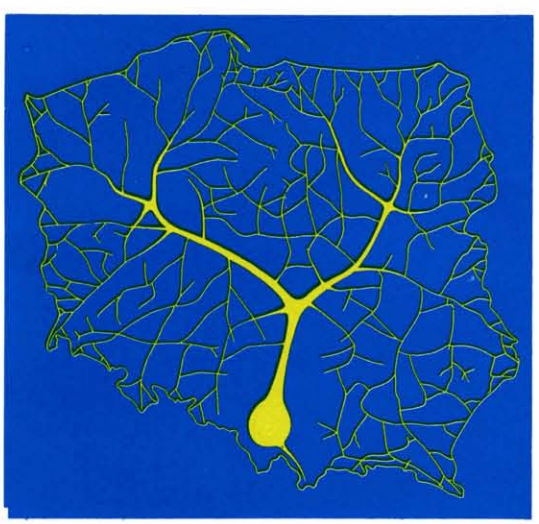
Acta Neurobiol. Exp. 1999, 59: 181-270

FOURTH INTERNATIONAL CONGRESS of the POLISH NEUROSCIENCE SOCIETY

ABSTRACTS



Gdańsk, 2-5 September 1999

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Session 1 - Satellite Symposium: Phenotype dependent susceptibility of cholinergic neurones to neurotoxic insults

1.1 EVIDENCE FOR A CENTRAL MUSCARINIC CHOLINERGIC DEFICIT IN THE DEVELOPING NERVOUS SYSTEM IN CHRONIC HYPERAMMONEMIA

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Exposure of the developing mammalian nervous system to high levels of ammonia results in serious neurological dysfunction and irreversible CNS damage. Hyperammonemia may result from such conditions as Reye Syndrome, congenital deficits of urea cycle enzymes or from intractible epileptic seizures. Congenital ornithine transcarbamylase (OTC) deficiency is the most common inborn error of urea cycle enzymes in humans resulting in chronic hyperammonemia. A large percentage of survivors of neonatal OTC deficiency suffer severe developmental disorders, including seizures, mental retardation and cerebral palsy. Neuropathological studies reveal ventricular enlargement, cerebral atrophy and delayed myelination, as well as Alzheimer type II astrocytosis. Using the sparse-fur (*spf*) mouse model of chronic hyperammonemia resulting from congenital OTC deficiency, studies of central cholinergic integrity revealed a developmental delay in choline acetyltransferase activity and of high-affinity [^{3}H]-choline uptake in several brain structures. Subsequent studies of muscarinic cholinergic binding site distribution showed a widespread loss of M_1 sites, consistent with cholinergic cell loss. These alterations are similar to those reported in Alzheimer's disease, suggesting that the severe cognitive dysfunction in congenital OTC deficiency may at least partly result from a muscarinic cholinergic lesion. Possible mechanisms involved in the pathogenesis of cholinergic cell loss in congenital OTC deficiency include ammonia-induced inhibition of pyruvate and a-oxoglutarate oxidation, resulting in decreased synthesis of acetyl CoA and a cerebral energy deficit, as well as NMDA reeptor-mediated excitotoxicity. Treatment of *spf* mice with acetyl-*L*-carnitine (ALCAR) results in partial recovery of the developmental choline acetyltransferase deficit, suggesting a potential therapeutic benefit of ALCAR in congenital OTC deficiency. Other therapies currently used include ammonia-lowering strategies (using sodium benzoate of sodium phenylacetate) and, in severe cases, liver transplantation. (Funded by The MRC Canada)

1.2 DOWNREGULATION OF ACETYLCHOLINE SYNTHESIS IN ALZHEIMER'S DISEASE: THE ROLE OF BETA AMYLOID AND METHODS OF PREVENTION

Blusztajn, J.K.

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Two characteristic features of Alzheimer's disease (AD) are deficits in cholinergic transmission of the basal forebrain cholinergic neurons, and the presence in the brain of β-amyloid plaques. We examined the effects of synthetic Aβ peptides at subcytotoxic concentrations (< 1 μM) on the cholinergic properties of a mouse cholinergic cell line, SN56. Aβ 1-42 and Aβ 1-28 reduced the acetylcholine (ACh) content of the cells in a concentration-dependent, saturable fashion, whereas Aβ 1-16 was inactive. The reductions in ACh levels caused by Aβ 1-42 and Aβ 1-28 were accompanied by proportional decreases in choline acetyltransferase (ChAT) activity. In contrast, acetylcholinesterase activity was unaltered, indicating that Aβ specifically reduces the synthesis of ACh in SN56 cells. The reductions in ACh content caused by Aβ peptides could be prevented by cotreatment with all-*trans*-retinoic acid or by the tyrosine kinase inhibitors, genistein and tyrphostin A25, compounds shown to increase ACh synthesis in SN56 cells. These results suggest that the ACh-reducing effect of Aβ in SN56 cells is dependent on protein tyrosine phosphorylation. In contrast to the actions of Aβ, overexpression of human APP_{751} in SN56 cells, and in transgenic mice, is associated with increased ChAT activity and ACh content, suggesting that cellular APP or sAPPα may upregulate the cholinergic function. These results suggest that a suppressive effect of Aβ on ACh synthesis may contribute to the cholinergic deficit in AD and that several pharmacological approaches might prevent the anticholinergic actions of Aβ peptides in AD.

1.3 ACETYLCHOLINE SYNTHESIS AND QUANTAL RELEASE RECONSTITUTED BY TRANSFECTION OF CHOLINE ACETYLTRANSFERASE AND MEDIATOPHORE cDNAs.

Bloc, A., Roulet, E., Bugnard, E., Falk-Vairant, J., Loctin, F., Israël, M.* and Dunant Y.

*Department Pharmacologie, Centre Médical Universitaire, CH-1211 Genève 4, Switzerland and *Laboratoire Neurobiologie cellulaire et moléculaire, C.N.R.S., F-91198 Gif-sur-Yvette, France.*

N18TG-2 cells can neither synthesise nor release acetylcholine (ACh) and do not express some of the proteins involved in transmitter storage and vesicle fusion. We restored some of these functions by transfecting N18TG-2 cells with cDNAs of either rat choline acetyltransferase (ChAT), or *Torpedo* mediatophore 16 kDa subunit, or both. ChAT-transfected cells synthesised but did not release ACh. Mediatophore-transfected cells expressed Ca^{2+}-dependent ACh release provided they were previously loaded with the transmitter. Cell co-transfected with ChAT and mediatophore released ACh that was endogenously synthesised. Synaptic-like vesicles were not found either in native N18TG-2 cells, nor in ChAT-mediatophore co-transfected clones, where all the ACh content was apparently cytosolic. Restoration of release did not result from enhanced acidification of intracellular organelles, and thereby enhanced ACh-proton exchange, since *Torpedo* 16 kDa subunit transfection decreased the V-ATPase-driven proton transport. Using ACh-sensitive *Xenopus* myocytes for real-time recording of evoked release, we found that transfected cells released ACh in quantal manner. Comparison of time characteristics, quantal size and rate of ACh release between ChAT-mediatophore co-transfected clones and mediatophore-transfected clones (passively loaded with ACh) will be presented. Our results will be discussed in the light of a possible functional coupling between ACh synthesis and release.

1.4 ACETYL-COA METABOLISM IN CHOLINERGIC NEURONS AND THEIR SUSCEPTIBILITY TO NEUROTOXIC INPUTS.

Szutowicz A., Tomaszewicz M., Jankowska A., Madziar B., Bielarczyk H.

Department of Clinical Biochemistry, Medical University of Gdańsk, Gdańsk, Poland.

Cholinergic neurones, unlike other brain cells, utilize acetyl-CoA not only for energy production but also for acetylcholine (ACh) synthesis. Therefore, suppression of acetyl-CoA metabolism by different neurotoxic inputs may be particularly harmful for this group of cells. Retinoic acid and dbcAMP caused additive increase of choline acetyltransferase (ChAT) activity and ACh content along with morphological differentiation of SN56 septal hybrid cell. On the other hand they decreased pyruvate dehydrogenase (PDH) activity, pyruvate utilisation and acetyl-CoA level in these cells. Differentiated cells (DC) were more susceptible to acute and chronic influences of NO, Al and β-amyloid. Short-term exposure of DC to NO caused in following days decrease of PDH, ChAT activities and growth inhibition of DC but did not affect non-differentiated cells (NC). NO decreased acetyl-CoA level and increased ACh release in DC but did not affect NC. In DC cultured with Al, the decrease of acetyl-CoA content and activation of ACh release were found. Such changes did not occur in NC grown with Al. Additive effects of Al and NO were observed in DC but not in NC. Also β-amyloid exerted more evident suppressory effects on ACh and acetyl-CoA metabolism in DC than in NC. One may suppose that relative deficiency of acetyl-CoA in highly differentiated cholinergic neurones could make them particularly susceptible to neurotoxic insults involved in development of different cholinergic encephalopahies. Supported by KBN project 4 P05A 044 12.

1.5 **IDENTIFICATION OF A CHOLINERGIC-SPECIFIC PROMOTER FROM THE MOUSE VAChT/ChAT GENE LOCUS**

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Two of proteins required to express the cholinergic phenotype are: choline acetyltransferase (ChAT), the rate-limiting enzyme in the synthesis of acetylcholine (ACh), and the vesicular ACh transporter (VAChT), the protein responsible for translocating cytoplasmic ACh into synaptic vesicles. The entire coding region of the VAChT gene is located within the first intron of the ChAT gene. The nested arrangement of these two genes, and the regulatory elements required for their transcription, has been defined as the VAChT/ChAT gene locus. In order to identify a promoter region of the mouse cholinergic locus, we have evaluated DNA fragments from this region to direct expression of a reporter gene (LacZ) in transgenic mice. A 6417 bp DNA fragment contains a set of regulatory elements that restrict the expression of LacZ to cholinergic neurons of transgenic mice. This 6417 bp DNA fragment encompasses: 633 bp of the 5'-flanking region of the mouse VAChT gene, the entire open reading frame of the VAChT gene, sequences upstream of the initiation codon of the ChAT gene, and the first 46 bp of the coding region. This region of the mouse VAChT/ChAT locus might serve as a cholinergic specific promoter for the expression of both VAChT and ChAT genes *in vivo*. The identification of this cholinergic-specific promoter will be useful to evaluate the mechanisms of diseases characterized by dysfunction of particular groups of cholinergic neurons, and will be valuable in designing and exploring strategies directed to treat such neurological disorders.

1.6 **EFFECTS OF PARTIAL AND COMPLETE BASAL FOREBRAIN CHOLINERGIC IMMUNOLESION ON CHOLINERGIC PHENOTYPE AND NEOCORTICAL CHOLINERGIC NEUROTRANSMISSION**

Roßner, S., Schliebs, R. and Bigl. V.
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Studies on the contribution of cortical/hippocampal cholinergic mechanisms to learning and memory formation rely on specific lesioning tools for cholinergic basal forebrain neurons. The antibody 192IgG recognizes the p75NTR in rats and is internalized into basal forebrain cholinergic neurons after intracerebroventricular infusions. Utilizing these properties of 192IgG, a selective toxin to cholinergic basal forebrain neurons was developed by chemical coupling of 192IgG to the ribosome inactivating protein saporin (192IgG-saporin). Intracerebroventricular infusions of 192IgG-saporin induce a dose-dependent loss of cholinergic basal forebrain neurons, while sparing both, cholinergic neurons outside the basal forebrain and noncholinergic neurons within the basal forebrain. Our data indicate, that 192IgG-saporin results in cholinergic cell death, rather than supression of cholinergic markers, as observed in other cholinergic lesion paradigms. Characteristics of this cholinergic cell loss in the basal forebrain include reduction of presynaptic cholinergic markers such as high-affinity choline uptake, choline acetyltransferase activity and acetylcholine release in the cholinergically deafferented neocortex/hippocampus. After partial cholinergic immunolesion, treatment with nerve growth factor restores neocortical/hippocampal presynaptic markers and increases the diameter but not the number of the remaining cholinergic basal forebrain neurons. Taken together, 192IgG-saporin represents a powerful tool to produce specific cholinergic basal forebrain lesions and to test therapeutic strategies aimed at stimulating cholinergic function.

