preliminary report

Mikolajczak P.L.¹, Boczon W.², Okulicz-Kozaryn I.¹, Wojciechowska M.², Kaminska E.¹, Szulc M.¹

¹Department of Pharmacology, Poznan University of Medical Sciences, Poland; ²Laboratory for Chemistry of Heterocycle Compounds, Adam Mickiewicz University, Poznan, Poland

Nicotine has well documented effects on cognitive and motor function, but some novel nicotine analogues may exert anxiolytic, antidepressant and antipsychotic activity (Singh et al. 2004). The aim of this study has been to investigate some aspects of pharmacological profile of new nicotine analogue – (-)-2'-thionicotine (2'-THN). In acute toxicity test 2'-THN up to 300 mg/kg (i.p.) was found to be a safe compound, as it did not produce any mortality in mice. Subsequently, male Wistar rats were given a single injection of 2'-THN (3, 30, 300 mg/kg, i.p.) and their sedative activity, motor coordination, anxiety-related reactions and cognitive function were assessed using actinometer, "chimney test", elevated-plus maze and passive avoidance test, respectively. It was found that 2'-THN produced dose-dependent lowering of spontaneous activity of rats. Improvement of motor coordination was shown at the lowest dose of 2'-THN, whereas such effects were not observed in higher doses. The compound affected neither the cognitive function nor anxiety-related reactions. Concluding, it seems that 2'-THN, especially in the lowest dose, shows an agonistic-antagonistic activity, yet further studies may need to be carried out to confirm this hypothesis.

P1.27 Brain zif-268 expression under immunization in rats

Myslinska D., Tokarski J., Cecot T., Trojnar W.

Department of Animal Physiology, University of Gdansk, Poland

Mapping of brain activatory process as measured by zif-268 expression following systemic administration of LPS ($n=6$) and Maloney-virus-induced lymphoma cells YAC-1 ($n=6$) was done on the male Wistar rats. The results obtained indicate that immunization with both LPS and YAC-1 cells caused significant enhancement of neural activity mainly in the diencephalon. The highest density of Zif-268 positive cells in both experimental groups was found in the paraventricular (LPS: 213%; YAC-1: 97% above the control), supraoptic (respectively: 177%; 122%) and anterior (240%; 210%) hypothalamic nuclei. A significantly higher zif-268 expression was found in almost all nonspecific, i.e., midline and reticular nuclei and limbic thalamic nuclei and also in the perirhinal cortex, medial part of amygdala and the bed nucleus of stria terminalis. High basal (saline injection; $n=6$) zif-268 expression was observed in the limbic cortex, amygdala and the bed nucleus of stria terminalis as well as in the extrapyramidal structures, motor and sensorimotor cortex with no differences between both experimental and control groups. No traces of Zif-268 protein were found in the cerebellum and brain stem structures. Data obtained indicate that under immunization besides nonspecific brain structures the activity of the hypothalamic-pituitary-adrenal axis is and structures related to it substantially increased.

P1.28 Influence of MPEP on certain behaviors in rats subjected to experimental episodes of hypoxia

Nadlewska A., Car H., Wisniewska R.J.

Department of Pharmacology, Medical University, Bialystok, Poland

The influence of the selective blockade of mGluR5 by MPEP on some behavioral effects was tested in control groups of rats and in rats that underwent short-term hypoxia once and repeated episodes of hypoxia. We used the following methods: the open field test and the passive avoidance test. MPEP (1 mg/kg i.v.) significantly enhanced locomotor and exploratory activity, improved the consolidation and retrieval in the passive avoidance situation. Single short-term hypoxia significantly inhibited motility of rats and profoundly impaired consolidation and retrieval processes, but the positive effect of MPEP on retrieval was preserved. The repeated episodes of short-term hypoxia were induced for five consecutive days and it also inhibited motility of rats, but did not influence on consolidation and retrieval processes. The episodes of hypoxia significantly diminished beneficial effect of MPEP on consolidation and retrieval, and locomotor and exploratory activity. MPEP used after the repeated episodes of short-term hypoxia, did not change behavioral activity of rats. Summary: MPEP used before the single episode of hypoxia only, had beneficial effect on retrieval.

P1.29 Prenatal exposure to lead influences the synthesis rate and level of the biogenic amines in the striatum and prefrontal cortex after amphetamine and/or 7-nitroindazole challenge in adult rats

Nowak P., Szczerbak G., Bortel A., Dabrowska J.B., Brus R.

Department of Pharmacology, Medical University of Silesia, Zabrze, Poland

Lead (Pb) is a highly neurotoxic agent, in both mammals and human. It is a major contaminant of the civilized environment, due to its high natural abundance, its massive industrial use and its being a component of paint and a pollutant of crude gasoline. In this study the effect of lead intoxication (Pb 250 ppm) during pregnancy of rats was studied on the biogenic amines and their metabolites level in prefrontal cortex and striatum in adult offspring after amphetamine and/or 7-nitroindazole challenge. Additionally, dopamine and serotonin synthesis rate was determined. The obtained results indicated that prenatal exposure to lead influences the dopamine level and metabolism predominantly in the striatum, whilst serotonin and norepinephrine were almost unaffected. We have also shown that in animals prenatally intoxicated with lead, inhibition of the brain nitric oxide synthesis influences in a different manner the amphetamine action on dopaminergic system in comparison with control animals. This study was supported by the grant from the State Committee for Scientific Research, Warsaw, 2 P05D 066 27.

P1.30 Central noradrenergic system lesion by DSP-4 prevent quinpirole ontogenically sensitization to quinpirole-induced yawning in rats

Nowak P.¹, Labus L.¹, Kostrzewa R.M.², Brus R.¹

¹Department of Pharmacology, Medical University of Silesia, Zabrze, Poland; ²Department of Pharmacology, James H. Quillen College of Medicine, Johnson City, USA

It is known that dopamine (DA) receptor can be sensitized by repeated treatment with quinpirole during postnatal development. It is manifested by increasing of locomotor activity and yawning behavior in adult animals. This study was undertaken to confirm whether low-dose quinpirole treatments may sensitize dopamine D2/D3 central receptor complex to quinpirole-induced yawning behavior. Additionally effect of DSP-4 on this behavior was examined. Newborn male rats were treated daily with quinpirole or saline during 10 days of postnatal life. DSP-4 (50.0 mg/kg, s.c.) was injected on the day 1st and 3rd of life. In 8 weeks old rats the level of biogenic amines and their metabolites was estimated in the frontal cortex, hippocampus and striatum of the brain. DSP-4 decreased NE level in the frontal cortex and hippocampus. DSP-4 injected neonatally prevents the dopamine D2/D3 receptor priming, and in experimental rats number of yawns after quinpirole challenge was as in control group. Above findings showed that for priming of the central dopamine D2/D3 receptor complex intact central noradrenergic system is necessary. Supported by Medical University of Silesia, grant NN-1-002/03.

P1.31 The influence of magnesium on the action of the antidepressant drugs in swim test in mice

Poleszak E.¹, Kedzierska E.¹, Wlaz P.², Nowak G.^{3,4}, Fidecka S.¹, Pilc A.^{3,5}

¹Department of Pharmacology and Pharmacodynamics, Medical University School, Lublin, Poland; ²Department of Animal Physiology, Institute of Biology, Maria Curie-Skłodowska University, Lublin, Poland; ³Department of Neurobiology, Institute of Pharmacology, Polish Academy of Sciences, Krakow, Poland; ⁴Department of Pharmacobiology, Collegium Medicum, Jagiellonian University, Krakow, Poland; ⁵Institute of Public Health, Collegium Medicum, Jagiellonian University, Krakow, Poland

Magnesium is an essential mineral that is needed for a broad variety of physiological functions. Deficiency of magnesium has been linked to depression and mood disorders. In animals magnesium, being a potent inhibitor of the NMDA receptor, exhibits antidepressant-like effects. The present study was aimed to investigate the interaction of magnesium with antidepressants in the forced swim test in mice. Mice were injected with imipramine, citalopram or reboxetine alone, and in combination with magnesium. Low, ineffective *per se* doses of imipramine and citalopram, but not reboxetine were active in the swim test in mice when administered together with ineffective doses of magnesium. Our results indicate that antidepressant-like activity of magnesium may be connected with the serotonergic neurotransmission.

P1.32 Brain-derived neurotrophic factor gene expression in rats following repeated treatment with mirtazapine

Rogoz Z., Skuza G., Dudys D., Cizek M., Legutko B.

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

Recent studies suggest a role of the brain-derived neurotrophic factor (BDNF) in the pathophysiology of depression. It is shown that serum BDNF levels are decreased in depressed patients. Moreover, antidepressant treatment increases serum BDNF levels and is positively correlated with a response to medication. In addition, repeated administration of classic antidepressant drugs (ADs) induces an increase in rat hippocampal or cortical BDNF gene expression. Furthermore, BDNF itself is sufficient to produce an antidepressant response. Since the most potent effect of classic ADs on BDNF gene expression was found after prolonged treatment, in the present study we investigated the influence of repeated treatment (twice daily for 14 days) of a new AD, mirtazapine (5 or 10 mg/kg), on BDNF mRNA level (the Northern blot) in rat hippocampus and cerebral cortex. To control experimental conditions, we also demonstrated an effect of the classic AD imipramine. The experiment was carried out on male Wistar rats. The tissue for biochemical assays was collected 24 h after the last dose of mirtazapine and imipramine. The obtained results show that mirtazapine, like imipramine, increases the BDNF gene expression in both the examined brain regions, and suggest that the enhancement of BDNF may be essential for the clinical effect of mirtazapine.

P1.33 Chronic co-administration of imipramine and amantadine induces hippocampal BDNF gene expression in rats

Rogoz Z., Skuza G., Legutko B.

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

The problem of antidepressant-resistant depression has been the subject of extensive studies, yet with no apparent therapeutic success. We found previously that joint administration of imipramine (IMI) and amantadine (AMA), a non-competitive NMDA receptor antagonist, produced a more potent antidepressant effect in the forced swimming test than did treatment with either drug alone. Our studies also indicated that co-treatment of IMI and AMA to drug-resistant, unipolar depressed patients led to clinical improvement. Despite more than 40 years of research, the mechanism of antidepressant action has not yet been fully elucidated. The majority of adaptive changes, proposed to be responsible for neurochemical antidepressant mechanisms, are not common to all antidepressants therapies. Since the most potent effect of ADs on BDNF gene expression was found after prolonged treatment, in the present study we investigated the influence of chronic treatment with IMI and AMA on BDNF mRNA level (the Northern blot) in the hippocampus. The obtained results showed that joint administration of IMI and AMA induced a more potent increase in the level of BDNF mRNA than did treatment with IMI or AMA alone; moreover, they suggested that BDNF may be involved in the mechanism of the synergistic antidepressant effect of IMI and AMA in drug-resistant depressed patients.

P1.34 Effects of lipopolysaccharide, interleukin-1beta and chronic mild stress on nociception in mice

Sadowski B., Wolak P.M., Juszcak G.R., Sliwa A., Tymosiak-Zielinska A., Swiergiel A.H.

Dept. of Animal Behavior, Inst. of Genetics and Animal Breeding, PAS, Jastrzebiec, Poland

Sickness behavior (SB) is regarded as an animal model of depressive behavior related to immune disturbances. However, the symptoms of SB and those of depression are not intimately correlated. One of the differences is nociception. Lipopolysaccharide (LPS) and interleukin-1beta (IL-1) produce SB and hyperalgesia in rats. Depressed humans can be either hypersensitive to pain or experience hypoalgesia. Male mice selected for high (HA) and low (LA) swim stress-induced analgesia (SSIA) were subjected to: (1) injections of LPS and IL-1; or (2) chronic mild stress (CMS), which is established animal model of depression. To assess pain sensitivity the animals were tested on a hot plate (HP) and latency of a paw withdrawal or its licking was scored. (1) SSIA was not altered by LPS injected before the swim. (2) LPS did not affect latency on the HP at 56 and 52 but at 49 deg C it produced hypoalgesia that was more pronounced in HA than in LA mice. (3) IL-1 produced hypoalgesia in HA but not in LA mice. (4) CMS produced hyperalgesia in HA mice but no in LA mice. Changes in nociception in animal models of depression may depend on the species, the genotype or the depression-producing procedure used.

Supported by KBN Grant 3PO4C 0582 and the EU Framework 6 NEWMOOD Integrated Project to AHS

P1.35 Effects of lipopolysaccharide, interleukin-1 and lactoferrin on intake of sweet solutions by mice

Sliwa A., Wolak P.M., Juszcak G.R., Tymosiak-Zielinska A., Swiergiel A.H.

Dept. of Animal Behavior, Inst. of Genetics and Animal Breeding, PAS, Jastrzebiec, Poland

Lipopolysaccharide (LPS) or interleukin-1beta (IL-1) produce depression-like behaviors including disturbed feeding and anhedonia. Mechanisms underlying anhedonia have not been fully elucidated but interaction between cytokines, opioids and brain reward system may play a role. Decreased consumption of palatable liquids is considered a possible measure of anhedonia. Satiated male mice from lines selected for high and low swim stress-induced analgesia (HA and LA lines) and differing in activity of opioids systems were trained in: (1) two-bottle test (water vs. sweetened milk or 2 or 5% sucrose or 0.15% saccharine solutions) to drink during 30 min or 4 days, (2) operant cages to obtain drops of milk as reinforcement. It was found that: (1) HA mice exhibited lower intake of the fluids than LA mice but higher 2% sucrose vs. water preference in the 4 days test, (2) injections of IL-1 and LPS depressed drinking in both lines but HA mice displayed a tendency for a more pronounced decrease in intake as compared to LA mice, (3) lactoferrin injected 30 min before LPS caused greater decrease of sweet milk intake than LPS alone but LF administered 24 h before LPS attenuated LPS-induced hypophagia.

Supported by KBN Grant 3PO4C 0582 and the EU Framework 6 NEWMOOD Integrated Project to AHS

P1.36 Anxiolytic activity of metabotropic glutamate receptor group II and III ligands in rat hippocampus: The putative role of neuropeptide YSmialowska M.¹, Wieronska J.M.¹, Domin H.¹, Zieba B.¹, Obuchowicz E.²¹Department of Neurobiology, Institute of Pharmacology, Krakow, Poland; ²Department of Clinical Pharmacology, Silesian University School of Medicine, Katowice, Poland

Our previous studies indicated that metabotropic glutamatergic receptor (mGluR) ligands may have anxiolytic activity and that neuropeptide Y (NPY) neurons in the amygdala were engaged in that effect. In the present study we tried to determine if similar effects may also be found in the hippocampus. Rats were injected intrahippocampally with the mGluR group II agonist L-CCG-I, or the group III agonist L-SOP. Some rats received additionally Y1 or Y2 receptor blocker BIBO or BIIE, respectively. Anxiolytic effects were tested in a plus-maze. The effects of ligands on NPY neurons were studied using NPY radioimmunoassay and *in situ* hybridization (NPYmRNA) methods. A significant anxiolytic effect was observed after L-SOP injection into the CA1 region, or after L-CCG-I injection into the GD of the hippocampal formation. The former effect was inhibited by BIBO, the latter by BIIE. Both L-SOP and L-CCG-I induced an increase in NPY level and NPYmRNA expression in the hippocampus. The obtained results indicate that mGluR agonists display anxiolytic activity in the hippocampus, and they suggest a different site of action and NPY mechanisms in the case of group II and III agonists.

P1.37 Naloxone (NX) antagonizes the enhancement of plasma beta-endorphin (B-END) level under immobilization (IMB) stress in pigs

Stojek W., Ciepiewski Z., Komarowska I., Borman A., Hirsz A., Tokarski J.

Department of Animal Physiology, University of Gdansk, Poland

The experiments were carried out on chronically catheterised (vena jugularis exterior) piglets divided into 3 groups: NN and Nn – stress resistant, nn – stress susceptible. The level of B-END and cortisol (COR) (both determined by RIA) in the course of 4h restraint with or without i.v. naloxone (NX, 1 mg/kg) administration was evaluated. The stress-induced augmentation of B-END level was significantly suppressed by NX: NN – 51.40% under comparison of maximal stress effects (without NX – 160.38 ± 7.67 pg/ml, with NX – 77.94 ± 12.51), Nn – 59.22% (without NX – 229.68 ± 9.59, with NX – 93.67 ± 8.90) and nn – 35.30% (without NX – 212.41 ± 9.20, with NX – 137.43 ± 11.11). Typical increase in COR level, characterizing stress reaction, was not significantly suppressed by NX and its changes were not significantly correlated with plasma B-END level. Naloxone-related, stress-induced increase in B-END is surprising. These effects indicates that "opioid form of stress" is dependent not only on the opioid receptor system (common view), but also on the high release of endogenous opioids, particularly intensive in stress susceptible (recessive homozygous) and resistant to stress heterozygous pigs.

P1.38 Behavioral alterations after 8-OHDPAT and WAY-100635 administration into the dorsal raphe nucleus in rats

Strzelczuk M., Koprowska M., Krotewicz M.

Laboratory of Neurophysiology, Lodz, Poland

The effects of 8-OHDPAT (300 ng), 5-HT_{1A} receptor agonist and WAY-100635 (500 ng), 5-HT_{1A} receptor antagonist co-administration into the dorsal raphe nucleus (DRN) on fear behavior in the light-dark transitions test were examined. Administration of 8-OHDPAT alone evoked anxiolytic effect revealed as an increase in the number of head dipping from the dark to the illuminated part of experimental chamber, but administration of WAY-100635 alone evoked anxiogenic effect revealed as a decrease of time out from the illuminated to the dark part of chamber and a decrease of time of locomotor activity in the illuminated part of chamber. Co-administration of WAY-100635 and 8-OHDPAT, in sequence WAY-100635 3 min before 8-OHDPAT, evoked anxiogenic effect identical symptoms as evoked WAY-100635 alone. As a result of the competence antagonism between the equimolar doses of WAY-100635 and 8-OHDPAT prior occupancy of 5-HT_{1A} receptor by WAY-100635 precludes 8-OHDPAT action. These results provide one more proof on specific and key role of 5-HT_{1A} receptors in the central regulation of fear behavior.

P1.39 Effect of oral administration of octopamine on the expression of predatory behaviour in workers of the ant *Formica polyctena*

Szczuka A., Godzinska E.J.

Nencki Institute of Experimental Biology PAS, Warszawa, Poland

As shown by us earlier, the expression of predatory behaviour in workers of the wood ant *F. polyctena* is retained not only in whole colonies, but also in groups composed solely of workers. However, the group size must exceed the threshold size of about 30, 40 workers. The effects of group size on the degree of completeness of sequence of predatory behaviour were flexible and reversible. To throw more light on neurobiological mechanisms of that phenomenon, we investigated the effect of chronic oral administration of octopamine (OA), a neurochemical implicated in the mediation of many behavioural and physiological processes in social Hymenoptera, and, in particular, in the control of the transition nurse-forager in the honeybee. OA (at a successively increasing dose of 2, 5 or 10 mg/ml of carbohydrate food) was offered to workers of *F. polyctena* kept in groups counting about 25 individuals. OA treatment had a significant effect on expression of predatory behaviour by increasing the incidence of seizing, transport and retrieval of insect prey. This implies that oral OA treatment has a stimulatory effect on the expression of predatory behaviour in small groups of workers of *F. polyctena*. However, that effect is not strong enough to induce the expression of the complete sequence of predatory behaviour in a durable way and in all treated groups.

P1.40 Effects of acute restraint on intake of palatable food by mice

Tymosiak-Zielinska A., Sliwa A., Swiergiel A.H.

Dept. of Animal Behavior, Inst. of Genetics and Animal Breeding, PAS, Jastrzebiec, Poland

Effects of restraint, which causes strong stress and induces sickness behavior (SB), on feeding were studied. Decreased intake of palatable foods is considered a measure of SB and, possibly, of anhedonia. Consumption of high motivational value sweetened milk was investigated in two lines of mice selected for high and low swim stress-induced analgesia (HA and LA lines) and differing in activity of opioid systems. Satiated males were trained in two-bottle test (water vs. milk) to drink as much as they could during 30 min once a day for at least 7 days. The animals were then restrained in small cylinders and after 10 or 30 min returned to their cages and presented with milk. Restraint strongly depressed drinking and HA mice drank significantly less than LA mice. It is concluded that in response to emotional stress, HA mice display a tendency for a more pronounced anhedonia than LA mice. The results agree with other observations suggesting that HA mice are susceptible to stress and display anxiety- and depression-like behavioral patterns. Experiments with anxiolytics and antidepressants that could affect restraint-induced anhedonia are in progress.

Supported by KBN (Polish Committee for Scientific Research) Grant 3PO4C 0582 and the EU Framework 6 NEWMOOD Integrated Project to AHS

P1.41 Interactions between depression and anxiety – effects of depression-inducing treatment on behavioral and neurochemical measures of anxiety

Wieczorek M.¹, Sliwa A.², Juszcak G.R.², Swiergiel A.H.²

¹Dept. of Neurobiology, Lab. of Neurophysiology, University of Lodz, Lodz, Poland; ²Dept. of Animal Behavior, Inst. of Genetics and Animal Breeding, PAS, Jastrzebiec, Poland

The aim of the present study was to determine the effect of the forced swim test (FST) on anxiety-like behavior in mice. The subjects were males belonging to the 59th generation of Swiss-Webster selectively bred for divergent magnitude of swim stress induced analgesia and displaying long (HA) and short (LA) post-swim nociceptive latencies. Animals were divided into 4 groups: HA-FST, LA-FST, HA-noFST and LA-noFST and then subjected to the open field (OF) and elevated plus maze (EPM) tests. It was found that the FST increased anxiety-like behavior that was line-dependent. Furthermore, the neurotransmitter systems activity was assessed. The FST produced an increase in MHPG/NE and 5-HIAA/5-HT ratios in several brain structures. It is concluded that the FST produces anxiety responses and affects brain neurotransmitter systems activity. Also, the effect of genetic lines on neurochemical activity was observed.

Supported by KBN Grant 3PO4C0582 and the EU Framework 6 NEWMOOD Integrated Project to AHS

P1.42 Activity of ligands of mGluRs in water maze in rats after hypoxia

Wisniewska R.J., Car H.

Department of Pharmacology, Medical University, Bialystok, Poland

The role of group I metabotropic glutamate receptors (mGluRs) in water maze is still unclear. The group I/II mGluR specific agonist, ACPD, and an antagonist, MCPG, were reported to have detrimental effects on learning in water maze. The present investigation assessed the effects of the selective group I mGlu receptor antagonists: AIDA, MPEP and LY367385, and APDC, an agonist of group II mGluRs, as well as L-AP4, an agonist of group III mGluRs on spatial learning in the Morris water maze in rats after hypoxia. AIDA, MPEP and APDC did not influence acquisition of spatial memory but reduced activity of rats in a free-swim trial. LY367385 and L-AP4 prolonged significantly distance and swim speed at first session of training and did not influence probe trial performance. Hypoxia produced disturbances in excitatory transmission and significantly impaired of spatial learning, but none of ligands of mGluRs used diminished hypoxia effect. Hypoxia changed activity of AIDA and L-AP4, only. We suggest that spatial learning probably is not mediated by single administration of these ligands of mGluRs, but effects of AIDA and L-AP4 are sensitive to hypoxia.

P1.43 Involvement of rimonabant, a cannabinoid cb1 receptor antagonist, in the behavioral effects of cocaine

Zaniewska M.¹, McCreary A.C.², Wydra K.¹, Nowak E.¹, Filip M.¹, Przegalinski E.¹

¹Institute of Pharmacology, Polish Academy of Sciences, Krakow, Poland; ²Solvay Pharmaceutical Research, Weesp, the Netherlands

Recent data indicate that cannabinoid CB1 receptors may play a crucial role in the brain reward system (Cohen et al. 2002, Behav Pharmacol; Lesscher et al. 2005, Eur Neuropsychopharmacol). In the present study we used the selective CB1 receptor antagonist rimonabant (SR141716) to evaluate the role of CB1 receptors in cocaine discrimination and reward. In rats trained to discriminate cocaine (10 mg/kg) from saline, SR141716 (5 mg/kg) administered in combination with cocaine (1.25–5 mg/kg) reduced the discriminative stimulus effects of cocaine. In rats trained to self-administer cocaine (0.5 mg/kg/infusion), SR141716 (5 and 10 mg/kg) did not affect cocaine reinforcement, but attenuated the cocaine-seeking behavior evoked by either the priming dose of cocaine (10 mg/kg) or the drug-associated cue-induced relapse. The present study describes the role of CB1 receptors in the subjective effects of cocaine and cocaine- and cue-primed seeking behavior. In contrast, current findings demonstrate that CB1 receptors are not necessary to maintain cocaine self-administration. These data are consistent with the presumption that SR141716 may be a potential medication in the case of cocaine cessation.

SYNAPTIC TRANSMISSION AND EXCITABILITY

P2.01 Electrophysiology of optic nerve input to the intergeniculate leaflet neurons in rats – *in vitro* studies

Blasiak A., Lewandowski M.H.

Institute of Zoology, Jagiellonian University, Krakow, Poland

Neurons in the mammalian intergeniculate leaflet (IGL), an element of the circadian timing system, receive direct photic input from the retina. Till now there is no data about the electrophysiological and pharmacological properties of the retinal input to the IGL. The purpose of this study was to analyze responses of the rat IGL in slice preparation, to the optic nerve stimulation and to identify neurotransmitters released from the terminals of retinal ganglion cells in this structure. Following optic nerve stimulation most of the responding IGL cells were excited and minority were inhibited. Neurons that were activated, were tested in the presence of APV-NMDA receptor antagonist. In the half of activated cells, responses were blocked completely in the presence of APV. In the remaining neurons, responses were partially inhibited in the presence of APV. Complete blockage of excitatory response was achieved by adding to the incubation fluid AMPA/kainite receptor antagonist-CNQX. Inhibitory responses were blocked in presence of bicuculline in the ACSF. The major conclusion of this study is that the glutamate is the main neurotransmitter mediating optic nerve input to the IGL, and that glutamate acts mainly via ionotropic receptors. Results of this study also indicate that GABAA receptors are involved in passing photic input to the IGL. Supported by grant 2PO4C 00327 and BW/IZ/30a

P2.02 Bafilomycin blocks vesicular histamine transport in *Drosophila*

Borycz J.A.¹, Borycz J.¹, Lu Z.¹, Pyza E.², Meinertzhagen I.A.¹

¹Dalhousie University, Halifax, Canada; ²Jagiellonian University, Krakow, Poland

Photoreceptors in the fruit fly *D. melanogaster* use histamine as a transmitter, and must replenish their synaptic vesicles to sustain high release rates. Vesicular transport requires the action of proton pumps such as vacuolar (V-)ATPases, which are specifically inhibited by bafilomycin. In the *Drosophila* brain, an antibody against the B-subunit of dV-ATPase immunolocalizes exclusively to the optic lobe, where post-embedding immun-EM reveals that most 10-nm gold particles lie close to membranes in freeze-substituted first neuropile, or lamina. Flies that drank bafilomycin A1 (0.05–0.4 mM in 4% aqueous glucose) showed dose-dependent increases of between 12.6% and 42.2% in HPLC-determined total head histamine. We examined histamine in fractions from centrifuged fly head homogenates. Viewed by EM, pellet fractions contain synaptosomes. In Western blots, anti-cysteine string protein, a synaptic vesicle marker, recognizes a 32–34 kDa band only in this pellet fraction, indicating that it is enriched in synaptic vesicle membranes. Pellet fractions from control *Drosophila* heads contained 72.5% of the histamine; the remaining 27.5%, presumed to be non-vesicular, was in the supernatant. Bafilomycin (0.1 mM) reduced the amount in the pellet, changing this ratio of 2.64/1 to 1.67/1, suggesting that histamine transport into synaptic vesicles depends on V-ATPase activity.

P2.03 Neurochemical alterations after 8-OHDPAT and WAY-100635 administration into the dorsal raphe nucleus in rats

Koprowska M., Krotewicz M., Strzelczuk M.

Laboratory of Neurophysiology, Lodz, Poland

The effects of equimolar doses of 8-OHDPAT and WAY-100635 alone and co-administration of both drugs into the dorsal raphe nucleus on monoamines (NA, DA, 5-HT) and their metabolites (MHPG, DOPAC, 5-HIAA) alterations in the hypothalamus (HPT), amygdala (AMY), midbrain (MID), hippocampus (HIP) and pons (PO) were examined. Administration of 8-OHDPAT (300 ng) alone evoked a decrease of 5-HT turnover in the HPT, HIP and PO, an increase of NA turnover in the HIP and PO and an increase of DA level in the HPT, AMY, and HIP. Administration of WAY-100635 (500 ng) alone evoked an increase of 5-HT level in the PO and an increase of 5-HIAA level in the MID but a decrease in the HIP, and an increase of NA turnover in the HIP and a decrease of DA turnover in the MID and PO. Co-administration of WAY-100635 and 8-OHDPAT (WAY-100635 3 min before 8-OHDPAT) evoked an increase of 5-HT turnover in all investigated structures, an increase of NA turnover in the AMY and an increase of DA turnover in the HPT and MID. These results prove the "serotonergic hypothesis" of fear/anxiety mechanism to be right and also indicate specific and fundamental role of the 5-HT_{1A} receptors in this mechanism. The influence of both drugs on the NA and DA systems activity is discussed.

P2.04 Cyclodextrin affects the GABAergic currents in cultured rat hippocampal neurons

Mozrzykmas J.W., Pytel M., Mercik K.

Lab. of Neuroscience, Dept. of Biophysics, Wroclaw University of Medicine, Poland

Cyclodextrins are potentially potent tools in e.g. drug delivery, modeling the catalytic enzymes, modifying the membranes (interaction with cholesterol) and increasing solubilization of lipophilic structures. CDs are endowed with hydrophobic nanocavities designed as inclusion complex for low-molecular-weight compounds. However, it is likely that CDs interact with various components of cellular membranes and modulate their functions. In the present study we investigated the effect of CDs on neuronal GABA_A receptors in rat hippocampal cultures. Current responses to rapid GABA applications were recorded in control conditions and in the presence of beta-CD. Interestingly, CD increased the amplitude of currents elicited by saturating GABA concentration. Deactivation of currents evoked by brief pulses of saturating GABA was slowed down by CD. Desensitization of GABA_ARs was studied by applying long pulses of saturating [GABA] and CD was found to decrease the rate and extent of desensitization. Recovery process studied in the paired-pulse experiments was accelerated by CD. These data indicate that CD strongly affect neuronal GABA_A receptors most likely by interfering with the receptor gating. The molecular mechanism of this effect is not clear but it can be speculated that it involves alterations in lipid-protein interactions.

Supported by grant PBZ-MIN-001/P05/28.

P2.05 Synaptic transmission in somatosensory cortical slices of genetic epileptic WAG/Rij rats

Pitra P.¹, Grzegorzewska M.¹, Hess G.^{1,2}

¹Institute of Pharmacology, PAS, Krakow, Poland;

²Jagiellonian University, Krakow, Poland

Rats of the WAG/Rij strain are regarded as a genetic model for generalized absence epilepsy in humans on the basis of neurological, behavioural and pharmacological findings, but the cellular mechanisms of hyperexcitability of the cortex of WAG/Rij rats are not fully understood. In the present study extracellular recordings were obtained from brain slices containing the perioral and the dorsal parts of the somatosensory cortex of WAG/Rij and control Wistar rats. Field potentials were evoked by paired electrical stimuli (20 ms interpulse interval) in the vertical (layer V–layer II/III) and in the horizontal (intralaminar, layer II/III) pathways. The amplitudes of responses to the first pulse of a pair were generally not different between WAG/Rij and control rats over wide range of stimulus intensities. However, in WAG/Rij rats, a smaller degree of paired-pulse depression of responses was observed in the vertical pathway of the peri-oral cortex, but not of the dorsal cortex. Interestingly, in the same slices there was no difference between paired-pulse response ratio in the horizontal pathway. These results are suggestive of a local and selective deficit in certain intracortical GABA-ergic connections in WAG/Rij rats.