Session 2 - Satellite Symposium: Neurobiology of pain

2.1 **IS PAIN COMPLEX? NEW CHALLENGES IN MODELLING A DYNAMIC PROCESS THAT SPANS ORDERS OF MAGNITUDE IN SPACE AND TIME**

Daniel B. Carr; *New England Medical Center, Boston, USA*

Advances in understanding the analgesic cascade have caused a shift of the "stress" concept away from pituitary- and sympatho-adrenal axes and instead to the spinal cord. Spinal stress responses are intracellular in contrast to the extracellular (i.e., secretory) responses of classical stress axes. Dorsal horn stress responses to nociceptive input display a more tenacious memory than pituitary- adrenal responses, manifest as long-term changes in responsivity and even structural remodelling. Study of the latter responses holds promise for analgesic design and delivery so as to target specific steps in the spinal cord algesic cascade. Further, recognition that cells within the dorsal horn form a complex, coupled, nonlinear dynamic system suggests that the mathematical and physical formalism developed recently to describe and predict the behavior of such systems can be applied to better understand how analgesics work at the spinal level. In this perspective, what is important about spinal cord function is not just that it is capable of existing in several modes (basal, sensitized..) but rather that it is an organ evolved to exhibit programmed instability and prompt, stereotypical transitions between these modes. Like other biological systems, within each mode the dorsal horn exists in a state of self-organized criticality. Transitions between dynamic modes reflect replacement of the attractor for one mode by that for the next. Ultimately, all agents that inhibit hyperalgesia or persistent pain after acute injury must do so by impeding such transitions. A key challenge is to integrate these advances in dynamic modeling into formal models of dorsal horn function accessible to clinicians and preclinicians interested in nociception. (For example, such a model may explain the perception of periodic lancinating pain experienced after stable nerve injury, as the output of a chaotic oscillatory process whose substrate is a linked neural network in the dorsal horn). Yet as such integration is accomplished, a second challenge will emerge, namely, how to meet the need for distinct models of causality to apply to processes occurring at different spatial and temporal scales. This general need (recognition of which lies at the heart of 20th century physics) underlies the inadequacy of descriptions of phenomena at one scale, such as the single neuron exposed in situ to an opioid, to describe and predict phenomena at greater temporal or spatial scales, such as reflex withdrawal or aversive behavior. Clearly, meeting the first challenge is relevant to the second challenge. A third, related but distinct challenge lies in expanding our growing knowledge of nociception to a still-larger scale that involves aggregates of individuals. Here, the goal is to construct evidence-based algorithms and practice guidelines that accommodate "nocioeconomic" pressures. Again heeding 20th century physics, policymakers may have to adopt a "complementarity principle". In such, the merit of one approach to causal inference, such as meta-analysis of randomized controlled trials, becomes overshadowed by the merit of another approach, such as outcomes tracking during continuous quality improvement, as the number of individuals involved and the duration of observation increase.

2.2 **ROLE OF OPIOIDS IN NEUROPATHIC PAIN**

Barbara Przewłocka
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Opioid are potent analgesics in various kind of pain, however neuropathic pain which developed after nerve injury are poorly controled by currently used opioids. The recent discovery of endomorphins, a highly selective endogenous ligands of μ opioid receptors, opens up a new pathways to study the efficacy of opioids in the neuropathic pain.

We studied spinal analgesic and antiallodynic effects of intrathecal (i.t.) administration of endomorphin-1 and endomorphin-2 in comparison with DAMGO or morphine in Wistar rats chronically implanted with intrathecal cannulas. In the neuropathic pain model (sciatic nerve crushing), endomorphin-1 and -2 (2.5, 5, 10 μg i.t.) had a significant antinociceptive effect on the tail-flick latency, the cold-water tail flick latency and both peptides antagonized allodynia in a dose-dependent manner. In contrast, morphine (5μg, i.t.) was found to be ineffective in those tests. Interestingly, endomorphin-1 showed in our study, clear analgesic activity in morphine-tolerant rats, but not in rats tolerant to DAMGO. Thus pretreatment with morphine did not result in the expression of cross-tolerance to endomorphin-1 analgesia.

The study suggest, that different μ-opioid receptor subtypes may mediate effects of morphine and endomorphins in neuropathic pain, or that molecular characteristic of μ-opioid receptor is modified by nerve injury. Further, the obtained results indicate that endomorphins may be regarded as effective drugs in neuropathic pain therapy.

Supported by the KBN grant 4 P05A 093 15

2.3 PAIN CONTROL BY IMMUNE CELLS

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Klinik für Anaesthesiologie und operative Intensivmedizin, Universitätsklinikum Benjamin Franklin, Berlin, Germany.

Intrinsic pain control in peripheral inflamed tissue can be mediated by an interaction between immune cell-derived opioids and opioid receptors localized on peripheral sensory nerve endings. A prominent opioid peptide involved in peripheral pain control is beta-endorphin (END). END is present and synthesized in circulating immune cells. END-producing lymphocytes home to inflamed tissue where, under environmental stressful stimuli or in response to releasing agents (corticotropin releasing factor), they secrete the opioids to inhibit pain. Afterwards they travel to the regional lymph nodes, depleted of the peptide. This migratory pattern is reminiscent of memory type T-cells and END was indeed found mostly in this type of cells. Extravasation of immune cells to the inflamed tissue is a multistep process (rolling, adhesion, transmigration), involving the sequential activation of various adhesion molecules (selectins, integrins, immunolgobulins) located on immune cells and vascular endothelium. Interruption of the leukocyte-endothelial cell cascade (e.g. by antibodies against adhesion molecules) can block immune cell extravasation and influence endogenous pain control in inflammation. Anti-selectin treatment results in a blockade of the infiltration of immunocytes containing END, a consequent decrease of the END content in the inflamed tissue and abolishes endogenous opioid analgesia. These findings indicate that endogenous pain control involves pathways traditionally used by the immune system for mounting a host response to pathogens (i.e. cell migration). This has implications for the understanding and treatment of pain in immunosuppressed patients with cancer or AIDS.

STRUCTURAL DETERMINANTS OF THE δ- AND μ- OPIOID RECEPTOR – G PROTEIN INTERFACE 2.4

Georgoussi[1], Z., Megaritis[1], G. Zioudrou[1], C., Milligan[2], G. and Merkouris[3], M.
[1]Institute of Biology, N.C.S.R "Demokritos" 153 10 Ag. Paraskevi, Athens, Greece, [2]Mol. Pharmacol. Group, IBLS University of Glasgow, Scotland, UK, [3]University of Chicago, Dept. of Pediatrics, Chicago, USA

The opioid receptors are classically described as coupling to members of the Giα/Goα family. Activation of opioid receptors results in the inhibition of adenylate cyclase and/or the regulation of a variety of ion channels and other effector systems. A wealth of information has recently shed light in our laboratory about the structural determinants involved in opioid receptor-G protein coupling domains. This was achieved using a series of receptor derived peptides corresponding to amino acid sequences of the second, third intracellular loop and the cytoplasmic tail of the murine δ-opioid receptor. To test whether these peptides could mimic or inhibit receptor interactions with G proteins we measured initially their ability to modify ligand stimulated G protein activation and ^{3}H agonist binding to the receptor in membranes from Rat-1 fibroblasts and $Neuro_{2A}$ cells stably transfected to express the δ- and μ-opioid receptor respectively. Moreover, we examined the ability of these peptides to interfere in adenylate cyclase coupling. We provide evidence that indicates that the amino-terminal potrion of the third intracellular loop and part of the carboxyl-terminal tail serve as major contact sites with G proteins, whereas the entire third intracellular loop is responsible for adenylate cyclase inhibition. Collectively, our data provide novel information about the molecular and structural determinants governing opioid receptor/G protein/adenylate cyclase interface. These receptor contact sites, should serve as a useful starting point, for the development of specific activators or inhibitors of G protein signaling.

2.5 Influence of preemptive administration of pentoxifiline on nociception. Experimental and clinical studies

J. Wordliczek, B. Przewłocka, A. Szczepanik, J. Dobrogowski
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Recent evidence suggests that cytokines play an important role in the process of nociception following an injury or a surgery. It has been shown that, among others, activation of proinflammatory cytokines following an injury intensifies the process of nociception.

The aim of the investigation was to assess the influence of pentoxifiline (PTX, a non-specific cytokine inhibitor) administered through preemptive analgesia on the development of nociception.

Experimental investigations, involving formalin-induced pain, were conducted on rats. It was established that preemptive intraperitoneal PTX, significantly enhances the nociceptive threshold for mechanical stimuli. An inhibitory influence of preemptive PTX on pain-related behaviour was also observed. The above findings were correlated with a significantly lower TNFα level in the serum of animals receiving preemptive PTX.

In clinical investigations preemptive PTX was administered intravenously before elective cholecystectomy. Patients who received preemptive PTX showed significantly lower opioid requirements in the early postoperative period as compared with patients from the control group. At the same time, the level of TNFα and IL6 monitored in blood serum was significantly lower in the PTX group.

The results obtained in this study confirm the hypothesis as to the possibility of modulating the process of nociception through the administration of a cytokine inhibitor through preemptive analgesia.

PERIPHERAL OPIOID RECEPTORS - BASIC AND CLINICAL ASPECTS 2.6

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Klinik für Anaesthesiologie und operative Intensivmedizin, Universitätsklinikum Benjamin Franklin, Berlin, Germany.

Peripherally applied opioids have antinociceptive and antiinflammatory actions. These effects are mediated by opioid receptors on peripheral sensory nerve terminals in rats and in humans. Opioid receptors are synthesized in the dorsal root ganglia, they are axonally transported towards the nerve terminals and they can be activated by exogenous agonists as well as by endogenous opioid peptides expressed in inflammatory cells. In humans, locally applied exogenous agonists (e.g. morphine) as well as immune cell-derived opioids can produce clinically measurable tonic pain inhibition. This presentation will review mechanisms of antinociceptive and antiinflammatory effects of peripherally applied opioids and will discuss clinical implications.

2.7 Intraarticular administration of fentanyl in chronic pain patients

J. Dobrogowski, J.Wordliczek
Department of Pain Research and Treatment, Collegium Medicum, Jagiellonian University, Kraków, Poland

The aim of this study was to compare analgesic effect of lidocaine, fentanyl or mixture of these substances given intraarticulary in chronic pain patients suffering from osteoarthritis of the hip or knee.

Methods: We studied 38 patients with confirmed diagnosis of osteoartritis of the hip (22 patients) or knee (16 patients) suffering from chronic pain resistant to conservative treatment. The study was performed using double-blind, randomised, cross-over , three period design and patients received intra-articulary injection of either 1% lidocaine (10 ml) – Group A, fentanyl (25 mcg in 10 ml of normal saline) – Group B or mixture of 1% lidocaine (10 ml) + fentanyl (25 mcg) – Group C. Seven and fourteen days later, patients had second and third randomly chosen injection.

Results: Pain relief was obtained in 12 patients with arthitis of the knee (%) and 14 patients with arthrosis of the hip (%). In the remaining (%) patients no analgesic effect was observed independently of the administered drugs. Mean duration of pain relief in a Group A was 2 hours (1 –24h), in Group B =11 hours (1 – 36 h) and in Group C = 48 hours (1 – 136 h). No side effects were reported.

Conclusion: Intraarticular administration of mixture of lidocaine and fentanyl had significantly longer duration of analgesic effect in comparison to analgetic action of these drugs given alone. This is probably connected with the influence of conformational changes in peripheral opioids action in arthritic joint caused by lidocaine.

2.8 NOVEL PERIPHERAL PEPTIDIC KAPPA AGONISTS.

Pierre J-M. Rivière, Todd W. Vanderah, Frank Porreca*, Richard Houghten#, Claudio Schteingart, Jerzy Trojnar, Jean-Louis Junien. Ferring Research Institute, San Diego, USA, *Dept. of Pharmacology, University of Arizona, Tucson, USA, #Torrey Pines Institute for Molecular Studies, San Diego, USA.

Novel and selective peptidic κ-ligands were recently identified by using positional scanning format mixtures of a tetrapeptide combinatorial library screened in opioid receptor radioligand binding assays (rat brain and guinea pig cerebellum membrane preparations). The best compound identified by positional scanning was an all D-amino-acid tetrapeptide, ff(D-Nle)r-NH2 (FE 200041), totally unrelated to dynorphins. New analogs of FE 200041 were prepared and tested. The activity of three tetrapeptide analogs, FE 200041, FE 200665 and FE 200666 is reported. This chemical series showed high affinity and higher selectivity than reference compounds for κ- versus μ- or δ-receptor subtypes, including when measured at cloned human opioid receptors.

	Affinity (Ki, nM) for cloned human opioid receptors		
	hKOR	hMOR	hDOR
FE 200041	0.15	4,570	-
FE 200665	0.24	4,050	-
FE 200666	0.08	7,090	>100,000
Enadoline	1.25	272	-
Asimadoline	0.17	581	322

All three tetrapeptides were potent κ-agonists both in vitro and in vivo. FE 200666 stimulated [^{35}S]GTPγS binding in a hKOR transfected cell line with an EC_{50} of 0.08 nM versus 0.50 nM for enadoline. FE 200041, FE 200665 and FE 200666 inhibited in a dose-related manner acetic acid-induced writhing in mice (A_{50}: 0.030, 0.007 and 0.012 mg/kg, i.v., respectively), being almost as potent as enadoline (A_{50}: 0.002 mg/kg, i.v.) and more potent than asimadoline (A_{50}: 0.313 mg/kg, i.v.) or morphine (A_{50}: 0.162 mg/kg, i.v.). All three peptides had a markedly decreased ability to penetrate the brain as suggested by their limited ability to induce sedation in the mouse rotarod assay. The ratios of A_{50}s between mouse rotarod and writhing assays after i.v. administration were 2 for enadoline, 4 for asimadoline, 88 for FE 200041, 647 for FE 200665 and 92 for FE 200666. Altogether, this new tetrapeptide κ- agonist series shows unprecedented affinity and selectivity for κ-opioid receptors, analgesic potency and peripheral selectivity. The anticipated overall therapeutic window of this chemical series is about 20- to 300-fold wider than that of κ-agonists currently under clinical development or previously discontinued for lack of safety.

2.9 NEUROMODULATORY PEPTIDES DERIVED FROM FOOD PROTEINS

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Many kinds of opioid peptides have been isolated from enzymatic digests of food or natural proteins. From the tryptic digests of various proteins, we isolated a couple of peptides which showed anti-opioid and contracting activities for guinea-pig ileum. These peptides have a common sequence, Hydrophobic residue-X_1-Leu-X_2-Arg, at their carboxyl termini and proved to be agonists for complement C3a receptor. Casoxin C (Tyr-Ile-Pro-Ile-Gln-Tyr-Val-Leu-Ser-Arg), which was derived from bovine κ-casein antagonized analgesic effect of morphine and U-50488H, the μ and κ agonists, respectively, but DTLET, the δ agonist after icv administration in mice. Casoxin C improved amnesia induced by scopolamine administration or ischemia. Complement C3a exhibited essentially the same effect. This meants that C3a, which has been regarded as an immunopeptide is also a neuropeptide. Based on the structure of C3a agonist peptide drived from rice albumin, we designed an orally effective peptide which showed antianalgesic and antiamnesic effect.

Previously, based on the structure of opioid antagonist peptides derived from food proteins, we found methylester of retro-opioid peptides showed opioid antagonist activity. We synthesized methylester of retro-nociceptin. The peptide showed affinity for nociceptin receptor (IC_{50} = 10^{-5}M) and analgesic activity after icv administration in mice. Furthermore, the nociceptin antagonist peptide improved learning performance in passive avoidance test using step-throuth apparatus.

Session 3 - Technical Workshop: Confocal microscopy and stereology in neurobiology

3.1 BIO-RAD - APPLICATION OF MULTI-PHOTON FLUORESCENCE MICROSCOPY IN NEUROSCIENCE. AN OVERVIEW

Fine, E.
National Institute for Medical Research, Mill Hill, UK

Not received

3.2 BIO-RAD - MICRO-RADIANCE 2000 – NEW SYSTEM IN CONFOCAL TECHNIQUE.

Davidovici, D.
Bio-Rad Laboratories GmbH, Munich, Germany

Not received.

3.3 OLYMPUS POLSKA - CONFOCAL MICROSCOPY AND FAST FLUORESCENCE IMAGING IN NEUROBIOLOGY

Olympus invites you to attend the presentation of our latest microscopic technology for fluorescence and transmitted light imaging:

(1) The **confocal laser scanning microscope FluoView** dedicated for 3D optical sectioning and time course image acquisition. The presentation will include the upgrade possibility of the FluoView for Multi-Photon Microscopy.
(2) The **imaging system developed by T.I.L.L. Photonics GmbH** (Germany) for high speed and precisely timed image acquisition. Key components of the system are the Polychrome Monochromator light source, the Imago CCD cameras, and the TILLvisION acquisition and analysis software.
In the workshop we explain the different technologies of both system solutions. 3D image stacks and time course image series will illustrate the wide range of morphological and physiological applications, e.g. multi-fluorescence labeling, Green Fluorescence Protein (GFP) detection, ion imaging incl. ratio analysis.
During the Congress the FluoView and the T.I.L.L. imaging system will be available for detailed demonstrations. You are invited to bring your own samples. We can use standard slides with samples covered by coverslip as well as samples in petri dishes with cover slip bottom or equivalent sample holders.
For special requests please contact Mr. Jacek Reinhold at Olympus Polska.

Session 4 - Opening Lecture

4.1 IDENTIFICATION OF THE BRAIN CELLS AND PROTEINS RESPONSIBLE FOR ALZHEIMER'S DISEASE (AD)

H.M. Wisniewski, Institute for Basic Research in Developmental Disabilities, 1050 Forest Hill Road, Staten Island, NY 10314

During the last 20 years, the proteins responsible for the formation of characteristic AD lesions, Aß plaques and neurofibrillary tangles, have been identified. They are known as amyloid-ß (Aß), which forms senile or Aß plaques and affects vessels with amyloid angiopathy, and tau protein, which is responsible for formation of neurofibrillary tangles. In persons with extensive accumulation of Aß and abnormally phosphorylated tau protein, AD develops. Nerve cells and microglia are the source of Aß protein forming the diffuse and neuritic plaques. Myocytes and perivascular cells are the cells producing Aß responsible for amyloid angiopathy. At the pre-fibrillized stage accumulation of the Aß and tau, there is no evidence of abnormal function of the neurons or structural pathology of the neuropil. Plaques and tangles develop when fibrillization of Aß and tau takes place. Conditions controlling the processes of fibrillization of the AD amyloidogenic proteins both *in vivo* and *in vitro* will be presented.

Session 5 - Parallel Symposium: Glia-derived neuroprotective and neurodegenerative signals in brain pathology

5.1 NEURONAL-GLIAL INTERACTIONS AFTER GLOBAL CEREBRAL ISCHEMIA AND AFTER EXPOSURE TO NEUROTOXIN

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In the human brain neurons degenerate after acute insults (e.g., stroke, cardiac arrest, exposure to neurotoxins, trauma). The effective strategies to prevent or limit neuronal damage remain elusive predominantly because of an incomplete understanding of the mechanisms of neuronal death. Therefore, the animal models of neurodegeneration are crucial to gain insight into these mechanisms. The cascade of events that follows brain insults is probably a dynamic interplay among various cells, including neurons and glial cells. Particular interest revolves recently around the role of astrocytes. This role has been viewed both as detrimental and beneficial in the processes of neurodegeneration.

This presentation will highlight and discuss our recent data obtained on two animal models. The first is an experimental model of complete cerebral ischemia and clinical death in rats, induced by 10 min cardiac arrest followed by resuscitation. The second is a model of brain intoxication and to induce it we exposed rats to a known environmental neurotoxin, trimethyltin (TMT). Both insults produce a distinct pattern of neuronal degeneration, especially in the hippocampus, accompanied by an intense gliosis. The focus of this presentation will be on the role of cytokines, neurotrophic factors and selected transcription factors produced by activated astroglia cells, in the processes of neurodegeneration. We conclude that the balance between their pro- and anti-apoptotic effects might influence the fate of neurons. This points to glial cells as a potential target for therapeutic manipulations.

5.2 ORIGIN OF OLIGODENDROCYTES IN AMNIOTES

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We focus our interest on the origin of oligodendrocytes (OL), the myelin forming cell in the vertebrates. The *plp* gene encodes the proteolipid protein and its alternatively spliced product, DM-20, major proteins of CNS myelin. In the mouse, *plp/dm-20* transcripts are expressed beginning at embryonic day 9.5 by restricted foci of germinative neuroepithelial cells. To address the question of the identity of the neural precursors expressing *plp/dm- 20*, a zeomycin resistance gene fused to the *lacZ* reporter was expressed in transgenic mice under the control of the *plp* regulatory sequences. In three different lines generated, the pattern of β-galactosidase expression was similar, and superimposable on the expression pattern of endogenous *plp/dm-20*. Following zeomycin selection, a dramatic enrichment in pre-OL was observed in cultures derived from E12.5 transgenic embryos. This enrichment indicates the oligodendroglial specification of neural precursors which continuously express *plp/dm-20*. Early *plp/dm-20* expressing precursors appear, however, to be a separate population from previously described *PDGFR*α OL precursors as shown by the striking differences in their i) patterns of distribution and ii) responsiveness to PDGF. These data suggest that OL have a plural origin and that early *plp/dm-20* defines one of the neural lineages generating OL.

In the chick embryonic brain, we have examined the spatio-temporal emergence of OL. During embryonic development, *plp/dm-20* and *PDGFR*α transcripts, and the O4-reactive antigen showed a similar segmental pattern of expression. However, *plp/dm-20*$^+$ cells were already observed, in the ventricular layer, at E2.5, i.e., 2 days before the appearance of O4$^+$ and *PDGFR*α^+ cells, suggesting that OL precursors arise nearly simultaneously with neurons. We therefore propose that, in the chick embryonic brain, the ventricular *plp/dm-20* expressing cells define the restricted territories of the germinative neuroepithelium where OL precursors originate. In addition, from E5, within the subventricular and mantle layers facing the *plp/dm-20*$^+$ ventricular foci, a dichotomy in the oligodendroglial population was observed between O4$^+$/*plp/dm-20*$^+$ and O4$^+$/*PDGFR*α^+ cells, suggesting a precocious segregation of OL.

5.3 **OXIDATIVE OLIGODENDROCYTE DAMAGE**

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Oxidative stress of oligodendrocytes emerges as a critical factor in acute leukoencephalopathies following ischemic, traumatic and inflammatory insult to the CNS. In this study, oxidative stress was induced in cultured oligodendrocytes by a brief exposure to reactive oxygen species (ROS) that are generated in large amounts during inflammatory and/or ischemic episodes, i.e., hydrogen peroxide, peroxynitrite and hypochlorite. Following the exposure, the cells were incubated in normal growth medium, and analyzed at different time points. Although no changes were observed during the exposure period, there was a progressive morphological degeneration and depletion of adherent cells during the postexposure period up to 72 h. The expression of genes encoding myelin-specific proteins was rapidly downregulated. For example, the steady-state levels of the messages dropped by 70% within 2 h following 15 min exposure to 4 mM hydrogen peroxide. The downregulation of myelin genes persisted even in the surviving cells for up to 72 h. Cell death was preceded by structural alterations in the nuclear envelope, and an orderly fragmentation of nuclear DNA resulting in the liberation of chromatin loops - a hallmark of programmed cell death. The results demonstrate that pathologically relevant ROS may cause delayed oligodendrocyte death and ensuing myelin loss. In addition, the ability of surviving oligodendrocytes to support myelin sheaths may be profoundly impaired by ROS leading to further demyelination.

5.4 H_2O_2-INDUCED DEATH IN OLIGODENDROGLIA-LIKE, OLN-93 CELLS, WITH ALTERED PHOSPHOLIPID COMPOSITION

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Over production of free radicals (FR) of oxygen have been implicated in the sequel of oxidative stress in mammalian cells. The molecular details of the genes and their products generated as a result of FRs and their involvement in apoptotic/necrotic cell death are gradually emerging. Lesser information is available on the role of the plasma membrane lipid components in transducing signals related to cell death. In this study we investigated the consequences of oxidative stress on cell death in actively proliferating or serum-deprived cultures of spontaneously transformed oligodendroglia cells before or after membrane polyunsaturated fatty acids (PUFA) and polar head groups (PHG) modification. OLN-93 were grown in the presence of various PUFA including ethyl-docosahexaenoic acid (Et-DHA) along with N-Me-ethanolamine (Me-Ea) or related N-bases. After 72 h incubation, marked modifications in PHG and fatty acid composition were detected by TLC and GC techniques. Lipid peroxides levels after treatment with 0.1 mM H_2O_2 (HP) in the presence of 50 μM divalent Fe was measured by the appearance of thiobarbituric acid reactive substances (TBARS). After 30 min incubation with HP, most of the TBARS were released into the culture medium and only about 10% were detected in the cell lipid extract. Modification with Et-DHA caused a two-fold increase of TBARS production compared to control cultures, in parallel to an increase in lipid-esterified DHA. Incubation with Me-Ea caused more than a 50% reduction in the production of TBARS. Me-Ea-modified cells expressed a substantial elevation of activated MAP kinase indicating that the observed effect may be due to stimulation of a signaling transduction cascade. Cell death monitored by several techniques including FACS, indicated an increase in cell death after Et-DHA cell feeding. This effect was reduced by administration of N-bases. These results raise the possibility that the nature of the polar head group and the degree of unsaturation may determine the ultimate resistance of oligodendroglia cells to oxidative stress and cell death. *Supported by a grant from the Gulton Foundation, NY.*

5.5 **REGULATION OF MITOGEN ACTIVATED PROTEIN KINASES (MAPKs) - MEDIATED PATHWAYS IN RELATION TO PROTEIN KINASE C (PKC) AND POSTISCHEMIC APOPTOSIS OF CA1 NEURONS.**

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Different neurotrophic factors and cytokines apparently linked to multiple effectors initiate signals that converge on common elements, particularly on pathways leading to activation of extracellular signal/stress activated protein kinases (ERK/SAPK) of MAPKs family and on transcriptional factors like NFκB and AP1. Transient brain ischemia in gerbils rapidly activates calcium-dependent signaling with series of kinases (between them PKC) and proteases (calpains) on the one hand and the above mentioned tyrosine receptors-associated effector proteins (MAPKs) and transcriptional factors on the other. The mechanistic correlation indicates that PKC may play an important role in regulation of ERK/SAPK cascades after ischemia. It has been shown that at 3 –4 days after the insult a strictly defined group of neurons in CA1 hippocampus undergoes apoptosis. Concomitantly, a profound down-regulation of PKC, an increase of calpain-mediated proteolysis and the changes in phosphorylation/activity of the MAPKs family members have been observed. This latter consists inhibition of ERK and activation of SAPK pathway. In effect, a relative concentration of phosphorylated c-Jun in transcriptional factor AP1 dimmer increased as compared with control or early reperfussion time. Similarly, in the experiments *in vitro*, utilizing N2a cell culture, the PKC down-regulation facilitated adverse signaling leading to apoptosis, while its activation sensitized neurons to trophic support provided by serum. Also MAPKs have changed in the manner similar to that described for postischemic apoptosis *in vivo*. Supported by SCSR 6 P04A 01014.