P2.06 Synaptic integration in cortical neurons under natural and artificial high-conductance states

Piwkowska Z.¹, Badoual M.¹, Rudolph M.¹, Destexhe A.¹, McCormick D.A.², Bal T.¹

¹Unite de Neurosciences Integratives et Computationnelles, CNRS, Gif-sur-Yvette, France; ²Department of Neurobiology, Yale University School of Medicine, New Haven, USA

To study the integrative properties of cortical neurons in "high-conductance states" receiving intense background synaptic bombardment, we combine two approaches: (1) intracellular recording of neurons in ferret cortical slices displaying spontaneous recurrent network activity (up-states) similar to *in vivo* observations (during sleep or anaesthesia); (2) mimicking high-conductance states by "conductance injection" with a dynamic-clamp technique into intracellularly recorded neurons. A simplified model of background synaptic conductance is run on a real-time Neuron simulator and interacts with a biological neuron in the slice. We developed and validated in our slices an analytical method allowing to extract parameters for the model from recorded background activity: these parameters allow us to re-create in the neuron Vm fluctuations following the same distribution as fluctuations seen during up-states. We are currently using both approaches to determine how layer 5 pyramidal neurons process very distal, apical inputs during on-going background synaptic activity. Preliminary results indicate that synaptic responses evoked by layer I electrical stimulation can be boosted and re-shaped by up-states occurring spontaneously in the slice.

BIOCHEMISTRY OF ADDICTION**P3.01 1-Methyl- 1,2,3,4-tetrahydroisoquinoline attenuates ethanol, cocaine and morphine addiction in behavioral models: neurochemical correlates**

Antkiewicz-Michaluk L.¹, Filip M.¹, Kostowski W.², Patsenka A.¹, Popik P.¹, Przegalinski E.¹, Wrobel M.¹

¹Institute of Pharmacology, PAS, Krakow, Poland; ²Institute of Psychiatry and Neurology, Warsaw, Poland

1-Methyl- 1,2,3,4-tetrahydroisoquinoline (1MeTIQ) is an endogenous compound synthesized in the mammalian brain. Our earlier reports have shown its neuroprotective properties against rotenone-induced neurotoxicity as well as its neurochemical activity on dopaminergic and glutaminergic system. The present study was aimed to analyze its antiaddictive properties in rodents. The results show that 1MeTIQ produced: (1) reduction in cocaine discrimination (with 5–10 mg/kg of cocaine) and elicited (about 47%) substitution, when given alone (50 mg/kg) to rats trained to discriminate cocaine, (2) inhibition of both, the acquisition and expression of conditioned place preference (CPP) induced by morphine in mice, without producing either CPP or aversion itself, (3) dose-dependent (25, 50 and 75 mg/kg, i.p.) reduction of ethanol consumption in rats selected to alcohol preference without changing of total fluid intake. Biochemical HPLC study showed a slight increase of DA metabolism in the striatum after acute application of 10% ethanol. Co-administration of 1MeTIQ with ethanol significantly modified its biochemical effect. In conclusion we suggest that 1MeTIQ may be considered as the potential antiaddictive agent.

P3.02 The effect of opioid receptor blockade on nicotine-induced antinociception and dependence in mice

Biala G., Budzynska B.

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

It has been shown that several behavioural and pharmacological actions are shared by nicotine and morphine. Additionally, nicotine can produce many of its behavioural effects by activating the opioid system. The present study was designed to evaluate the implication of the opioid system in nicotine-induced antinociception and physical dependence in mice. The results indicate that, at a dose of 3 mg/kg, nicotine produced a significant antinociception in the hot plate test in mice. Moreover, the opioid receptor antagonist naloxone dose-dependently attenuated this effect. Our second experimental protocol used consisted of intermittent administration of nicotine, 2.5 mg/kg, four times daily for 7 days. In attempt to precipitate nicotine abstinence, mice were given one injection of the nicotinic acetylcholine receptor antagonist mecamylamine or naloxone, one hour after the last nicotine injection. Interestingly, our findings revealed that both drugs precipitated somatic withdrawal signs such as: front paws tremor, jumping, wet dog shakes, body tremor, ptosis, piloerection and chewing. These data support a pivotal role of an interaction between opioid and cholinergic mechanisms in mediating acute antinociceptive effect of nicotine as well as aversive consequence of nicotine withdrawal in mice.

P3.03 Genetically determined susceptibility to stress and long-lasting changes in plasma beta-endorphin (B-END) in pigs

Ciepielewski Z., Stojek W., Borman A., Tokarski J.

Department of Animal Physiology, University of Gdansk, Poland

In experiments carried out on 15 chronically catheterised cross-bred Pietrain piglets the level of plasma B-END and cortisol (COR) (both determined by RIA) were measured during 24 h of restraint. The animals were divided on the basis of halothane (phenotypic) and genetic-DNA assay (molecular analysis of RyR1 gene) into 3 groups: NN and Nn – stress resistant, nn – stress susceptible. It was found that in all pigs long-lasting stress evoked phasic changes in plasma B-END level: firstly an evident elevation with maximum at 2nd hour (by 106%, 60%, and 73% in nn, Nn and NN pigs, respectively) and then return to the baseline at 4th hour in stress susceptible nn pigs and at 8th hour in phenotypic stress resistant pigs (Nn and NN). At 16th hour the dramatic increase in B-END level in stress susceptible (nn) pigs occurred (by 492% above baseline) which persisted until the end of the experiment with maximum at 24th hour (by 747%). Typical increase in COR level, characterizing stress reaction, habituated in the course of long-lasting loading. These changes were not significantly correlated with plasma B-END level. Thus, susceptibility to stress is connected with high B-END release, especially expressed by "late endorphin excessive release" in recessive homozygous pigs (nn). Moreover, these data point to genetic basis and determination of opioid form of stress.

P3.04 Influence of C-terminal fragment of CART peptide on behavioral actions of morphine

Dylag T.¹, Kotlinska J.², Rafalski P.², Grzebisz A.², Silberring J.¹

¹Faculty of Chemistry and Regional Laboratory, Jagiellonian University, Krakow, Poland; ²Dept. of Pharmacology and Pharmacodynamics, Medical Academy, Lublin, Poland

Cocaine- and amphetamine-regulated transcript (CART) peptide is involved in feeding, stress, pain and drug dependence. The structure of CART peptide suggests the C-terminal region might be crucial for its activity. We tested whether C-terminal fragment of CART peptide, L-Abu-DCPRGTS-Abu-NSFLLKCL, is able to influence morphine-induced locomotor sensitization and withdrawal syndrome. Aminobutyric acid (Abu) residues replace naturally occurring cysteine residues to prevent their pairing. In the morphine sensitization test, mice were divided into two groups which received either morphine (10 mg/kg, s.c.) or saline, five times at 3-days intervals. On day 20, animals previously treated with morphine received a challenge dose of morphine (10 mg/kg, s.c.) and the tested peptide (0.05, 0.1 or 0.25 µg, i.c.v.). Withdrawal signs (escape jumps) were precipitated by naloxone (4 mg/kg, i.p.) in morphine-addicted mice. We found that CART (85-102) inhibits the expression of morphine-induced sensitization at the doses of 0.05 and 0.1 µg but does not influence spontaneous locomotion alone. The peptide (0.1 µg) also attenuated the withdrawal syndrome as measured by the number of escape jumps. The results suggest that CART system might be able to modify the behavioral effects of morphine.

P3.05 Operant self-administration of ethanol in Warsaw alcohol-preferring rats

Dyr W.¹, Rok-Bujko P.¹, Kostowski W.^{1,2}

¹Institute of Psychiatry and Neurology, Warsaw, Poland; ²Medical University, Warsaw, Poland

WHP and WLP rats were bred in our laboratory to obtain lines that differ in their preference for ethanol (EtOH) solution. This study examined the oral self-administration of EtOH under a lever-pressing procedure in WHP and WLP EtOH-naive rats. Rats were initiated to self-administer 10% ethanol, on a fixed ratio 1 (FR1) schedule of reinforcement in daily 30 min sessions. Then, rats were trained to self-administer EtOH on a FR-2 and FR-3 schedules. Further, the extinction responding for EtOH in the absence of EtOH reinforcement was determined.

After completion of the results, the EtOH intake of WHP and WLP rats under the home-cage two-bottle EtOH-water choice procedure was assessed. Lever pressing for EtOH on FR-1 schedule of reinforcement did not significantly differ between WHP and WLP rats. Contrary to WHP rats, WLP rats failed to acquire and maintain EtOH self-administration on a FR-3 schedule. Finally, as expected, WHP rats displayed high daily EtOH intake (6–8 g/kg/day) when exposed to the two-bottle choice. In contrast, mean EtOH intake of WLP rats was lower than 1.0 g/kg/24 h. This result suggests that EtOH acts as a stronger reinforcer in WHP rats than in WLP rats. This study was supported by the grant Nr 64, 2004-05, Inst. of Psychiatry and Neurology, Warsaw, Poland.

P3.06 Ethanol- and saccharin-induced changes in proenkephalin and prodynorphin expression in the mouse brain

Gieryk A.¹, Korkosz A.², Rzymaska I.², Ziolkowska B.¹, Bienkowski P.², Kostowski W.², Przewlocki R.¹

¹Institute of Pharmacology, Krakow, Poland; ²Institute of Psychiatry and Neurology, Warsaw, Poland

There is increasing evidence that alcohol reward involves the endogenous opioid system. Repeated exposure to ethanol may lead to changes in activity of that system, which could underlie alcohol dependence and addiction. We have used the technique of *in situ* hybridization to assess the influence of long-term ethanol drinking on the proenkephalin (PENK) and prodynorphin (PDYN) gene expression in the areas of the C57BL mouse brain implicated in addiction. Moreover, the effect of ethanol, a chemical reinforcer, was compared to that of a natural reinforcer, saccharin. Ethanol or saccharin was self-administered by mice for 30 days in the two bottle choice paradigm, while the third (control) group was exposed only to water. Alcohol drinking significantly influenced PENK gene expression only in the striatum when compared to water-drinking control. However, significant differences in the expression of the opioid genes were detected between alcohol- and saccharin-exposed animals also in other brain regions. The observed disparities between effect of ethanol and saccharin may underlie the difference between these substances in their propensity to produce addiction.

Supported by grant PBZ-KBN-033/P05/2000 (Warsaw, Poland).

P3.07 Hyperactivity of rats with the ventral hippocampal lesion is reversed by chronic cocaine self-administration

Kolomska P.¹, Wyszogrodzka E.¹, Wierzba-Bobrowicz T.¹, Kostowski W.^{1,2}, Stefanski R.^{1,2}

¹Institute of Psychiatry and Neurology, Warszawa, Poland; ²Warsaw School of Medicine, Poland

Accumulating evidence has indicated that the ventral hippocampus importantly modulates the mesolimbic dopamine system and is involved in motivated behavior. Therefore, we investigated the effect of ibotenic-acid lesions of the ventral hippocampus on locomotor activity and instrumental behavior reinforced by intravenous cocaine injection. Male, Sprague-Dawley rats were used as subjects. Rats were randomly assigned to receive either bilateral sham operations or ibotenic-acid lesions of the ventral hippocampus. Three months after surgery, ventral hippocampus-lesioned rats showed a 70% enhancement of the locomotor activity and this effect was reversed by chronic cocaine self-administration. Self-administration sessions were conducted in standard operant chambers equipped with two nose-poke operanda. Lesioned rats responded at higher rates during acquisition and maintenance of cocaine self-administration and tended to acquire self-administration faster. The present data suggest that the ventral hippocampus is involved in acquisition and maintenance of cocaine self-administration. It may also be involved in the pathophysiology of attention-deficit/hyperactivity disorder, since increased locomotor activity caused by the ventral hippocampal lesion was ameliorated by cocaine self-administration.

P3.08 Modulation of the expression of opioid inducible genes using RNA interference

Ligeza A., Wawrzczak-Bargiela A., Kaminska D., Korostynski M., Przewlocki R.

Institute of Pharmacology, PAS, Krakow, Poland

RNA interference (RNAi) is a phenomenon leading to a specific degradation of target mRNA. We employed RNAi technology in order to control the expression of selected transcription factors. CREB and Elk-1 were chosen as earlier studies have shown that opioid treatment led to their activation. These transcription factors bind to the promoter regulatory elements CRE and SRE and transactivate the transcription of target genes regulated by opioids. Using siRNA expression vector (pSilencer 2.1-U6 hygro, Ambion) we transfected HEK293 MOR cells expressing mu-opioid receptor. We obtained stably transfected cell lines expressing proper silencing constructs, capable of the degradation of target transcription factor mRNA. RT-PCR analysis revealed about 50% for CREB and 60% for Elk-1 reduction of the expression. For these cells in comparison to control HEK293 MOR cells we observed significant expression differences of some IEG after induction of PKC signaling pathway. These expression changes were measured for Arc and Fos-B, both genes implicated in the development of opioid dependence and tolerance. These preliminary results show that even 60% knock-down of transcription factor expression may result in a considerable target gene expression changes and possibly that system may be employed in further studies on molecular mechanisms of addiction.

Supported by KBN grant no. 6 P05A 107 20

P3.09 Morphine suppresses of immunityMoroz V.¹, Lelevich V.², Shejbak V.¹¹Grodno State Medical University, Grodno, Belarus; ²Institute of Biochemistry NASB, Grodno, Belarus

A change of immunoreactivity in patients with narcotisms is one of the reasons of development somatic and infectious diseases. We determined parameters of the condition of immune system after i.p. administration of 1% morphine hydrochloride solution in a growing dosage under the circuit, since 10 up to 40 mg/kg in 7, 14 and 21 day of introduction. Tests of the first level (Petrov et al. 1980) were used. Since 7 day of introduction decrease in total amount of leukocytes, which for 14 day reaches 70% with simultaneous decrease in absolute amount lymphocyte on 50%, comes to light were revealed. Decrease in absolute amount of the B-lymphocytes and the tendency to reduction of complement activity and circulating immune complexes in whey of blood is defined also. Thus, the chronic morphine intoxication results in significant infringements of immune system that is expressed in suppression of proliferate and functional activity of peripheral blood leukocytes with simultaneous reduction of absolute amount of lymphocytes and B-lymphocytes in particular. Earlier (Portales et al. 1995) it has been shown, that formation of narcotic dependence is accompanied by changes of total amount and function of leucocytes, however changes in amount B-lymphocytes not revealed.

P3.10 Cocaine-induced AP-1 transcription factor is regulated by ERKRadwanska K.¹, Valjent E.², Caboche J.², Kaczmarek L.¹¹Nencki Institute of Experimental Biology, Warsaw, Poland; ²University Pierre et Marie Curie, Paris, France

Extracellular signal-regulated kinases (ERKs) and AP-1 transcription factor have been functionally linked to addiction. It has also been shown that ERKs activation can regulate cocaine-induced expression of c-Fos and FosB protein components of AP-1. A direct link between ERKs and AP-1 activation has, however, remained unexplored. In this study we investigated the role of ERKs in the regulation of DNA-binding activity and composition of AP-1 induced in the mouse caudate putamen by cocaine treatment. We have found that pre-treatment with ERK pathway inhibitor, SL327 compound, has no influence on cocaine-induced DNA-binding activity of AP-1, 1 hour after acute cocaine treatment. This phenomenon is the result of a simultaneous decrease of c-Fos protein level and increase of JunB and deltaFosB protein levels, composing AP-1 complex. SL327 pre-treatment, however, reduces the DNA-binding activity of the AP-1 induced 6 hours after acute cocaine treatment and 1 hour after the last of the chronic cocaine injections, which could be explained by the concomitant reduction of all cocaine-induced proteins (c-Fos, FosB, deltaFosB, JunB). Our results support the notion that ERK pathway inhibitors can be a valuable tool to obliterate cocaine-induced molecular changes and thus probably the development of addiction.

P3.11 Rat brainstem mao after morphine administrationTsydik V.¹, Lelevich V.², Zimatkin S.¹¹Grodno State Medical University, Grodno, Belarus; ²Institute of Biochemistry NASB, Grodno, Belarus

Monoamine oxidase (mao) is a key enzyme of a biogenic amines-neuromediators exchange that suggests its value in narcotic drugs effects. But practically there are no researches at a cellular level by histo- or immunohisto-chemistry.

We determined by own modification of a peroxidase method activity and izozymic structure mao in neurons of a various neuromediators systems, and also in a number of a glial cells of a barrier structures of a brain of not purebred white male rats after morphine administration, as well as in dynamics of a withdrawal syndrome. A 1% solution morphine hydrochloride was administered i.p. in a growing dosage under the circuit, since 10 up to 20 mg/kg within 7 day, and with 20 up to 40 mg/kg within 7 and 14 day. "Polyamine" in a doze of 500 mkg/kg was administered i.p. 7 days of withdrawal. We have found out significant changes of mao condition in the majority structures in 1 hour after morphine administration. Changes at a withdrawal syndrome are expressed in decreases in activity of enzyme and mao a/mao b ratio after 1 day of a withdrawal. It is interesting, that at increase in a dosage and duration of morphine administration changes of mao condition are expressed to a lesser degree. "Polyamine" normalizes functional condition mao in dynamics of a withdrawal syndrome in majority of the investigated brain structures.

P3.12 1MeTIQ an endogenous neuroprotective compound prevents morphine addiction: Comparison with neurotoxic analog, 1BnTIQ

Wasik A., Romanska I., Patsenka A., Antkiewicz-Michaluk L.

Laboratory of Receptor and Neuromediator Metabolism, Institute of Pharmacology PAS, Krakow, Poland

The central dopaminergic system plays an important role in motor activity, motivation, learning and addictions. Tetrahydroisoquinolines (TIQs) are endogenous substances present in the mammalian brain and acting as natural antidopaminergic regulators. We compared the influence of two derivatives of TIQs: 1MeTIQ and 1BnTIQ on morphine mechanism of action. The present results have shown, that 1MeTIQ possesses strong anti-addictive activity, and prevents the development of morphine tolerance and dependence. Biochemical studies have shown, that 1MeTIQ significantly decreased the rate of dopamine and serotonin metabolism whereas, 1BnTIQ dramatic increased it in morphine-dependent rats. *In vivo* microdialysis study has shown, that 1MeTIQ given peripherally did not change the release of dopamine in the striatum, but causes the strong increase of its extraneuronal metabolite, 3-MT in synaptic cleft. In contrast, 1BnTIQ decreased the concentration of dopamine in the striatum and elevated the concentration of dopamine metabolites. In conclusion, we may suggest that anti-addictive effect of 1MeTIQ may be connected with its mechanism of action on dopaminergic system, which is completely different from the action by neurotoxic analog, 1BnTIQ.

P3.13 Operant cocaine self-administration in rats is abolished by the neonatal 5,7-dihydroxytryptamine treatment

Wyszogrodzka E.¹, Kolomanska P.¹, Rok P.¹, Krzascik P.², Stefanski R.^{1,2}, Kostowski W.^{1,2}

¹Institute of Psychiatry and Neurology, Warszawa, Poland; ²Warsaw School of Medicine, Poland

A large body of evidence supports the hypothesis that the reinforcing effect of cocaine depends on its ability to block not only dopamine but also serotonin (5-HT) transporters. The present study examined the effect of neonatal serotonergic lesions with the 5-HT-selective neurotoxin 5,7-dihydroxytryptamine (5,7-DHT) on intravenous cocaine self-administration. Three days old Sprague-Dawley rats were pretreated with desipramine followed by an intracisternal injection of 5,7-DHT on each side (control group received DMI and appropriate volume of vehiculum). Three month after surgery, rats were allowed to acquire self-administration of cocaine at the dose of 0.3 mg/kg/injection. Early postnatal 5-HT lesions completely blocked the acquisition of cocaine self-administration in lesioned rats. In contrast, control animals easily acquired cocaine self-administration. As indicated by neurochemical data, neonatally lesioned rats had decreased 5-HT and 5-hydroxyindoleacetic levels in the brain. These results suggest that destruction of 5-HT in early period of life strongly influences incentive motivation for cocaine. Our study provides insight into the involvement of the 5-HT system in the reinforcing effects of cocaine.

P3.14 The effects of contingent and non-contingent cocaine administration on the expression of proenkephalin, prodynorphin and dopamine D2 receptor in the rat brain

Ziolkowska B.¹, Stefanski R.², Mierzejewski P.², Kostowski W.², Przewlocki R.¹

¹Institute of Pharmacology, Polish Academy of Sciences, Krakow, Poland; ²Institute of Psychiatry and Neurology, Warsaw, Poland

Chronic exposure to drugs of abuse leads to neuroadaptations that underlie addiction. In animal models, however, some effects of the drugs may depend on whether they are self-administered or non-contingently administered to animals. In the present study, the influence of chronic response-contingent and non-contingent cocaine administration on the expression of several genes was assessed by *in situ* hybridization in the rat brain regions associated with reward and reinforcement. The expression of proenkephalin gene was not affected by exposure to cocaine in either animal group. Levels of prodynorphin mRNA were increased in the dorsal striatum both in rats self-administering cocaine and receiving non-contingent injections. In contrast, expression of the dopamine D2 receptor was increased in the ventral tegmental area only in the rats self-administering cocaine, but not those receiving the drug non-contingently. Our results indicate that the change in the D2 receptor expression was associated with motivational processes driving cocaine self-administration, whereas the effect on prodynorphin gene expression resulted from direct pharmacological actions of cocaine.

Supported by grant PBZ-KBN-033/P05/2000 (Warsaw, Poland).

BRAIN OSCILLATIONS AND RHYTHMS

P4.01 One-compartment neuron models of LGN and PGN cells in the primary visual pathway of the cat

Baszczak M., Kasicki S.

Nencki Institute of Experimental Biology, PAS, Warsaw, Poland

Two one-compartment neuron models, built in Simulink/Matlab, were adjusted to the properties of the cells in the lateral geniculate nucleus (LGN) and the perigeniculate nucleus (PGN). The values of parameters were based on experimental data obtained from adult cat. Thalamo-cortical relay neurons display two modes of action potential generation: (1) burst firing in which action potentials occur in the high-frequency burst riding on a low threshold Ca²⁺ spike, and (2) tonic, single spike activity in which action potentials are generated in trains, the frequency of which depends on the strength of depolarization. Electrophysiological data shows that these two modes depend on various ionic currents (I_{Na}, I_{NaP}, I_K, I_C, I_M, I_{AHP}, I_H, I_A, I_{K2}, I_T, I_L, I_{Kleak}, I_{Naleak}). Our neuron models are equipped with all these currents, and the cells replicate both modes of action potential generation in the thalamo-cortical relay cells, when adequately stimulated. These neuron models were connected to each other as in a real LGN-PGN loop. LGN and PGN cells interacted by excitatory (AMPA, NMDA) and inhibitory (GABAA, GABAB) synapses. A tonic stimulation of the LGN cell induced in the network spindle oscillations, as observed during experiments. This basic network will be used for creating a simple model of primary visual pathway.

P4.02 Anatomical contribution to the hippocampal theta amplitude and frequency in freely behaving cats

Bocian R., Golebiewski H., Eckersdorf B., Konopacki J.

Department of Neurobiology, University of Lodz, Poland

Occurrence of the hippocampal theta depends on the integrity of pathway originating in the brainstem reticular formation. It has been demonstrated that reticular influences are relayed to the limbic cortex *via* the posterior hypothalamus (PH) and medial septum (MS). Numerous data indicate that MS in the rats is involved in the modulation of theta amplitude and theta frequency is determined by the supramammillary nucleus (SuM) of the hypothalamus. In the present study we attempted to establish an anatomical substrate to hippocampal theta amplitude and frequency in the freely behaving cats. The following drugs were administered into MS and PH: procaine, atropine, and muscimol. Intraseptal and intrahypothalamic injections of these drugs blocked hippocampal theta rhythm. A substantial difference in the recovery time course between amplitude versus frequency of HPC theta was observed. While theta frequency was almost at the control level upon the first appearance, the amplitude showed a gradual recovery. These findings indicate that in the freely behaving cats, in contrast a freely moving or anesthetized rats, not only the MS region but also the PH itself are engaged in the modulation of the hippocampal theta amplitude. The question arises as to the locus of the neuronal substrate responsible for encoding of the frequency of the hippocampal theta in the cat.

P4.03 The effect of gap junction blockage on hippocampal theta activity

Golebiewski H., Eckersdorf B., Konopacki J.

Department of Neurobiology, University of Lodz, Poland

Regular slow activity (RSA, theta rhythm) is the most prominent, semi-sinusoidal field potential spontaneously generated by the mammalian brain. Recorded mainly in the hippocampal formation (HPC) RSA is the electroencephalographic pattern involving basic mechanisms underlying neuronal oscillation and synchrony. Current anatomical and electrophysiological evidences strongly suggest the existence of gap junctions (GJs) in the HPC, especially in CA1, CA3 and DG area. All this regions are known as intrinsic theta generators. The accumulating data have revealed that electrical communication between neurons through gap junctions determine important mechanisms of synchronization of the cell firings. The role of the gap junctions in maintenance of spontaneous as well as sensory and electrically induced hippocampal theta activity in freely moving cat we investigated in the present study. Intraperitoneal injection of the gap junction blockers (carbenoxolone or quinine) temporarily suppressed spontaneous and evoked HPC rhythmical activity. The suppression lasted a few hours and than amplitude and power of the hippocampal RSA showed a progressive increase. Theta frequency, in contrast, did not differ from the control level. The results provide evidence for the involvement of electrical coupling in production and maintenance of theta oscillations in limbic cortex *in vivo*.

P4.04 Cyclic expression of the alpha subunit of the Na/K-ATPase in the brain of *Drosophila melanogaster*

Gorska-Andrzejak J.¹, Wszolek A.², Gorlich A.², Semik D.², Pyza E.²

¹Zoological Museum, Institute of Zoology, Jagiellonian University, Krakow, Poland; ²Department of Cytology and Histology, Institute of Zoology, Jagiellonian University, Krakow, Poland

The Na⁺/K⁺-ATPase is an integral plasma membrane protein that maintains the intracellular concentrations of Na⁺ and K⁺ in the cytosol of almost all animal cells. In neurons and glia it is necessary for volume regulation, action potentials, and secondary active transport. The enzymatic activity of this pump requires at least two subunits, a catalytic alpha subunit and a regulatory beta subunit. Combinations of different isoforms of these subunits can alter the kinetics of enzyme activity dependent on a wide range of regulatory mechanisms and pathways. In the fly's visual system, temporal changes of cell plasticity induced by both external light stimuli and internal signals from a circadian clock might be dependent on Na⁺/K⁺-ATPase activity. Our previous studies showed that in the second optic neuropil (medulla), the level of expression of the beta subunit cycles in a circadian manner. It was detected as oscillations in the whole head GFP fluorescence in a transgenic line of *Drosophila* expressing GFP under control of the beta subunit gene *Nervana 2*. Here, we report the data indicating that also the expression of the catalytic alpha subunit exhibits circadian changes in the optic lobe and in the central part of *D. melanogaster* brain.

P4.05 Hemispheric information processing – slow ultradian and asymmetric rhythms

Iskra-Golec I.

Department of Management Psychology and Ergonomics, Jagiellonian University, Krakow, Poland

There have been demonstrated ultradian and asymmetric rhythms in physiological indices of brain hemisphere activation. The aim of this study was to test the following hypotheses: (i) speed of shallow and semantic encoding of laterally exposed stimuli changes daily in an ultradian manner and (ii) ultradian rhythms of stimuli processing speed addressed to the left hemisphere differs in the period length from those addressed to the right hemisphere. During 24-hours constant routine experiment information processing performance of 30 students were measured 8 times, every 2.5–3 hours starting from 06.30 A.M. Parallel sets of words and pictures were exposed at a random order in either the left or the right visual field on the computer screen by purposely-designed program. The subjects were to press one of two buttons reacting to picture or word or answering the question concerning stimuli meaning. The time series of each subject's processing speed underwent cosinor analysis. Two significant ultradian components were found. Dominant periods were analysed using three factorial ANOVA (factors were level of processing, visual field, and stimulus). There have been found that ultradian rhythm of processing speed in the left hemisphere had longer average period than rhythms of processing speed in the right hemisphere. These means an asymmetry in the rhythmicity of processing speed of brain hemispheres.

P4.06 Hippocampal theta rhythm after strychnine microinjection into the rostral part of nucleus reticularis pontis oralis in rats

Kroplewski M., Jurkowlaniec E.

Department of Animal Physiology, University of Gdansk, Poland

Nucleus reticularis pontis oralis (RPO) is thought to participate in the regulation of hippocampal theta rhythm, but involvement of rostral and caudal part of this structure is different. The caudal part is effective in producing of the theta rhythm. As indicate our previous procaine studies the rostral part of RPO may contain neurons, which inhibit the theta rhythm, possibly by GABA or glycinergic transmission. In the present study the effect of glycine receptor blockade in the rostral part of RPO on the hippocampal theta rhythm was examined in urethane-anesthetized rats. The animals ($n=8$) were implanted with recording electrodes in the dorsal hippocampus and received microinjection of glycine receptor antagonist, strychnine (15 mM, 0.5 μ l) to the rostral part of RPO. The theta rhythm was induced by sensory stimulation (60 s tail pinch) in the control condition and every 10 min after strychnine. The effect of strychnine on theta rhythm was observed for 1 hours.

Strychnine either did not influence the sensory-elicited theta rhythm or evoked 1–2 min episodes of the theta without any stimulation. The results obtained together with previous observations that procaine in the rostral RPO releases the theta indicate that rostral RPO may contain a group of neurons, at least partially glycine-sensitive, whose inhibition could facilitate the theta activity.

P4.07 Wavelet mapping of sleep spindles

Latka M.¹, Jernajczyk J.¹, Kozik A.², Jernajczyk W.³, West B.J.⁴

¹Institute of Physics, Wrocław University of Technology, Wrocław, Poland; ²Video EEG Lab, Department of Child Neurology, Wrocław, Poland; ³Sleep Disorders Center, Institute of Psychiatry and Neurology, Warsaw, Poland; ⁴Mathematical and Information Science Directorate, Army Research Office, Research Triangle, USA

Spindles are characteristic signature of human stage 2 sleep and consequently play a major role in polysomnography. We demonstrate that complex continuous wavelet transforms faithfully capture the wave-packet nature of sleep spindles. We use the wavelet power normalized by the variance of the electroencephalographic signal to characterize the distribution of spindle intensity across EEG channels. We dub this novel procedure wavelet mapping of sleep spindles. The study of 23 juveniles and children with focal epilepsy reveals strong localization of spindle intensity. Moreover, the mean maximum normalized wavelet power is significantly larger than the mean power calculated at the epileptic foci. Thus, the results of the study provide first direct indication of the pathological influence of epilepsy on spindle generation.

P4.08 Injections of substance P and its analog, DPDPT, into the pedunculopontine tegmental nucleus suppresses hippocampal theta rhythm in urethane-anesthetized rats

Leszkowicz E., Trojnar W.