Session 6 - Parallel Symposium: Metabotropic glutamate receptors: neurotoxicity, neuroprotection and therapeutic perspectives - part 1

6.1 DESIGN, SYNTHESIS, AND PHARMACOLOGY OF NOVEL mGluR LIGANDS

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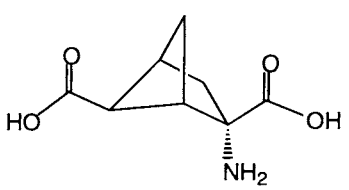
The amino acid glutamate (Glu) plays a pivotal role in biological processes ranging from memory and learning to neuronal degeneration. This major excitatory amino acid (EAA) acts through disparate Glu receptors, which can be categorized into the ionotropic and metabotropic glutamate receptors. The metabotropic Glu receptors (mGluRs) are coupled to cellular effectors through GTP-binding proteins. To date, eight mGluR subtypes have been identified. To better understand the roles of the individual mGluRs in physiological and pathophysiological processes, there is an important need to learn more about the structural features relevant to the design of novel, high affinity ligands that are family and subtype specific. This lecture will highlight recent progress in the search for novel mGluR ligands, such as ABHxD-I.



ABHxD-I

6.2 Modulation of Glutamate Receptors in Search of Protection from Excitotoxic-induced Neuron Death

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Glutamate, the main excitatory neurotransmitter in brain, can be toxic to neurons. In particular, tissue damages occurring during a variety of acute and chronic neurological diseases are thought to result from intense stimulation of ionotropic and metabotropic receptor pathways leading to elevated cytoplasmic calcium concentrations. Many cytoplasmic calcium-dependent enzymes such as (NO)synthase and the protease calpain have been proposed to mediate excitotoxicity. Alternatively, reactive oxygen species may be the ultimate determinant of cell death. Metaboptropic glutamate receptors, in particular, appear to interfere with these processes in two different ways. Group I mGluRs agonists and antagonists amplify and attenuate, respectively, neuronal death induced by excessive activation of iGluRs or oxygen glucose deprivation. Agonists at Group II/III exhibit neuroprotective properties. We have been engaged in the development of new agents able to disrupt the chain of events linked to neurodegeneration without impairing excitatory neurotransmission. The results obtained have been useful to improve the understanding of the process implicated and have also opened the way to possible therapeutic applications. An outline of these researches, together with recent results aimed at the discovery of new subtype selective modulators of mGluRs, as well as new free radical scavengers will be presented.

6.3 NEUROPROTECTIVE EFFECTS OF mGlu RECEPTOR AGONISTS.

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At Eli Lilly & Co. we have recently discoverd novel molecules that are selective agonists of group II mGlu receptors (LY354740, LY379268 and LY389795) and this has allowed us to examine the role of mGluR group II agonists on NMDA-induced neurotoxicity *in vitro* and in global cerebral ischaemia *in vivo*.

LY354740, LY379268 and LY389795 produced concentration-related neuroprotective effects in day 12 rat cortical neuronal cultures treated with NMDA under both rapid and slowly triggered cell death paradigms and this protection was significantly enhanced in the presence of glia. The agonists also reduced NMDA-induced nucleosome formation at low nanomolar concentrations.

Further evaluation of the neuroprotective effects of *in vivo* showed that in a gerbil model of transient 3 min global cerebral ischaemia LY354740 prevented both CA1 hippocampal cell loss and apoptosis measured by TdT fragment end labelling of DNA. In contrast, treatment with LY389795 and LY379268, more potent agonists of mGluR2 and mGluR3 provided robust neuroprotection against a 5 min occlusion. For example, LY379268 (10mg/kg i.p.) provided significant protection when administered at 30 min (88%), 60 min (39%) and 120 min (30%) post-occlusion. LY379268 also blocked the ischaemia-induced apoptosis in the CA1 hippocampal region.

In conclusion these results indicate that the mGluR2/3 agonists provide neuroprotection against NMDA-induced neurotoxicity *in vitro* and ischaemic brain damage in a model of global cerebral ischaemia *in vivo* and therefore, may be therapeutic agents for the treatment of neurodegenerative diseases.

6.4 NAAG AS AN AGONIST OF METABOTROPIC GLUTAMATE RECEPTOR - mGluR3

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N-acetylaspartylglutamate (NAAG) is a prevalent neuropeptide in the mammalian central nervous system. NAAG meets the criteria for a neurotransmitter. Recently, we have shown that in cultured cerebellar granule cells and astrocytes NAAG activates group II metabotropic glutamate receptors. Using mammalian cell lines expressing individual mGluRs (1-6 and 8), we demonstrated that NAAG selectively activates mGluR3 receptor. Presently, NAAG is the only endogenous agonist exhibiting selectivity against individual mGluR. Using cultured cortical cells, we were able to show that NAAG protects neuronal cells from NMDA-induced neurotoxicity through the activation of mGluR3. We have cloned and expressed NAAG peptidase gene from a rat hippocampal library. This carboxypeptidase type 2 hydrolyzes NAAG to glutamate and N-acetylaspartate. The peptidase is blocked by the structural analog of the peptide β-NAAG. We discovered that β-NAAG not only blocks the peptidase activity but also is a selective antagonist of mGluR3 receptor in cultured cerebellar granule cells and in cell lines expressing individual mGluRs. In rat hippocampal slices, we found that NAAG completely suppresses LTP in the dentate gyrus, while β-NAAG prevents this blockade. Furthermore, β-NAAG (100 μM) applied alone causes an increase in the baseline excitation of hippocampal neurons and causes the spontaneous formation of LTP.

Session 7 - Parallel Symposium: Transport phenomena in the brain: cross-talk between blood brain barrier-astrocytes-neurones

7.1 **Functional distribution of neurotransmitter transporters in mammalian brain.** Nathan Nelson, Frantisek Jursky, Ayelet Sacher and Hannah Nelson Department of Biochemistry, Tel Aviv University, 69978 Tel Aviv, Israel

Neurotransmitter transporters are involved in termination of the synaptic neurotransmission and are implicated as the sites of action of antidepressant medicines and illicit drugs. In addition to their function in neurotransmission, neurotransmitter transporters play a key role in neuroregulation and brain development. γ-Aminobutyric acid (GABA) is a major inhibitory neurotransmitter in the mammalian brain and is widely distributed throughout the nervous system. Molecular cloning studies have resulted in the isolation and characterization of cDNAs encoding four different GABA transporters, GAT1, GAT2, GAT3 and GAT4. While GAT1 exclusively transports GABA the other transporters are capable in addition to transport β-alanine and/or taurine. Consequently, β-alanine and taurine inhibit GABA transport by GAT2, GAT3 and GAT4, but have no effect on GABA transport by GAT1. Our results suggests a close transient relationship of selective GAT4 expressing radial glial elements with GAT1 expressing axons and GAT1 positive areas during the late brain embryogenesis. Because the beginning of expression for both GAT1 and GAT4 correlates with the expression of the adult GABA receptor, transporters seems to be connected with the maturation of GABAergic inhibitory system in the brain. Using immunocytochemical localization, the distribution of the glycine transporters GLYT1 and GLYT2 in the developing mouse brain, was studied. The appearance of GLYT1 and GLYT2 immunoreactivity begins during the period of fiber outgrow and synaptogenesis. The distribution of these two glycine transporters implies that GLYT1 and GLYT2 operate in concert within the area where both are present. The functional distribution of neurotransmitter transporters will be discussed in terms of cooperation between neuronal and glial cells for an effective termination of neurotransmission as well as damage control from neurotransmitters toxicity.

7.2 **Molecular characterization of monocarboxylate transporters mediating transfer of lactate between astrocytes and neurons**

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To support the neuronal signal transmission, astrocytes supply neurons with energy metabolites and neurotransmitter precursors and remove neurotransmitters and waste substances. Two different models of brain energy metabolism are currently discussed. The classical view suggests that both astrocytes and neurons derive their energy exclusively from glucose. A recently developed model, in contrast, suggests that glucose is metabolized in astrocytes mostly to lactate, which is then transferred to neurons as an energy metabolite. Neurons may derive as much as 50% of their energy demand from lactate under physiological conditions. In agreement with the modified model we detected different isoforms of monocarboxylate transporters in astroyctes and neurons. To facilitate lactate release, astrocytes express the low-affinity monocarboxylate transporter MCT1. The released lactate is then taken up by neurons via the high-affinity transporter MCT2. The regulation of monocarboxylate transporter expression in cultured cells supports the notion that MCT1 is preferentially used for efflux, whereas uptake is mediated by MCT2. Both transporters are H^+/monocarboxylate cotransporters linking monocarboxylate transport to the pH-regulation in the brain. The expression pattern of monocarboxylate transporters supports the model of a transfer of lactate between astrocytes and neurons.

7.3 Protective effect of glial cells against LPS-mediated blood-brain barrier injury.

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Numerous infections of the central nervous system are characterized by altered blood-brain barrier functions leading to brain damage. In order to better understand the mechanisms leading to BBB disruption in such pathologies, we used a an *in vitro* BBB model consisting of a coculture of brain capillary endothelial cells and glial cells. Here, we report that an activation of differentiated brain capillary endothelial cells with lipopolysaccharides (LPS) added in the luminal compartment for 15 hours, results in an increase in the permeability of the monolayers. As it has been shown that the microenvironment, i.e. the glial cells surrounding the brain capillaries, appears to be of prime importance in specifying at least certain cellular properties, we investigate whether glial cells are able to modulate this endothelial cells response to LPS. When endothelial cells were incubated with LPS added luminally for 15 hours, but in the presence of glial cells in the abluminal compartment, surprisingly, in this case, LPS did not exhibit any effect on the endothelial cell monolayer permeability, suggesting a protective effect of the glial cells on the LPS-mediated injury. Further experiments performed with purified astrocytes revealed that microglial cells and/or oligodendrocytes are essential for the complete protection of the endothelial cell monolayers integrity.
All these results provide for the time, direct evidence for a modulatory effect of glial cells on brain capillary endothelial cell response to inflammatory mediators such as LPS.

7.4 **CARNITINE TRANSPORT AND ITS UNIQUE FUNCTIONS IN BRAIN**

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Carnitine (4-trimethylamino-3-hydroxybutyric acid), a compound known to facilitate transport of fatty acyl moieties to mitochondria in the peripheral tissues, thus facilitating their β-oxidation, accumulates also in brain. In neurons carnitine and its acyl derivatives seem to fulfill a different role: Formation of acetylcarnitine promotes synthesis of acetylcholine, whilst its long-chain acyl derivatives, especially palmitoylcarnitine were observed to regulate differentiation and the activity of protein kinase C in neuroblastoma NB-2a cells. The mechanism of carnitine transport through the blood-brain barrier, was studied *in vitro* with cultured brain capillary endothelial cells (the primary culture of porcine cells and the transfected, immortalized rat cell line, RBE4). Uptake of carnitine through the apical membrane was inhibited by butyrobetaine. In the absence of carnitine the endothelial cells are capable to release about 50% of carnitine, the process affected by SH-group reagents. Carnitine transport in rat cerebral cortical neurons was found to be sodium dependent. The initial velocity of this process was decreased by GABA. Out of various GABA transporters inhibitors tested, only NO-711 and nipecotic acid decreased carnitine accumulation, while betaine, taurine and β-alanine had no effect. Due to the fact that carnitine did not reveal any effect on the accumulation of GABA, an involvement of GAT1 has been excluded. In conclusion, different mechanisms of carnitine transport in endothelial cells and neurons have been postulated.

Session 8 - Parallel Symposium: Calcium binding proteins in CNS

8.1 PHENOTYPE OF PARVALBUMIN NULLMUTANT MICE

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The calcium-binding protein parvalbumin (PV) is expressed in specific neurons which are often inhibitory and GABAergic and it has been suggested that PV plays a role in the control of their efficiency. We have generated mice lacking PV by homologous recombination. No obvious morphological alterations were observed in the PV-deficient mice and the frequency of homozygous animals was approximately 25%. The contribution of PV to the inhibitory functions of GABA cells was tested in two different paradigms. On one hand, we investigated the extent of intersite frequency coupling in the cortex of PV-KO mice. Local field potentials were recorded from temporal cortical regions using 4 independently targeted microelectrodes. We used bispectral analysis to measure the level of non-linear coupling (quadratic phase coupling, QPC) of spectral frequency components. Control animals showed mainly high frequency (>65 Hz) components indicating prevalent local processing and short range functional interactions. Homozygous animals were characterized by an abnormally high proportion of low frequency QPC, thus suggesting interactions at longer range than in wild type animals. In the second model we tested the seizure susceptibility of PV-KO mice after pentylenetetrazol-induced seizures. PV-KO animals had a shorter latency of onset of seizures and they reached status epilepticus (SE) significantly ($p < 0.05$) faster than wild type controls. Our results indicate that the lack of PV compromises some of the inhibitory functions, thus increasing the synchronization in the cerebral cortex and the susceptibility to seizures.

8.2 FLUORESCENCE ANALYSIS OF RETINAL *NCS* PROTEINS.

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Guanylyl cyclase activating proteins (GCAP1 and GCAP2) and recoverin are homologous Ca^{2+}-binding proteins of *Neuronal Calcium Sensors* family, recently found in vertebrate retina. They share a number of structural features: N-terminal myristoylation, presence of four EF-hand motifs of which 2-3 are functionally active, and have molecular weights about 23.5 kDa. GCAPs have been shown to regulate activity of retinal guanylyl cyclase in a calcium-dependent manner. Recoverin has been posulated to be a calcium-dependent regulator of rhodopsin kinase. All three proteins posses two conservative tryptophan residues (31 and 104 in recoverin, 21 and 94 in GCAP1, 27 and 99 in GCAP2) that are located close to the "silent" EF1 and "active" EF3, respectively. Other tryptophan residues are differently located in each protein. We recorded fluorescence spectra of each indicated protein at different free calcium concentrations. The fluorescence emission spectra of both GCAPs show characteristic decrease of intensities that correspond with increase in $[Ca^{2+}]_{free}$, however, we didn't observe shift in λ_{max} of emission. Emission spectra of recoverin show decrease of intensities but also red shift of λ_{max} when free calcium concentration increases. Obtained results suggest that recoverin and GCAPs, although structuraly related, change their conformation under calcium binding differently.

8.3 VISININ-LIKE PROTEINS (VILIPs): INTRACELLULAR NEURONAL CALCIUM SENSOR PROTEINS INVOLVED IN THE REGULATION OF CYCLASE ENZYMES

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Intracellular neuronal Ca^{2+} sensor (NCS) proteins constitute a rapidly growing family of EF-hand Ca^{2+}-binding proteins. They are involved in the regulation of Ca^{2+}-dependent intracellular signaling cascades, including signal transduction in photoreceptor cells or olfactory receptor neurons (Braunewell & Gundelfinger, 1999, Cell Tiss. Res 295, 1-12). VILIPs are a sub-family of NCS proteins differentially expressed in distinct subsets of CNS neurons. VILIP-1 is widely expressed in the vertebrate brain. In contrast VILIP-3 displays a more restricted distribution with most abundant expression in the cerebellum. Within the cerebellum, VILIP-1 is expressed in a subset of granule cells whereas VILIP-3 is strongly expressed in Purkinje cells. VILIP-1 is completely absent from these latter cells. Both proteins interact Ca^{2+}-dependently with the cell membrane. This membrane insertion appears to be mediated by a Ca^{2+}-dependent myristoyl switch. Transfection studies with C6 glioma cells have shown that VILIP-1 regulates basic and forskolin-stimulated cAMP production when ectopically expressed in these cells – probably by direct or indirect interaction with adenylyl cyclase. The effect depends on myristoylation of the molecule and acylation-deficient VILIP-1 mutants act in a dominant negative way. In other neural cell types VILIP-1 appears to regulate guanylyl cyclase activity. This suggests that VILIPs may be involved in the cross-talk of Ca^{2+}-dependent and cyclic nucleotide-mediated intraneuronal signaling pathways.

8.4 WHAT IS THE FUNCTION OF CALRETININ AND CALCYCLIN IN THE BRAIN?

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Of many calcium binding proteins (CaBPs) in the brain, only a few have known functions. The majority of CaBPs are described as proteins that are able to bind calcium ions, but it remains to be established if this is all they are able to do or if they are also involved in Ca-sensitive pathways and interact with other proteins. We work on two EF-hand CaBPs: calcyclin (CY) and calretinin (CR), which are expressed in a variety of cells in the brain. CR is a neuronal protein, while CY is present in neurons and some glial cells. I will present the data of several approaches indicating that both proteins seem to be Ca-sensors rather than Ca-buffers. I will show the properties of the calcyclin-binding protein (CacyBP) which was identified, purified and characterized in our laboratory. This novel and unique protein represents a possible physiological target of CY. The CacyBP amino acid sequence, deduced from a cDNA clone we recently isolated, reveals no known functional or structural domains. The antibodies against this protein used on brain sections, and the method of hybridization in situ, suggest a neuron specific expression of this protein. I will also show that the expression of CR (as a fusion protein with Green Fluorescent Protein) does not change the Ca-buffering capacity of C6 glioma cells. These data, together with our biochemical data, indicate a search for CR' physiological target is justified. Both CR and CY have cell-specific distributions in the brain and this further supports the hypothesis regarding their specific Ca-dependent function in the brain. I will present our data on the mechanisms involved in the regulation of their gene promoters.

8.5 Insights into the physiology of neuronal calbindin D28k from calbindin nullmutant and transgenic mice

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To study the physiological role of neuronal calbindin D28k we have generated mice nullmutant for this gene. Previous analysis has revealed abnormalities in two brain regions. Whereas hippocampal pyramidal and granule cells display an unexpected increased resistence to anoxia, cerebellar changes are subtle and, as far as known, restricted to changes in synaptically evoked calcium transients in dendrites of Purkinje cells which are known to contain high amounts of calbindin. Associated is a deficit in motor coordination which is revealed only when the animal has to adopt and learn novel patterns of limb coordination.We are currently trying to learn more about the neuronal populations involved. Following a complementation/rescue strategy, transgenic mouse lines expressing calbindin under heterologous promoters (e.g. neuron specific enolase) are crossed onto the calbindin null background. Data on the expression of calbindin and behavioral performance of these mice will be presented. Data obtained so far demonstrate improved motor coordination in some but not all of the compound lines. As an alternative strategy we are creating a conditional knock-out of the calbindin gene using Cre/loxP methodology. This should allow us to eliminate calbindin expression in selected neuronal populations, such as Purkinje cells.

Session 9 - Parallel Symposium: Metabotropic glutamate receptors: neurotoxicity, neuroprotection and therapeutic perspectives - part 2

9.1 FUNCTIONAL HETEROGENEITY AND NEUROTOXIC PROPERTIES OF PHOSPHOLIPASE C-COUPLED METABOTROPIC GLUTAMATE RECEPTORS

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Group-I metabotropic glutamate receptors (mGluR1 and mGluR5) activate phospholipase C but induce distinct patterns of intracellular Ca^{2+} $[Ca^{2+}]_i$ mobilization. mGluR1 receptors trigger transient peaks followed by sustained $[Ca^{2+}]_i$ elevation, whereas mGluR5 produce $[Ca^{2+}]_i$ oscillations. Also mGluR1, but not mGluR5,receptors expressed in CHO cells show a rapid time-course of agonist-induced IP_3 formation. Group I mGluRs appear to contribute to glutamate neurotoxicity. We examined the viability of CHO cell lines with stable expression of mGluR1 and mGluR5 receptors. Cells transfected with mGluR1 showed a decreased life span after reaching confluency in contrast to cells expressing mGluR5 and to untransfected cells. DNA analysis revealed a pattern of fragmentation typical of apoptosis in mGluR1-transfected cells but not in cells expressing mGluR5. In addition, cells expressing mGluR1 showed a decreased rate of protein biosynthesis and increased accumulation of lactate, suggesting a profound alteration of oxidative metabolism. The signal transduction and neurotoxic properties of mGluR1 receptors appear to be controlled by a single aspartate residue (Asp854). Mutation of this residue to threonine yields a receptor (mGluR1D854T) which exhibits the properties of mGluR5. This includes the oscillatory pattern of $[Ca^{2+}]_i$ responses, a slow time-course of IP_3 formation, and the lack of apoptotic cell death in a CHO cell line expressing the mutated receptor. These results suggest the existence of functional differences between the signal transduction mechanisms of the two phospholipase C-coupled mGluRs and point to mGluR1, rather than mGluR5, as the receptor contributing to apoptotic cell death.

9.2 METABOTROPIC GLUTAMATE RECEPTORS COUNTERACT MUSCLE RIGIDITY INDUCED BY HALOPERIDOL

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The aim of the study was to determine whether any metabotropic glutamate receptor (mGluR) ligands evoke an antiparkinsonian effect in the haloperidol-induced muscle rigidity. Four ligands of mGluRs were used: the potent and selective mGluR1 antagonist (RS)-1-aminoindan-1,5-dicarboxylic acid, the mixed Group II agonist/Group I antagonist (S)-4-carboxy-3-hydroxyphenyl-glycine ((S)-4-C3H-PG), the potent Group II agonist (+)-2-aminobicyclo[3.1.0.]hexane-2,6,-dicarboxylic acid (LY354740 and a selective Group II agonist 3-amino-3,5-pyrrolidinedicarbo-xylic acid (2R,4R)-APDC. Only the LY354740 penetrated into the brain from the periphery. Therefore all other drugs were injected bilaterally into the rostral striatum. Muscle tone was recorded by a mechanomyographic/electromyographic method which measured the resistance of the rat's hind foot and the reflex response of its muscles to passive movements.

(S)-4C3H-PG (5 and 15 μg/0.5μl) and LY354740 (5 and 10 mg/kg) diminished the muscle rigidity induced by haloperidol (1 mg/kg) whereas (2R,4R)-APDC (7.5 and 15 μg/0.5μl) and AIDA (0.5 and 2 μg/0.5μl) did not. Our results suggest that stimulation of mGluRs of Group II may be important to diminution of parkinsonian-like muscle rigidity. However, it seems that not only striatal mGluRs participate in the antiparkinsonian effect of the mGluRs ligands.

9.3 **POTENTIAL ANTI-ANXIETY EFFECTS OF GROPU II GLUTAMATE METABOTROPIC RECEPTOR AGONISTS IN ANIMAL MODELS.**

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Despite several biochemical data about metabotropic glutamate (mGlu) receptors, our knowledge of behavioural effects of mGlu receptor agonists/antagonists is still inadequate. LY 354740 is a systemically active agonist of group II mGlu receptors. After peripheral administration, LY 354740 produced anxiolytic-like effects in conflict drinking test in rats and a four-plate test in mice. It was also found that LY 354740 decreased spontaneous locomotor activity in mice, but did not disturb motor coordination. In behavioural models of depression including despair test and a tail suspension test, LY 354740 did not produce antidepressant-like effects. Another agonist of group II mGlu receptors and antagonist of group I mGlu receptors (S)-4-carboxy-3-hydroxyphenyl-glycine (S-4C3HPG) after intrahippocampal administration produced a dose-dependent anticonflict effect in rats, which was unrelated to reduced perception of stimulus or to an increased thirst drive. The above results indicate that agonists of group II mGlu receptors may play a role in the therapy of anxiety and/or drug-dependence states. The brain sites of action of LY 354740 need to be identified and the mechanism of both the above described effects remains to be elucidated.

9.4 **ROLE OF mGlu RECEPTORS IN THE MECHANISMS OF NEUROTOXICITY**

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Not received.

Session 10 - Parallel Symposium: Plasticity of adult cerebral cortex

10.1 **FUNCTIONAL PLASTICITY FOLLOWING SUSTAINED INHIBITION OF ADULT CEREBRAL CORTEX**

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A powerful tool for dissecting out the functions of cerebral cortical areas is to inactivate an area and observe the resulting functional deficits. Currently available inactivation methods are difficult to monitor and either lead to irreversible damage or are useful over only very short durations. We have used polymer implants, releasing the $GABA_A$ receptor agonist muscimol, to achieve sustained, localized and reversible blockade of intrinsic cortical activity. Elvax polymer sheets containing 75mM muscimol (200μm thick, up to 300mm^2 in area) were implanted sub-durally over adult ferret cortex and gave an even level of drug release (110pmol/mm^2/day) for implants lasting between 2 and 16 weeks. Autoradiographic tracing of released [^{3}H]muscimol following cortical implantation showed a highly localized distribution of muscimol around the implant. The spatiotemporal extent of functional activity blockade was monitored by extracellular electrophysiological recording of responses to single visual stimuli at 0, 2, 6 and 16 weeks after implantation of Elvax sheets over adult ferret visual cortex. Visual cortical responses were blocked throughout the depth of the cortex and blockade was reversible. Adult ferrets that had received unilateral implants of muscimol Elvax over auditory cortex exhibited significant sound localization deficits in auditory space contralateral to the inactivated cortex. In the majority of animals, these deficits reduced with repeated testing during activity blockade, indicating functional plasticity within the adult auditory system. *Supported by The Wellcome Trust, UK*

10.2 EXPERIENCE-DEPENDENT PLASTICITY IN DEVELOPMENT OF BARREL CORTEX OF RODENTS.

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One of the methods of evoking substantial plasticity in the barrel cortex is by univibrissae rearing, where all whiskers, but D1 are removed for some time by steady tension applied to the base of the vibrissa. Plasticity-of this kind involves both potentiation of spared vibrissae responses and suppression of deprived vibrissae responses in adolescent animals. In adult animals, only potentiation of spared vibrissae responses was detected. There seems to be no critical period for potentiation in superficial layers of barrel cortex, but plasticity cannot be evoked in layer IV later than the first postnatal week. There is a critical period for suppression however, which cannot be evoked and possibly cannot be maintained in adult rodents. In adolescent animals substantial suppression was observed as early as one week after deprivation. This precedes potentiation of the spared vibrissae input by at least one week suggesting that either mechanisms of potentiation and suppression are independent, or suppression is, for some reason, a prerequisite for potentiation in adolescence but not in adulthood. Potentiation depends on the ability of α-CamKII to get autophosphorylated and to a lesser extent on the presence of α/δ–CREB, but only in adult animals.
Taken together, barrel cortex displays early plasticity, characterised by its presence even in layer IV; adolescent plasticity, with downregulation of the deprived input and adult plasticity, distinguished by an absence of depression and a dependence on α-CamKII and α/δ–CREB.