Dept. of Animal Physiology, University of Gdansk, Poland

Among the reticular generators of the hippocampal theta rhythm the pedunculopontine tegmental nucleus (PPN) is one of most important. Its cholinergic, GABA-ergic and glutamatergic components are known to be involved in theta generation. Here we present data indicating that tachykinin system of the PPN may also be a part of this circuitry. In urethane-anesthetized rats implanted with bilateral recording electrodes in the dorsal hippocampus and with an injection cannula unilaterally into the PPN, the hippocampal theta rhythm was elicited by a tail pinch and the theta peak power (Pmax) and the corresponding peak frequency (Fmax) were assessed in the pre- and postinjection conditions. Intra-PPN injection of substance P (SP) and its analogue, [d-Pro2, d-Phe7, d-Trp9]-Substance P (DPDPT), caused suppression of the theta activity in both hippocampi. SP led to approximately a 50% (10 min post-SP) to 30% (60 min post-SP) decline of Pmax, with no effect on Fmax. DPDPT reduced Pmax by approximately 70% (10-60 min post-DPDPT), and Fmax by 0.4 Hz (10 min post-DPDPT). In conclusion, our study suggests that tachykinin system in the PPN may exert inhibitory influence on hippocampal theta rhythm, probably *via* activation of GABA interneurons which may tonically inhibit PPN outputs to the other theta-relevant structures.

P4.09 Opioid system in the pedunculopontine tegmental nucleus (PPN) suppresses cholinergic component of the hippocampal theta rhythm in urethane-anesthetized rats

Leszkowicz E., Matulewicz P., Trojnar W.

Dept. of Animal Physiology, University of Gdansk, Poland

Cholinergic neurons in the PPN are crucial for the induction of the theta rhythm in the hippocampus. Here we provide evidence that the opioid system in the PPN may modulate cholinergically-mediated hippocampal theta. In urethane-anesthetized rats implanted with bilateral recording electrodes in the hippocampus and with an injection cannula unilaterally into the PPN, the hippocampal theta rhythm was elicited by injections of: cholinergic agonist carbachol (CA, 10 μ g) alone, in mixture with an opioid agonist morphine (CA+MF, 10 μ g +5 μ g), or in mixture with both MF and an opioid antagonist naloxone (CA+MF+NAL, 10 μ g +5 μ g +5 μ g). The latency and duration of the theta were analyzed. Intra-PPN CA induced the bilateral theta with a latency and duration of 2 min and 40 min, respectively. MF prolonged the latency and shortened the duration of the theta to 7 min and 18 min, respectively, in the CA+MF group ($P<0.05$). NAL blocked MF-evoked theta suppression: in the CA+KA+NAL group the theta appeared after 1 min and lasted 44 min ($P<0.05$ comparing to the CA+MF group). Taken together, these findings suggest that the opioid system in the PPN suppresses cholinergically-dependent hippocampal theta rhythm (theta 2), possibly through opioid receptors present on the PPN neurons.

P4.10 The effect of gap junction blocker, carbenoxolone, on theta rhythm in anesthetized rats

Matulewicz P.¹, Konopacki J.², Jurkowlaniec E.¹

¹Department of Animal Physiology, University of Gdansk, Poland; ²Department of Neurobiology, University of Lodz, Poland

Gap junctions (GJs) are thought to play an important role in central mechanisms of oscillations and synchrony. In the present study the effect of GJs blockage on the hippocampal formation theta rhythm was examined in urethanized rats. The animals ($n=5$) were implanted with recording hippocampal electrodes. Theta rhythm was induced by sensory stimulation (4 min tail pinch). Carbenoxolone was administered i.p. in a dose of 400 mg/kg. The effect of carbenoxolone was observed for 3 h after the injection. Carbenoxolone induced clear suppression of theta rhythm: it was manifested by a gradual reduction of the theta frequency and power and an increase in the power of the delta band. This effect developed 30 min after the i.p. injection of carbenoxolone and lasted for at least 3 h. No recovery of theta rhythm was observed. The results demonstrate a critical role of electrical coupling in the generation of theta oscillations. The interaction between carbenoxolone and urethane anesthesia is also addressed.

Supported by Department of Animal Physiology, University of Gdansk and grant no. 0081/PO4/2002/23.

P4.11 Circadian rhythm of Aquaporin-4 water channel protein expression in the mouse retina

Romek M., Musialik M., Karasinski J., Pyza E., Semik D.

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

The high water permeability of mammalian cell membranes is known to be caused by a family of channel proteins aquaporins, which freely permit movement of water but not other small molecules. Aquaporin-4 (AQP4) was found to be preferentially expressed in the rodent retina. Thus, we analysed circadian rhythms in the expression of AQP4 in the retina of 12-weeks old C57Bl mice maintained in the light/dark cycle (LD 12/12), in constant darkness (DD) or continuous light (LL). Rabbit anti-AQP4 polyclonal antibody was employed and the intensity of immunohistochemical reaction against AQP4 was quantified using confocal microscopy and image analysis software. The immunohistochemical results were confirmed by Western blott analysis. AQP4-immunoreactivity in the mouse retina extends from the inner to the outer limiting membrane and is concentrated along vitreal surface and outer and inner plexiform layers. Immunostained profiles belonged to Muller cells and astrocytes. We found that the expression of AQP4 in the mouse retina was lower at Zeitgeber Time 1 (ZT 1, ZT 0 – the beginning of the day) compared with ZT 13 (ZT 12 – the beginning of the night) in LD. C57Bl mice held in DD maintained oscillations in AQP4 expression. These results indicate that the expression of AQP4 in the mouse retina is regulated endogenously by a circadian clock.

P4.12 Inhibition of neuronal activity in the rat intergeniculate leaflet by serotonergic projection from the dorsal raphe nucleus

Siejka S.¹, Blasiak T.¹, Raison S.², Pevet P.², Lewandowski M.H.¹

¹Jagiellonian University, Krakow, Poland; ²Universite Louise Pasteur, Strasbourg, France

The intergeniculate leaflet (IGL) is a part of mammalian circadian timing system. IGL receives photic information from retinal ganglion cells and non-photoc information from nuclei located in the brain stem. Among them serotonergic (5-HT) input from the raphe nuclei has the most significant effect on mammalian circadian timing system. Our previous experiments indicate that dorsal raphe nucleus (DRN) modulates neuronal activity in the rat IGL. The aim of present study was to check whether 5-HT axon terminals from the DRN are involved in this modulatory effect. The data were collected from anaesthetised rats. A lesion of 5-HT terminals in the IGL was carried out by a local injection of the 5,7-DHT. After 10 days of survival time animals were deeply anaesthetised, mounted in a stereotaxic frame and the extracellular recording/stimulating procedures were performed. In the control animals (intact 5-HT fibres) DRN stimulation inhibited neuronal firing of the IGL cells and DRN lesions induced a strong increase in the level of neuronal firing. In animals with destroyed 5-HT fibres the DRN stimulation or lesion did not affect neuronal activity of the IGL. The obtained results show that 5-HT terminals in the IGL plays basic role in modulation of the IGL neuronal activity.

Supported by IZ grant no. BW/IZ/9b/2004.

P4.13 IGL-like neuronal oscillations in the rat pretectum, similarities and discrepancies

Szkudlarek H.J., Lewandowski M.H.

Institute of Zoology, Jagiellonian University, Krakow, Poland

The pretectum is responsible for a control of various oculomotor and visuomotor functions, modulation of nociception and responsiveness to somatosensory stimuli. Some evidences indicate its involvement in the circadian regulation, mainly because of connections with the suprachiasmatic nucleus (SCN) and the intergeniculate leaflet (IGL), structures regulating circadian rhythmicity, and its innervation by the melanopsin containing retinal ganglion cells (mRGCs), a plausible circadian photoreceptor. Firstly in the SCN and later in the IGL, slow oscillations in the neuronal activity were observed. Looking for an answer whether this type of activity is characteristic of all structures innervated by the mRGCs or involved in the circadian regulation, we performed experiments on urethane anaesthetized male rats. Our results show occurrence of the slow bursting activity in rat pretectum, similar to that observed in the IGL. However, in most cases, this type of activity does not disappear in darkness. Returning to the previous light condition causes temporal disturbances in the oscillations. The intravitreal injection of tetrodotoxine (TTX) to the ipsilateral eye causes small decrease in the firing rate, and subsequent contralateral retina blockage elicits loss of this type of activity. In the case of the IGL only later effect was observed.

Supported by Institute of Zoology Grant no. BW/IZ/3B/2004

P4.14 Recovery of carbachol-induced theta in hippocampal slices after i.p. administration of carbenoxolone – *in vivo/in vitro* approach

Kowalczyk T., Golebiewski H., Konopacki J.

Department of Neurobiology, University of Lodz, Lodz, Poland

Since our first demonstration of cholinergically induced theta rhythm recorded from the hippocampal (HPC) slice preparation mechanisms underlying the oscillation and synchrony in the HPC neuronal network have been successively studied in the *in vitro* conditions. In our recent work we have presented evidences for the contribution of the electrical communication between neurons through gap junctions (GJs) in the production of theta rhythm recorded in the hippocampal formation maintained *in vitro*. The purpose of the present study was to investigate the pattern of recovery of carbachol-induced theta recorded from the hippocampal slices obtained from the carbenoxolone (GJs blocker) pretreated rats. Animals were i.p. injected with 100 mg/kg carbenoxolone 1, 2, 3, 4, 6, 8, 10, and 12 h prior to the dissection. Cholinergically induced (carbachol, 50 μ m) EEG activity was recorded from the CA3c area of the hippocampal formation slice. The i.p. injection of carbenoxolone resulted in abolishment of cholinergically induced *in vitro* theta. Longer time between the GJs blocker administration and the dissection (at least 4–6 h) resulted in well-synchronized theta recorded from the hippocampal slice. Involvement of electrical coupling in production of *in vitro* theta rhythm is discussed.

P4.15 Is sleep disturbed by absence seizures

van Luijtelaar E.¹, Bikbaev A.²

¹Biological Psychology, NICI, Radboud University Nijmegen, Nijmegen, the Netherlands; ²International School of Neuroscience, Bochum, Germany

We investigated the organisation of sleep in WAG/Rij rats in order to access whether the sleep cycle is affected by spike-wave discharges (SWD). A second aim was to test an assumption of the cortico-reticular theory that anterior sleep spindles are controlled by the same mechanisms as SWD. EEG recordings were made during the beginning and end of the light period in 4 and 6 months old WAG/Rij and control (ACI) rats. Large strain differences were found in the length of (non-REM) sleep cycle (WAG/Rij>ACI) and REM (ACI>WAG/Rij). Also time-of-day (longer at the beginning of the sleep period) and age-related (shorter in older rats) effects were found. The sleep cycle showed a most striking outcome: the (non-REM) sleep cycle was seriously shortened in WAG/Rij rats but only in recordings made at the end of the light period and only in older rats. SWD and posterior sleep spindles were strain dependent, anterior spindles not. It can be concluded that the sleep cycle and phasic events are under genetic control. Also time of day and age control sleep related variables. SWD and anterior sleep spindles are differently controlled by genotype, age and time of day and this is not compatible with the cortico-reticular theory of absence epilepsy. The sleep cycle is disrupted by absence seizures but only in fragile periods when drowsiness and light slow wave sleep dominate.

P4.16 Are there any differences in the activity of 5-HT cells of the rat MRN depending on the zeitgeber time?

Werhun K., Lewandowski M.H.

Institute of Zoology, Jagiellonian University, Krakow, Poland

Serotonin (5-HT) is involved in the modulation of the mammalian circadian clock mechanism located in the suprachiasmatic nuclei (SCN). This clock is synchronised by light (photic input) and by non-photic inputs. The last one are notably conveyed to the SCN by a direct 5-HT pathway arising from the median raphe nucleus (MRN). Despite the well characterized effect of 5-HT on the SCN neurons, little is known regarding the endogenous regulation of the 5-HT activity in the circadian clock. 5-HT neurons display circadian variation in their tryptophan hydroxylase (TpH) content, TpH mRNA level and in the release of 5-HT in the SCN. In relation to the TpH variations obtained in LD and DD, the aim of the present study was to determine if there are any time-dependent differences in the neuronal activity of the MRN 5-HT cells. *In vivo* measurement of extracellular spontaneous firing of the single 5-HT neurons has been performed in the rat MRN at different zeitgeber time (ZT). At the moment we do not have enough data to conclude whether there exist any differences, in whole day/night cycle, in the activity of the MRN 5-HT cells depending on ZT. However, our preliminary results indicate that from 7 to 16, there is no relationship between ZT and the pattern of neuronal activity or firing rate. Supported by grant obtained from the Institute of Zoology of the Jagiellonian University BW/IZ/3b/2004

SENSORY SYSTEM

P5.01 Interhemispheric asymmetry of the N20o subcomponent latency: New parameter of median nerve somatosensory evoked potentials assessment in healthy adults¹

Kinalski R.¹, Pietrzak D.², Jamiolkowski J.³, Pogorzelski G.², Domian K.²

¹Clinical Neurophysiology Chair, CM UMK, Bydgoszcz, Poland; ²Rehabilitation Dept., Sniadecki Hospital, Bialystok, Poland; ³Health Public Dept., AM, Bialystok, Poland

Signalized earlier (Post Rehab, 2004, 18: 41.) these are first in Poland measurements of latency of the time point of the N20 peak onset, signed as N20o. Latency of N20o was measured using method recommended by Sonoo et al. (Electroenceph Clin Neurophysiol, 1996, 100: 319.) In healthy adult 11 men and 13 women (21 right-handed and 3 left-handed) median nerve was stimulated first on right (Rstim) and then on left (Lstim) side. Somatosensory evoked potentials were recorded (using CP-Fz montage) simultaneously from contralateral (CPc) and ipsilateral (CPi) scalp upon SI cortex area 3b. Latency mean values (LMV) calculated for N20 and N20o were compared to the sides of stimulations and recordings. Height (H) was correlated (x) with LMV of N20o for CPc and CPi. StatSoft Polska "Statistica 6.0" program was used. Results: As compared with LMV calculated for N20 irrespective to stimulation side the LMV calculated for N20o were significantly longer to ipsilateral CP(<CP). (1) RSTIM: LMVCPc ($P=0.001$). (2) Lstim: LMVCPc<LMVCPi ($P=0.008$). Irrespective to stimulation side the LMV of N20o only with height correlated. Rstim: (1) HxCPc: $r=0.53$; $P=0.008$. (2) HxCPi: $r=0.52$; $P=0.007$. Lstim: (1) HxCPc: $r=0.68$; $P=0.0004$. (2) HxCPi: $r=0.55$; $P=0.007$.

P5.02 Functional differences between distinct regions of rat somatosensory thalamus revealed with principal component analysis

Swiejkowski D.A., Kublik E., Wrobel A.

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

Insight into average responses of large groups of neurons is an advantage of the evoked potential (EP) technique, but its low spatial resolution results in interference of local and distant influences. In our experiments on the contextual modulation of thalamic somatosensory EPs recorded in awake rats, we faced such interpretational problems due to short distances between recording electrodes and low signal-to-noise ratio. The EPs elicited by vibrissae stimulation were recorded by means of several electrodes implanted chronically to different sub-regions of the ventral postero-medial (VPm) and the medial posterior (POM) nuclei. EPs recorded when an animal was habituated to the experimental conditions differed significantly from those obtained when it was aroused by additional aversive stimuli. Application of the principal component analysis allowed us to differentiate EPs recorded at close thalamic locations and to find that the contextual EP difference was pronounced in ventro-lateral and posterior VPm and posterior POM. We conclude that specific sub-regions of both VPm and POM are differently involved in tactile information processing when an animal is quiet or aroused.

Supported by the State Committee for Scientific Research grant no. 2P04C 046 27

P5.03 Spatio-temporal receptive field structure and direction preference of neurons in cat's superior colliculus

Wypych M.¹, Ghazaryan A.², Borkowski W.¹, Wrobel A.¹, Waleszczyk W.J.¹

¹Nencki Institute of Experimental Biology, PAS, Warsaw, Poland; ²State Medical University after M. Heracy, Yerevan, Armenia

The aim of this study was to elucidate the relationship between spatio-temporal structure of the receptive fields and preference of direction of stimulus movement of visual neurons from superficial layers of the cat's superior colliculus. Two models of directional selectivity have been proposed: (1) time shift between excitatory inputs to the cell, and (2) spatial asymmetry of delayed inhibition. To investigate the mechanism of direction preference in collicular neurons we recorded extracellular activity of single units from anesthetized cats during presentation of flashing or moving light spot stimuli along horizontal axis of their receptive fields. Spatio-temporal receptive field profiles obtained with randomly flashing spots showed that: (1) most of the units had longer response latencies in the part of receptive field which was located farther away from area centralis; (2) some units showed suppression of evoked activity in the receptive field region located closer to area centralis, whereas preferred direction of motion was away from area centralis. Our results are opposite to known models and suggest that some other mechanism of direction preference of stimulus movement might take place in the superior colliculus.

Supported by KBN grant no. 3 P04C 082 22.

MOTOR SYSTEM

P6.01 Ca²⁺-binding calmyrin is a monomeric protein highly expressed in motoneurons of rat spinal cord

Blazejczyk M.¹, Sobczak A.¹, Piszczek G.², Kreutz M.R.³, Kuznicki J.¹, Wojda U.¹

¹International Institute of Molecular and Cell Biology, Warsaw, Poland; ²NHLBI/NIH, Bethesda, USA; ³Leibniz Institute for Neurobiology, Magdeburg, Germany

Ca²⁺-signaling mediated by Ca²⁺-sensor proteins plays a pivotal role in neuronal processes. Our recent data demonstrated a Ca²⁺-sensor calmyrin protein (KIP) expression in certain regions of human brain. Here we analyzed structural features of KIP recombinant protein and its localization in rat central nervous system. Using SDS-PAGE, gel filtration and analytical ultracentrifugation, we have shown that *in vitro* KIP exists in a dynamic equilibrium of 21.9 kDa monomer and a 43.8 kDa covalent dimer. Dimerization constant was calculated as $K_a = 1.78 \times 10(3) (M)^{-1}$ at 6°C. However, only monomeric KIP was detected by western blotting in several human tissues and in rat brain, and crosslinking did not preserve any dimeric endogenous KIP, in contrast to recombinant KIP, indicating that *in vivo* KIP is mainly monomeric. Immunostaining of KIP in rat forebrain was rather weak and similar as we observed previously in human brain. In contrast, the most intense KIP immunoreactivity in rat central nervous system was found in motor neurons of the spinal cord, where somata, neurites, and axonal processes were strongly labelled. In summary, our data suggest that calmyrin monomer might be a prominent Ca²⁺-sensor in motor neurons of the spinal cord.

P6.02 Different effects of Riluzole treatment on survival of motor units of fast and slow muscles

Cabaj A.², Lapinska I.¹, Majczynski H.¹, Slawinska U.¹

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

In newborn rats the sciatic nerve crush (SNC) leads to a considerable death of motoneurone due to glutaminergic toxicity. Riluzole is a presynaptic inhibitor of glutamate release with neuroprotective properties. We investigated whether Riluzole treatment enhances the survival of MUs of two types of muscles: a slow (soleus, Sol) and a fast (extensor digitorum longus, EDL) after injury carried out at various times after birth (24 h vs. 6 h) in newborn rats. When the rats were tested three months later, the Riluzole treatment was found to improve recovery of Sol but not of EDL in the late SNC (24 h). In Sol muscle the higher number of MUs was obtained compared to saline treated rats while the integrated EMG activity was at the same level as in intact muscle. In EDL, the reduction of MUs number induced by SNC was similar in Riluzole and saline treated rats while their iEMG was lower. In early SNC (6 h) rats the positive effects of Riluzole were obtained in MUs number of Sol as well as EDL muscles. Moreover, in Riluzole treated rats the iEMG activity of Sol was at the same level while in EDL was even higher than in control muscles without SNC. We conclude that the neuroprotective effects of Riluzole depend on the rate of motoneurone maturation that is not synchronous in rat hindlimb muscles.

P6.03 Motor unit action potentials in the medial gastrocnemius muscle of young and old rats

Ciechanowicz I., Krutki P., Lochynski D., Celichowski J.

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

The motor unit action potentials (MUAPs) were investigated in the medial gastrocnemius muscle of 4 groups of rats: the control (5–10 months old) and three groups of old animals (20, 24, 28 months old). The contractile properties of motor units, as well as the amplitude, duration, peak-to-peak time, and number of turns within MUAPs were estimated for 236 units, under isometric conditions. The amplitude of MUAPs increased in aged animals, whereas time parameters prolonged for all types of motor units. In 28 months old animals more phases of MUAPs were noticed. For fast motor units of the oldest rats (24, 28 months old), the contraction force and amplitude of MUAPs decreased, in parallel to the decrease of muscle mass. Within all 4 studied populations of motor units, correlations between amplitudes of action potentials and contraction forces were found. In old animals the number of motoneurons innervating muscles decreases and denervated muscle fibres can be reinnervated by sprouting axons of surviving motoneurons. This process may be responsible for the observed increase in contraction force and amplitude of MUAPs. Prolongation of MUAPs and the increase of phases of this potential is likely the result of the decrease in muscle fibre conduction velocity and/or the reinnervation of additional muscle fibres by axon collaterals.

P6.04 Differences between male and female motor unit properties of the medial gastrocnemius muscle in rat

Drzymala H., Celichowski J.

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

The study shows differences of contractile properties and proportions of motor units located in the hind limb locomotor muscle between male and female rats. Experiments performed on Wistar rats under general anaesthesia were based on functional isolation and electrical stimulation of single motor units of the medial gastrocnemius muscle. Composition of motor units was different for male and female subjects, with higher number of the fast fatigable type and lower number of slow type in male animals. The contraction and the half-relaxation times were significantly longer in male motor units, what might be due to differences in muscle length. The slower contraction of male motor units suggests lower firing rates of their motoneurons. On the other hand, no significant differences between sexes were observed with respect to force parameters of motor units (twitch and maximal tetanus forces), except the fast resistant type (higher force values in male muscles). The mass of the whole muscle was approximately 1.5 times higher in male rats. However, the mean ratio of motor unit tetanus force to the muscle mass was almost twice smaller in this group, what suggests that muscles of male rats are composed of higher number of motor units. Finally, values of the fatigue index were different between sexes only for fast resistant units (significantly higher in female rats).

P6.05 Comparison of rat and cat motor unit properties in the medial gastrocnemius muscle

Krutki P., Celichowski J., Lochynski D., Pogrzebna M., Mrowczyński W.

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

Motor unit contractile properties were compared between two most frequently studied mammals: rats and cats. Experiments were performed on functionally isolated motor units of the medial gastrocnemius muscle. Considerable interspecies differences concerned composition of the muscle; FF motor units predominated in cats (68%), FR units in rats (52%). The contraction and relaxation times in the cat were longer than in the rat and the border values for fast/slow motor units division amounted to 44 and 20 ms, respectively. The mean values of twitch and tetanic forces were lower in rats (7–8 times for fast, 2–5 times for slow motor units), but variability between the strongest and the weakest units within each type was 10–60 times in cats, whereas only 3.5–14 times in rats. Summation of twitches into the tetanus was comparable for fast units, but for S units was more effective in the cat. Evident interspecies differences concerned sag appearance and profiles of unfused tetanic contractions of FF and FR units. Differences of contractile properties are influenced by the size, number and innervation ratios of motor units in the cat and rat muscle, as well as their biochemical variability. Various composition of motor unit types and uneven mechanisms of force development may reflect biological adaptation to variable behaviour of cats and rats.

P6.06 Effects of ageing on the regulation of force in the rat medial gastrocnemius motor units

Lochynski D., Krutki P., Celichowski J.

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

Influence of the ageing process on motor units basic properties and the force-frequency relationship was studied in the rat medial gastrocnemius muscle. Experiments were performed on 5 months old (control) and on 20, 24, 28 months old rats. The continuous decrease of average muscle mass (atrophy) was observed. The proportion of FR and S type units increased and FF type units decreased in aged rats. The significant increase of both twitch and tetanus forces was revealed in all types of aged motor units, for FF units especially in 20 months old group. The fatigue index of FR units was lower in aged rats but FF units became more resistant to fatigue. The force frequency curve was shifted to the left, mainly for fast units. This shift of the curve was reflected in a prolongation of the twitch and relaxation times. Except FR and S units of the oldest group of rats, 60% of the maximal force was achieved at lower frequencies of stimulation and the curves were steeper (higher force augment per 1 Hz increase of stimulation rate) for all three types of motor units in old rats. In the oldest group of animals, the shift of the curve was reversed for all types of units. It is likely that motoneurons operate at lower firing rates to optimally regulate the contraction force with ageing.

P6.07 Deterioration of locomotor movements following initial improvement after spinal cord hemisection in rats

Majczynski H., Kurowski P., Lapinska I., Nosecka E., Gorska T., Slawinska U.

Nencki Institute of Experimental Biology, PAS, Warsaw, Poland

The aim of our experiments was to study the spontaneous recovery of unrestrained locomotion of adult rats after hemisection of spinal cord at low thoracic level. We analyzed velocity of locomotion and various measures from footprint recordings (i.e., step length, base of support, symmetry of hindlimb and forelimb stepping, hindlimb abduction, distance between prints of homolateral fore- and hind-paws). The footprint testing started 1–2 weeks after injury when rats regained hindlimb plantar walking and hindquarters support. One month after hemisection all of the measured indices showed improvement of locomotor performance, and some of them were similar to those before surgery. However 2 months later the deterioration of locomotion was observed. Although hindlimb plantar walking and hindquarters support were preserved, the symmetry of hindlimb stepping and distance between homolateral footprints were severely altered. Smaller changes were observed in base of support and step length. The results of our study show, that the recovery of locomotor movements after spinal cord hemisection of rats is not permanent and locomotor performance may be significantly deteriorated few months later.

Supported by MNII grant nr 2PO5A 09227 and statutory funds for the Nencki Institute.

P6.08 Graft-induced restoration of hindlimb functions is mediated by 5HT₂ receptors

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

¹Nencki Institute of Experimental Biology, PAS, Warsaw, Poland;

²Institute of Biocybernetics and Biomedical Engineering, Warsaw, Poland

An intraspinal transplantation of embryonic serotonergic neurons below the level of total transection was previously found to induce an improvement in hindlimb locomotor functions in adult spinal rats. The aim of the present study was to investigate the nature of the reestablished innervation using pharmacological agents that interfere with 5HT transmission. The hindlimb motor functions in grafted and in control (ungrafted) spinal rats were estimated by testing bipedal treadmill hindlimb locomotion and several neurological reflexes with simultaneous recordings of the EMG activity from extensor (soleus) and flexor (tibialis anterior) muscles. In grafted rats, a 5HT₂ antagonist, Cyproheptadine (i.p.) resulted in an impairment of hindlimb movements and some reflexes with accompanied decrease of integrated EMG activity. During locomotion, the EMG amplitude and the duration of extensor burst activity were also reduced. The subsequent i.p. Quipazine (5HT₂ agonist) treatment reversed these indices to almost pre-drug level. In control spinal rats the same pharmacological manipulations impaired much less hindlimb abilities that were, in general, very limited. Our results show that the graft-induced restoration of hindlimb functions in rats is brought about by the new serotonergic inner-

vation through the 5HT₂ receptors.

P6.09 Force-frequency relationship of rat and cat motor units in the medial gastrocnemius muscle

Mrowczynski W., Celichowski J., Krutki P.

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

Relationship between motor unit force and frequency of stimulation was studied in the medial gastrocnemius muscle of rats and cats. The tetanic contractions of functionally isolated motor units were evoked in both species at increasing frequencies under isometric conditions. Significant interspecies differences were revealed with respect to the course of force-frequency curves for motor units belonging to fast fatigable (FF) and fast resistant to fatigue (FR) types. The curves for the cat muscle were shifted to the left in relation to results obtained for rat motor units. This showed evidently that feline FF and FR motor units reached higher level of tetanic forces at lower ranges of stimulation frequency in comparison to rat motor units. Curves for the cat were steeper (3.2 and 3.6 mN of the force increase per 1 Hz, for FF and FR units) than in rat (1.9 for FF and 2.5 mN for FR units). These results corresponded to lower values of twitch to tetanus ratio in the cat. Moreover, the relationship between the stimulation frequency corresponding to 60% of maximal force and the contraction time revealed lack of correlation when the data for cat and rat motor units were presented together. It is likely that two various mechanisms of force generation during tetanic contractions exist in both species studied.

P6.10 Postural stability assessment in patients with Parkinson's disease

Orawiec R.¹, Blaszczyk J.W.^{1,2}, Klodowska-Duda G.³, Opala G.^{1,3}, Jasinska-Myga B.³

¹University School of Physical Education, Katowice, Poland;

²Nencki Institute of Experimental Biology, Warsaw, Poland;

³Central Clinical Hospital of Silesian University School of Medicine, Katowice, Poland

Parkinsonism is a common neurological disorder; it affects about 2% in the elderly groups. Clinical observations revealed major problems with postural control in PDs with a forward shift in center of gravity. The postural sway represented by center-of-foot pressure COFP while standing quiet was measured in two experimental groups: first consisted of 55 patients with idiopathic PD (20 females and 35 males, age range 42–82 years, mean age 64.9 ± 8.9 years) and in the equal size control (C) group consisting of age and gender matched healthy subjects. Postural sway was recorded by a force platform (Medicap France) via the 16 bit A/D interface (sampling frequency 22 Hz) in eyes open (EO) and eyes closed (EC) conditions. The analysis revealed significant increase of the sway path length in EC condition in both groups studied. In contrast to controls the in the PDs there was no clear correlation between the patient's age and ranges of postural sway. Mediolateral sway range strongly correlated with a stage of the PD. Additional analysis of the data involved comparison of sway fractal dimension (Df, Higuchi algorithm). The results of this analysis also clearly support the difference between groups.

P6.11 Effect of unilateral lesion of the nucleus accumbens on behaviour evoked by stimulation of the A10 neuronal group in the contralateral hemisphere

Plucinska K., Jankowska B., Jankowski M., Prabucka I., Trojnar W.

Department of Animal Physiology, University of Gdansk, Poland

Unilateral electrolytic lesions of the region of the A10 dopaminergic neurons facilitate behavioral responses evoked by electrical stimulation of the homologous A10 group in the contralateral hemisphere. Anatomical specificity of this "contralateral facilitation effect" was tested in the present study. The nucleus accumbens (Acb) – terminal field of the A10 neurons was lesioned unilaterally and electrical stimulation-induced feeding evoked from the contralateral A10 group was measured in the stimulation frequency/reaction latency curve shift paradigm. The effects of the Acb lesion were bidirectional. A decrease in the stimulation frequency threshold (facilitation) was found in rats with lesions localized in the middle Acb involving partly its core and shell areas. Impaired stimulation-induced feeding (increase of the frequency threshold) accompanied lesions of the posterior Acb core/shell region with marked destruction of the shell. Misplaced lesions in the ventral pallidum had no effect. Brain expression of *c-fos* and *zif-268* genes were assessed in the mesolimbic structures and compared in both behavioral groups. The results obtained indicate that "the contralateral facilitation effect" is anatomically specific but it may involve both somatodendritic and terminal regions of the mesolimbic system.

P6.12 The influence of endurance training on contractile properties of motor units in the rat medial gastrocnemius muscle

Pogrzebna M., Celichowski J.