10.3 **MECHANISMS OF LESION-INDUCED PLASTICITY IN THE ADULT VISUAL CORTEX**

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Neuronal reorganization in the primary visual cortex is observed after homonymous photocoagulator lesions of the central retina (10 degrees in diameter) as well as after cortical lesions (2-4 mm diameter). The long-term effects of both types of lesions include changes in receptive field topography as well as changes in RF size and substructure. This topographical reorganization leads to filling-in of the scotomata in both cases, based on plasticity of different intracortical pathways. However, recent evidence indicates that both kinds of visual cortical plasticity are accompanied by similar local patterns of events promoting changes of synaptic efficacy within the neuronal network: an increase of single neuron excitability observed with both lesion models in vivo seems to be caused by local up-regulation of the excitatory (glutamatergic, NMDA-receptor mediated) and down-regulation of the inhibitory (GABAergic, GABAA+B receptor mediated) synaptic transmission observed at cortical lesions in ex vivo in vitro experiments. Long-term potentiation is significantly facilitated in cells recorded in the same cortical region.
Two completely different types of lesions (one several afferent neurons away the other local) trigger very similar events in the visual cortex that can promote synaptic plasticity. Increased glutamatergic excitation, reduced GABAergic inhibition., increased excitability, and facilitated long-term potentiation might be the common early mechanisms underlying local neuronal reorganization in the adult visual cortex.
Supported by grants from the DFG (Ey 8/23 and SFB 509, TP C4).

10.4 SHORT-TERM PLASTICITY OF THE RAT'S BARREL CORTEX INDUCED BY CONTEXTUAL VARIATION.

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Event related potentials evoked (EPs) by vibrissa stimulation in rat barrel cortex are of two distinct classes according to the relative contribution of their principal components. These components can be attributed to activation of different pyramidal cell populations: supra- and infragranular. With well-habituated stimuli EPs are dominated by a component related to the supragranular cells (class 1). However, the first reinforcement of vibrissa stimulation in the classical aversive paradigm favours the appearance of EPs dominated by a component characteristic for infragranular cells (class 2). Similar dynamic changes of the relative occurrence of the two EP classes follow other aversive stimuli, including pressing the animals ear and restraining a whisker. The DC level of local field potential preceding class 1 EP is lower then measured before the occurrance of class 2 EPs.

The hypothesis is proposed that neuromodulatory action elicited by contextual stimulation on the whole principal barrel column activates mostly those neurones which provide an output to the surrounding barrels. In the classical conditioning paradigm this mechanism may lead to experience-dependent changes within the intracortical network.

Session 11 - Plenary Lectures including Jerzy Konorski memorial lecture *by R.G.M. Morris*

11.1 REVERSIBLE INACTIVATION OF EXCITATORY NEUROTRANSMISSION REVEALS THE PARTICIPATION OF THE HIPPOCAMPUS IN DISTINCT MEMORY PROCESSES

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Studies of patients with circumscribed brain damage and of animals with experimental lesions have implicated the hippocampal formation and related neocortical structures in spatial, declarative/relational, episodic and other types of memory. These types of memory are, however, each thought to consist of a series of interdependent but potentially dissociable memory processes - encoding, storage, consolidation and retrieval. One purpose of the experiments to be described was to identify whether the hippocampus makes an independent contribution to these memory processes at different times. To do this, we examined the effects of acute and chronic inactivation of either AMPA receptor-mediated fast synaptic transmission or NMDA receptor-mediated synaptic plasticity at various times during or after training in a watermaze. Electrophysiological and 2-deoxyglucose autoradiographic techniques were first used to establish the temporal and regional specificity of this method of neural inactivation and its reversibility. A series of behavioural experiments were then directed at isolating dissociable memory processes. The results provide direct evidence that the hippocampus participates, at least for spatial memory, in both encoding and retrieval processes. Fast AMPA receptor-mediated synaptic transmission is required for both encoding and retrieval, NMDA receptor-mediated activity only for encoding. Studies of a cellular model of memory formation, long-term potentiation, also indicate that the input-specific persistence of LTP requires the synthesis of plasticity-related proteins, but these need not be synthesised in response to the events that trigger LTP at any one set of synapses. These findings have implications for memory consolidation. Other studies indicate that persistent hippocampal activity is required for the long-term storage of certain memory traces or their consolidation in neural circuits elsewhere in the brain. A key new concept to emerge is that the 'hippocampal-dependence' of a type of memory can be 'memory-process' specific.

11.2 **MELATONIN: NEW LIGHT IS SHINING ON THE OLD MOLECULE'S STORY**

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The pineal hormone melatonin (MEL) has been traditionally considered as a neurochemical transducer of the environmental photoperiod. By its pattern of biosynthesis and secretion, which is circadian in nature (with high values at night and low values during daytime), MEL conveys information about phase, duration and strength of the daily photoperiod for the organisation of seasonal and circadian physiology. In addition to the pineal gland, MEL is synthesized in retina of many vertebrates, where it functions as a local neuromodulator. During evolution, the mammalian pineal gland has lost the ability to directly detect light, and information about environmental lighting conditions reaches the gland via polysynaptic pathway originating in the retina. A crucial link in this pathway is a small region in the anterior hypothalamus containing the suprachiasmatic nuclei (SCN) - the site of a master biological clock. Recently, cDNA encoding arylalkylamine N-acetyltransferase (AA-NAT; a key regulatory enzyme in MEL synthesis) has been cloned and characterised, enabling deeper studies on the regulation of the enzyme activity. MEL exerts its biological actions through three types of specific receptors now designated: mt_1, MT_2 and Mel_{1c}. Although all these receptors are coded by separate genes they show similar pharmacological characteristics. Convincing evidence indicates that exogenous MEL - if suitably timed - may be a useful chronobiotic with which to affect (by means of phase shifting) the function of the endogenous biological clock. Based on this activity, MEL seems to be helpful in adaptation to simulated or actual time zone change (jet-lag) or shift work, as well as to treat so called chronobiological sleep disorders, particularly in geriatric patients. In addition to the mentioned properties, MEL has been shown to be endowed with free radical scavenging properties, as well as oncostatic or immunostimulating activity, but these features still are considered as debatable matters.

Session 12 - Poster Session: Development and aging

12.1 *THE CORRELATION BETWEEN MATURITY AND DYSGRAPHICALNESS OF HANDWRITING*

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The paper presents the results obtained by examining the maturity of handwriting in 89 dysgraphic children aged 8, 9, 10 and 11, with the normal neurological findings, normal sight and hearing, and average intellectual abilities. We have applied the scales for maturity assessment and dysgraphicalness of handwriting (Aziagiarera & Ozias, 1972).
By a qualitative analysis of the results, we have found that in 82.1% of the children with dysgraphia, the handwriting maturity was below expected for the age. By a statistical processing of the results, we have established that there exists a very high statistical significance between the maturity and dysgraphicalness of handwriting ($X2>9,210$, at the significance level of 0.001). It has also been established that there are no statistically significant differences in maturity and dysgraphicalness of handwriting between boys and girls ($X2<6.635$, at the significance level of 0.001).

12.2 PROSOMERES IN THE HUMAN EMBRYONIC BRAIN

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Division of the prosencephalon into several structures (isocortex, hippocampus, olfactory bulb, basal ganglia) indicates that there is an early regionalization of the forebrain.
Study was made on serially sectioned human embryos of developmental stages 13 to 23 (32 to 56 postovulatory days). Embryos are from the Collection of the Department of Anatomy in Poznań.
In embryos at stages 13 and 14 within the prosencephalic vesicle 5 neuromeres (prosomeres) are found: one telencephalic and four diencephalic. The telencephalic neuromere (T) is close to the nasal pit. During stage 14 primordia of the telencephalon medium and cerebral hemispheres are formed. The first diencephalic neuromere (D1) is characterized by optic vesicle, and D2 is marked by the hypophysial primordium. During described stage within neuromere D2 two additional neuromeres (synencephalon and parencephalon) are evident. The synencephalon is represented by the dorsal bulging of the posterior part of D2, and the remaining part of D2 becomes the parencephalon, which is divided into rostral and caudal parts. The rostral prosencephalon contains optic vesicle, infundibular region, and ventral thalamus. The caudal parencephalon includes the mammilary region and the dorsal thalamus. The subdivision into prosomeres is clear up to stage 17, after which all telencephalic neuromeres disappear.

12.3 THE LATERAL NUCLEUS OF THE OCULOMOTOR COMPLEX IN HUMAN EMBRYONIC BRAIN

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The aim of present study is to trace the lateral nucleus of the oculomotor complex on 61 serially sectioned human embryos from the Collection of the Department of Anatomy in Poznań.

The motor nuclei of the cranial nerves are formed in the primary efferent column which extends through the basal plate of the brain. The lateral nucleus of the oculomotor complex appears in the basal plate of the midbrain at stage 13. At stage 14 this nucleus presents well defined group of neurons at the cephalic flexure of the neural tube. Nerve cells of the nucleus are loosely packed in comparison to cells of the mantle layer. There is a large migration zone between the matrix layer and the nucleus. In stage 16 within the lateral nucleus two groups of cells (medial and lateral) can be distinguished. Posteriorly these groups of the lateral nucleus unite. At stage 19 the formation of the groups of neurons related to innervation of the particular muscles of the eye is observed. In this stage the migration zone between the matrix layer and the nucleus is very narrow. At stages 21 and 22 the lateral nucleus is located close to the midline. The medial and the lateral groups of the lateral nucleus are located in the same sagittal plane (medial lies more ventrally, lateral more dorsally).This allows to distinguish ventromedial and dorsolateral parts of the lateral oculomotor nucleus during the last week of the embryonic period.

12.4 ENHANCED NMDA-EVOKED MOBILISATION OF INTRACELLULAR Ca^{2+} IN THE HIPPOCAMPUS OF DEVELOPING RATS IN VIVO

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The aim of this study was to evaluate the role of intracellular Ca^{2+} pool(s) in generation of NMDA receptor-mediated Ca^{2+} signal in the hippocampal neurones of immature rats in vivo. Particular attention was focused on the involvement of Ca^{2+}-induced Ca^{2+} release (CICR) via ryanodine receptors, and a role of Ca^{2+} binding protein calbindin D_{28k}. Microdialysis experiments in the dentate gyrus (DG) of halotane-anaesthetised new-born rats at postnatal day (PND) 7, were combined with measurements of Ca^{2+} concentration in initially calcium-free medium, and ^{45}Ca efflux from the prelabelled endogenous pool(s) to calcium-containing medium. This way we estimated changes in the extracellular concentration of Ca^{2+} (Ca_e), and mobilisation of intracellular Ca^{2+} followed by its release from neurones, respectively. Application in the dialysis medium of 5 mM NMDA for 20 min induced a transient decrease in Ca_e, reflecting influx of extracellular Ca^{2+} to neurones, and simultaneous huge release of ^{45}Ca to dialysate. This effect in PND 7 rats was significantly enhanced as compared to the NMDA-evoked ^{45}Ca release in DG of the adult rats. Autoradiographic detection of ryanodine receptors in the brain of PND 7 rats visualised in DG a high expression of [^{3}H]ryanodine binding sites, similar to the adult level. On the other hand, immunocytochemical study in the brain of PND 7 rats, unlike in adult rats, demonstrated only negligible cabindin-like immunoreactivity. These results indicate that in the immature rat DG the NMDA-evoked release of ^{45}Ca, presumably due to CICR mechanism, is more pronounced than in adult rats. The role of calbindin in the mechanism of ^{45}Ca release in our microdialysis experiments is negligible. *Supported by KBN grant # 4.P05A.052.16.*

12.5 EARLY OPTIC NERVE FIBERS IN HUMAN EMBRYOS OF STAGE 16 AND 17

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Study was performed on 15 human embryos of developmental stages 15, 16 and 17 (32 to 43 days). Embryos are from Collection of Department of Anatomy in Poznań. Serial sections were stained with routine histological methods and impregnated with silver salts.
During stage 15 the lens vesicle forms and it contacts the surface ectoderm. Within the inner layer of the optic cup two zones (layers) can be distinguished: primitive nuclear and marginal layers.
At stage 16 the lens vesicle separates from surface ectoderm. Pigment granules can be observed in the external layer of the optic cup. Within primitive nuclear layer mitotic activity is observed. Groups of neuroblasts migrating from this layer into the marginal layer are noted. Some of these neuroblasts differentiate into retinal ganglion cells, axons of which traverse through the marginal layer to the optic stalk, where they bend and grow into internal layer of the optic stalk. The optic nerve fibers can be observed only in the posterior pole of the optic cup and in the retinal part of the optic stalk.
During stage 17 development of the optic nerve fibers proceeds anteriorly. The growing nerve fibers reach half of the length of the optic stalk. This process transforms optic stalk into the optic nerve. Neuroblasts migrating from the primitive nuclear layer accumulate in the marginal layer and form the internal nuclear layer. There is an increase in optic nerve fibers in marginal layer. These fibers form the optic nerve fiber layer.