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

The influence of 4-week endurance training on fast fatigable (FF), fast resistant to fatigue (FR) and slow (S) motor units of rat gastrocnemius muscle was studied. Four adult rats covered a daily distance of 1 000 m on a treadmill. Nine untrained rats formed the control group. Electrophysiological experiments were performed on 99 and 78 motor units of the control and trained group, respectively. The proportion of three types of units and their contractile properties were compared. As a result of the training the number of FF units decreased whereas the number of FR units increased. The time parameters of the twitch shortened in all three types of units. The twitch and tetanus forces in fast units increased significantly. Changes in resistance to fatigue were also observed, mainly in FR units, which displayed a shift of the fatigue index towards higher values. The study revealed that 4-week endurance training evoked adaptive changes in motor units of the medial gastrocnemius muscle. Larger number of FR units in the population of fast units might be due to the transformation of FF into FR units. The shortening of the twitch time parameters and increase of force of FR units supports this conclusion. The increased mean value of fatigue index of fast units was also a result of larger participation of FR units in the studied muscle.

P6.13 Motor cortex excitability threshold and silent period evoked by transcranial magnetic stimulation in spinocerebellar ataxia

Rakowicz M., Rola R., Derejko M., Zdzienicka E., Poniatowska R., Sulek A., Szirkowiec W., Inglot E., Niewiadomska M.

Institute of Psychiatry and Neurology, Warsaw, Poland

Cortical excitability could be abnormal due to degeneration of cerebellum and its connections in patients with hereditary spinocerebellar ataxias type 1 (SCA1) and type 2 (SCA2). Excitability and inhibition of motor cortex and corticospinal projections were investigated in 22 patients with SCA1 and 15 with SCA2. Transcranial magnetic stimulation (TMS) was used to evaluate cortical motor threshold (MT) at rest, silent period (SP) during voluntary contractions, central motor conduction time (CMCT) and amplitude of motor evoked potentials (MEPs) from hypothenar and extensor digitorum brevis (EDB) muscles. MRI was performed to measure atrophy of cerebellum. MT was elevated and MEPs amplitude decreased mainly in SCA1 patients for upper and lower limbs, while in 25% of SCA2 cases only in EDB. Lengthening of SP was more pronounced in hypothenar muscles in SCA1 and similarly in both entities in EDB. CMCT was prolonged in all SCA1 cases and in 30% of SCA2 patients. MRI revealed more evident cerebellar atrophy in SCA2. Our results documented that cerebellar atrophy activates inhibitory cortical interneurons and causes cerebellar excitation to motor cortex in another way in SCA1 and SCA2 patients. The research was supported by 3PO5B 019 24 grant from the State Committee for Scientific Research.

P6.14 Influence of adenosine A2A receptor stimulation on proenkephalin (PENK) and prodynorphin (PDYN) mRNAs expression in rat brain

Wardas J., Lenda T., Kuter K.

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

In the striatum, GABAergic medium spiny neurons form two main output pathways: striatopallidal (co-expression of enkephalin, dopamine D2 and adenosine A2A receptors) and striatonigral (with dynorphin, substance P, dopamine D1 and adenosine A1 receptors). The expression of the neuropeptide gene in these neurons is controlled by many neurotransmitters and their receptors, including dopamine and adenosine. A2A receptor antagonists reduce the PENK mRNA expression enhanced by 6-OHDA lesion or the blockade of D2 receptors. It has also been shown that in A2A knockout mice the level of PENK mRNA is slightly reduced. Moreover, adenosine A2A receptor agonists display behavioural and biochemical effects similar to those produced by neuroleptics. The aim of the present study was to find out whether adenosine A2A receptors are involved in the regulation of PENK and PDYN mRNA expression in the striatum, estimated by *in situ* hybridization. CGS21680, a selective A2A agonist, given acutely in a dose of 1 mg/kg did not influence PENK or PDYN mRNA, but its dose of 5 mg/kg increased the expression in both the dorsal and the ventral striatum. Summing up, these results demonstrate that the postsynaptic adenosine A2A receptors located on striatopallidal neurons regulate the expression of enkephalin, as well as – probably transsynaptically – that of dynorphin.

PLASTICITY

P7.01 Plasticity of thalamo-cortical connections in adult mouse slices

Boratynski P., Bekisz M., Kasicki S.

Nencki Institute of Experimental Biology, PAS, Warsaw, Poland

Stimulation of thalamo-cortical fibers evokes long-term potentiation (LTP) in barrel cortex in young rodents. After critical period of first 2 weeks it seems to be impossible, although plasticity can be observed in an adult cortex. The difficulty to induce LTP in thalamo-cortical connections may be caused by various factors. One of the reasons may be the interlaminar architecture of connections within a cortex, especially the influence of inhibitory interneurons. We investigated whether such processes might be responsible for lack of thalamo-cortical LTP in barrel cortex in adult mice. The experiments were performed on slices, in which field potentials, evoked by stimulation of fibers entering cortex, were recorded with tungsten electrodes placed in layers II/III, IV, V and VI. A possibility of inducing LTP and long term depression (LTD) was verified in 4 experimental situations differing with the agents applied to the bath: (1) bicuculline methbromide (GABA antagonist) and carbachol (cholinergic agonist); (2) only bicuculline; (3) only carbachol; (4) no additional agents. A weak amplification of response (LTP) was obtained after tetanic burst stimulation in infragranular layers of barrel cortex only in presence of both agents. Such amplification was not observed in supragranular layers. In all experimental situations a low-frequency stimulation induced LTD both in supra and infragranular layers.

P7.02 Midbrain dopaminergic cells after unilateral lesion and contralateral stimulation of the ventral tegmental area (VTA)

Cecot T., Jerzemowska G., Trojnar W.

Dept. of Animal Physiology, University of Gdansk, Poland

Unilateral electrolytic lesion of the VTA facilitates behavioral response induced by electrical stimulation of the contralateral VTA. For explanation the mechanism of this "contralateral facilitation effect" the number of tyrosine hydroxylase (TH)-containing neurons in the A10 and A9 groups was counted in rats subjected to unilateral lesion and then to electrical stimulation of the contralateral VTA. TH⁺ neurons were counted in brain slices taken from A10 and A9 neuronal groups and compared to rats subjected only to the unilateral electrical stimulation or only to unilateral damage of the VTA, and also to the naive group. Unilateral VTA lesion caused bilateral decrease in the number of TH⁺ cells in the middle A10 group. In rats subjected to unilateral VTA lesion and contralateral stimulation the TH⁺ cells in the lesioned hemisphere were equally numerous as those in the homologous hemisphere of the lesioned only rats and the TH⁺ cells in the stimulated hemisphere were equal to the number of those in the stimulated only rats. In the A9 group there was a tendency for increased number of the TH⁺ cells in front and decreased TH⁺ cells number behind the implanted electrodes. The results suggest that the "contralateral facilitation" effect may be connected with plastic changes in the target structures rather than the somatodendritic area of the A10/A9 neuronal groups.

P7.03 The role of Abl in regulation of AP-1 transcription factor in neuroplasticity

Detka D., Kaczmarek L.

Nencki Institute of Experimental Biology, PAS, Warsaw, Poland

AP-1 is a ubiquitous transcription factor which has been repeatedly shown to be involved in synaptic plasticity and learning. AP-1 can be a homodimer of Jun proteins (c-Jun, JunB, JunD) or heterodimer of Jun and Fos (c-Fos, FosB, Fra-1, Fra-2). AP-1 complex of proteins binds to specific sites in genes' promoters and regulates expression of the variety of genes depending on tissue and physiological context. Its functional diversity emerges from interaction with different co-regulators or possible variations of proteins in the complex. Using SOS-Recruitment System (SRS) we have screened cDNA library from the rat brain after two-way passive avoidance behavioural training to find proteins binding to AP-1. We have identified sequence which appeared to be N-terminal fragment of c-Abl tyrosine kinase. Here we provide evidence confirming that c-Abl can interact with AP-1.

P7.04 Expansion of row B whiskers cortical representation after sensory deprivation of the remaining barrel field: 2-deoxyglucose study on rats

Jablonka A., Zakrzewska R., Kossut M.

Nencki Institute of Experimental Biology, PAS, Warsaw, Poland

Barrel field is a cortical somatosensory representation of the rat whiskers. Barrels are arranged in the cortex in the same way as whiskers on a snout and are easily identified in layer four. We investigated expansion of the somatosensory representation of one intact whiskers row while the remaining whiskers were trimmed for a month. Unilateral whisker trimming apart from row B was performed a month before [¹⁴C] 2-deoxyglucose (2-DG) brain mapping. Before 2-DG injection animals were immobilized and whiskers of the non deprived side were also cut, sparing only the row B. After isotope injection the rows B of both sides were stimulated for 45 minutes and then the animals were sacrificed. Separated brain hemispheres were cut tangentially to the barrel field. Every second section was exposed to roentgen film. The remaining sections were stained for cytochrome oxidase (CO) activity. The autoradiograms were analyzed by a computer image analysis program. The autoradiograms and CO were compared and the range of the activation row B cortical representation measured by an automated computer procedure. Cortical representation width of the spared row of vibrissae doubled comparing labeling to the control of row B. Representation of active mechanoreceptors acquires more cortical representation at the expense of inactive inputs.

P7.05 Extracellular matrix metalloproteinase inhibitor S24994 abolishes LTP in CA1 field of rat's hippocampus

Okulski P.¹, Konopacki F.¹, Balcerzyk M.¹, Wilczynski G.M.², Kaczmarek L.¹

¹Nencki Institute of Experimental Biology, PAS, Warsaw, Poland; ²Warsaw Medical University, Warsaw, Poland

Change of synaptic function during long term potentiation (LTP) has been associated with structural and morphological transformations, which engage extracellular space reorganization. This could involve endopeptidases named matrix metalloproteinases (MMP), function of which is to cleave extracellular space allowing reorganization. Recently it was shown that MMP-9 is activated during plasticity of hippocampus evoked by kainate treatment. Moreover, recent experiments of our group on knockout MMP-9^{-/-} mice show hippocampus dependent learning dysfunction, as well as LTP decay in CA1 field of the hippocampus. In this experiment we wanted to check whether LTP in slices of rat's CA1 field activates MMP-9, and if the use of specific MMP-9 inhibitor S24994 will block LTP. Our results, obtained with hybridization *in situ* method, show that LTP in control slices, not treated with S24994, activates the proteolytic function of MMP-9 in puncta, which could be associated with synapses in the dendritic area of CA1 field. LTP in slices treated with the inhibitor can be evoked by titanic stimulation but, as opposed to control slices, potentiation shortly decays to baseline. Obtained results confirm the role of MMP-9 in neuronal plasticity and clearly indicate synapse-like puncta around dendrites as the site of endopeptidase action.

P7.06 Transcription factor Yin Yang 1 regulates *in vivo* MMP-9 expression in the rat hippocampus

Rylski M.¹, Bielinska B.¹, Saganek R.¹, Konopacki F.A.^{1,2}, Wilczynski G.M.^{1,3}, Kaczmarek L.¹

¹Laboratory of Molecular Neurobiology, Nencki Institute of Experimental Biology, PAS, Warsaw, Poland; ²School of Molecular Medicine, Warsaw, Poland; ³Department of Pathology, Medical University of Warsaw, Poland

MMP-9 (matrix metalloproteinase 9) is a protein involved in the formation of the long-term synaptic plasticity, and as a consequence also in the learning and long-term memory functioning. Its expression is regulated mainly transcriptionally. Despite of the essential physiological functions, there is no data concerning MMP-9 mRNA regulation in the normal brain. We found that transcription factor Yin Yang 1 (YY1) represses MMP-9 expression in the rat hippocampus *in vivo*. Two hours after neuronal excitation MMP-9 mRNA expression is strongly induced in the rat hippocampus. This molecular event is correlated with the release of YY1 from mmp-9 proximal promoter in the rat hippocampus *in vivo*.

P7.07 Role of group I metabotropic glutamate receptors in memory reconsolidation

Salinska E.

Medical Research Centre, PAS, Warsaw, Poland

Although reconsolidation of memory after reminder does not seem to be the simple reiteration of the sequential stages occurring during memory consolidation, nevertheless both phenomena probably employ similar mechanisms including activation of glutamate receptors and protein synthesis. It is known that group I metabotropic glutamate receptors (mGluRs GI) are involved in memory consolidation and modulation of protein synthesis. The aim of present study was to investigate the role of mGluR5 in memory reconsolidation and to determine whether inhibition of these receptors may affect protein synthesis in this process. The one-trial passive avoidance task on chicks was used as the experimental model of learning. Injection of mGluR5 antagonist MPEP into a specific chick brain region IMHV resulted in amnesia provided the injection was made in a short time either before or after training and approximately 4 h after training. This amnesia was permanent, resembling the effects of protein synthesis inhibitors. To the contrary MPEP injection immediately after reminder resulted in only a transient amnesia one hour later. Increased expression of Egr-1 and c-Fos 2 h after training was abolished bilaterally in chicks injected with MPEP, whereas after reminder MPEP affected gene expression only in right IMHV. These results demonstrate differential involvement of mGluR5 in the mechanisms of memory consolidation and reconsolidation in the chick brain.

P7.08 Short-term sensory learning does not alter Parvalbumin neurons in the barrel cortex of adult mice

Siucinska E., Kossut M.

Nencki Institute of Experimental Biology, PAS, Warsaw, Poland

Classical conditioning paradigm involving stimulation of a row of facial vibrissae produced expansion of the cortical representation of the activated vibrissae that was demonstrated by labelling with 2DG in the barrel field. We have also shown that functional reorganization of the S1 cortex is accompanied by an increase in the density of small GABAergic cells and GAD67(+) neurons in the hollows of barrels representing the trained row. In the present study we have examined GABAergic parvalbumin-containing interneurons in the cortical representation of trained facial vibrissae after short-term aversive training, in order to determine whether the observed changes in GAD(+) neurons are accompanied by changes in PV immunoreactivity. Using double immunofluorescent staining, it was found that following aversive training: (i) all PV(+) neurons in the barrel hollows were GAD(+); (ii) about 70% of GAD(+) neurons also contained PV; and (iii) the ratio of GAD(+) neurons to GAD/PV(+) neurons in trained barrel hollows was increased by 24% compared to controls. This study is the first to demonstrate that the density of double labelled GAD/PV-IR neurons does not alter during cortical plasticity, thus suggesting that some other population of GABAergic interneurons is involved in learning-dependent changes in layer IV of the barrel cortex.

P7.09 Involvement of FosB protein in various behavior

Solecki W.^{1,2}, Krowka T.^{1,2}, Kubik J.², Osikowicz M.¹, Mika J.¹, Wozniak G.³, Kaczmarek L.³, Przewlocka B.¹, Przewlocki R.^{1,2}

¹Institute of Pharmacology, Krakow, Poland; ²Institute of Applied Psychology, Krakow, Poland; ³Nencki Institute of Experimental Biology, PAS, Warsaw, Poland

The Fos family proteins play a role in mechanisms of brain plasticity and addiction. In our experiment we used mice lacking fosB gene to clarify the involvement of FosB in learning/memory, anxiety, depression, analgesia and tolerance. We compared the knockout vs. wild type mice behavior using the elevated plus maze, four-plate, Porsolt and T maze tests, as well as nociceptive thresholds (tail flick test), body temperature and locomotor activity after acute and chronic morphine administration. In the elevated plus maze test knockout mice displayed less ability to perform in spatial learning task than wild type mice. Knockout mice also had a tendency to exhibit shorter immobility time in the Porsolt test compared to wild type mice. The data obtained from the four-plate and locomotor activity tests failed to show any differences. Additionally, we observed lower analgesic effects and hypothermia in fosB knockout vs. wild type mice after a single, but not chronic morphine injection. Our results suggest a role of FosB in learning/memory, as well as in acute, but not chronic morphine effects.

Supported by statutory funds and PBZ-KBN-033/PO5/2000.

P7.10 Sighs of relief in rats

Soltysik S.¹, Jelen P.²

¹Nencki Institute of Experimental Biology, PAS, Warsaw, Poland; ²Medical University of Warsaw, Poland

A deep breath, i.e., a sigh, in mammals is a ubiquitous respiratory phenomenon, whose function is to prevent airlessness (atelectasis) in hypoventilated parts of lungs. Sighs are also correlated with emotions, such as anxiety, anger and resentment in humans and obviously, judging from the expression – sigh of relief – in many languages, with relaxation or relief. If sighs are indiscriminately associated with opposite emotions, their role in social communication is doubtful. If, however, there is a selective facilitation of sighs by either fear, anxiety or relief, then they might, in addition to their respiratory role, function also as a social signal of a particular mood. To induce fear a stimulus was paired with a tail shock (5 times in a daily session). To provide a relief, another stimulus, presented before the expected shock (also 5 times per session), was followed by the omission of shock. In 16 rats experiencing fear during a Danger Stimulus (predictor of tail shock) and a relief during the Safety Stimulus (predictor of the non-occurrence of expected shock) the rate of sighing was 7.5 times higher during relief (180/h) than during fear (24/h), and 20 times higher than between trials (9/h), with all differences highly significant ($P < 0.001$). This clear correlation of sighs with relief (from fear of the tail shock) supports our hypothesis that sighs in social mammals may function as signals of safety.

P7.11 Unilateral photothrombotic stroke in the frontal cerebral cortex causes bilateral impairment of skilled movements of the rat forelimb

Sulejczak D., Skup M., Strzalkowski R., Macias M., Czarkowska-Bauch J.

Nencki Institute of Experimental Biology, PAS, Warsaw, Poland

Unilateral damage to the frontal cortex produces sensorimotor deficits on the side contralateral to the lesion but it may also cause ipsilateral deficits. In this study we have examined the motor skills of the contra- and ipsilateral forelimb after unilateral, photothrombotic stroke damaging motor cortex. Skilled reaching movements were analyzed for detection of motor impairment. Prior to lesioning, rats were learned to retrieve a sunflower seed located on an outer shelf of a reaching box until they reached about 80% successes for both limbs. The rats were tested in the 3rd, 7th, 14th, 21st, and 28th postoperative day. Bilateral impairments of reaching were observed beginning from the 3rd postoperative day and were much more pronounced in the task which required reaching for the seed located laterally than for that placed in front of the box opening. These impairments progressed in postoperative time, particularly in more difficult task (lateral reaching). Surprisingly, we did not observe compensation or recovery of skilled movements, which was reported by other groups following devascularizing stroke of the frontal cerebral cortex.

Supported by State Committee for Scientific Research Polish-German grant PBZMIN-001/P05/14 and statutory funds for the Nencki Institute

P7.12 Sensory learning-induced enhancement of inhibitory synaptic transmission in mice barrel cortex

Tokarski K.¹, Urban-Ciecko J.², Kossut M.², Hess G.^{1,3}

¹Institute of Pharmacology, PAS, Krakow, Poland; ²Nencki Institute of Experimental Biology, PAS, Warsaw, Poland; ³Jagiellonian University, Krakow, Poland

In adult mice, aversive conditioning involving tactile stimulation of a row of vibrissae paired with electrical shock to the tail, induces expansion of cortical representation of stimulated vibrissae and elevation in the GABAergic markers. Using whole-cell recording we investigated spontaneous inhibitory postsynaptic currents (IPSCs) in layer IV excitatory neurons in *ex vivo* brain slices prepared from mice previously subjected to conditioning. The first, CS+UCS group, received tactile stimulation (conditioned stimulus, CS) paired with electrical shock to the tail (unconditioned stimulus, UCS). The second group received only the CS and the third, naive group was untreated. In the CS+UCS group the mean frequency of spontaneous IPSCs in neurons of the barrel corresponding to the vibrissa stimulated during training (barrel B) was significantly higher than in the control barrel of CS+UCS mice (barrel D). The latter was not different from the mean frequency of IPSCs in both barrels B and D either in the CS or in the naive groups. Differences in the mean amplitude of IPSCs between groups were unrelated to the training. These results demonstrate that a form of associative learning results in an enhancement of the GABAergic transmission within the cortical representation of a peripheral receptive zone.

P7.13 Intracortical transmission in mouse barrel cortex after behavioral training

Urban-Ciecko J.¹, Kossut M.¹, Hess G.^{2,3}

¹Nencki Institute of Experimental Biology, PAS, Warsaw, Poland;

²Institute of Pharmacology, Krakow, Poland; ³Jagiellonian University, Krakow, Poland

Pairing tactile stimulation of a row of whiskers with a tail shock induces an expansion of functional representation of stimulated whiskers and an increase in GABAergic markers within the somatosensory cortex of adult mice. In the present study we examined field potentials evoked in *ex vivo* slices of the barrel cortex, obtained from trained and naive mice. Pathways leading from layer VI to layer IV and from layer IV to layer II/III were investigated within and between the cortical column corresponding to the 'trained' vibrissae, which was stimulated during training, and in a control barrel column. Trains of eleven pulses at 2–40 Hz were applied. The amplitude of layer VI–IV responses in the "trained" column was reduced and a weaker depression of successive responses was evident at 40 Hz, but not at 2 Hz, as compared to control column and responses in naive mice. Intracolumnar responses in layer IV–II/III pathway were unchanged. In a transcolumar pathway from layer IV of "trained" column to layer II/III of the neighboring, "untrained" column, responses to trains of stimuli applied at 40 Hz were smaller and depressed faster. These data indicate that plasticity induced by associative learning selectively modifies intracolumnar and transcolumar pathways as well as short-term synaptic dynamics in the barrel cortex.

PHYSIOLOGY AND METABOLISM

P8.01 Membrane proteins, a key challenge in today's proteomics research

Bierzynska-Krzysik A.^{1,2}, Kang S.U.², Silberring J.¹, Lubec G.²

¹Jagiellonian University, Krakow, Poland; ²University of Vienna, Austria

New proteomic approaches are being developed continually in order to exemplify the greater coverage of proteome and complexity of protein networks. Loss of membrane proteins, especially those with multitransmembrane domains, all the more under-represented in conventional two-dimensional electrophoresis, remains a serious limitation of the technique. Major difficulties, concerning especially high molecular weight proteins, are due to their low solubility and tendency to self-aggregation during the first dimension (isoelectric focusing, IEF) or the equilibration step. This often leads to horizontal streaking, rendering mass spectrometry troublesome. Adaptation of the high resolving power, two-dimensional preparative polyacrylamide gel electrophoresis system, first introduced by MacFarlane, revolutionized the hydrophobic protein research, offering the possibility to visualize proteins with single- or multiple transmembrane domains as clearly distinct spots. In the present study, initial results employing discontinuous gel electrophoresis, firstly in an acidic buffer system using the cationic detergent benzyldimethyl-n-hexadecylammonium chloride (16-BAC) and subsequent SDS-PAGE, in conjunction with mass spectrometry, led to the identification of a vast number of integral, outer membrane and transporting proteins playing an important role in synaptic physiology.

P8.02 Alterations in dopamine beta-hydroxylase (DBH) activity, TAS (Total Antioxidative Status), Mg and Zn contents in human serum after Zn supplementation

Grabowska M.¹, Schlegel-Zawadzka M.¹, Walkowiak J.², Przyslawski J.²

¹Medical College, Jagiellonian University, Krakow, Poland;

²Medical Academy, Poznan, Poland

The aim of the study was to examine the effect of Zn supplementation on DBH activity, TAS, Mg and Zn contents in serum of healthy subjects (HS) and in patients with mucoviscidosis (PM). DBH activity ($\mu\text{mol}/\text{min}/\text{l}$) was determined spectrophotometrically. Antioxidant status of serum was measured by TAS (Randox test). Mg content was measured colorimetrically (Biochemtest, POCH Gliwice). Zn content in blood samples was measured by flame AAS. A consent for the study was obtained from the Ethical Committee, Medical Academy, Poznan. Group differences were assessed using the Student-t test for paired and unpaired groups. HS ($n=41$) and PM ($n=22$) were supplemented 50 days with daily dose 25 mg Zn+2/d. DBH activities were not change after Zn supplementation in HS ($75.9 \pm 25.5 \mu\text{mol}/\text{min}/\text{l}$, $82.6 \pm 26.8 \mu\text{mol}/\text{min}/\text{l}$) and in PM ($66.4 \pm 16.5 \mu\text{mol}/\text{min}/\text{l}$; $66.7 \pm 20.0 \mu\text{mol}/\text{min}/\text{l}$). TAS increased about 64.4% in HS ($1.6 \pm 0.4 \text{ mmol}/\text{l}$; $2.7 \pm 0.5 \text{ mmol}/\text{l}$; $P<0.05$), but not in PM ($1.6 \pm 0.5 \text{ mmol}/\text{l}$; $1.6 \pm 0.4 \text{ mmol}/\text{l}$). Mg content slightly decreased in serum of HS ($2.3 \pm 0.7 \text{ mg}/\text{dl}$, $2.1 \pm 0.8 \text{ mg}/\text{dl}$) in opposite to PM ($2.1 \pm 0.5 \text{ mg}/\text{dl}$; $2.3 \pm 0.9 \text{ mg}/\text{dl}$) after supplementation. Zn supplementation increased Zn contents in serum of HS about 10.1% ($0.9 \pm 0.1 \text{ mg}/\text{l}$; $1.0 \pm 0.3 \text{ mg}/\text{l}$; $P<0.05$) and in PM about 28.8% ($1.2 \pm 0.4 \text{ mg}/\text{l}$; $1.5 \pm 0.5 \text{ mg}/\text{l}$; $P<0.05$).

P8.03 Role of 3-mercaptopyruvate sulfurtransferase in the synthesis of sulfane sulfur-containing compounds in cultured astrocytes

Jurkowska H., Uchacz T., Dulinska-Litewka J., Wrobel M.

Institute of Medical Biochemistry, Jagiellonian University Medical College, Krakow, Poland

Non-oxidative L-cysteine metabolism, the source of metabolically active sulfane sulfur atoms, as been investigated in mouse cortical astrocytes. There are three sulfur-transferring enzymes on this desulfuration pathway: 3-mercaptopyruvate sulfurtransferase (MPST), gamma-cystathionase and rhodanese. In mouse cortical astrocytes, only a trace activity of gamma-cystathionase has been found, thus, MPST seems to be the only enzyme responsible for the production of sulfane sulfur. The expression of the gene for MPST has been confirmed and its activity has been determined. N-acetylcysteine (0.25–1 mM in the culture medium) and ribose-cysteine (2–5 mM), precursors of cysteine, have not been demonstrated to increase either the activity of MPST and rhodanese or the level of sulfane sulfur in these cells (12 h–72 h of incubation). N-acetylcysteine has increased the level of glutathione in the cells, but ribose-cysteine has not had this effect, what suggests that it either does not enter these cells or delivers a negligible amount of cysteine. A decreased sulfane sulfur level has been found to accompany increased cells proliferation, what confirms the suggested dependency between the level of sulfane sulfur and the proliferation of cells.

NEUROGENESIS AND DEVELOPMENT

P9.01 Binocular deprivation alters the stratification pattern and the morphology of the axons of large ganglion cells in cat retinaBurnat K.^{1,2}, Waleszczyk W.², Van der Gucht E.¹, Arckens L.¹¹Lab. of Neuroplasticity and Neuroproteomics, K.U. Leuven, Belgium; ²Lab. of Visual Perception, Nencki Institute of Experimental Biology, PAS, Warsaw, Poland

We examined the expression profile of neurofilament protein in discrete cell types in whole mounts and sections of adult binocularly deprived and control cat retina using a monoclonal antibody (SMI-32), which recognizes the non-phosphorylated epitope on the high molecular weight subunits of neurofilament proteins. A population of large retinal ganglion cells exhibited neurofilament protein expression in their soma and the proximal parts of the dendritic arbors. These immunopositive cells were distributed throughout the retina, but with the lowest density in the central retina. In retinas of control cats the immunoreactive dendrites branched specifically into sublamina of the inner plexiform layer, well described as the OFF inner plexiform sublamina. On the contrary in the binocularly deprived retinas these large ganglion cells branched throughout the whole inner plexiform layer. In addition, in deprived retinas the majority of optic nerve fibers had a beaded structure. In consequence, although previously unrecognized, the morphology of ganglion cells is profoundly affected by binocular visual deprivation.

P9.02 The role of nitric oxide in maturation of the cholinergic system in rat brain

Domek-Lopacinska K., Kaminska M., Kopczuk D., Strosznajder J.B.

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

Nitric oxide (NO), an important messenger molecule in the brain is synthesized mainly by the neuronal isoform of the nitric oxide synthase (nNOS) that is present with different extent in all brain regions. However till now little is known about the role of NO in cholinergic system development. Using immunolocalization of cGMP and cholinergic marker we have indicated that cGMP synthesis stimulated by NO donor occurs in all cholinergic fibers in the cortex and striatum, it is very high at 1 till 10 days after birth (1d to 10d) with subsequent lowering during maturation and aging. The aim of this study was to investigate NOS activity and correlate it with cGMP level. The activity of NOS was determined in the striatum, hippocampus and cerebral cortex during development (10d, 21d and 4 months). Our data indicated that NOS activity is decreasing with brain maturation. Comparing three investigated brain areas in p10 rats the highest activity of NOS was observed in the striatum. In 21d and in 4 months (adult) animals the NOS activity decreased in the hippocampus and striatum, comparing to 10d. This data on NOS activity correlate with our previous finding on age-related lowering of cGMP concentration in the brain cholinergic system. Our data suggest that NO/cGMP participate in the development of the cholinergic system.

P9.03 Influence of buspirone on neurogenesis in the aged gray short-tailed opossum, *Monodelphis domestica*

Grabiec M., Djavadian R., Turlejski K.

Nencki Institute of Experimental Biology, PAS, Warsaw, Poland

Buspirone is a 5-HT_{1A} partial agonist and is widely used in the treatment of anxiety. In the present study, we evaluated the influence of buspirone on neurogenesis in the olfactory bulb (OB), subventricular zone (SVZ) and dentate gyrus (DG) of the aged opossums. All animals received intraperitoneal injections of bromodeoxyuridine (BrdU, 150 mg/kg) to label the neural precursor cells. Half of the examined animals were injected with buspirone (3 mg/kg) one hour earlier. All animals were transcardially perfused with 4% paraformaldehyde 4 weeks after injections. Using BrdU immunolabeling we showed that pretreatment with buspirone significantly increased the numbers of BrdU-positive cell nuclei in the DG as well as in the OB and SVZ. Next we performed double immunofluorescent labeling for BrdU and cell-type specific markers: glial fibrillary acidic protein (GFAP) or neuronal cell nuclei marker, NeuN. Confocal microscopy was used to detect double labeled cells. Although large numbers of GFAP-positive cells were labeled in the OB and DG, we did not find colocalization of GFAP with BrdU, whereas the majority of BrdU-labeled nuclei colocalized with the NeuN labeling. These results show that buspirone influences on neurogenesis in all neurogenic structures of the opossum brain.