12.6 INFLUENCE OF CLORAZEPATE DIPOTASSIUM ON DEVELOPMENT OF FOETUSES AND EPINEPHRINE LEVELS IN PREGNANT FEMALES RAT BRAINS

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The aim of this experiment was to study of the influence of clorazepate dipotassium on development foetuses and epinephrine levels in pregnant females brains of Wistar rats.

Pregnant females received intraperitoneal /i.p./ clorazepate dipotassium in the doses 1,0, 1,25, 1,5 mg/kg b.m. on each days from 8 to 14 and from 14 to 20 of gestation. Three groups were controls: UC-untreated control, TC-treated with physiological saline i.p. 0,1 ml/kg b.m. and ST- treated with 0.26 mg/kg b.m. chlormethine hydrochloride i.p. as a standard teratogen. Pregnant females were euthanised on day 21 of gestation and caesarean sections were performed and implantation sites were recorded as live, dead and resorbed. The evaluation of birth defects of internal organs was carried out to Wilson's technique in Barrow's and Taylor's modifications. Obtained brains were fixed in liquid nitrogen, homogenised and tested with biochemical methods.

On the basis of these studies it has been found the highest incidents of malformed foetuses occurred after 1,25 mg/kg b.m. in organogenesis. A significant differences of epinephrine levels in females brains after dose 1,5 mg/kg b.m. administered from 14^{th} to 20^{th} day of pregnancy was noted.

12.7 DEVELOPMENTAL CHANGES IN REPRODUCTION OF TEMPORAL INTERVALS

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From research carried out over the last years, it has become apparent that the information processing is temporally segmented by sequential units of approx.2-3 s. Such segmentation reflects probably a temporal integration (TI) – one of hypothetical temporal mechanisms which binds successive events up to approx. a few s. A support for such operations comes among others from studies on temporal reproduction, where standards of approx. 2 – 3 s are reproduced correctly, because they are probably processed within a temporal window specific for operating of TI. Using this paradigm, we tested the effect of normal cognitive development, modality and the range of presented standards on the limit of TI. Sixty children aged 6 – 7, 9 – 10 and 13 – 14 years were asked to reproduce standard durations indicated by a light or by a tone. Presented durations ranged from 1- 5.5 s or from 1 – 3 s, in steps of 0.5 s. Response deviations from the standards were measured in terms of *accuracy* (reflecting an "internal clock") and *precision* (a "clock variability") associated with attention processes. *The accuracy data* showed that durations of approx. 2 s were reproduced correctly in the three age groups. Standards longer than 2.5 s were underestimated independently of the subjects' cognitive level, however, the accuracy for standards shorter than 2 s depended on the age: while both older groups displayed the accurate reproduction, the youngest group showed significant difficulties (overestimation). These relationships were independent of both the modality and the range of presented standards. *The precision data*, on the other hand, showed a significant improvement of response stability as results of cognitive child development. Moreover, a general trend toward more accuracy and more precision in reproduction of auditory than visual standards was found. Our observations may indicate that the upper limit of TI is a stable feature across the life span investigated in the present study, however, age-related changes within the border of operating TI seem to be strongly linked to cognitive child development. The prefrontal and parietal cortex seems to be a neuronal substrate for these developmental effects.
Supported by the KBN grant No 4.PO5E.09609.

12.8 DEVELOPMENTAL EXPRESSION OF AROMATASE AND ESTROGEN RECEPTOR-ß IN THE MALE AND FEMALE MOUSE HYPOTHALAMUS

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Estrogen is implicated in many aspects of neuronal differentiation. Sexual differentiation of the hypothalamus is the consequence of perinatal exposure to estrogen. In the present study, we have analyzed the pre- and early postnatal expression of the estrogen-forming enzyme aromatase and the recently cloned novel estrogen receptor-ß (ERß) in the male and female mouse hypothalamus by semi-quantitative RT-PCR. Both mRNAs were readily detectable on embryonic day (E) 15 in males and females. During prenatal development, expression of aromatase (**1**) and ERß (**2**) increased gradually in males showing a maximum at birth. In contrast, levels of transcripts in females did not change prenatally. Thus, significant higher levels of expression were found on E17 and postnatal day (P) 0 in males. Postnatally, aromatase expression returned to lower levels until P10 in both sexes, whereas ERß levels remained high in males only. In conclusion, these data indicate that sex differences in estrogen synthesis parallel those of ERß expression in the perinatal hypothalamus. These data suggest that signaling through ERß might be essential for the proper sexual differentiation of the male hypothalamus.

Supported by the Deutsche Forschungsgemeinschaft (Be 1444/2-2).

1. Karolczak et al., J. Neuroendocrinol. 10, 267-274 (1998)
2. Karolczak and Beyer, Neuroendocrinology 68, 229-234 (1998)

12.9 MAST CELLS AND CALCIUM DEPOSITS IN HUMAN PINEAL GLAND

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The pineal gland, through the synthesis and releasing of melatonin, plays the role of interface between the cyclic environment and the rhythmic vertebrate body. Recently, it has been suggested that pineal mast cells as a source of histamine may play an important role in melatonin secretion. Aim of the study was to examine the phenotype of mast cells in developing pineal gland of human fetuses and children and in adults used as a control group. The results showed that in the early development mast cells were tryptase positive and their number gradually increased. Mast cells disappeared from the pineal gland in children with bone marrow aplasia and the cells were numerous in children with leukemia. Those mast cells were tryptase and chymase immunopositive and were localized in the close vicinity of small calcified bodies. In the pineal capsule, some calcified bodies contained tryptase positive mast cells. In adult individuals, deposits of calcareous bodies were surrounded by numerous mast cells. Some of the cells were embedded within these deposits. Accumulation of calcium was found in cytoplasmic granules of mast cells. The results lead to the conclusion, that the increased number of mast cells in pathology is concomitant with the calcareous body formation and with the process of calcification in the human pineal gland.

12.10 POSTNATAL CHANGES OF MAP-2 IMMUNOREACTIVITY IN THE RAT HIPPOCAMPUS

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Microtubule associated proteins are considered to be a sensitive marker for dendritic damage or plasticity, particularly in the hippocampus.
A total number of 45 brains at various ages were examined. The care and treatment of the animals were in accordance with the guidelines of the local ethical committee. After perfusional fixation the brains were frozen, cut on the cryostat and stained immunohistochemicaly using MAP-2 antibodies. On the day of birth MAP-2 immunostaining was found in a few cell bodies and synaptic terminals in all layers of the cornu Ammonis as well as in the granular and polymorphic layer of the dentate gyrus. Labeled cells were observed in the stratum oriens and pyramidale of CA1 and CA3 sectors through all developmental stages and in stratum radiatum in CA3 till 17th postnatal day. Immunolabeled fibres and terminals were found in all layers of the hippocampus in each of the developmental stages. In the dentate gyrus up to the eighth days there were no immunostained cells in the granular and molecular layers, but there was high density of immunostained fibres instead. In the polymorphic layer some immunolabeled cells were observed at all stages. After eight days the density of positive fibers began to decrease, but high density of the synaptic terminals was still observed in all layers of the hippocampus.
Our results indicate that immunohistochemical characteristic of MAP-2 reactivity in the hippocampus and dentate gyrus differs according to the layer and the developmental stages. We suggest that it can be relevant to the ability of the neuronal structure to stabilize its neuronal network.

12.11 DISTRIBUTION OF THE 5-HT$_{1A}$ RECEPTOR IN THE DEVELOPING BRAIN OF THE SHORT-TAILED OPOSSUM *(MONODELPHIS DOMESTICA)*

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The important role of serotonin during development is well established. Family of serotonergic receptors in mammals consists of 7 types and 14 subtypes. Receptor 5-HT$_{1A}$ is one of the most important of them. In the adult animal it is involved in various physiological functions, such as regulation of feeding, thermoregulation, control of sexual behaviour, nociception. The role of this receptor in the development of the central nervous system was not investigated, but may be important. We studied distribution of the 5-HT$_{1A}$ receptor in the brain of the opossum *Monodelphis domestica* of different postnatal ages, using antibody against rat's 5HT$_{1A}$ receptor. In the brain of the new-born opossum, development of which corresponds to that of the 14-day-old rat embryo, no immunohistological staining for this receptor was visible. Clear 5-HT$_{1A}$ immunoreactivity appeared on the 9th postnatal day. The densest labeling was found in the hippocampus (fields CA1 and CA3), piriform cortex, raphe nuclei, and neocortex, especially the insular cortex. Initially, the stain was visible mainly on the axon hillocks. Later, the label appeared on the initial segments of axons and even on the cell bodies. The density of labeling increased with the age of animal until two months of life and then stabilized.
Thus, the number of the 5HT$_{1A}$ receptors increased linearly until the stage when the opossums are becoming independent.
This work was supported by KBN Grant No 4PO5A.0811.11.

12.12 EXPRESSION OF mRNA ENCODING MICROTUBULE ASSOCIATED PROTEINS (MAPs) IN THE DEVELOPING MOUSE BARREL CORTEX

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MAPs are a heterogenous group of developmentally regulated proteins referred to as immature and mature MAPs. MAP2 immature (MAP2c) and mature (HMW MAP2) isoforms arise from a single gene as a result of alternative splicing. This also refers to TAU isoforms. MAPs have been proposed to participate in neuronal plasticity. The rodent barrel cortex, a cortical representation of vibrissae, provides an excellent model to study both developmental and adult neuronal plasticity because it is arranged into anatomically distinguished cellular modules which undergo morphological and functional reorganisation upon modification of the sensory input. In the present study [^{35}S]-labeled oligonucleotides were used to detect HMW MAP2, MAP2c as well as immature and mature TAU mRNA variants in the barrel cortex of mice at postnatal days (P) 12, 21, 28 and 70. HMW MAP2 and MAP2c mRNAs showed a complementary pattern of developmental expression. HMW MAP2 mRNA level was lowest on P12 and increased significantly by P21. In contrast, the level of MAP2c mRNA was highest on P12 and dropped down to a much lower value on P21. By P28 both immature and mature isoforms gained an adult level which was sustained on P70. On P12 a differential laminar distribution of both immature and mature MAP2 mRNA was apparent with a lowest level in the layer V. On later developmental stages the hybridization signal did not differ between cortical layers. Age differences in mRNA expression were less prominent for TAU isoforms. Nevertheless, as in the case of MAP2, the switch from immature to mature variants was observed between P12 and P21. The present results show that during the first three postnatal weeks immature MAPs dominate over their mature counterparts and that the end of this period is characterized by a sudden switch between the two, manifested as a depression in the expression of immature isoforms and a peak in the expression of mature isoforms.
Supported by the State Committee fo Scientific Research grant 6 P04A00512.

12.13 THE POSTNATAL DEVELOPMENT OF THE RAT PARACLAUSTRAL RESERVOIR

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A distinct group of small cells lying in the ventral part of external capsule in the rat brain is clearly visible at the birth and is called by us as a "paraclaustral reservoir". Neuroblasts, which are generated in the neocortical neuroepithelium, migrate in the lateral cortical stream and accumulate in a paraclaustral reservoir. From there, they penetrate into the cerebral cortex and other structures of the basal telencephalon.
24 rat brains of various ages at postnatal days (P) 0, 1, 3, 4, 5, 7, 10, and 14 were studied. Animal care and treatment were in accordance with the guidelines of the local ethical committee. After perfusional fixation the brains were frozen, cut on the cryostat and stained using following methods: cresyl violet, immunohistochemistry (antibodies for microglia: OX-42, ED1 and GFAP for astroglia) and *in situ* DNA end labeling (TUNEL).
During the first postnatal week three cell populations: spindle-shaped or oval neurons, ED1/OX-42 positive ameboid microglia and GFAP positive astroglia coexist in the paraclaustral reservoir, although starting from about P4 the neuronal population appears to have reduced in size. Only a few scattered neurons are seen after P7. From the beginning of second postnatal week only glial cells were visible medially to the piriform claustrum. Accumulation of ED1/OX-42 positive ameboid microglia was observed in ventral part of external capsule up to P14; after this time distribution of microglial and astroglial cells did not differ from another part of external capsule.
The rather low number of TUNEL-positive nuclei in the paraclaustral reservoir suggests that apoptosis is not a crucial mechanism leading to decay of this structure.