P9.04 Mechanism of adult neurogenesis in mice lacking cyclin D2Kowalczyk A.¹, Wielkopolska E.², Filipkowski R.K.², Ryłski M.², Kaczmarek L.²¹Mossakowski Medical Research Centre, Warsaw, Poland; ²Nencki Institute of Experimental Biology, PAS, Warsaw, Poland

Cyclin D2 is a regulator of G1 phase in the cell cycle and, in particular, of neurogenesis in the brain of adult mice. Mice lacking cyclin D2 (D2 KO mice) are phenotypically unrecognizable from their wild type siblings. However, their morphometrical and morphological analyses show smaller brain with especially smaller olfactory bulb, dentate gyrus, cerebellum and cortex (Kowalczyk et al. 2004, J Cell Biol, 167: 209-213.). We have investigated in more detail the impact of cyclin D2 mutation on the structure of the adult brain. Almost all brain regions show differences in cell density and size of granular layers. The analysis of the cortex reveals the visual, auditory, sensory and motoric fields to be significantly smaller besides having proper localization. The whole cortex is thinner and cells in the layers II/III are less packed. It is surprising that D2 KO mice appear neurologically normal and are efficient in solving simple behavioral tests. It shows a large compensatory capacity of the brain. We prove the existence of two separate mechanisms of neurogenesis for development and for adulthood based on activity of different cyclins D.

P9.05 The comparison of morphometric analysis of cerebellum nuclei (nucleus dentatus and nuclei fastigii and nucleus interpositus lateralis and medialis) in the fetal life of cattle

Krakowska I., Matysek M., Boratynski Z.

Dept. of Animal Anatomy, Agricultural University, Lublin, Poland

The cerebellum of the cow fetuses at age 8, 12, 14, 16, 18, 20, and 23 weeks were used in this experimental research. Morphometric analysis of four cerebellum nuclei in fetal life of cattle were examined. The measurements of neurocyte and neuroblasts were done by morphometrical analysis using Multi Scan software. The material was stained according to the Kluver–Barrera method and with cresyl violet. In the 16th week of fetal life neurocytes were visible only in the nucleus fastigii. In others cerebellum nuclei we could observe neuroblasts. The neuroblast nuclei were visible after staining with violet cresyl while the cytoplasm was hardly visible. The nuclei were large, circular and surrounded with a small amount of the cytoplasm. In the 18th week of fetal life the average cell surface area was the largest in the nucleus interpositus medialis cerebelli. In the 20th and 23th weeks of fetal life the neurocyte surface area was the largest in the nucleus dentatus and the nucleus fastigii. At the end of pregnancy nucleus interpositus lateralis showed the smallest average cell surface area. In case of all four cerebellum nuclei the results indicate on slow cell development in cattle fetuses in the first part of pregnancy. In the second part of pregnancy, cell development is much faster.

P9.06 Developmental pattern of PSD-95 expression in the barrel cortex of mice

Nowicka D.

Nencki Institute of Experimental Biology, PAS, Warsaw, Poland

PSD-95 is a postsynaptic protein constituting the postsynaptic density. Apart from organizing and scaffolding function PSD-95 is thought to play a pivotal role in regulating synaptic plasticity. Several findings *in vitro* suggest that PSD-95 plays a role in synapse formation. Overexpression of PSD-95 in cultured hippocampal neurons results in promoting receptor clustering at postsynaptic sites and enhancing maturation of the presynaptic terminal. Yet, no systematic studies have been performed so far to investigate the expression pattern of PSD-95 during development *in vivo*. In our study we investigated PSD-95 protein expression in the developing mouse somatosensory cortex. Using immunofluorescence, we found that at all ages the staining was overwhelmingly punctate in character. It was observed exclusively in neuropil. At early ages the immunofluorescent staining of PSD-95 was low and homogenous throughout cortical layers, except the marginal zone, where it was prominent. During the first postnatal week the staining increased in all layers and barrels in layer IV became visible. The staining was present in barrel hollows in contrast to barrel septa which were devoid of immunofluorescence. At P14 the intensity of immunofluorescence was high in all layers and persisted into adulthood. Western blot analysis confirmed the progressive increase of PSD-95 protein level during development. This is in agreement with the developmental increase of synaptic contacts observed during the second postnatal week.

This work was supported by statutory funds from the Nencki Institute.

P9.07 Differentiation of glial cells from human umbilical cord blood-derived neural stem cell line: A potent role of growth factors and neuromorphogenes

Sypecka J., Buzanska L., Winiarska H., Domanska-Janik K.

Neurorepair Department, Medical Research Centre, Warsaw, Poland

Human cord blood-derived neural stem cell-like (HUCB-NSC) line was shown to be able to differentiate into main types of neural cells: in the presence of 2% serum cells spontaneously differentiated into neuron, astrocyte and oligodendrocyte-like cells in about 20%, 10%, and 1%, respectively. The aim of the presented study was to promote glial differentiation by the application of various growth factors and neuromorphogenes. The experimental procedure consisted of either 2 or 3 week cell culture followed by immunocytochemistry with stage-specific antibodies: NG2, O4, GalC for oligodendrocytes and S100b/GFAP for astrocytes. The most potent factors promoting oligodendroglial differentiation turned out to be T3, PDGF-AA and CNTF- their application resulted in up to 10-fold increase in GalC+ cell number. The application of either CNTF or the combination of PDGF-BB + RA upregulated the differentiation into astrocytes (~60% S100b+ cells). The influence of modulating factors on the cell in culture (growth rate, survival) was assessed by MTT test and Live/Dead Viability/Cytotoxicity Kit assays. The selected growth factors/neuromorphogenes promote differentiation of HUCB-NSC into glial cells with subsequent inhibition of their proliferation.

Supported by grant no. 28/E-32/SPUB/JRC/P-05/DIE 62/2005 and grant no. 22143-2004-06 F1ED/ISP/PL.

P9.08 Effect of time of prenatal mild or severe hypoxia on development of rat brain

Tjulkova E.I., Vataeva L.A.

I.P. Pavlov Institute of Physiology of the Russian Academy of Sciences, Sankt-Petersburg, Russia

In an attempt to establish more sensitive periods for prenatal hypoxic injury, pregnant Wistar rats were exposed to severe or mild hypobaric hypoxia (180 Torr) at different times of pregnancy (gestation days 14th to 16th or 18th to 20th). The impact of hypoxia in olfactory cortex of offspring was also studied through the measurement of phosphoinositide system activity, a biochemical determinant of cellular injury and subsequent maturation. It was revealed that the appearance of prenatal hypoxia effect on the activity of brain phosphoinositide system considerably depended on the time of its application. In adult, 1- and 15-days old offspring of rats exposed to hypoxia on gestation days 14–16 elevated level of polyphosphoinositids (phosphatidylinositol-5-phosphates and phosphatidyl-4,5-diphosphates) in brain cortex was detected, as compared to offspring of control rats and rats subjected to hypoxia on 18–20 days of gestation. Besides, only offspring of first group (15-days old and adult) demonstrated enhanced phosphoinositide response to glutamate. Fifteen days old pups of the second group (subjected to hypoxia on 18–20 days of gestation) showed a decrease of the response. The response of adult rats of this group was unaffected. The revealed changes of phosphoinositide system activity might reflect adaptive processes result in increase of tolerance of brain neurons to hypoxia.

P9.09 Effect of time of prenatal mild or severe hypoxia on behavior in rats

Vataeva L.A., Tjulkova E.I.

I.P. Pavlov Institute of Physiology of the Russian Academy of Sciences, Sankt-Petersburg, Russia

In an attempt to establish more sensitive periods for prenatal hypoxic injury, pregnant Wistar rats were exposed to severe or mild hypobaric hypoxia (180 or 360 Torr) at different times of pregnancy (gestation days 12th to 14th, 14th to 16th, or 18th to 20th). The effect of hypoxia on somatic, motor and cognitive development of offspring was then assessed. It has been found that the severe or moderate prenatal hypoxia result in a significant retardation of development as evidenced by indices of body weight and of somatic maturation. In the test of negative geotaxis significant differences in the behavioral patterns between the control and experimental groups were revealed in 3-day-old and 7-day-old pups. Differences between experimental and control pups in the test of the cliff-avoidance were evident in 7- and 9-day-old pups. In pups at the age of the 7th to 13th days signs of impairments of learning ability were more pronounced in rat pups exposed to prenatal hypoxia at the 14th to 16th days of gestation as opposed to pups subjected to hypoxia at the 18th to 20th days of gestation. Using Morris water-maze it was shown that the exposure to prenatal hypoxia affected behavior of males only. Most sharp changes were detected in learning of male rats whose mothers were subjected to hypoxia on 14–16 days of gestation.

NEUROANATOMY**P10.01 Topography in the inferior olive projections to the uvula and paramedian lobule in the rabbit**

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

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

Climbing fiber projections from the inferior olive (IO) to vermal lobule, uvula (IX) and paramedian lobule (PML) were studied in the rabbit by using of retrograde double labeling technique. Following unilateral injections of fluorescent tracers diamidino yellow in IX and fast blue in PML, different numbers of labeled neurons were distributed in restricted, spatially separated regions of contralateral IO subdivisions. The findings indicate that the olivary projections are organized according to the topographical pattern: medial and lateral regions of the medial accessory olive project to PML and IX, respectively; mainly lateral region of the dorsal accessory olive projects to IX, and medial one to PML with some areas of overlapping; medial and lateral regions of the beta nucleus and the dorsomedial cells column project to IX and PML, respectively; the dorsal cap projects to both lobules in rather diffuse manner; dorsal lamella of the principal olive sends fibers to PML; medial and lateral regions of ventral lamella of the principal olive supply PML and IX, respectively; medial portion of the ventrolateral outgrowth projects mainly to IX. The present findings suggest that the cortical as well as visual, vestibular and somatosensory information mediated by IO subdivisions may be selectively distributed to both cerebellar targets under study.

P10.02 Molecular determinants of presynaptic specialization in two structurally and functionally divergent neuromuscular junctionsJuraneck J.¹, Mukherjee K.², Rickmann M.³, Jahn R.²¹Warmia and Mazury University, Olsztyn, Poland; ²Max Planck Institute for Biophysical Chemistry, Goettingen, Germany; ³Georg August University, Goettingen, Germany

Vesicle exocytosis occurs in a specialized region in the chemical synapse called active zone, which is at ultrastructural level characterized as an electron dense cytoskeletal matrix. The cytomatrix at active zone (CAZ) is thought to play an essential role in neurotransmission by defining release site and coordinating synaptic vesicle cycle. Biochemical studies have identified six major proteins associated with CAZ, namely RIM, Munc13, Liprin, ERC, Bassoon and Piccolo, however the knowledge about function and distribution of the proteins in mammalian nervous system is still limited. In our study we have focused on distribution of CAZ proteins in two divergent peripheral neuromuscular junctions – motor endplate with distinguishable active zone and smooth muscle synapse en passant which lack the specialized region. The results demonstrate that in contradiction to previous report motor NMJ contains all studied CAZ proteins. Unexpectedly, synapse en passant is also found to contain two of the proteins, ERC2 and Bassoon. This is the first study on CAZ proteins revealing the presence of the proteins in neural structures devoid of active zone.

P10.03 Development of dopaminergic neurons in the mid-brain structures of FGFR1 transgenic miceKlejbor I.¹, Ludkiewicz B.¹, Dziwiatkowski J.¹, Stachowiak M.K.², Morys J.¹¹Dept. of Anatomy and Neurobiology, Medical University of Gdansk, Gdansk, Poland; ²Dept. of Pathology and Anatomical Sciences, States University of New York, Buffalo, USA

The mesencephalic dopaminergic system has been divided into two main functional units: the substantia nigra (SN) with nigrostriatal pathway involved in motor functioning and the ventral tegmental area (VTA) with mesocorticolimbic pathway related with the reward and motivation. In our study we wanted to determine whether reduced signaling of FGFR1 engaged in the proliferation and maturation of cells could affect the development of dopaminergic (DA) cells in SN and VTA. We generated transgenic mice that expressed dominant negative FGFR1 (TK-) from the tyrosine hydroxylase gene promoter. The material consisted of two groups of mice: wild type and homozygous [th-tk(-)/th-tk(-)] of various postnatal ages. The numerical density of DA cells in the SN and VTA was estimated. In both groups the age related changes in the density of dopaminergic were similar in the examined structures; however the presence of mutation lowered the density of TH-positive cells in SN and VTA of transgenic mice. In aged wild type mice the density of DA cells decreased to the level of transgenic ones what could be a result of degenerative processes. To conclude, we assume that the FGFR1 (TK-) mutation affects development of DA cells in SN and VTA.

P10.04 Colocalization of selected neuropeptides and calcium binding proteins in the claustral interneurons in the rat during the postnatal period

Kowianski P., Morys J.M., Dziewiatkowski J., Wojcik S., Narkiewicz O., Morys J.

Dept. of Anatomy and Neurobiology, Medical University, Gdansk, Poland

The occurrence of colocalization of neuropeptides with calcium binding proteins was studied by means of qualitative and quantitative methods in the postnatal period (P0 to P120). The colocalizations of neuropeptide Y (NPY) with three calcium binding proteins: calbindin D28k (CB), calretinin (CR) and parvalbumin (PV), as well as somatostatin (SOM) with CB and vasoactive intestinal neuropeptide (VIP) with CR are reported. The moments of occurrence of particular types of colocalizations are differentiated. VIP/CR colocalization is present at P0; NPY/CB type at P4. Two types of colocalizations – NPY/CR and SOM/CB are observed for the first time at P7, whereas NPY/PV type at P14. Age-dependent pattern of increase in the percentage of double labeled neurons was observed in NPY/CB and SOM/CB types. The highest percentage of colocalizing neurons occurs in VIP/CR type of colocalization (51%), whereas the lowest – in NPY/CR and NPY/PV types (9.5% and 9%, respectively). Studied types of colocalizations appear at various stages of postnatal maturation, differ in dynamic patterns of development and in its percentages. The presence of specific mechanisms, regulating the co-expression of studied substances in the claustral interneurons during maturation is suggested.

P10.05 Entorhinal cortex of the opossum *Monodelphis domestica*: Cytoarchitecture and connectivity

Olkowicz S., Turlejski K.

Nencki Institute of Experimental Biology, PAS, Warsaw, Poland

The entorhinal cortex (EC) is an interface between the neocortex and the hippocampal formation. We wanted to examine what is the structure and connectivity of EC in a marsupial species which has a primitive neocortical organization. Basic cytoarchitectonic pattern is similar to that described in other species and comprises six layers (four cellular layers and two cell-sparse layers: I and IV). From studies involving retrograde tracers major afferents to the EC may be divided into several groups. The most significant input reaches EC from olfactory structures like the main olfactory bulb, anterior olfactory nucleus, piriform cortex and endopiriform nucleus. There is also a robust projection from the orbitofrontal cortex. Other important pathways originate in the rhinal cortical areas like the contralateral EC and perirhinal cortex. Nucleus reuniens provides the only thalamic input to EC. There are substantial numbers of neurons projecting to the EC that derive from the hippocampal formation. These include the CA1 field, subiculum and presubiculum. Projections from the hippocampal formation are reciprocated and reach the molecular layer of the dentate gyrus and stratum lacunosum-moleculare of the hippocampus. Therefore, structure and connections of EC in the opossum are generally similar to those in eutherians.

P10.06 Brain c-fos expression after unilateral electrolytic lesion and contralateral electrical stimulation of the ventral tegmental area in rats

Prabucka I., Jerzemowska G., Cecot T., Trojnar W.

Department of Animal Physiology, University of Gdansk, Poland

Unilateral lesions of the ventral tegmental area (VTA), the key structure of the mesolimbic system, facilitate behavioral responses (feeding and exploration) induced by electrical stimulation of the VTA in the contralateral hemisphere. In search for a neuronal mechanism behind this "contralateral facilitation effect", the level of activation of the prosencephalic structures as measured by neuronal expression of c-fos protooncogene, was assessed in rats subjected to unilateral electrocoagulation and contralateral electrical stimulation (for 10 consecutive days) of the VTA (L/S group) in comparison to the unilaterally stimulated (S), unilaterally lesioned (L) and sham (Sh) groups. Increased c-fos expression was found in the septum (both medial and lateral), nucleus accumbens and cingulate cortex. The number of active neurons in the L/S group was the sum of that of the L and S groups. Interhemispheric differences were found only in the S group (higher c-fos expression in the stimulated hemisphere). C-fos expression in the Sh group was similar to that in the S group. Results obtained indicate that electrolytic lesion acts in a very similar way as the chronic brain stimulation and the "contralateral facilitation effect" may depend on the increased level of the activation of the limbic structures.

P10.07 The size of neurons in the nuclei of the mammalian amygdala: The comparative morphometric study

Rowniak M., Robak A., Sztejn S.

Department of Comparative Anatomy, University of Warmia and Mazury, Olsztyn, Poland

The size parameters of the neurons were analysed in the amygdala (CA) and their subdivisions of the common shrew, guinea pig, rabbit, fox and pig. Each neuron was characterized by a set of morphometric parameters: the length (the long axis of the soma), width (the short axis of the soma), size (the sum of both axes) and shape factor (the ratio of both axes). The intraspecific comparisons of the means indicated that CA in each of the studied species could be subdivided into the three compartments. The first one, formed by the lateral (LA), basolateral (BL) and basomedial (BM) nuclei, is characterised by the large neurons with the size values significantly higher than the mean for the species CA. In contrast, the intercalated (I), central (CE), medial (ME) and lateral olfactory tract (NLOT) nuclei represent amygdaloid areas where the size values are significantly lower from the mean. Only in the rabbit's CE and pig's NLOT the sizes of neurons are higher from the mean. The third region is represented by the cortical (CO) nucleus, in which the sizes of cells are in the most cases statistically similar to the mean for the species CA. Only in the rabbit and fox these values are respectively lower and higher from the mean. The interspecific comparisons of the means revealed the positive correlation between the size of CA and the size of the cells in it.

P10.08 The neuronal structure of the striatum in the common shrew (*Sorex araneus*): Golgi and Nissl studies

Wasilewska B., Najdzion J., Robak A., Szteyn S.

Department of Comparative Anatomy, Faculty of Biology, University of Warmia and Mazury, Olsztyn, Poland

The studies were carried out on telencephalons of the adult common shrew. In the striatum the following types of neurons were distinguished: (1) Medium-size spiny neurons are the most numerous in the neuronal population. Their perikarya have a polygonal and rounded shape. The cells have 2–6 smooth dendritic trunks. Most of them divide twice or three times. Sometimes the fourth bifurcation may be observed. The dendrites are covered with spines. They are differentiated as regards shape and they are bent at different angles to the mother branch. An axon emerges from the cell body, rarely from initial portion of the dendritic trunk. Sporadically the axonal collaterals were observed. (2) Medium-size aspiny neurons with perikarya that have a polygonal or rounded shape. They possess 3–6 long dendrites, which have a wavy course. Dendritic trunks are smooth, however the dendritic branches are covered with swellings. An axon usually emerges from the soma. (3) Large neurons with multipolar and triangular perikarya. The cells have 2–5 smooth dendritic trunks. Dendrites are poorly ramified and they possess irregular swellings. An axon originates either from the soma or from the initial portion of the dendritic trunk.

P10.09 Immunohistochemical and electrophysiological studies of CRF in the frontal cortex of rat brainZieba B.¹, Tokarski K.², Wieronska J.M.¹, Grzegorzewska M.², Hess G.², Smialowska M.¹¹Department of Neurobiology, Institute of Pharmacology, PAS, Krakow, Poland; ²Department of Physiology, Institute of Pharmacology, PAS, Krakow, Poland

Corticotropin releasing factor (CRF), a 41 amino acid neuropeptide, is localized in several structures of brain, including cerebral cortex. The aim of the present study was to investigate the localization of CRF in frontal cortex and the influence of intracortical application CRF on excitability of neurons. Immunohistochemical studies show that in the frontal cortex CRF-immunoreactive (CRF-ir) neurons are found mainly in the layers II and III. CRF-ir processes and varicose fibers are observed in all cortical layers. Electrophysiological studies show that CRF reduces the amplitude of sAHP and attenuates the spike frequency adaptation in identified pyramidal cells, without significantly affecting field potentials, which suggests that CRF increases the excitability of neuron in rat frontal cortex but does not modulate synaptic transmission in tested intra- and interlaminar connections. The increase in cell membrane resistance after application of CRF suggests that the reduction of sAHP and spike adaptation is due to the inhibition of the Ca²⁺ activated K⁺ channel. Our data indicate that: (1) CRF-ir neurons are distributed in rat frontal cortex, and (2) CRF can modulate the excitability and functional state of the pyramidal neurons in that structure.

NEUROIMMUNE BIOLOGY**P11.01 NGF administration affects MHC class I and PDGFRalpha expression in brain progenitor cells of EAE rats**

Bacia A., Triaca V., Aloe L.

Institute of Neurobiology and Molecular Medicine, CNR, Rome, Italy

Nerve growth factor (NGF) is present in the subventricular zone (SVZ) of experimental allergic encephalomyelitis rats (EAE), an animal model of multiple sclerosis (MS). The major histocompatibility complex (MHC) antigen class I is an important factor for the regulation of the brain immunoprivileged status and it is expressed by nerve cells. We have investigated the effect of NGF on MHC class I and PDGFRalpha in the SVZ of EAE rats. EAE Lewis rats received i.c.v. injections of saline, NGF or TGFbeta and 48 h later the animals were sacrificed and brain sections immunostained to localize trkA, MHC I and PDGFRalpha positive cells. The results of these studies showed that NGF and to a lesser extent TGFbeta, enhanced trkA expression in cells of the SVZ and adjacent areas in EAE rats. Moreover, following NGF administration, we found co-expression of MHC I and trkA in these brain areas, while NGF and TGFbeta injection enhanced MHC I and PDGFRalpha positivity in oligodendroglial progenitors (OPC). This latter effect was more evident after TGFbeta-treatment. Our findings indicate that NGF influences the presence of trkA, MHC I and PDGFRalpha in brain progenitor cells, suggesting that NGF through these effects contributes to the dynamic changes of NGF-receptive progenitor cells, including OPCs.

Supported by Project "Cellule Staminali" CS 23, ISS to Luigi Aloe

P11.02 Different effects of repeated and continuous chronic restraint stress on NK cytotoxic activity in pigs

Borman A., Ciepielewski Z., Stojek W., Tokarski J.

Department of Animal Physiology, University of Gdansk, Poland

Cross-bred Pietrain piglets were subjected to the restraint stress in a hammock for 4 hours during 5 consecutive days or continuously for 24 hours. Repeated stress results in progressive and sustained suppression of peripheral blood natural killer cell cytotoxicity (NKCC, 51Cr-release assay against K-562 target cells, baselines in consecutive days: 31.8 ± 2.6%; 24.3 ± 2.1; 12.5 ± 1.3; 10.7 ± 0.7; 9.9 ± 0.4). The effects observed during continuous restraint were different: bidirectional NKCC changes at early period of stress were followed by its gradual return to the baseline (baseline – 27.8 ± 1.9; 15th min of stress – 33.2 ± 4.0; 2nd or 4th h – 14.6 ± 1.6; 24th h – 26.2 ± 3.1). Stress-induced plasma cortisol changes habituated in the course of repeated restraint (RIA, maximum – 1st day: 206.4 ± 17.9 ng/ml; 5th day: 122.5 ± 10.3) as well as during continuous stress (baseline: 40.8 ± 3.7; 2nd h – 189.4 ± 19.1; 24th h – 43.3 ± 4.5) and were considerably independent of NKCC changes. Thus, relatively normal NKCC levels at the end of 24 h stressing were opposite to a deep suppression as a result of repeated day-by-day 4 h restraint. These data indicate a possibility of long-term summation of multiple immunosuppressive effects contrary to habituation – transient changes caused by continuous stress.

P11.03 Inflammation related apoptosis can be prevented by inhibition of nitric oxide synthesis

Czapski G.A., Cakala M., Gajkowska B., Strosznajder J.B.

Medical Research Centre, Warsaw, Poland

Till now, little is known about the role of constitutive isoforms of nitric oxide synthase (cNOS) in brain during systemic inflammation. The aim of this study was to analyse the expression and activity of particular isoforms of NOS and their roles in oxidative stress, apoptosis and neuronal degeneration in mice brain after systemic administration of lipopolysaccharide (LPS; 1 mg/kg b.w.). Our results indicated that LPS enhances exclusively iNOS expression and activity in substantia nigra, but not in other brain parts. However, by using of cNOS inhibitors we have indicated that cNOSs are also involved in LPS-evoked cascade. N-nitro-L-arginine and 7-nitroindazole prevented LPS-induced free radical dependent lipid peroxidation, NAD depletion and apoptosis inducing factor (AIF) translocation from mitochondria to nucleus. NOS inhibitors did not have any effect on poly(ADP-ribose)polymerase (PARP) activity, indicating that energetic disturbances and AIF release may be directly related to NO-evoked mitochondria disturbances. Pathological alterations of mitochondria on ultrastructural level were also observed in electron microscopic examination of neurones in substantia nigra. Our results indicate that constitutive isoforms of NOS beside of iNOS are involved in detrimental cascade evoked in the brain by LPS administration.

This study was supported by grant PBZ-MIN-001/PO5/16.

P11.04 Nitric oxide and prostaglandins in the lipopolysaccharide-induced pituitary-adrenal response during stress

Gadek-Michalska A., Spyryka J., Bugajski J.

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

The role of prostaglandins (PG) and nitric oxide (NO), generated after peripheral lipopolysaccharide (LPS) administration, in the adaptation of hypothalamic-pituitary-adrenal (HPA) axis under stressful circumstances have not been elucidated. The aim of the present study was to assess the effect of chronic repetitive restraint and social crowding stress on the involvement of NO and PG in the LPS-induced HPA axis response. Male Wistar rats were restrained or crowded for 7 days prior to treatment. Cyclooxygenase and nitric oxide synthase (NOS) inhibitors were injected 15 min before LPS. Two h after injection LPS increased significantly ACTH and corticosterone secretion. Repeated restraint impaired more potently than crowding stress the LPS-induced HPA-response. Indomethacin considerably reduced the LPS-induced HPA response in non-stressed rats and to a lesser extent diminished this response in repeatedly restrained or crowded rats. Neuronal and inducible NOS inhibitors, N omega nitro-L-arginine and aminoguanidine, respectively, decreased the LPS-induced HPA response, more potently in crowded than control rats. These results indicate that prostaglandins and NO generated by neuronal and inducible NOS are involved in the LPS-induced HPA axis response under basal conditions and in its adaptation under chronic social stress.

NEUROTOXICITY AND NEURODEGENERATION

P12.01 Inhibitory effect of alpha-synuclein and non-amyloid beta component of Alzheimer's disease amyloid on dopamine transporter function in rat striatal synaptosomes:

Relationship to oxidative stress

Adamczyk A., Kazmierczak A., Cakala M., Strosznajder J.B.

Dept. of Cellular Signaling, Medical Research Center, Polish Academy of Sciences, Warsaw, Poland

Alpha-Synuclein (ASN) accumulates in dopaminergic neurons as Lewy bodies, which are characteristic of Parkinson's and other age-related diseases. Moreover, neurotoxic fragment of ASN, non-amyloid beta component of Alzheimer's disease (AD) amyloid (NAC) was found in senile plaques in AD. The aim of this study was to determine the role of brain aging, ASN and NAC in striatal dopamine transporter (DAT) function. Moreover, the involvement of free radicals in DAT activity was evaluated. Radiochemical and spectrofluorimetric methods were used in this study. The results showed that aging, ASN and NAC (10 μ M) inhibited [3H]DA uptake in striatal synaptosomes by 25%, 50%, and 30%, respectively. Analysed peptides stimulated by 57% intrasynaptosomal generation of reactive oxygen species (ROS) measured by using fluorogenic probe, 2'-7'-dichlorofluorescein diacetate. Oxidative stress evoked by FeCl₂ (25 μ M) in the presence of ascorbic acid (250 μ M) and by nitric oxide (NO) donor, sodium nitroprusside (SNP) (10 μ M) significantly decreased [3H]DA uptake. We suggest that ROS generated during brain aging and by toxic peptides may be involved in alteration of DAT activity and dopaminergic system degeneration.

Supported by grant no. 3PO5A12724.

P12.02 Age-dependent hyperphosphorylation changes cellular compartmentalization of tau protein in cholinergic neurons

Bakalerska-Pazera M., Niewiadomska G.

Nencki Institute of Experimental Biology, PAS, Warsaw, Poland

We hypothesize that the age-related degeneration of cytoskeleton in basal forebrain cholinergic neurons (BFCNs) renders the NGF-TrkA signaling system non-functional and thereby impairs trophic support. Comparing young (4 months) and aged (28 months) rat brain, we examined immunohistochemically the compartmentalization of phosphorylated Tau protein using antibodies against: P-Tau404, P-Tau231, and GSK3 beta kinase, as well expression of NGF and its P-TrkA receptor. We also characterize the efficiency of retrograde axonal transport of BFCNs. Retrograde labeling of BFCNs after injection of fluorogold into multiple sites in cortex and hippocampus revealed a significantly lower number of fluorogold positive cells in aged brain. Despite a lower density of P-TrkA immunoreactivity in cortex and hippocampus of aged rats, there was no difference in NGF expression. In young animals P-Tau, GSK3 beta immunoreactivity was observed mainly in neuronal fibers with minimal staining in soma in the main subdivisions of basal forebrain. By contrast, Tau and GSK3 beta labeling was confined to the cell bodies in aged rats. This is confirmation that aging leads to a redistribution of cytoskeletal proteins in BFCNs. Since a somatic localization of P-Tau is indicative of cytoskeletal breakdown, we suggest that failure of axonal trafficking may be responsible for the lack of trophic support in aged BFCNs.

P12.03 Effects of zinc and cadmium on morphology and plasticity of neurons in the visual system of *Musca domestica*

Borowska J., Pyza E.

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

The central nervous system of insects provides an excellent model to study basic processes occurring in neurons. Using the housefly's brain, toxic effects of zinc (Zn) and cadmium (Cd) on neuronal morphology and plasticity was examined in the first order interneurons of the fly's optic lobe. These neurons, L1 and L2 monopolar cells, exhibit a circadian rhythm of size changes, swelling during the day and shrinking by night. In the present study larvae were reared on a diet containing commercial rabbit food, milk powder and water mixed with different concentrations of Zn or Cd. The following concentrations were used: (1) control, existing in commercially available rabbit pellets used for preparing rearing media, (2) low, detected in soil of heavy polluted areas in Poland, and (3) high, sub-lethal concentrations. Analysis of L1 and L2 axon sizes showed that zinc has only minor effects on L1 and L2 morphology and on their daily rhythms in changes of axon sizes. In turn Cd decreases or abolishes daily differences in Cd treated flies. Moreover, in Cd exposed cells changes in axon sizes were concentration dependent, increased after low but decreased after high dose of Cd.

P12.04 Age and gender influence on astrocyte activation in model of Parkinson's disease (PD)Ciesielska A.^{1,2}, Joniec I.², Przybylkowski A.², Kurkowska-Jastrzebska I.¹, Czlonkowska A.^{1,2}, Czlonkowski A.²

¹Institute of Psychiatry and Neurology, Second Department of Neurology, Warsaw, Poland; ²Medical Academy, Department of Experimental and Clinical Pharmacology, Warsaw, Poland

Most studies investigating the role of astrogliosis in the development of PD rarely ever take into consideration age and sex as variables. There is growing evidence indicating distinct effects of these factors on the astroglial reactivity. Western blotting were conducted to evaluate changes in the astrocytic marker-glia fibrillary acidic protein-GFAP content in the striatum male and female C57BL/6 mice after (3 and 12 months old) 6 h; 1, 3, 7, 14, 21 days post 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-MPTP intoxication. After MPTP intoxication, in addition to the normal 50 kDa GFAP, small bands of degraded GFAP (40–48 kDa) were seen. GFAP degradation plays an important role in astrogliosis. In aged and young male we detected maximal increase of 50 kDa GFAP expression after 3 days post intoxication, and this increase was greater in aged *versus* young male. 50 kDa GFAP level was reduced in young and aged male at later time points. In aged and young female the maximum of 50 kDa GFAP expression was detected at 7day time points, however this increase was continued at the same level to 21-day time points only in aged female. Total of GFAP (degraded + 50 kDa GFAP) content was higher in both sexes of aged mice than in young.

P12.05 The neuroprotective effect of MTEP, a potent and highly selective mGluR5 antagonist, on excitotoxic neuronal deathDomin H.¹, Kajta M.², Palucha A.¹, Smialowska M.¹

¹Department of Neurobiology, Institute of Pharmacology, PAS, Krakow, Poland; ²Department of Experimental Neuroendocrinology, PAS, Krakow, Poland

Recent evidence suggests that metabotropic glutamate receptors (mGluRs) can modulate glutamatergic transmission in the central nervous system. Experimental studies have shown that the blockade of group I mGluRs, or the activation of group II and III mGluRs may have neuroprotective effect. The present study attempted to determine whether the novel, highly selective mGluR5 antagonist 3-[(2-methyl-1,3-thiazol-4-yl)ethynyl]-pyridine (MTEP) had neuroprotective action against kainate-induced excitotoxicity in mouse neocortical and hippocampal neurons. In order to evoke toxic effects, primary neuronal cultures were exposed to 150 μ M kainate for 24 h (hippocampus) and for 48 h (neocortex). MTEP (1, 10, and 100 μ M) was applied 30 min before, or 30 min after kainic acid. Kainate neurotoxicity was measured by lactate dehydrogenase (LDH) efflux from the damaged cells into the culture media. We found that both pre- and post-treatment with MTEP reduced excitotoxic neuronal cell death in mouse cortical and hippocampal cultures. The above results indicate that MTEP may have a neuroprotective potential.

This study was supported by KBN grant no. 2P05A 11428

P12.06 Group I metabotropic glutamate receptors and ischemic preconditioning

Duszczyk M., Gadamski R., Ziembowicz A., Lazarewicz J.W.

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

Antagonists of group I metabotropic glutamate receptors (mGluRs GI) are known to protect neurons in some models of brain ischemia. Also preconditioning to ischemia by brief episode of a sublethal insult induces tolerance to the subsequent lethal ischemic insult. The role of glutamate receptors in the induction of ischemic tolerance has been suggested, but involvement mGluRs GI in this process is unknown. In this study we examined effects on ischemic preconditioning of two mGluRs GI antagonists selective to mGluR1 and mGluR5, EMQMCM and MPEP, respectively. The global forebrain ischemia was induced in Mongolian gerbils by bilateral occlusion of common carotid arteries. The tolerance to injurious 3 min global forebrain ischemia was evoked 48 h earlier by preconditioning 2 min ischemia. Drugs at doses of 5 mg/kg known to induce biological effects *in vivo* were administrated i.p. one hour before preconditioning ischemia. The brain temperature was measured with telemetric equipment. Loss of CA1 pyramidal neurons was assessed 14 days after 3 min ischemia. Our experiments demonstrate that ischemic preconditioning reduces neurodegeneration from about 75% to 20–50%, and that application both EMQMCM and MTEP before preconditioning does not inhibit induction of ischemic tolerance. This points to lack of mGluRs GI involvement in the ischemic preconditioning.

P12.07 NG2 expressing cells survive neurotoxic insult *in vitro* and retain the ability to divide

Dzwonek K., Figiel I.

Nencki Institute of Experimental Biology, PAS, Warsaw, Poland

NG2 chondroitin sulfate proteoglycan expressing cells comprise a unique class of glia, present in adult CNS, distinct from astrocytes, oligodendrocytes and microglia. Due to their ability to differentiate into mature oligodendrocytes they are now referred to as oligodendrocyte progenitor cells (OPCs). However, a large number of NG2 positive cells persists in immature stage throughout a lifetime and responds to various types of injury. In order to investigate OPCs reactivity *in vitro*, a model of trimethyltin evoked neurodegeneration was used. The study applied immunocytochemical staining with specific antibodies. The results demonstrate that, in mixed neuronal-glia cultures of hippocampal dentate gyrus, NG2 expressing cells survive treatment with neurotoxin in a concentration that injures most of neurons. They strongly upregulate NG2 proteoglycan expression and undergo morphological changes. Moreover, expression of a marker for proliferating cells – PCNA reveals that OPCs retain the ability to divide after neurotoxic insult. It remains to be established what is the role of NG2 positive cells in neurodegeneration since in the model applied in the present study mature oligodendrocytes appear very rarely.

P12.08 Multipotent character of NG2 positive glial cells activated in neurodegenerative conditions

Fiedorowicz A., Figiel I., Dzwonek K., Zaremba M., Oderfeld-Nowak B.

Nencki Institute of Experimental Biology, PAS, Warsaw, Poland

NG2 chondroitin sulfate proteoglycan positive glial cells present in adult CNS have been generally recognized as oligodendroglia progenitor cells (OPCs), due to their ability to differentiate into oligodendrocytes upon isolation into culture. Their role was mainly emphasized in demyelinating conditions. Recently, however, since the number of NG2+ cells highly increases after various kinds of traumatic injuries, it has been proposed that the production of new oligodendrocytes is not their only role within the adult CNS. It has been shown that these cells can generate also other cell types: astrocytes and neurons (Belachew et al. 2003, J Cell Biol). This presentation summarizes our most recent data concerning the novel properties of a subpopulation of activated NG2+ cells appearing around the apoptotic region of murine dentate gyrus neurons after intoxication with trimethyltin. The data point to the possibility that these cells may generate yet another, macrophage-like, cell type. While expressing also O4 and nestin, characteristic for OPCs, they acquire typical amoeboid, macrophage shape and present macrophage antigens recognized by specific antibodies (ED1 and OX42). Their spatial relation to apoptotic granular neurons and the presence of apoptotic bodies of dying neurons inside NG2+ cells, strongly speaks in favour of their phagocytic role.

P12.09 TNF-alpha receptor type 1 upregulation in neuronal-glia cultures of hippocampal dentate gyrus following neurotoxic insult

Figiel I., Dzwonek K.

Nencki Institute of Experimental Biology, PAS, Warsaw, Poland

Tumor necrosis factor-alpha (TNF-alpha) is a potent cytokine, which has been shown to play an important role in sustaining and modulating a neurodegenerative event or to promote cell survival. The pleiotropic biological properties of TNF-alpha are signaled through two distinct cell surface receptors, TNFR1 (p55) and TNFR2 (p75). The former has a cytoplasmic death domain and is responsible for cytotoxic effects of TNF-alpha. Recent evidence indicates that all cell types in the CNS express TNF-alpha receptors. However, it is unknown whether distribution of these receptors is changed after neurotoxic insult. In previous studies, performed on mixed neuronal-glia cultures of hippocampal dentate gyrus treated with glutamate (GLU) or trimethyltin (TMT), we reported that both pathogenic factors induced neuronal apoptosis accompanied by an increased expression of TNF-alpha. However, TMT was more potent reducing cell viability up to 90%. The main purpose of the current study was to evaluate changes of TNFR1, using double fluorescent immunocytochemistry, in glial and neuronal cells in the cultures exposed to GLU or TMT. We observed enhanced TNFR1 immunoreactivity in both cell types only in cultures treated with TMT, what was confirmed by Western blot technique. Our results indicate that more severe neurodegeneration correlates with increased expression of TNFR1.

P12.10 L-DOPA-induced free radicals generation in rat striatum

Golembiowska K., Kowalska M., Dziubina A.

Institute of Pharmacology, Krakow, Poland

The current therapy of Parkinson's disease is based on L-DOPA. However, the toxicity of L-DOPA is a controversial issue, since oxidative stress due to high L-DOPA-derived DA level may be associated with a progression of degenerative process. Therefore, we attempted to study the effect of local and peripheral L-DOPA administration on free radicals generation in rat striatum. P-hydroxybenzoic acid (PBA, 2.5 mM) was infused through microdialysis probes for free radicals measurement in dialysates from rat striatum. The reaction product of PBA with hydroxyl radical, 3,4-dihydroxybenzoic acid (3,4-DHBA) was assayed with HPLC-ED. Direct intrastriatal infusion of L-DOPA (50 µM) or its peripheral administration (100 mg/kg) together with benserazide (50 mg/kg) markedly increased the dialysate level of DA and of 3,4-DHBA. Similar effect on DA and 3,4-DHBA levels was produced by methamphetamine (1 mM). Mitochondrial complex II inhibitors malonate (5 mM) and 3-nitropropionic acid (5 mM), which are devoid of potent effect on DA release did not impair redox state of striatal cells. It is concluded, that high DA concentration related to L-DOPA therapy seems to be a major risk when looking for a source of free radicals generation in Parkinson's disease.

P12.11 The effects of peripheral nerve extracts versus BDNF on retinal ganglion cells survival following injury

Golka B.¹, Swiech-Sabuda E.¹, Larysz-Brysz M.¹, Gorka D.¹, Golka D.², Marcol W.¹, Lewin-Kowalik J.¹

¹Department of Physiology, Medical University of Silesia, Katowice, Poland; ²Department of Pathomorphology, Medical University of Silesia, Katowice, Poland

Retinal ganglion cells (RGCs) of adult rats are unable to regenerate their axons following optic nerve injury and soon they enter the pathway of apoptosis. Our previous studies revealed that protein extracts obtained from peripheral nerves predegenerated for 7 days were capable of evoking a regenerative process within the optic pathway. We compared effects of peripheral nerve extracts and BDNF on survival of RGCs and regeneration of their axons in adult rats. Autologous connective tissue chambers filled with fibrin, the fractions or BDNF were implanted into the transected optic nerve. BDNF and 7-day predegenerated nerve extracts enhanced RGCs survival rates and the nerve fibers outgrowth. The features of regeneration were less prominent in the group with non-predegenerated nerve extracts and almost absent in the control group.

P12.12 Effects of prenatal alcohol exposure on activity, anxiety and learning in young adult Wistar rats

Jakubowska-Dogru E.¹, Dursun I.¹, Uzbay T.²

¹Middle-East Technical University, Dept. of Biological Sciences, Ankara, Turkey; ²Department of Medical Pharmacology, Gulhane Military Medical Academy, Ankara, Turkey

The objective of the present study was to examine the effects of prenatal exposure to alcohol on sensorimotor coordination, emotionality, learning and memory in 3 months old Wistar rats. Alcohol was delivered to the pregnant dams intragastrically, throughout GD 7–20, at the dose of 6 g/kg/day resulting in peak BAC of 340 mg/dl as assessed on GD 20. A pair-fed isocaloric and untreated control groups were included. Alcohol exposed rats were not impaired in the rotarod/accelerod tests. Their behavior in the open field and plus maze suggested some increase in the anxiety level. Hyperactivity was not observed. In cognitive tasks, alcohol treated rats showed slightly slower rate of initial place learning in the Morris water maze but eventually they reached the same asymptotic performance as controls. Memory retention after 1 and 10-day delay, reversal learning, as well as working memory capacity were the same in alcohol exposed and control rats. The results of this study confirm that effect of prenatal alcohol intoxication on behavior is age-dependent, and functional recovery during maturation refers equally to both motor and cognitive aspects of the behavior. Elucidation of the mechanisms of this recovery requires further investigations.

P12.13 Antiapoptotic effects of memantine on staurosporine-induced cell damage in neocortical cultured neurons

Jantas-Skotniczna D., Kajta M., Lason W.

Institute of Pharmacology, Krakow, Poland

Both, N-methyl-D-aspartate receptor (NMDAR) and protein kinase C (PKC) activation have been shown to represent an essential element controlling excitotoxicity in the brain. In order to elucidate a possible role of NMDA receptor in PKC-dependent apoptosis, we investigated effect of memantine – a clinically useful uncompetitive antagonist of NMDA receptor, on staurosporine-induced caspase-3 activity and lactate dehydrogenase (LDH)-release in primary neocortical cell culture on different days *in vitro* (DIV). Present study has indicated that the vulnerability of neuronal cells to staurosporine-induced caspase-3 activity is higher in immature neurons (7DIV) than in mature ones (12DIV). Memantine (0.05–2 μ M) did not induce any cytotoxic effects but attenuated staurosporine-induced caspase-3 activity and LDH-release in neocortical neurons. Additionally we found that memantine (0.5–10 μ M) did not affect staurosporine evoked apoptosis in SHSY5Y cell line, which does not express functional NMDA receptors. These results indicate that memantine in low concentration, which are specific for blocking NMDA receptor, attenuates the PKC-dependent apoptosis in neocortical neuronal cultures.

P12.14 Sex-dependent differences in the iNOS protein expression in a murine model of Parkinson's disease

Joniec I.¹, Ciesielska A.^{1,2}, Przybylkowski A.¹, Kurkowska-Jastrzebska I.², Czlonkowska A.^{1,2}, Czlonkowski A.¹

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

In the present study we investigated the influence of gender on iNOS in a murine model of PD induced by MPTP. iNOS protein expression was assayed by Western Blot methods in the striatum of young and old male and female C57BL/6 mice (2 and 12 months old), after 6 h; 1, 3, 7, 14, 21 days post MPTP intoxication. The relatively quick rise of iNOS expression in both, male and female mice, groups followed by MPTP intoxication was observed. A significant differences in the iNOS protein expression was achieved: in group of young male compared to young female mice as well as in group of old male compared to old female mice at 14 day and was observed to the 21 day. Our results indicated on the part of iNOS in processes of neurodegeneration, lying at bases' of PD. Additionally it was displayed that the pattern of changes in iNOS protein expression is different in both male and female. The increase of iNOS in both young and old male mice group was observed to the 21 day but in female mice the level of iNOS protein was decreased after 14 days. The data indicate that there is a sex-dependent difference in the iNOS expression. These observations may help in the better understanding the gender differences which exist in PD

P12.15 Estrogen interaction with apoptotic effects of tetrachlorodibenzo-p-dioxin in primary neuronal cell cultures

Kajta M., Jantas-Skotniczna D., Lason W.

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

Apoptosis of neuronal cells is particularly intensive at early stage of development and dioxins disturbing this process could cause damages leading to genetic defects. Since dioxin-mediated transcription can be affected by estrogens, we investigated apoptotic effects of dioxin and its interaction with estrogen in neuronal cell cultures. The vulnerability of mouse neuronal cells to TCDD (tetrachlorodibenzo-p-dioxin; 10-500 nM) was most prominent at earlier culture stages. A substantial, 48–56%, induction of caspase-3 was inhibited by AcDEVDCCHO and found within first 6 hours after treatment. The caspase activation was followed by 15–36% decrease in cell survival. Continuous and multiple exposure to 17 beta-estradiol was not cytotoxic, but increased the cell responsiveness to TCDD in a dose dependent manner resulting in 15–22% cell death. These effects were more pronounced in a presence of alpha-naftoflavone, thus pointing to the prevalence of non-aryl hydrocarbon receptor-mediated effects in response to TCDD, possibly due to disturbance of neuroprotective mechanisms related to estrogens. Since the toxicity of TCDD was inhibited by SB216763 and BIO-acetoxime, we suggest an involvement of glycogen synthase kinase-3beta in interactive actions of dioxins and estrogens on apoptotic processes in neuronal cells.

P12.16 The high resolution morphological study of MMP-9 and its mRNA in the rat hippocampus

Konopacki F.A.^{1,2}, Wilczynski G.M.³, Wilczek E.³, Lasiecka Z.^{3,4}, Kaczmarek L.²

¹School of Molecular Medicine, Warsaw, Poland; ²Nencki Institute of Experimental Biology, PAS, Warsaw, Poland; ³Warsaw Medical School, Warsaw, Poland; ⁴Warsaw University, Warsaw, Poland

Matrix metalloproteinase 9 (MMP-9), a member of matrix metalloproteinases (MMPs), a family of endopeptidases requiring Zn²⁺ for enzymatic activity. Their major targets are proteins of extracellular matrix (ECM), such as collagens, elastin, ECM glycoproteins and others. They take part in various physiological and pathological processes, such as tissue- and organ development, wound healing, inflammation, tumor invasion. Recently their role in neuronal plasticity was proposed. In the rat kainate model an upregulation of MMP-9 (both protein and mRNA) was demonstrated in hippocampal dentate gyrus (DG). Since this structure undergoes prominent plastic change, it was hypothesized that MMP-9 in stimulated granule cells contributes to their plastic response. Our study show that in control rats both MMP-9 immunoreactivity and in situ hybridization signals are present in the form of small, discrete foci, distributed sparsely in neuronal cell-bodies, and within the neuropil (suggesting local translation). Double labelling showed them to colocalize with dendrites and synapses. After kainate, a dramatic increase of MMP-9 and its mRNA occurred in dendrites/synapses in DG. As an outcome, the results suggest a role for MMP-9 in neuronal plasticity.

P12.17 Simvastatin did not protect dopaminergic neurons from damage caused by MPTP

Kurkowska-Jastrzebska I.¹, Joniec I.², Balkowiec-Iskra E.², Czlonkowski A.², Czlonkowska A.^{1,2}

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

Since the inflammatory reaction evoked during neurodegeneration is discovered to be harmful to neurons and aggravates the damage or even contributes to the pathological process, the anti-inflammatory medicines are considered as a new treatment strategy in neurodegenerative disorders. Statins are suggested to have immunomodulatory properties that may play a role in reducing inflammatory response during neurodegenerative processes. We investigated the effect of simvastatin on dopaminergic neurons injured with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). C57Bl mice received 2, 5, 10, 20, and 50 mg/kg simvastatin in one dose daily for 3 days before MPTP administration and for 7 following days after MPTP. On day 7, dopamine level, and the number of TH positive cells were assessed. Simvastatin treatment had however no influence on the decrease of the dopamine level and of the number of dopaminergic cells observed after MPTP treatment. In conclusion, simvastatin fails to prevent dopaminergic cells damage following MPTP administration.

P12.18 The influence of acute and chronic paraquat administration on catecholaminergic systems in rat frontal cortex

Kuter K.¹, Nowak P.², Wieronska J.M.³, Zieba B.³, Dabrowska J.², Bortel A.², Kwiecinski A.², Zapala M.¹, Smialowska M.³

¹Department of Neuropsychopharmacology, Institute of Pharmacology, PAS, Krakow, Poland; ²Department of Pharmacology, Medical University of Silesia, Zabrze, Poland; ³Department of Neurobiology, Institute of Pharmacology, PAS, Krakow, Poland

Parkinsonian motor symptoms are often accompanied by cognitive dysfunction involving frontal cortex. We have examined an influence of paraquat (PQ), a putative parkinsonism-inducing pesticide, on the cortical catecholaminergic systems. Rats were treated with paraquat (10 mg/kg i.p.) acutely and chronically. The levels of catecholamines and their metabolites were measured using HPLC. Immunohistochemical detection of tyrosine hydroxylase immunoreactive (THir) neurons was performed. Acute paraquat treatment increases levels of HVA and MOPEG and enhances norepinephrine turnover in the frontal cortex. Chronic PQ treatment increased dopamine metabolism and turnover between 4th and 12th week of the treatment. Moreover, the levels of MOPEG and norepinephrine turnover were increased after 4 to 8 weeks of treatment. THir neurons appeared in the cortex after 8 and 12 weeks of PQ treatment. The above results suggest that PQ activates catecholaminergic systems in the frontal cortex.

The study was supported by KBN, grant no. PB2-MIN-001/PO5/18.

P12.19 Imaging genomics. MRI morphometry reveals the pathoanatomic basis of SCA17Lasek K.¹, Wolters A.³, Klein C.¹, Hagenah J.¹, Walter U.³, Zuehlke C.², Nitschke M.¹, Rolfs A.³, Binkofski F.¹¹Dept. of Neurology, UKSH, Campus Luebeck, Luebeck, Germany; ²Inst. of Human Genetics, UKSH, Campus Luebeck, Luebeck, Germany; ³Dept. of Neurology, Univ. of Rostock, Rostock, Germany

Spinocerebellar ataxia (SCA 17) is a rare genetic disorder characterized by dementia and extra- and pyramidal symptoms. We aimed at identifying the pathoanatomic basis of this disorder by applying *in vivo* MRI voxel-based morphometry (VBM) to 12 patients and 12 controls. Data analysis was performed using SPM2 (FIL, London) and optimised VBM protocol with additional correlation of the morphometric data with the Mini Mental Score (MMSE; dementia). The comparison between the two groups revealed a pattern of degeneration of the grey matter, centred around the left-sided mesial cerebellar structures, including the worm. This finding correlates well with the leading clinical feature of SC17 patients – ataxia. Furthermore, atrophy was found in the occipito-temporal and -parietal structures, the bilateral anterior putamen and parts of the motor network, mirroring the other pyramidal- and extrapyramidal symptoms. Most interestingly, a highly significant correlation was found between the MMSE and atrophy of the Ncl. accumbens and less significant the right putamen and right dorso-lateral prefrontal cortex, accounting for the leading psychiatric symptoms.

P12.20 Toxicity of tetrahydroisoquinolines (TIQs) in the HEK293 cell lines expressing dopamine and organic cation transportersLenda T.¹, Schulze G.², Rommelspacher H.², Bonish H.³, Bojarski A.¹, Lorenc-Koci E.¹¹Institute of Pharmacology, PAS, Krakow, Poland; ²Section of Clinical Neurobiology, Charite-CBF, Berlin, Germany; ³University of Bonn, Germany

MPP⁺, a parkinsonism-inducing toxin is transported into neurons by dopamine transporter (DAT) and organic cation transporters (OCTs). The aim of the study was to examine the contribution of DAT and OCTs to the toxicity of endo/exogenous, potential parkinsonism-inducing toxins – TIQ derivatives. Experiments were performed on the HEK293 cell lines expressing DAT, OCT1-3 transporters. Cell viability was estimated using cell proliferation assay. The cultures were exposed to: TIQ (A), 1-methyl-TIQ (B), 2-methyl-TIQ (C), 1-(3',4'-dihydroxy-benzyl)-TIQ (D), 1,2[N]dimethyl-6,7-dihydroxy-TIQ (E) and MPP⁺ at a concentration range of 1 μ M – 1 mM. The uptake of [3H]DA in the presence of these compounds was measured in DAT-expressing cells. Compounds A, B, C were not toxic up to 1mM in all cell lines. Compound D showed toxicity in wild type (WT), DAT and OCT-expressing lines. Compound E was toxic only in OCTs and DAT lines. The data on [3H]DA uptake suggest that both D and E might be taken up by DAT. Our results show that only E compound may be selectively toxic towards dopaminergic neurons, while the toxicity of D seems to be unspecific although DAT may be involved. Supported by statutory activity of IF PAS and BMBF,01GZ0309.

P12.21 Presenillin 1 overexpression in bacterial systems – looking for the source of recombinant membrane protein for structural studies

Lewandowicz A., Kuznicki J.

International Institute of Molecular and Cell Biology, Warsaw, Poland

The presenillin 1 (PS1) is the main part of g-secretase complex comprising an active site cavity for proteolysis of amyloid precursor protein yielding toxic Ab42 peptide. Determination of PS1 structure is important to explain early onset familial Alzheimer's disease mechanism and find an effective treatment based on interfering with g-secretase activity. The elucidation of membrane protein structure remains tremendous challenge due to problems with the yield, purification and crystallization. Recently, the *Bacillus subtilis* integral membrane protein – "mistic" has been overexpressed in *E. coli* and its structure determined. This protein autonomously integrates into membrane and was successfully used for overexpression of fusion constructs with some membrane proteins (Roosild et al. 2005, Science 307). We report here our results of PS1 expression with the aid of mistic protein tag. We have successfully used the C43 *E. coli* strain to overexpress mistic protein – PS1 construct (4 h, 37°C under 0.9 mM IPTG induction at 0.6 OD600). The band of around 70 kDa revealed by anti-loop PS1 antibody, corresponds to mistic protein – PS1 fusion with His-Tagged mistic sequence part attached to the N-terminal end of presenillin 1. However, the expression level is low and more appropriate conditions are being optimized for further studies.

P12.22 Cu/Zn-SOD overexpression impairs regeneration and aggravates neuropathic pain after sciatic nerve injuryLewin-Kowalik J.¹, Kotulska K.^{1,2}, London J.³, LePecheur M.³, Paly E.³, Golka B.¹, Larysz-Brysz M.¹¹Medical University of Silesia, Department of Physiology, Katowice, Poland; ²Medical University of Silesia, Department of Neurology, Katowice, Poland; ³University Paris 7 Denis-Diderot, Paris, France

Cu/Zn-Superoxide Dismutase (SOD1) is a key enzyme in the metabolism of free radicals in mammals. It was showed to play important role in neurodegeneration and inflammation. The aim of this study was to examine the role of SOD1 overexpression in peripheral nerve regeneration and neuropathic pain-related behavior in mice. Sciatic nerves of SOD1 overexpressing and wild type mice were transected and immediately resutured. Then, the regeneration was assessed functionally (SFI) and morphologically (growth cones, Schwann cells, and macrophages number in the distal stump of the transected nerve). Autotomy, an animal model of neuropathic pain, was also measured. We found markedly worse SFI outcome, more significant atrophy of denervated muscles and decreased intensity of histologic features in SOD1 overexpressing animals as compared to wild-type ones. Neuroma formation at the injury site was also more prominent in this group. Autotomy was markedly worse in SOD1 transgenic mice than in wild-type animals. SOD1 overexpression seems to be deleterious for nerve regeneration processes. This can be at least partly ascribed to disturbed inflammatory reactions at the injury site.

P12.23 Functional impairment of nerve regeneration in *trkB*^{+/-} mice

Lewin-Kowalik J.¹, Kotulska K.^{1,2}, Marcol W.¹, Larysz-Brysz M.¹, Malinowska-Kolodziej I.¹, Golka B.¹

¹Medical University of Silesia, Department of Physiology, Katowice, Poland; ²Medical University of Silesia, Department of Neurology, Katowice, Poland

This study examines how the *trkB*, the member of the family of neurotrophin receptor tyrosine kinases, influences the regeneration of peripheral nerves. Sciatic nerves of wild-type and *trkB*^{+/-} adult mice were transected and immediately resutured. The regeneration progress was accessed functionally throughout 4 weeks long follow-up. We observed significantly worse sciatic functional index outcome as well as calf muscles mass decrease in *trkB*^{+/-} mice when compared to wild-type ones. However, the calf circumference loss in denervated limb was markedly stronger in wild-type group. Toe-spread test revealed better functional recovery in wild-type mice than in *trkB*^{+/-} animals. The results suggest that *trkB* receptor plays important role in the process of regeneration in peripheral nerves. However, the discrepancies between muscle size and mass need further examination.

P12.24 Increase in prosaposin mRNA expression in the rat model of temporal lobe epilepsy

Lukasiuk K.¹, Kontula L.², Pitkanen A.²

¹Nencki Institute of Experimental Biology, PAS, Warsaw, Poland; ²A.I. Virtanen Institute for Molecular Sciences, University of Kuopio, Kuopio, Finland

Prosaposin as a precursor of saposins regulates activity of lysosomal hydrolases. It can also promote neuritic outgrowth and nerve regeneration. Upregulation of prosaposin mRNA has been shown in the brain following nerve injury, ischemia and stab wound. Here we show prosaposin mRNA expression in the brain in rat models of temporal lobe epilepsy. Epilepsy was induced by status epilepticus evoked either by electrical stimulation of the amygdala, or by kainic acid. Status epilepticus and appearance of spontaneous seizures were monitored. Animals displaying spontaneous seizures following recovery from status epilepticus were considered epileptic. Fourteen days after induction of epilepsy, rats were perfused. Coronal sections were used for *in situ* hybridization with radioactive oligonucleotide probe detecting prosaposin mRNA. When compared to controls, epileptic animals had marked increase in prosaposin mRNA in the temporal lobe structures adjoined to areas of extensive neuronal damage. Additionally, upregulation of prosaposin expression in the hilus, stratum lacunosum moleculare and CA3 of the hippocampus of some epileptic animals was observed. It remains to be determined if alterations in prosaposin gene expression underlie neuronal damage, recovery from the damage or neuronal plasticity.

P12.25 Ligands of metabotropic glutamate receptors are neuroprotective in rat model of perinatal asphyxia

Makarewicz D.¹, Wroblewski J.T.², Danysz W.³, Lazarewicz J.W.¹

¹Medical Research Centre, Polish Academy of Sciences, Warsaw, Poland; ²Department of Pharmacology, Georgetown University, Washington DC, USA; ³Merz Pharmaceuticals GmbH, Frankfurt/M, Germany

Participation of the metabotropic glutamate receptors (mGluR) in the ischemic brain insult has been well established, but the role of their subtypes, particularly in developing brain is unclear. In this study we use the model of perinatal asphyxia in 7-day old rats neuroprotective efficacy of ABHxD-I, the agonist of mGluR mainly of groups II/III (G II/III), and of the antagonists of mGluR group I subtypes mGluR1 and mGluR5, EMQMCM and MTEP, respectively. Hypoxia/ischemia (H/I) was induced by unilateral carotid occlusion followed by 65 min exposure to hypoxia. The drugs were administered i.p. 30 min after hypoxia, ABHxD-I in one dose of 30 mg/kg while MTEP and EMQMCM were injected three times every two hours in doses of 1.25, 2.5 and 5.0 mg/kg. The brain damage was evaluated two weeks after H/I as weight deficit of the ipsilateral hemisphere. These results demonstrate that ABHxD and EMQMCM, but not MTEP applied systemically have significant neuroprotective effect in the rat model of birth asphyxia. These ligands do not affect the rectal body temperature of the animals recovering after H/I. Thus, agonists of mGluRs GII/III and antagonists of mGluR1 are promising candidates for treatment of perinatal asphyxia.

P12.26 Modeling human neurodegenerative diseases in *Drosophila*

Michno K.^{1,2}, Boulianne G.L.^{1,2}

¹Program in Developmental Biology, The Hospital for Sick Children, Toronto, Canada; ²Department of Molecular and Medical Genetics, University of Toronto, Toronto, Canada

Human neurodegenerative diseases are characterized by progressive neuronal cell loss often resulting in memory and cognitive decline, motor dysfunction and ultimately premature death. Despite the prevalence of these diseases, there are no effective cures. Insight into many of these syndromes has come from the identification of single gene mutations that are associated with inherited forms of the disease. For example, mutations in the presenilin 1 or presenilin 2 gene are linked to familial forms of Alzheimer's disease (FAD). Here, we describe how we have taken advantage of the tools available in the fruit fly *Drosophila melanogaster* to establish a model to study presenilin function and to identify novel presenilin interacting partners. Our studies have revealed novel genetic interactions between presenilin and several known regulators of intracellular calcium homeostasis. This is exciting because calcium dysregulation is thought to be involved in the pathogenesis of AD. We are currently characterizing these interactions to determine how presenilin function can impact calcium homeostasis.

P12.27 Effect of introduction of ICER (inducible cAMP early repressor) on neuronal death in hippocampal organotypic culture

Mioduszevska B., Jaworski J., Kaczmarek L.

Nencki Institute of Experimental Biology, PAS, Warsaw, Poland

The acronym ICER (inducible cAMP early repressor) refers to a group of four proteins produced from the CREM/ICER gene due to use of an internal promoter placed in an intron of the CREM (cAMP responsive element modulator) gene. The ICER proteins contain DNA binding/leucine zipper domains that make them endogenous inhibitors of transcription driven by CREB (cAMP responsive element binding protein) and its cognates, CREM and ATF-1 (activating transcription factor-1). ICER expression is inducible in the brain and in neuronal culture by a variety of stimuli. As a CREB antagonist, ICER appears to be of pivotal importance in neuronal programmed cell death. We have reported previously that ICER delivered in adenoviral vector evokes programmed neuronal death in primary cultures. Here we report results drawn from an organotypic hippocampal culture, which is more similar to *in vivo* conditions. These experiments confirm our former results and strongly supports notion that ICER induces neuronal death both *in vitro* and in semi-*in vivo* conditions.

P12.28 Participation of bone marrow stromal cells in repair of cerebral cortex injury

Pasiut S.¹, Opydo M.²

¹Department of Clinical Rehabilitation, Academy of Physical Education, Krakow, Poland; ²Department of Animal Physiology, Jagiellonian University, Krakow, Poland

In recent years there has been an increasing interest in bone marrow stromal cells (MSCs) due to their potential therapeutic function, also connected with repair of brain injury (Chen et al. 2001, Stroke 32: 1005-1011. Mahmood et al. 2001, J Neurosurg 94: 589-595.). The aim of this study was to investigate the effect of MSC on microglia/macrophages and astrocytes response and on glial scar formation after traumatic brain injury (TBI). Animals in experimental group received marrow stromal cells directly to the injured site after mechanical injury. One week after injury animals were sacrificed, the injured cortex were removed and prepared for histological and immunocytochemical staining. Treatment of brain injury with stromal cells showed significant increase in the number of reactive astrocytes (GFAP-positive), localized only around the lesion area. Nevertheless, the formation of glial scar was only slight greater than in the control animals. The amount of microglia/macrophages (lectin-positive) was reduced and they were observed mainly within the newly formed glial scar. New vessels were also found in the site of scar formation. The results suggest that implantation of MSC to the injured site have the influence on cellular response after TBI and can promote repair of cerebral cortex injury.

P12.29 Differential effect of photothrombotic stroke on D1 dopamine and beta-1 adrenergic receptors in rat cerebral cortex

Rogozinska K., Skangiel-Kramska J.

Nencki Institute of Experimental Biology, PAS, Warsaw, Poland

The effect of focal photothrombotic stroke (Rose Bengal technique) on the distribution of D1 dopamine and beta-1 adrenergic

receptors was examined in different cortical areas in the rat brain with quantitative receptor autoradiography using [3H]SCH23390 and [3H]dihydroalprenolol (respectively) as ligands. The lesion was located in the right hemisphere in the primary somatosensory cortex in the vicinity of the barrel field. Analysis was performed in the lesion core, penumbra, frontoparietal cortex (motor and somatosensory areas) and striate cortex 4 hours, 1, 4, 7, 28, and 60 days after stroke. In the lesion core a significant drop in receptor binding sites was found as soon as 4 hours after stroke for D1 receptors and not before 4 days in case of beta-1 adrenergic receptors. This decrease was maintained up to 7th day for both beta-1 and D1 receptors. 28 days after stroke, in the lesion core D1 receptor binding level returned to the control value, while beta-1 receptors were upregulated, also in the homotopic region in the contralateral hemisphere. In penumbra and other examined regions no alterations in D1 receptor level were found in any studied time points. This was also true to homotopic regions in the contralateral hemisphere. The increase of D1 receptor binding in the lesion core after initial post injury decrease may be due to the shrinkage of the lesion volume. Thus, we hypothesize that D1 receptors are not involved in the reorganization processes occurring after photothrombotic stroke. Beta-1 adrenergic receptors were upregulated both in the ipsilateral and contralateral hemispheres in penumbra and frontoparietal cortex (motor and somatosensory areas) 1 day and 28 days after lesion induction. This significant increase may result from reactive astrogliosis (the existence of beta-1 receptors on glial cells has been proved). Our results suggest that beta-1 adrenergic receptors may contribute to some compensatory or/and adaptive mechanisms that might develop in the injured cortex. Supported by Polish-German MSRI grant no. K 057/P05/2003.

P12.30 Neuroprotective potential of methylnicotinamide in homocysteine-induced neurotoxicity *in vitro*

Slomka M., Zieminska E., Lazarewicz J.W.

Medical Research Centre, PAS, Warsaw, Poland

Our recent data point to the role of excitotoxicity mediated by both the NMDA receptors and group I metabotropic glutamate receptors in the neurotoxicity of homocysteine (HCY), while a conventional explanation is based on a potent S-adenosylhomocysteine-evoked inhibition of transmethylases. Nicotinamide (PP) was reported to have neuroprotective potential *in vitro* and *in vivo*. This effect might be partially attributed to its methylated metabolite, N-methylnicotinamide (NMN). Biosynthesis of this compound may be suppressed in hyperhomocysteinemia. In this study we compared the neuroprotective potentials of the different concentrations of PP and NMN in HCY-induced toxicity in cultured rat cerebellar granule cells (CGC). Acute neurodegeneration was induced by treatment with 25 mM D,L-HCY for 30 minutes, while subchronic insult was evoked by incubation with 2.5 mM HCY for 24 hours, then neurotoxicity was evaluated using the propidium iodide staining. Our results demonstrated a partial, statistically significant neuroprotection after 24 h preincubation with NMN at 10⁻³ M and with PP or NMN at 10⁻² M concentrations in acute HCY toxicity or by their simultaneous administration with HCY in the subchronic toxicity of HCY. These results indicate that NMN may be considered as putative neuroprotective agent in hyperhomocysteinemia.

P12.31 Astroglia in lead toxicity – neuroprotective or neurotoxic?

Struzynska L.¹, Sulkowski G.¹, Chalimoniuk M.², Lenkiewicz A.¹

¹Laboratory of Pathoneurochemistry, Medical Research Centre, Polish Academy of Sciences, Warsaw, Poland; ²Department of Cellular Signaling, Medical Research Centre, Polish Academy of Sciences, Warsaw, Poland

The main function of astroglia is controlling of glutamate homeostasis in brain. Astrocytic glutamate transporters – GLAST and GLT-1 protect cells against potentially neurotoxic glu. It is suggested that glutamatergic component underlies lead-induced toxic effects and astrocytes serve as a cellular Pb deposition site. Thus, it was of interest to investigate the role of astroglia in adult rat brain after short-term exposure to Pb. Molecular evidence for overexpression of GLAST mRNA and protein were found with simultaneous decrease of GLT-1 expression in brain homogenates of Pb-exposed rats. Additionally, the expression of glutamine synthetase (GS), which converts potentially toxic glutamate to nontoxic glutamine, was doubly enhanced. The transporters provide glutamate not only as a substrate for GS but also for synthesis of cellular glutathione (GSH). Western blot analysis with anti-GSH abs revealed enhanced expression of GSH-protein complexes at the level of 40 and 20 kDa suggesting activation of S-glutathionylation processes and indirectly, increased GSH pool. The results indicate activation of astrocytic processes regulating glutamate homeostasis in brain of Pb-exposed rats. The response is rather of neuroprotective character, however the protection may be insufficient considering downexpression of GLT-1 transporter.

P12.32 Neurons and glia upregulate Flk1 following various cortical injuries

Sulejczak D.¹, Nosecka E.¹, Macias M.¹, Skup M.¹, Czarkowska-Bauch J.¹, Walski M.², Frontczak-Baniewicz M.²

¹Nencki Institute of Experimental Biology, PAS, Warsaw, Poland; ²Medical Research Center, Warsaw, Poland

Flk1, the receptor of VEGF, is traditionally linked to epithelial cells. Recent reports indicate that Flk1 may be expressed also by neurons and suggest its involvement in neuroprotection. This implicates that Flk1 is upregulated following brain injury. We aimed to investigate the brain expression of Flk1 following various types of cerebral cortex injuries. Three models were employed: devascularization, photothrombosis and mechanical damage of rat cerebral cortex. Adult male Wistar rats were used. Flk1 distribution and expression levels were estimated immunohistochemically on brain sections, obtained at 1, 4 and 7 days postsurgery. Non-operated animals showed weak Flk1 IR in the cortical (mainly layer V) and striatal neurons with no staining of glial cells. Starting from 1 postlesion day we observed many heavily stained neurons in cortical penumbra in all experimental groups. In the 4th and 7th postlesion days also Flk1 IR astrocytes were found in the same areas. These observations were confirmed and extended by immunoelectron studies. Our data point to potential involvement of Flk1 in neuronal response to the injury and add new aspect to neuronal-glia interactions.

Supported by SCSR grants: PBZ-MIN-001/P05/14 and 2P05A 02828, statutory funds for the Nencki Institute and for the Medical Research Center

P12.33 Glutamate excitotoxicity in pathogenesis of multiple sclerosis

Sulkowski G.¹, Struzynska L.¹, Dabrowska-Bouta B.¹, Kwiatkowska-Patzer B.², Rafalowska U.¹

¹Laboratory of Pathoneurochemistry, Department of Neurochemistry, Medical Research Centre, PAS, Warsaw, Poland; ²Department of Neuropeptides, Medical Research Centre, PAS, Warsaw, Poland

Multiple sclerosis (MS) is a chronic autoimmune neurological disorder targeting the white matter of the central nervous system. The etiology of MS has not yet been fully elucidated, however glutamate is suggested to be a factor contributing to MS pathogenesis. The present studies were performed using the model of experimental autoimmune encephalomyelitis (EAE). We have focused specifically on the expression of group I metabotropic glutamate receptors (mGluR1) and excitatory amino acid transporters (EAATs), which are membrane bound proteins localized mainly in astroglial cells and glutamatergic nerve endings. The clearance of extracellular glutamate by EAAT proteins is a mechanism protecting neurons from its toxicity. Western blot analysis revealed the increased expression of mGluR1, mGluR 5 protein in homogenates from EAE rat brain. Simultaneously, the expression of EAAT 2 and 3 proteins was significantly enhanced as compared to the control. Changes in glutamate transport in synaptosomal fraction were also observed. The results suggest that mGluRs, like glutamate transporters, may play a role in the complex processes associated with the progressive brain damage in multiple sclerosis.

P12.34 Apoptosis of astrocytes induced by glutamate can be inhibited by FK506

Szydłowska K., Zawadzka M., Kaminska B.

Nencki Institute of Experimental Biology, PAS, Warsaw, Poland

L-Glutamate (GLU) is a major excitatory neurotransmitter in the CNS. After ischemia neurons are depolarized and excessively release GLU what causes death of neurons. Astrocytes influence these processes by GLU uptake, but an excess of glutamate can cause death of astrocytes. In the present work, we demonstrated that GLU concentrations higher than 50 mM affect survival of astrocytes (MTT metabolism test and cell cycle analysis), induces apoptotic alterations in nuclear morphology *in vitro* (DAPI and TUNEL), causes mitochondrial potential disruption (JC-1) and activation of caspase-9 and -3. FK506 (Tacrolimus) is an inhibitor of calcineurin, and exerts neuroprotective action in traumatic brain injury, focal and global ischemia. We found that 1 μM FK506 inhibits glutamate-induced decrease in astrocytes viability, DNA fragmentation, changes in mitochondrial potential, and activation of caspases. We have demonstrated that FK506 administered at 1 mg/kg, 60 min after MCAo is neuroprotective (Zawadzka and Kaminska 2005, *Glia*). To determine whether FK506 blocks astrocytes death, we performed a double staining for GFAP – astroglial marker and DAPI/TUNEL labeling on brain tissue sections. Our findings suggest that astrocytes are targets for FK506 and modulating of glutamate-induced astrocyte death early after reperfusion may be a novel mechanism of FK506-mediated neuroprotection in ischemia.

P12.35 Axotomy does not induce apoptosis in some sympathetic neurons innervating the porcine uterus

Wasowicz K., Podlasz P., Bukowski R.

Division of Animal Anatomy, Department of Functional Morphology, University of Warmia and Mazury, Olsztyn, Poland

To study the effect of axotomy on induction of apoptosis in sympathetic neurons innervating the porcine uterus 16 juvenile female pigs were used. In 8 animals (group A) the fluorescent tracer fast blue (FB) was injected into the right uterine horn and in 8 animals (group B) into the uterine cervix. After 3 weeks in 4 animals of group A the right uterine horn with ovary and oviduct was excised, while in 4 animals of group B the total ovariectomy was performed. Remaining animals served as controls. After one-week survival period animals were sacrificed. Frozen sections from formalin-fixed inferior mesenteric ganglia (IMG) were subjected to immunohistochemistry with antisera for Bax, Bcl-2, galanin (GAL) and apoptosis detection with TUNEL. The presence of studied substances was determined in FB-positive neurons assumed to innervate the respective part of the uterus. No apoptosis signal was seen in FB-positive neurons of either experimental, or control animals of groups A and B. No differences in the immunostaining for Bax and Bcl-2 were visible between FB-positive neurons of groups A and B, either. However, strong induction of GAL expression was visible in FB-positive neurons after axotomy.

P12.36 Prion protein gene polymorphisms are not a risk factor for Alzheimer's diseaseZekanowski C.^{1,2}, Gacia M.^{2,1}, Religa D.³, Safranow K.⁴, Dziedzicko V.⁴, Jakubowska K.⁴, Barcikowska M.², Kuznicki J.¹

¹International Institute of Molecular and Cell Biology, Laboratory of Neurodegeneration, Warsaw, Poland; ²Medical Research Center, Polish Academy of Sciences, Department of Neurodegenerative Disorders, Warsaw, Poland; ³Karolinska Institutet, Department of Neurotec, Section of Experimental Ge, Novum, Stockholm, Sweden; ⁴Pomeranian Medical University, Department of Biochemistry and Chemistry, Szczecin, Poland

Alzheimer's disease (AD) is the most common form of dementia. Several lines of evidence suggest that PRNP gene polymorphisms could be a risk or modulatory factor in AD. We analyzed the coding region and 5' UTR of PRNP gene in a group of 50 EOAD patients, 114 LOAD patients, and 220 age-matched controls (>65 years). In the 5' regulatory region we detected a common polymorphism -102 c/g, and a novel polymorphism +113c/a in one allele. In the coding region we detected only common polymorphisms, including M129V substitution. When alleles and genotypes frequencies were compared by the Chi2 test, no significant differences between EOAD, LOAD and controls were found. Only in the case of M129V polymorphism in LOAD group $P=0.06$, which could suggest a weak positive tendency, but not a statistical correlation. It could be concluded that analyzed polymorphisms in the PRNP gene are not an independent risk factor for AD in the studied groups.

P12.37 Caspase 3 and homocysteine-induced excitotoxicity

Ziemska E., Stafiej A., Kozłowska H., Lazarewicz J.W.

Medical Research Centre, PAS, Warsaw, Poland

Previously we demonstrated the obligatory synergy of the group I metabotropic glutamate receptors (mGluRs GI) and NMDA receptors in mediating acute and chronic HCY excitotoxicity in cerebellar granule cells (CGC). Mechanisms of excitotoxicity induced by glutamate (GLU) have been identified with calcium imbalance, mitochondrial dysfunction and activation of caspases. In this study we tested if these mechanisms are involved in HCY neurotoxicity. Primary cultures of rat CGC were incubated for 30 min in the presence of 25 mM HCY or 1 mM GLU. Neurotoxicity was evaluated after 24 h using the propidium iodide staining, the uptake of extracellular calcium was measured with radioactive calcium, changes in the intracellular calcium concentration (Cai) were estimated using Fluo-3 and confocal microscope, cytochrome c release to cytoplasm was evaluated with immunocytochemical methods, and activation of caspase 3 was monitored with immunoblotting. HCY and GLU induced comparable neurodegeneration, however HCY-evoked calcium uptake, an increase in Cai and cytochrome c release were much lower than induced by GLU. Both GLU and HCY induced comparable activation of caspase 3. These data indicate that although caspases seems to play a role in GLU and HCY neurotoxicity, the mechanisms of their activation may differ, HCY toxicity being less dependent on calcium and mitochondrial dysfunction.

P12.38 Neonatal cerebral hypoxia-ischemia: Involvement of FAK-dependent pathwayZiemka-Nalecz M.¹, Makarewicz D.², Zalewska T.¹

¹Medical Research Institute, NeuroRepair Department, Warsaw, Poland; ²Medical Research Institute, Neurochemistry Department, Warsaw, Poland

Focal adhesion kinase (FAK) is a non-receptor tyrosine kinase thought to play a major role in transducing extracellular matrix (ECM)-derived survival signals into cells. Thus, modulation of FAK activity may affect the linkage between ECM and signaling cascade to which it is connected and may participate in a variety of pathological settings. In the present study we investigated the effect of neonatal cerebral hypoxia-ischemia (HI) on levels and tyrosine phosphorylation of focal adhesion kinase and the interaction of this enzyme with Src protein tyrosine kinase and adapter protein p130Cas, involved in FAK-mediated signaling pathway. The total amount of focal adhesion kinase as well as its phosphorylated form declined substantially to about 50% of the control between 24–48 h after the insult. Concomitantly a decreased association of FAK with its investigated molecular partners, Src kinase and p130Cas protein has been observed. This early response to brain hypoxia-ischemia was attenuated during prolonged recovery with almost complete return to control values at 7 days. These data are indicative of an involvement of FAK-dependent signaling pathway in the evolution of HI-induced neuronal degeneration.

MOLECULAR BASIS OF BRAIN FUNCTIONS

P13.01 c-Fos and TrkA-immunoreactivity after open field stress in the rat hypothalamus

Badowska-Szalewska E., Klejbor I., Ludkiewicz B., Domaradzka-Pytel B., Morys J.

Dept. of Anatomy and Neurobiology, Medical University, Gdansk, Poland

The hypothalamus is a structure involved in response to stress stimulation. Changes of c-Fos protein expression in activated neurons as well as TrkA (receptors of NGF) immunoreactivity following acute and chronic open field stress were studied immunohistochemically in the periventricular zone of hypothalamus. The material consisted of adult rats divided into three groups: nonstressed (control) animals, rats exposed to acute and chronic open field stress. In the control animals single c-Fos and a lot of TrkA positive cells in studied nuclei were observed. They were relatively homogeneously distributed. Exposition to the acute open field stress caused increase of c-Fos-positive cells in examined nuclei. TrkA-positive neurons were strongly activated in response to acute stress. Chronic stress caused decrease of the number of c-Fos-positive cells predominantly in paraventricular and arcuate nuclei, whereas in the supraoptic nucleus the relatively high level of c-Fos immunoreactivity was maintained. Similarly, chronic stress resulted in maintaining high TrkA activity in the supraoptic nucleus while its reduction in the paraventricular and arcuate nuclei. Our results suggested that hypothalamic nuclei showed different level of adaptation in response to stress probably due to the differences in their neurosecretory activity.

P13.02 Neuron-specific eGFP expression from lentivectors in rat brain

Duniec K.^{1,2}, Mioduszevska B.¹, Serre A.², Kaczmarek L.¹, Mallet J.²

¹Department of Molecular and Cellular Neurobiology, Nencki Institute of Experimental Biology, Warsaw, Poland; ²LGN, CNRS, Paris, France

Lentiviruses have the ability to introduce their genes to nondividing cells, such as neurons. Thus they are potentially of great value in gene therapy, and in investigating the function of neurons in the brain. Yet for such purposes it is crucial to limit transgene expression to adult neuronal cells. In order to meet this aim we have prepared constructs containing the eGFP gene driven either by the control promoter PGK, or one of the neuron-specific promoters: NSE, CaMKII alpha, calretinin or synapsin 1 promoter. These constructs were incorporated into replication deficient lentiviral vectors, which were tested *in vitro* in rat neuronal primary cultures and *in vivo* by stereotaxic injections of the lentivectors into adult rat brain (hippocampus and striatum), which were then followed by immuno-reactions identifying neurons by NeuN marking. Further we compare the chosen promoters by measuring the eGFP fluorescence and analyzing its persistence 5 weeks and 6 months post injection. Preliminary results show that synapsin 1 promoter gives the highest eGFP fluorescence and is most neurospecific from all the tested promoters. The ability to provoke transgene overexpression exclusively in neurons is essential to avoid severe disturbance of nonneuronal cells' function and in so doing to improve the lentivectors gene transfer tool.

P13.03 Inducible gene expression in rat neurons

Konopka W.¹, Duniec K.¹, Owczarek D.¹, Wawrzyniak M.², Mioduszevska B.¹, Maleszewski M.², Kaczmarek L.¹

¹Nencki Institute of Experimental Biology, PAS, Warsaw, Poland; ²Warsaw University, Warsaw, Poland

To advance our understanding of the central nervous system there is a need for refined approaches to control gene expression in neuronal culture as well as in the brain. We have applied tetracycline inducible system to rat neurons from cerebral cortex cultured *in vitro* and to transgenic rats. Cortical neurons were transfected with combination of Tet regulatory proteins: tTR (tetracycline TransRepressor) and rtTA (reverse tetracycline TransActivator) driven either by neuronal specific promoter of CaMKII gene or by constitutive hCMV promoter. Expression of EGFP driven by inducible Tet promoter was quantitatively measured with the aid of laser scanning cytometry. Cultures were stimulated with 1 µg/ml of doxycycline. Very high dox-stimulated induction was observed when tTR and rtTA were driven by the CaMKII promoter. However, introduction of the human CMV promoter resulted only in a mediocre neuronal gene expression, unless the cells were treated, either in culture or *in vivo*, with depolarizing concentrations of KCl. Next, we have developed transgenic rats that carry EGFP transgene under control of Tet promoter (TR-GFP). Tet regulatory protein (rtTA) was introduced into TR-GFP rats either by lentiviral vector or by crossbreeding with another transgenic line with rtTA.

P13.04 Effects of immune stimulation on avoidance responses in mice

Wolak P.M.¹, Werka T.², Juszcak G.R.¹, Sliwa A.¹, Tymosiak-Zielinska A.¹, Sadowski B.¹, Swiergiel A.H.¹

¹Dept. of Animal Behavior, Inst. of Genetics and Animal Breeding, PAS, Jastrzebiec, Poland; ²Nencki Institute of Experimental Biology, PAS, Warsaw, Poland

It is hypothesized that peripheral administration of lipopolysaccharide (LPS), known to induce sickness behavior, produces learning and memory deficits. Effects of LPS on two-way avoidance conditioning were assessed in male mice selected for high (HA line) and low (LA line) swim stress-induced analgesia. Avoidance training (7 session, 30 trials each) was performed in a shuttle-box. A trial started with the conditioned stimulus onset (CS, white noise). Five seconds later the unconditioned stimulus (US, footshock) was added. During the intertrial intervals (ITI, 14–26 s duration) mice were allowed to cross between the compartments (intertrial responses, ITR). In the 6th session all mice were treated with saline or LPS, 120 minutes before testing. Avoidance performance was clearly impaired in HA in comparison to LA mice. However, after LPS administration, the avoidance was increased in HA and decreased in LA subjects. Moreover, LPS decreased ITR rate, but only in LA mice. Between lines differences in LPS effects and in the avoidance learning are discussed.

Supported by KBN Grant 3PO4C0582 and the EU Framework 6 NEWMOOD Integrated Project to AHS

LEARNING AND MEMORY

P14.01 AM281 alters recognition memory in rats

Bialuk I., Kosiorok P., Hryniewicz A., Zbucki R., Kalinowska A., Winnicka M.M.

Department of General and Experimental Pathology, Medical University of Bialystok, Poland

Cannabis sativa L. is a source of cannabinoids (CB) responsible for many side effects. CB act through G protein coupled receptors: CB1 and CB2. The peripheral receptor CB2 is present mainly on cells of the immune system. CB1 receptor is located predominantly in the CNS and is one of the most abundantly expressed neuronal receptors. CB are known to attenuate learning and memory processes in both humans and animals. In rodents, disruptive effect of CB on memory, reversed by SR141716A, was shown in behavioral tests based on conditioning. The present study assessed the influence of AM281, structurally similar to SR141716A, a CB1 receptor antagonist/inverse agonist, on recognition memory evaluated in an object recognition test. Moreover, because CB may alter motor function, the influence of AM281 on psychomotor activity was tested in an open field. AM281 at all doses (0.1, 0.5, 1.0 mg/kg) given i.p. 15 min before presenting an object A (learning trial), significantly improved recognition memory, measured by the difference in exploration of object B and a duplicate of object A (A'), presented 2 h later, as compared to a control group. Moreover, AM281 did not alter psychomotor activity in an open field. Pro-cognitive effects of AM281 indicate that this compound exerts activity of CB1 receptor inverse agonist.

This study was supported by AMB grant No. 3-24668L

P14.02 Somatosensory cortex activation during classical conditioning training in mice

Cybulska-Klosowicz A., Zakrzewska R., Kossut M.

Nencki Institute of Experimental Biology, PAS, Warsaw, Poland

Learning is associated with alertness, a state mobilizing the organism to detect novel stimuli and subsequently adequately responding to them. During the process of learning, alertness and attention may modify responsiveness of neuronal pathways. We mapped brains of mice with 2-deoxyglucose (2DG) autoradiography during the 1st and the 3rd session of classical conditioning training involving whiskers stimulation on one side of the snout paired with aversive or appetitive unconditioned stimulus. Such sensory pairing results in an enlargement of functional cortical representation of the involved vibrissae in the contralateral hemisphere, visible after the training. In the present experiment, during 1st session, an increased labeling was seen in the barrel cortex in both hemispheres. The effect was the same independently of the applied unconditioned stimulus. In the 3rd session activation of the barrel cortex was unilateral, as expected after unilateral whisker stimulation. We have shown that unilateral sensory stimulation in the initial stage of conditioning activates primary sensory area in both hemispheres. The sensory pathway from vibrissae to barrels is completely crossed and the two barrel fields are linked by fibres of the corpus callosum. The results suggest that during the early phase of conditioning, the interhemispheric interactions are enhanced.

P14.03 The role of neurogenesis in learning: Studies of cyclin D2 knock-out mice

Filipkowski R.K.¹, Kiryk A.¹, Knapska E.¹, Jaholkowski P.¹, Kowalczyk A.^{1,2}, Piechal A.³, Blecharz-Klin K.³, Widy-Tyszkiewicz E.³, Kaczmarek L.¹

¹Nencki Institute of Experimental Biology, PAS, Warsaw, Poland;

²Mossakowski Medical Research Centre, Warsaw, Poland;

³Medical University of Warsaw, Poland

Cyclin D2 knock-out mice (D2 KO mice) exhibit no neurogenesis in the adult brain (Kowalczyk et al. 2004, J Cell Biol 167: 209-213.). However, their developmental neurogenesis, although slightly affected, still allows for formation of the brain, with all the major structures present, albeit, some of them smaller. This allows testing cyclin D2 KO mice to determine the importance of adult neurogenesis in, e.g., learning and memory. We have investigated D2 KO mice and their wild type siblings as controls in several behavioral paradigms in which the role of neurogenesis is postulated like trace fear-conditioning and Morris water maze. We also studied their adaptation to a new environment, place preference, place avoidance and place relearning in IntelliCages (NewBehavior). In all these tasks, we have found D2 KO mice to display mild deficits in learning and memory. It cannot be excluded that these deficits are mostly attributed to developmental changes leading to alterations in size of some of the critical brain structures, including the hippocampus, olfactory bulb, cerebellum and neocortex.

P14.04 The hippocampal piramidal cell synapses simulator

Gorzalanczyk E.J.^{1,5}, Huflejt M.², Kniat J.², Murakowski J.³, Wozniak P.⁴

¹Department of Neuropsychology and Behavioral Genetics, Institute of Psychology, Kazimierz Wielki University, Bydgoszcz, Poland; ²Poznan University of Technology, Poznan, Poland; ³Department of Electrical and Computer Engineering, University of Delaware, Delaware, USA; ⁴SuperMemo Research, Kolobrzeg, Poland; ⁵Sue Ryder Care Home, Bydgoszcz, Poland

The new version of hippocampal piramidal cell synapses simulator was constructed. The theoretical base of this simulator is based on alternative splicing as the explanation of long-term memory. A model of mechanisms involved in the process of memory at the level of glutamate synapse is presented. This process is described by means of two variables – retrievability and stability. The tool for the simulation of a synapse is written in C++ and environment Borland C++ Builder. Alternative splicing is proposed as the base of regulation of the synapse strength. In the model the population of the NMDA (N-methyl-D-aspartate) and AMPA (amino-3-hydroxy-5-methyl-4-isoxazolepropionic) receptors is changing proprieties during the memorization process. A mathematical description and computer application of the glutamate synapse is used. The computer model, in accordance with the two independent variables of memory makes it possible to change the activity of the group of synapse parameters. This is a new original explanation of the plasticity mechanisms involved in memory formation at the molecular level.

P14.05 Neuropsychological evaluation of memory deficits in Parkinson's disease

Gorzelańczyk E.J.^{1,2,3}, Gryz J.¹, Harat M.¹, Laskowska I.², Litwinowicz A.¹, Michalak M.², Olzak M.^{1,2}, Rolinska P.², Zukiewicz K.²

¹Department of Neurosurgery, Military Hospital, Bydgoszcz, Poland; ²Department of Neuropsychology and Behavioral Genetics, Institute of Psychology, Kazimierz Wielki University, Bydgoszcz, Poland; ³Sue Ryder Care Home, Bydgoszcz, Poland

It has been observed that Parkinsonian patients (PD) demonstrate memory deficits in various domains. To examine to what extent PD patients are impaired in memory tasks in comparison with healthy individuals, we investigated 24 patients with PD. A control group was included. Assessment of the following memory aspects was conducted: short-term visuospatial memory (BVRT), visuospatial working memory (TMT), verbal working memory (MMSE, Stroop Test). PD patients demonstrated significant cognitive decline compared to controls according to all examined functions. The most significant differences were revealed in MMSE, TMT A&B and Stroop Test part B ($P < 0.01$), less relevant in BVRT ($P < 0.05$). Surprisingly, reading velocity was significantly better in PD than in the control group (Stroop Test part A, $P < 0.01$). Patients' age differentiated the execution of only two tasks (Stroop Test part B and BVRT errors; $P < 0.01$) to the younger's (under 62) advantage. These data reinforce the probability of frontostriatal pathway contribution in memory functions

P14.06 Differential involvement of the central amygdala in appetitive versus aversive learning

Knapska E.¹, Walasek G.¹, Nikolaev E.², Neuhaeuser-Wespy F.³, Lipp H.P.³, Kaczmarek L.², Werka T.¹

¹Department of Neurophysiology, ²Department of Molecular and Cellular Neurobiology, Nencki Institute of Experimental Biology, PAS, Warsaw; ³Institute of Anatomy, University of Zurich, Switzerland

Understanding the function of the distinct amygdaloid nuclei in learning comprises a major challenge. In the present study, we employed c-Fos immunolabeling to compare the engagement of the lateral and central nuclei of the amygdala in appetitively and aversively motivated behaviors. This experiment was designed to extend our previous results with appetitive and aversive instrumental training in rats, which showed that the central amygdala (CE) responded, surprisingly, selectively to the appetitive conditioning only. Therefore, the present experiment was carried out on different species, mice, trained either for place avoidance or place preference (aversive vs. appetitive conditioning) in an automated learning system (INTELLICAGE). Again, much more intense c-Fos expression was observed in the CE after the appetitive training as compared to the aversive training. These data, obtained in two species and by means of novel experimental approaches balancing appetitive vs. aversive conditioning, strongly suggest that the central nucleus of the amygdala is specifically involved in appetitively motivated learning processes.
Supported by NCCR "Neural Plasticity & Repair".

P14.07 Individual fear reaction to aversive context

Lehner M.¹, Skorzewska A.¹, Taracha E.¹, Maciejak P.^{1,2}, Wisłowska-Stanek A.², Zienowicz M.², Turzyska D.¹, Sobolewska A.¹, Plaznik A.^{1,2}

¹Dept. of Neurochemistry, Institute of Psychiatry and Neurology, Warsaw, Poland; ²Dept. of Experimental and Clinical Pharmacology, Warsaw Medical University, Poland

Rats differing in sensitivity to pain pretested in flinch-jump test (MS more sensitive, LS less sensitive) were subsequently examined in a conditioning fear test with simultaneously measured vocalization. The MS group showed enhanced freezing reaction in comparison with control group (rats exposed to the testing box only) and very low scores of vocalization. In LS group vocalization scoring was increased whereas freezing response was comparable to control group reaction. There appeared differences in the expression of c-Fos protein between MS and LS in CA1, DG layers of the hippocampus and medial frontal cortex (mFrCx). In CA1 layer, MS group had increased c-Fos expression, whereas in mPFC and dentate gyrus (DG) there was decreased c-Fos expression. Moreover, in the basolateral (BLA) and medial amygdala (MeA) enhanced c-Fos expression appeared in MS animals compared with control group. Additionally, using 5-HT immunostaining 5-HT content was examined in some brain regions. We detected that LS rats had more 5-HT than MS group in mPFC while in BLA serotonin content was greater in LS group. The behavioral and immunocytochemical results suggest the occurrence of differences in processing of fear-memory reaction correlated with sensitivity to pain stimuli.

P14.08 Memory impairment as a possible effect of rapamycin therapy

Gawrys L., Kaczmarek L.

Nencki Institute of Experimental Biology, PAS, Warsaw, Poland

Cellular consolidation theory posits that conversion from short-term memory (STM) to long-term memory (LTM) is based on transformation of temporary changes in synaptic transmission into persistent modification of synapses, which requires synthesis of proteins *de novo*. There is a growing body of evidence that protein translation at dendrites enables specificity of plastic changes. The translational signaling pathway regulated by protein kinase mTOR is suggested to play a key role in local dendritic translation, and thus contributes to long-term memory. mTOR is specifically inhibited by rapamycin. Some studies using LTP and LTF models showed that rapamycin can block long-term plastic changes. Rapamycin is also commonly used in medical treatment of patients after organ transplantation as an immunosuppressing drug. The research goal is to investigate the effects of rapamycin therapy on long-term memory formation in patients after transplantation. Our hypothesis holds that patients taking rapamycin show specific deficits in long-term memory compared to patients in control groups.

P14.09 The dentate gyrus is important for segregating relevant and irrelevant associations

Malinowska M., Sadowska J., Wesierska M.

Nencki Institute of Experimental Biology, PAS, Warsaw, Poland

The hippocampus represents associations but a selective deficit in a place avoidance task indicated hippocampus also coordinates neural activity associated with selective activation and suppression of relevant and irrelevant representations (Wesierska et al. 2005, *J Neurosci* 25: 2413). In that work, tetrodotoxin (TTX) infusions into one hippocampus only impaired place avoidance when relevant associations with distal room cues had to be segregated from irrelevant associations with local arena cues. That Room+Arena-(R+A-) task required rats on a slowly rotating arena to associate shock with a place in the room and segregate the relevant room associations from irrelevant associations with arena cues. We made permanent lesions of hippocampal sub-fields on one side to test whether the dentate gyrus (DG) is specialized for segregating relevant and irrelevant associations. R+A- avoidance was tested for several days after neurotoxic or electrolytic lesion of one hippocampus. Damage to the DG was more impairing than hippocampal damage that did not include DG. Avoidance was similar in sham operated controls and rats with large unilateral CA1 or combined CA1 and CA3 lesions that excluded DG. The data suggest that the DG is specialized for segregating relevant and irrelevant associations.

Support: KBN grant No. 68, 3P04C 028 23.

P14.10 The role of satiation and smell of the food reward in performance and extinction of the instrumental response in rats with limited early taste experience

Stasiak M., Domagalska D., Walasek G.

Nencki Institute of Experimental Biology, PAS, Warsaw, Poland

Twelve Long-Evans rats were used. Eight rats deprived of a variety of food tastes in early life, i.e., rats with limited early taste experience, were fed with one of two kinds of standardized food: the food with beef (B) or the food with liver (L). Four rats were fed with only B both during the first 3 months and after that period, i.e., during the acquisition of the instrumental response. Four other rats were fed with only L. Four nondeprived additional rats were fed with a variety of foods, after 3 months, these rats were fed with only one kind of food: two rats were fed with only B and two rats were fed with only L. After finishing the acquisition of the instrumental response, the stages with alternated food, and the acute extinction sessions, there was a "satiation" phase of the training. In this phase the rat had been satiated before the experimental session. Altogether, there were 8 sessions: 4 with the B reward and 4 with the L reward. An "extinction with a smell of the food reward" was the last phase of the experiment. In this phase only "the smell" of the reward was available to the rat. There were 2 sessions, one with the B smell and one with the L smell. The results of "the satiation" and "the smell" phases showed no differences between the deprived and the nondeprived rats.

P14.11 Chronic thioacetamide-induced liver failure affects idiothetic but not allothetic memory in ratsWesierska M.¹, Klinowska H.², Fresko I.², Albrecht J.²¹Nencki Institute of Experimental Biology, PAS, Warsaw, Poland; ²M. Mossakowski Medical Research Centre, Warsaw, Poland

Hepatic encephalopathy (HE) in humans is characterized by memory deficits. Rats treated with 2 i.p. doses of 250 mg/kg of thioacetamide (TAA) at 24 h intervals and examined 60 days later show liver injury, changes in brain metabolism typical of HE, and impaired attention processes and non-associative learning. In the present study allothetic (Room+, Arena-) and idiothetic (Arena+) memory acquisition in the place avoidance (PA) task were tested in 12 HE and 8 control rats. In the PA test rats receive shock whenever entering the 60-degree part of circular arena, and low number of entrances is an index of memory acquisition. In allothetic task rotation of arena dissociates distal Room and local Arena cues, the to-be-avoided place is in a fixed position to room cues. In idiothetic task, in darkness, this place is in a stable location vs. arena cues. HE rats showed impaired idiothetic, but not allothetic memory. The results support the notion that HE affects cortical rather than hippocampal function. We suggest that appropriately designed analysis of idiothetic memory may become a routine psychometric test in patients with minimal HE.

Support: SCSR grants 68, 3P04C 028 23 (MW); 6P05A 00321 (JA).

P14.12 Alleviation by *Hypericum perforatum* of the stress-induced impairment of spatial working memory in rats

Trofimiuk E., Walesiuk A., Braszko J.J.

Medical University of Bialystok, Department of Clinical Pharmacology, Bialystok, Poland.

The aim of the present study was to further substantiate the hypothesis that St John's wort (*Hypericum perforatum*) protects against stress-induced cognitive disorders. Previously we found that the plant, administered at the dose 350 mg kg⁻¹ for 21 days orally, prevented the deleterious effects of both, chronic restraint stress (2 h daily for 21 days) and long-term corticosterone treatment on recognition and the spatial reference memory. In this study, we tested effects of St John's wort on spatial working memory in a water maze and also on recall of the passive avoidance behaviour (PAB) as well learning of the conditioned avoidance responses (CARs) in rats. We found that *Hypericum perforatum* significantly enhanced spatial working memory as well as recall of the PAB, but had no effect on the acquisition of CARs. Similar results were obtained in stressed and corticosterone treated (5 mg kg⁻¹) group. Noteworthy, the severity of dysfunction caused by stress was much higher than that caused by corticosterone. None of our treatments produced significant motor coordination impairments as tested in 'chimney' test. In conclusion, the results of the present study indicate that *H. perforatum* has appreciable stress alleviating as well as memory improving activity.

P14.13 Participation of D2 dopamine receptors in the memory improvement caused by angiotensin IV (Ang IV) and Ang II(3-7) pentapeptide

Braszko J.J.

Medical University of Bialystok, Department of Clinical Pharmacology, Bialystok, Poland

Important role of angiotensin IV (Ang IV) in the processes of learning and memory has now been well established. We previously found that intracerebroventricular (ICV) administration of Ang IV as well as Ang II(3-7) pentapeptide enhances learning of conditioned avoidance responses (CARs), facilitates recall of a passive avoidance (PA), and improves object recognition (OR) in rats. Since dopaminergic system is crucial for the cognitive processes in this study we sought for the dopaminergic D2 mediation of these effects using remoxipride (Remox) as a selective D2 receptor antagonist. Male Wistar rats (180-200 g), pretreated with Remox {(S)-(-)-3-Bromo-N-[(1-ethyl-2-pyrrolidinyl)methyl]-2,6-dimethoxybenzamide hydrochloride} 5 mmol/kg intraperitoneally, were 2 h later given Ang IV or Ang II(3-7) (1 nmol ICV) and then tested in the above cognitive paradigms as well as in the open field and elevated 'plus' maze to control for the unspecific, respectively motor and emotional, effects of our treatments. Both, Ang IV and Ang II(3-7) effectively enhanced learning of CARs ($P < 0.001$), recall of PA ($P < 0.001$), and improved OR ($P < 0.001$). Pretreatment with Remox abolished all these cognitive effects of both peptides. Moreover, in a radial maze, Remox abolished the improvement of working memory caused by Ang IV and Ang II(3-7). Remox, Ang IV, and Ang II(3-7), given at the same doses and routes as in the cognitive tests, did not significantly influence crossings, rearings and bar approaches in the open field as well as the parameters measured in the elevated 'plus' maze making thus major contribution of the unspecific effects of our treatments to the results of the memory tests improbable. In conclusion, these results point to a major role of the D2 dopaminergic receptors in mediation of the cognitive effects of Ang IV and Ang II(3-7).

P14.14 Ginkgo biloba abolishes stress-induced impairment of retrieval of passive but not acquisition of active avoidance behaviour in rats

Walesiuk A., Trofimiuk E., Braszko J.J.

Medical University of Bialystok, Department of Clinical Pharmacology, Bialystok, Poland

Exposure to chronic stress has been shown to alter cognitive functions such as learning and memory and has been linked to the mood and anxiety disorders. Anti-anxiety or sedative agents used for the management of stress have several disadvantages and ill effects. Therefore, in this study we investigated efficacy of a natural medicine, the extract of Ginkgo biloba (EGB 761) in prevention and treatment of the post-stress memory dysfunctions. We used restraint rat model of stress which involves no painful stimulation and combines both emotional and physical components simulating physiological stress. The purpose of the present study was to assess whether EGB 761 has the potential of alleviating stress-induced impairment of aver- sively motivated learning and recall in rats. Male Wistar rats (160-180 g) subject to a 21 day (2 h daily) restraint stress were then tested for their ability to learn conditioned avoidance responses (CARs) and to recall a pre-learned passive avoidance behaviour (PAB). A corticos-

terone (5 mg/kg s.c.; daily) group was run in parallel to ascertain the contribution of the stress cortisolemia to its cognitive effects. Stressed or corticosterone treated rats learned CARs at similar rates to these of controls. However, recall of PAB was significantly ($P < 0.05$ and $P < 0.01$, respectively) attenuated in both groups. EGB 761 changed rates of CARs acquisition in neither naive nor stressed or corticosterone treated groups. It however, completely abolished impaired recall in both, stressed as well as corticosterone treated group. In conclusion, the present findings indicate, that simultaneous administration of EGB 761 prevents chronic stress-induced impairment of information retrieval in rats.

PAIN AND ANALGESIA

P15.01 Prepulse inhibition of the acoustic startle in mice selected for magnitude of swim analgesia

Blaszczyk J.W.¹, Sadowski B.²

¹Nencki Institute of Experimental Biology, PAS, Warsaw, Poland;

²Institute for Genetics and Animal Breeding, Wolka Kosowska, Poland

Acoustic startle response (ASR) and prepulse inhibition (PPI) of ASR was examined in male Swiss-Webster mice selectively bred for high and low swim-stress induced analgesia. Randomly bred mice served as unselected control line. Startle stimuli were 112 dB SPL 20-ms white noise tones with 2-ms rise time. The mouse was presented with four trial types: one consisting of the startle stimulus alone, and three others of startle stimulus preceded by a 73, 83 or 89 dB 20-ms white noise prepulse against 46 dB background. The delay between the prepulse and the startle stimulus was fixed at 100 ms (onset to onset). Dizocilpine maleate (0.15, 0.25 or 0.5 mg/kg) or saline was administered i.p. 30 min before the ASR session. ASR magnitude was the highest in the high analgesia (HA) line as compared to the control (C) and the low analgesia (LA) line, and was higher in C than in LA mice. LA mice manifested only slightly and nonsignificantly less PPI as compared to the other lines. Dizocilpine at all doses profoundly disrupted PPI in the HA, but was not effective in the C and LA lines. The results show that ASR magnitude and the glutaminergic mechanism of PPI are genetically determined traits. The selected mice can serve as a convenient animal model of sensorimotor gating deficit observed in schizophrenic patients.

P15.02 Expression of opioid genes in neuropathic and inflammatory pain

Korostynski M., Kaminska D., Rodriguez Parkitna J.M., Obara I., Makuch W., Przewlocka B., Przewlocki R.

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

The aim of our study was to profile the expression of opioid genes in rat model of neuropathic (sciatic nerve injury) and inflammatory (injection of Freund's adjuvant) pain. We observed two-fold increase in the abundance of prodynorphin in the lumbar spinal cord (L4-L6) 3 days after injury or inoculation, respectively. Furthermore we found three-fold decrease of spinal proopiomelanocortin mRNA only after nerve injury. Opposite transcriptional regulation of prodynorphin and proopiomelanocortin mRNAs at the spinal level corresponds to lower

opioid antinociceptive efficacy, which can be due to non-opioid pronociceptive action of prodynorphin system. Dramatic and persistent increase in the abundance of prodynorphin mRNA in dorsal root ganglia (DRG) was observed exclusively after nerve injury. Contrary to some of the earlier reports, we found no significant changes in the abundance of mRNAs corresponding to mu, delta and kappa opioid receptors, as well as proenkephalin in the spinal cord. Our results suggest that the increase of gene expression level for prodynorphin in the DRG is likely to be the critical factor for the development and maintenance of neuropathic pain.

Supported by EPILA QLK6-1999-02234.

P15.03 Antinociceptive effects of morphine in neonatal 5,7-dihydroxytryptamine lesioned rats: The role of nitric oxide

Krzascik P.¹, Kolomanska P.², Zajda M.¹, Papasz A.¹

¹Warsaw School of Medicine, Warszawa, Poland; ²Institute of Psychiatry and Neurology, Warszawa, Poland

Recent studies indicate that serotonin antagonists as well as serotonin neuron destruction reduce morphine analgesia. The same effect was observed after nitric oxide synthase inhibitors. We have studied the influence of neonatal serotonergic (5-HT) lesion (5,7-dihydroxytryptamine i.c.v. injections in the 3rd day after birth) on behavior of adult rats (Kostowski and Krzascik 2003, Krzascik and Kostowski 2004). Neonatal lesion induced hypoactivity in forced swimming test was reversed by the NG-nitro-L-arginine (L-NA) given prior to the 5,7-DHT lesion. Similarly, the antinociception effect of morphine tested in the hot plate test was also reversed by L-NA given prior to the 5,7-DHT lesion. Our results suggest, that the nitric oxide is involved in neuronal plasticity after the neonatal serotonergic neuron destruction.

P15.04 The involvement of NOS inhibitors in effects of morphine and endomorphin-1 in neuropathic pain

Makuch W., Sieja A., Przewlocka B.

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

In our previous study we demonstrated that antinociception after various mu opioid receptors agonists, including morphine, was enhanced by inhibition of nitric oxide synthase (NOS) in acute pain in rats. In contrast to morphine, endomorphin-1 (EM-1) and endomorphin-2 (EM-2) analgesia remained unchanged after N6-Nitro-L-arginine methyl ester hydrochloride (L-NAME) administration. The analgesic potency of morphine, but not endomorphins, is attenuated in neuropathic pain. Mechanism of this difference is unknown. Therefore, we investigated the possible role of NOS inhibitors: L-NAME, 7-Nitroindazole (7-NI), 1-(2-Trifluoromethylphenyl)imidazole (TRIM) in morphine- or EM-1-induced analgesia in acute and neuropathic pain (Bennett's model) in rats. All tested NOS inhibitors when administered (i.t.) 10 min prior to morphine, potentiated its antialloodynic effect. In contrast, the antinociceptive effect of EM-1 was slightly inhibited or not changed after pretreatment with NOS inhibitors. These results suggest that nitric oxide is involved in spinal nociception, and that the increased production of nitric oxide following the nerve injury may diminish the efficiency of morphine antinociception in the spinal cord.

Supported by statutory activity.

P15.05 The involvement of glycogen synthase and cyclin dependent kinases in morphine analgesia in rats

Obara I., Wawrzczak-Bargiela A., Rodriguez Parkitna J.M., Makuch W., Przewlocki R., Przewlocka B.

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

Identification of a precise mechanism of morphine analgesia appears essential for effective treatment of pain. It has been shown that glycogen synthase (GSK) and cyclin dependent kinase (CDK) are involved in the signal transduction by the opioid receptor. Therefore, in the present study, we investigated the role of GSK and CDK kinases in the mechanism of a single and chronic morphine administration in rats. The influence of a intrathecal (i.t.) injection of GSK/CDK inhibitors, SB216763 (SB), roscovitine (ROSC) and BIO-Acetoxime (BIO), on the pain threshold and on the development of morphine tolerance was measured by tail-flick test in rats. Our studies showed that acute and chronic injection of SB, ROSC and BIO did not change the pain threshold in naive rats. Repeated co-administration of morphine with SB, ROSC and BIO prevented the development of morphine tolerance. A single injection of the GSK/CDK inhibitors restored morphine analgesic potency in morphine tolerant rats. Above results were parallel to the level of the phosphorylated GSK suggesting that the GSK and CDK kinases may be involved in the mechanisms of opioid tolerance, and inhibitors of the kinases may be an important target of research.

Supported by grant PBZ-KBN-033/P05/2000 (Warsaw, Poland).

PSYCHOPHYSIOLOGY AND NEUROPSYCHOLOGY

P16.01 The study of the instrumental responses reinforced by sexual or exploratory behaviour in male rats

Beck J.¹, Kostowski W.^{2,3}

¹Department of Physiology, Medical University, Warsaw, Poland; ²Department of Pharmacology, Medical University, Warsaw, Poland; ³Department of Pharmacology and Physiology of Nervous System, Institute of Psychiatry and Neurology, Warsaw, Poland

We reported previously that during testing the instrumental sexual responses, male rats immediately after the copulatory contact with female tend to return spontaneously to the start compartment. We have elaborated the instrumental response aimed to return to the start compartment. The apparatus consisted of maze and goal compartment. The first as well as the last divisions of the maze were connected with goal compartment by guillotine doors. The goal compartment and the last division of maze were equipped with bars. The opening of each door took place after 5–10 bar presses. During the test, male rat was placed in the goal compartment where the estrous female was tethered. After the mount bout (the cluster of copulatory events), the male performed the instrumental response enabling him the departure from the female and the exploration of the maze. Subsequently the male performed the instrumental responses reinforced by the contact with estrous female. We suggest that the analysis of these instrumental responses may give some information about the role of drive satisfaction (or hypothetical "antidrive") and/or opponent processes that act during the sexual preparatory and consummatory activities.

P16.02 The effects of socialization on intermale behavior in old rats

Boguszewski P., Meyza K., Zagrodzka J.

Nencki Institute of Experimental Biology, PAS, Warsaw, Poland

It is known that high level of anxiety affects social behavior diminishing the frequency and duration of social contacts. In our previous experiment though, we found that old rats, in spite of their increased level of anxiety, did not show any differences in number and time spent on interactions when compared to young adults. Aggressive episodes in old group were not observed at all (Boguszewski and Zagrodzka 2002). The present experiment was designed to examine whether the lack of high anxiety effect on social interactions was age-related or rather connected with socialization process – the period of socialization being much longer in the old group (24 months old) than in the young (4 months old). The experiments were performed on old (24 months old) male rats housed individually or in 3–5 home cage groups. Each experimental rat was placed in the test arena for 10 min and then confronted for the next 10 min with a stranger rat of the same age. The encounters were video recorded and different behavioral events were encoded by two independent observers using computer-based method (Behavior Viewer, Boguszewski P.). Statistical analysis has revealed that the number and time of pro-social and aggressive events is significantly higher in individually housed animals. These results indicate that with age the impact of anxiety on social contacts decreases. Longlasting socialization however inhibits intermale aggressive behaviors.

P16.03 Sensitivity to chemical toxins after exposure to non-chemical stressors

Dyzma M., Lutz P., Gralewicz S.

Nofer Occupational Medicine Institute, Department of Toxicology and Carcinogenesis, Lodz, Poland

Exposure to a stressor may persistently alter the CNS functional state and change the organism's response to neurotoxic chemicals. Recently we have demonstrated that pretreatment with footshock decreases the rat's susceptibility to the effects of the amphetamine (AMPH), a psychostimulant, and chlorphenvinphos (CVP), an organophosphate pesticide. The purpose of the present work is to compare effects of two other nonchemical stressors: social defeat (SDE) and immobilization (IMMO) on the rat's responsiveness to AMPH and CVP. Male Wistar rats are being used. SDE involves repeated (5 times) encounter of the subject with a conspecific. IMMO consists in 2 h incarceration inside a plastic tube. Stress level is evaluated by determination of serum corticosterone (CORT) concentration. Blood acetylcholinesterase (AChE) activity is also measured. The results obtained so far show that SDE and IMMO, both cause a dramatic increase in CORT concentration subsiding within 60 min. Also AChE activity exhibits considerable increment. Unlike the footshock, both stressors appear to augment the locomotor response to AMPH given two weeks after stressing. Studies concerning the second toxic agent (CVP) are in progress and will be reported soon. The results support the supposition that organism's response to neurotoxic chemicals may be differently affected by earlier exposure to nonchemical stressors.

P16.04 Functional brain asymmetry of motor function in left- and right-handersGut M.¹, Grabowska A.¹, Urbanik A.², Forsberg L.³, Binder M.⁴, Sobiecka B.², Kozub J.²

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

The aim of the presented study was to assess the brain activity patterns for motor behavior in left- and right-handers (LH and RH). Twelve RH and 17 LH participated in the experiment. Subjects performed two types of tasks during fMRI scanning: simple movements (flexion-extension movements of an index finger) and complex movements (sequential tapping in proper order) both with right and left hand in sequence. The results show that during simple movements of either hand in either group activation was exclusively contralateral and focused in S1 and M1 cortex. Simple movements of the right hand resulted in activation of a wider area in the left hemisphere in RH than in LH, whereas simple movements of the left hand in activation of a wider area in the right hemisphere in LH than in RH. In contrast to simple movements, during complex movements a bilateral activation was observed in both groups. However, the groups differed as to the size of the activated areas in the ipsilateral vs. contralateral sides. The obtained results suggest that functional brain lateralization of motor function depends on handedness and complexity of a task.

P16.05 Age differences in the subjective accentuation task

Kolodziejczyk I., Szelag E.

Nencki Institute of Experimental Biology, PAS, Warsaw, Poland

The aim of our study was to investigate age-related differences in subjective accentuation task. Young (aged 19–25 years), elderly (65–67) and very old (95–103) people listened to series of identical sounds presented at 5 different frequencies, ranged from 1 to 5 sounds/s. The task was to create a subjective rhythm by the accentuation of every 2nd, 3rd or nth tone, thus, to integrate sounds into groups containing 2, 3 or more elements. The analyzed variable was the number of sounds integrated into one group for each frequency. The number of integrated sounds decreased with the presentation rate, however, this relationship weakened with subjects' age. Moreover, for the slowest tempo centenarians integrated more sounds than subjects in younger groups. Age-related differences in this task may be interpreted in terms of temporal aspects of information processing. Specifically, these differences may suggest the existence of age-related changes either in the frequency of attentional peaks (Jones' dynamic attending theory) or lengthening of the upper limit of hypothetical integration mechanism (Poeppel's hierarchical model of time perception).

Supported by the KBN grant no. PBZ-KBN-022/PO5/1999.

P16.06 c-fos expression in rats hyper- and hypo-responsive to novelty in basal conditions and after chronic electric stimulation of the ventral tegmental area (VTA)

Krzyzak A., Ledochowski P., Jerzemowska G., Trojnar W.

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

In search for a central mechanism behind individual differences in behavioral reactivity to various environmental stimuli the level of activity of the prosencephalic structures (as measured by c-fos expression) was compared in rats showing high (HR) and low (LR) locomotor reactivity to novelty. Two groups of male Wistar rats were used: not subjected to any particular external stimulation ($n=11$) and subjected to 14-day unilateral electrical stimulation of the VTA ($n=9$), which produces behavioral signs of psychomotor activation. Even in the basal conditions the HRs showed a higher than the LR number of Fos+ neurons in the majority of the tested structures, particularly in the motor structures, retrosplenial cortex, corticomедial and basolateral amygdala and some hypothalamic and thalamic nuclei. In the stimulated group the number of Fos+ nuclei was markedly increased, more in the stimulated hemisphere both in HRs and LR. HR>LR differences concerned the same structures as in control group and additionally nucleus accumbens, some limbic thalamic and septal nuclei. These results point to an increased excitability of wide brain areas in subjects showing behavioral hyperactivity.

P16.07 The role of amygdala galanin in coping with psychological stress

Lioudyno V., Tsikunov S., Klimenko V.

Institute for Experimental Medicine, RAMS, St-Petersburg, Russia

The neuropeptide galanin is involved in mechanisms of learning and memory. The effects of galanin on aversive emotional learning suggest its participation in adaptation to stressful stimuli and habituation to repeated stresses. It is known that amygdala plays a key role in processing of the biological relevance of events and in forming appropriate behavioral responses. The amygdala as well as the hippocampus undergo plasticity during coping with stress, and many affective disorders are associated with increased excitability of amygdala. The reactions of amygdala galaninergic system in response to psychological stress were evaluated with RT-PCR method. The rats were exposed to a predator (python), which induced long lasting changes in neuronal activity, as well as promoted the development of depressive behavior. Our study revealed strain-specific differences in pre-progalanin mRNA expression in amygdala immediately after predator exposure. A significant stress-induced increase in pre-progalanin mRNA level was found only in rats resistant to emotional disorders. Also, the changes in reactivity of amygdala galanin to subsequent stress were still present six days after predator exposure. The increase of pre-progalanin mRNA expression in amygdala in response to forced swimming was greater in stressed compared to non-stressed rats.

P16.08 P300-related cortical inhibition can not be demonstrated with more complex stimuli

Milner R., Gierych E., Michalski A.

Nencki Institute of Experimental Biology, PAS, Warsaw, Poland

Threshold regulation hypothesis was tested using event-related potentials in humans (Elbert and Rockstroh 1987, *J Psychophysiol* 4: 317-333.). According to this hypothesis the income of important stimulus inhibits irrelevant cortical activity. In previous studies, cortical responsiveness after target and non-target oddball stimuli was measured using additional, probing stimuli (Michalski 2001, *Acta Neurobiol Exp (Wars)* 61: 93-104.). Responses to probes were inhibited after targets. In these experiments, blinks of color LED diodes were used for all stimuli. The theory suggests however, that more complex stimuli, engaging more neurons should increase the probability that responses to probes would interact not only with inhibited but also with excited parts of cortex. This should affect purely inhibitory interactions. In present experiments, computer generated images were used instead of LED diodes. The results showed that P300-related inhibition of probe responses could not be demonstrated for 6 deg × 6 deg color images. It was insignificant even with uniformly colored squares of this size. Such results support the prediction that inhibitory interactions can only be demonstrated if there is little overlap between the assemblies of neurons responding to oddball and probe stimuli.

P16.09 The effect of age and gender on the perception of temporal order

Szymaszek A., Szelag E., Sliwowska M.

Nencki Institute of Experimental Biology, PAS, Warsaw, Poland

The existing evidence suggests that the temporal order (TO) of two acoustic stimuli can be properly recognized if their onsets are separated by a temporal gap of at least 40 ms. The aim of the present study was to investigate age- and gender- related differences in the perception of TO. Two groups of subjects were studied: young (aged 20–29 years) and elderly (60–69). Two methods of stimulus exposition were used: (1) inter-hemispheric – two 1-ms clicks were presented one to each ear; (2) intra-hemispheric – two 10-ms tones of 400 and 3 000 Hz were exposed to both ears. In both methods the stimuli were exposed with various onset asynchronies. The task was to report the TO of stimuli. The temporal order threshold (TOT) was defined as the minimum asynchrony required to report this order at 75% of correct responses. The results showed that: (1) the younger subjects displayed lower TOT than the older ones (mean TOTs 48 vs. 82 ms, respectively); (2) women showed higher TOT than men (78 vs. 52 ms); (3) inter-hemispheric presentation was more difficult than intra-hemispheric one (77 vs. 54 ms). In older subjects the deterioration of performance may be caused by slowing of the information processing. Gender differences may be explained by the differences in gray/white matter ratio in the brain or a rate of the pacemaker in the hypothetical "internal clock".

Supported by the KBN grant no. PBZ-MIN/001/PO5/06

P16.10 Does callosal maturation affect the crossed minus uncrossed reaction time difference (CUD) in children? Yes, but not in the expected way

Wolski P.

Jagiellonian University, Krakow, Poland

Relatively late myelination of callosal fibers had been hypothesized to increase the crossed minus uncrossed difference in simple reaction time (CUD) in children. Although the original results of Brizzolara and coauthors (Brizzolara et al. 1994, *Behav Brain Res* 64: 179-184.) conformed with the hypothesis, subsequent replications have failed. The present study totaling 102 seven year-olds have shown the grand average of the children's CUD to equal the meta-analytic estimate of the adult value: 3.1 ms. Although no different from those of the grown-ups, the children's CUDs did however show an interesting peculiarity – their observed individual variation was of purely random character, whereas the same measurement in adults showed reliable individual differences. Such results seem to suggest predominantly structural source of the CUD effect in children, while in the adults some dynamic, possibly attentional, factors might be involved too.

P16.11 Information processing accuracy – alternation of shallow and deep strategies

Iskra-Golec I.

Department of Management Psychology and Ergonomics, Jagiellonian University, Krakow, Poland

There have been shown different recall scores depending on the time when the stimuli were acquired. That has been attributed to daily changes in hemispheric dominance. The aim of this study was to find out the 24-hour endogenous trends of recognition accuracy of stimuli processed at shallow and deep levels while exposed to the left or to the right visual fields. During the 24-hours constant routine memory performance of 30 subjects was measured 8 times starting from 06.30 hour. The stimuli were exposed and reactions were recorded by purposely-designed software program. During recognition, immediately following stimuli processing, all already presented stimuli plus new ones were shown in the middle of the computer screen. The subjects were to press appropriate buttons while reacting to the already seen or the new stimuli. Four factor (measurement time, level of processing, visual field, and stimulus) analysis of variance have been performed on the accuracy data. There have been found significant interaction of measurement time, visual field, and level of processing on recognition accuracy. The results show alternating periods of the left hemisphere predominance over the right one in recognition accuracy. The period of predominance in recognition of stimuli processed at the semantic level was followed by the period of predominance in recognition accuracy of stimuli processed on the shallow level.