12.14 UNBIASED STEREOLOGY IN THE STUDY OF THE MATURATION OF THE AMYGDALO-CORTICAL CONNECTIONS

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The aim of this study was to evaluate the method for quantification of changes in the developmental and maturational nervous tissue. The simple unbiased stereological methods were used for the study of the morphological changes in the connections of the basolateral amygdala with the frontal areas.
20 rats clustered in 4 groups of various postnatal ages (P14, P21, P45, P90) were studied. The care and treatment of the animals were in accordance with the guidelines of the local ethical committee.
The unbiased stereology was based on the analysis of systematic random sections containing fluorescently labeled cells by means of the optical disector/fractionator method with the usage of the ocular grid. The following set of parameters were estimated: numerical density and total number of neurons, the percentage of labeled neurons, the distribution of labeled neurons along the rostrocaudal extent of the amygdala. Coefficient of error for some estimates was calculated as well as the biological variability was evaluated.
Finally even such a simple unbiased method can be very powerful tool for the precise and objective study of the morphological developmental changes.

12.15 THE QUANTITATIVE BUT NOT QUALITATIVE CHANGES IN THE MATURATION OF THE CLAUSTROCORTICAL CONNECTIONS IN THE RAT

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The claustrum in the various adult animals possesses the reciprocal connections with the cerebral cortex. These connections are always topographically arranged.
Because there is no information concerning the development of these connections, the main aim of our study was to assess the topographic and quantitative changes in the motor and somatosensory cortical projection zones of the claustrum during the postnatal life.
30 rats divided into 6 groups of various postnatal ages (P0, P7, P14, P21, P45, 90) were studied using a 2% water solution of the retrograde fluorescent tracer (Fluoro-Gold). The care and treatment of the animals were in accordance with the guidelines of the local ethical committee.
The motor and somatosensory projection zones in the claustrum are detected in all groups under study, occupying the whole anteroposterior extend of the dorsal claustrum. No significant changes in the topography can be reported with the age. The motor projections are more numerous than the somatosensory ones in all studied groups. The highest intensity of the motor projection is situated more anteriorly in the claustrum than that of the somatosensory projection. The intensity of the claustral projections both to the motor and somatosensory cortices decreases with the age of the animals.
In conclusion we state that although the claustrocortical projections are well developed at the moment of birth, the considerable quantitative changes take place in the first three month after birth, which adjust these connections to the proper functioning in the mature CNS.

12.16 DEVELOPMENTAL ASPECTS OF TEMPORAL SEGMENTATION IN MOTOR BEHAVIOUR

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Substantial experimental evidence suggests the temporal segmentation, falling within a 2-3 s time window, which is observed not only in different perceptual and cognitive tasks, but also in spontaneous motor behaviour. Such temporal segmentation reflects probably an underlying temporal integration (TI) mechanism, which automatically links together elementary events in the 2-3 s units. In the present experiment we tested whether (1) cognitive development of the child effects the temporal segmentation in the above time domain in the evoked behaviour in children, and whether (2) the temporal segments in such a task are of the similar length as reported in earlier studies on spontaneous behaviour in adults.
Fifty five elementary school pupils classified into three age groups: 6-7-, 9-10-, 13-14-year-olds were studied. They were asked to produce everyday activities such as: waving, scratching, patting, stroking, „sh.....", „a fig for you", etc. The requested actions were classified into „rhythmically repeated" behaviours (RRB) and „non-repetitive" ones (NRB). The results showed that the action units produced by children were temporally segmented in a narrow time span which usually did not exceed a „3-second-window", similarly as in spontaneous behaviour in adults. Moreover we found shortening of the action lengths as results of child cognitive development: the 6-7-year-olds demonstrated significantly longer action units than the two older groups. The RRB as more complex were significantly longer than NRB, independently of the subjects age . From these observations we conclude, that the limits of TI change with child development, older children demonstrated the shorter integration.
Supported by the KBN grant No 4.PO5E.09609.

12.18 **CHARACTERISTICS OF THE MICROVESSEL NETWORK IN THE DEVELOPING HUMAN HIPPOCAMPUS**

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The purpose of this study was to evaluate the characteristics of developmental vascularization of the normal human hippocampus during prenatal period. Vascular development was studied by means of lectin histochemistry using a Ulex Europaeus Agglutinin (UEA). The number of vessels per square milimeter, their internal cross-sectional area and diameter were measured in the pyramidal layer in the hippocampal sectors CA1-4. The diameter of vessels is rather uniform during the development of the hippocampal formation, but numerical density of vessels increases. The vascularization of the Ammon's horn presents regional differences depending on the stage of maturation of sectors. At the developmental age of 28 weeks the value of numerical vessel density in all sectors of stratum pyramidale is close (changes from 101 to 118 vessels/mm^2). In mature newborn there are remarkable differences in the vessel density between the sectors. The high vessel density in CA2 (245 vessels/mm^2) followed by CA3 (213 vessels/mm^2) is notable. Sectors CA1 and CA4 are less vascularized fields. The lower numerical density of vessels in the CA1 sector than in other sectors, and greater neuronal density in this sector suggest that the maturation of neurons is independent of the development of vascular network. The decrease of numerical vessel density resulting in less advanced blood supply to CA1 sector may predipose to the anoxic-ischemic lesions in this region of developing hippocampus in cerebral hypoperfusion.

12.19 SUBSTANCE P AND PHRENIC MOTONEURON ACTIVITY DURING PERINATAL LIFE OF RAT

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Experiments were performed *in vitro* on foetal (18 to 21 days *post coitum*; E18-21) and newborn (postnatal days 0 to 3; P0-3) rat brainstem spinal cord preparations to analyse the perinatal developmental changes in the effects induced by substance P (SP). SP induced significant increase in the respiratory frequency of newborn rats (10^{-9}M), whereas concentration up to 10^{-7}M induced no change in foetal preparations.

A whole-cell current-clamp mode was used to record the unitary activity of phrenic motoneurons. In newborn and E20-21 foetal rats SP-containing a CSF depolarised the phrenic motoneurons, increased their input resistance, reduced the rheobase current and shifted the Intensity/Frequency curves leftwards. In E18 foetal rats, no change was evoked by SP.

A peptidase inhibitor mixture was used to block the enzymatic degradation of endogenous SP. This mixture was ineffective in changing the respiratory frequency in newborn and foetal preparations. In newborn rat phrenic motoneurons, the peptidase inhibitor mixture induced changes similar to those caused by SP but no change was induced in foetal rats.

These results indicate that SP may modulate : 1) the activity of respiratory rhythm generator in newborn but not foetal rats; 2) the activity of phrenic motoneurons at E20, E21 and newborn rats but not at E18.

Results obtained using the peptidase inhibitor mixture suggest that endogenous SP is probably not involved in the control of the respiratory rhythm in the prenatal period, but may influence the activity of the phrenic motoneurons after birth.

Branches of the vestibular ganglion to membranous labyrinth in staged human embryos. 12.17

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Study was performed on 34 serially sectioned human embryos, of developmental stages 15 to 23 (36 to 58 postovulatory days) from the collection of the Department of Anatomy in Poznań.

In embryos at stage 15 the vestibular ganglion is placed posteriorly to the cochlear ganglion and indications of the ampullar nerves are seen. Axons of the vestibular ganglion cells enter the common afferent tract.

During stages 16 and 17 the common trunk of the anterior and lateral ampullar nerves is found. The posterior ampullar nerve forms separate ramus.

In stages 20 and 21 the structure of the ampullar nerves does not change and the vestibular ganglion is divided into superior and inferior parts.

During last embryonic stages (22 and 23) the utriculo-ampullar, posterior ampullar, and saccular nerves are observed. However, the most evident are the ampullar nerves. The least developed is saccular nerve. In these embryos the utricule and saccule are clearly separated.

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13.1 **IMMEDIATE EFFECTS OF VAGOTOMY ON FUNCTIONAL RELATIONS BETWEEN RENAL SYMPATHETIC NEURONS**

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The effect of dopaminergic mechanisms on interactions between sympathetic neurons (Lupa et al. 1998) suggests that the pattern of their functional connectivity can be modified. In the present experiments we studied the effect of vagotomy on interations between the renal sympathetic neurons. In rabbits anaesthetized with urethane + chloralose the ongoing sympathetic discharge was recorded in bundles of the renal nerve. The activity of several single neurons was separated by computer. The type of interaction was determined by crosscorrelograms (CCs) before and immediately after bilateral setion of the vagus nerves in 71 pairs of neurons. The shared inhibitory input in control conditions was seen in CCs of 29 pairs of neurons (40.8%) and its incidence decreased after vagotomy to 22 neuron pairs (31%). Vagotomy did not materially affect the incidence of the shared input combined with direct excitatory synaptic connection [14 neurons pairs (19.7%) before and 12 neurons pairs (16.9%) after section] and the rate of occurrence of direct excitatory synaptic connection [3 pairs of neurons (4.2%) both before and after section]. On the other hand, severing the vagus nerves increased from 25 (35.2%) to 34 (47.9%) the incidence of the pairs of neuron pairs which did not show any interaction. These data suggest that the vagus aferents, most probably activated during rhythmic respiratory movements, decide the pattern of the neuronal connectivity between the renal sympathetic neurons.

13.2 NEURAL ACTIVITY IN TESTIS UNDER PHYZJOLOGICAL AND EXPERIMENTAL CONDITIONS IN THE PIG.

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This study investigated effect of active immunisation against GnRH and ageing on neuronal activity of the testis. Three groups of boars were used: juvenile 10 week-old (J; n=4), intact (IA; n=3) and immunocastrated (IC; n=8). Immunocastration was accomplished by two successive vaccinations with synthetic GnRH. Testes were collected from 26-week-old animals. Immunohistochemistry was applied to localise PGP 9.5, SNAP-25, DβH, NPY, VIP and LH-receptors within testicular structures. **In testes of IA,** PGP-9.5, SNAP-25, DβH-IR nerve fibres were associated only with blood vessels within tunica albuginea and parenchyma. Very scarce VIP and NPY-IR nerves were associated also with blood vessels but only within tunica albuginea. **In testes of J and IC boars**, PGP-9.5, SNAP-25, DβH-, VIP- and NPY-IR nerve terminals were much more numerous. DβH-IR fibres formed dense network distributed within structures of the capsule and interstitium, and some of them were located very close to Leydig cells. Many VIP and NPY-IR nerve fibres surrounded blood vessels in the tunica albuginea and parenchyma and some of them were observed between seminiferous tubules. In contrast, distinctly smaller amounts of LH-receptor within interstitial cells of IC and J pigs were observed as compared to that found in active testes (i.e. IA group). These findings suggest that nervous system plays an important role in regulation of metabolism of Leydig cells in the prepuberty. In the active gonads metabolism of those cells is probably regulated mainly by hormonal way.

Supported by: grant P06K 025 13

13.4 **Vesicular acetylcholine transporter-immunoreactive (VAChT-IR) nerve supply to the male and female genital organs in the pig.**

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VAChT belongs to the group of so-called "docking-proteins" comprising different molecules that are capable to bound transmitter molecules within the synaptic vesicle. In the present study we combined retrograde tracing and double-immunolabelling to determine the distribution, chemical characteristics and origin (only for chosen organs) of nerve fibres supplying female (n=6 animals) and male (n=6 animals) genital organs in the juvenile pig. In the gonads, VAChT-IR axons were scarce (testes) or moderate in number (ovaries) and they were addressed predominantly to the vasculature. In the oviduct, uterus and the vagina, VAChT-IR fibres supplied both vascular and non-vascular smooth muscles, and a small proportion of them was often distributed beneath the epithelium and in the vicinity of uterine glands. Vas deferens received the most prominent VAChT-IR nerve supply. These axons were found predominantly in the lamina propria and they were less numerous in the muscle coat. In accesory genital glands and in the penis, VAChT-IR nerve terminals were scarce, and they were associated only with arterial blood vessels. In all the organs studied, VAChT colocalized with VIP, SOM and/or NPY. This study has disclosed the presence and different distribution patterns of cholinergic nerve fibres in the genital organs of the juvenile pig.

Supported in part by the grant KBN P06K 021 09

13.3 **The distribution and neurochemical coding of the rectum-projecting neurons in the inferior mesenteric ganglion (IMG) of the pig.**

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Close anatomical and physiological similarities were found between human and porcine neural elements controlling the gastro-intestinal tract. Although intraganglionic distribution and chemical phenotypes of C-SMG neurons supplying both the small and large intestine in the pig are well known, there is, however, a paucity of data dealing with the contribution of the IMG to the innervation of the large bowel. Therefore, combined retrograde tracing and double-immunolabelling was used to disclose the distribution and chemical properties of IMG neurons projecting to the rectum in the pig (n=3 animals). Retrograde tracing revealed that rectum-projecting (R-P) neurons formed one large elongated cluster distributed along the dorsal ganglionic border and a smaller centre located close to the caudal colonic nerves (CCN) output. As revealed by immunofluorescence, the largest subset of R-P neurons consisted of TH/NPY-immunoreactive (TH/NPY-IR) perikarya, while the TH-IR neurons exhibiting immunorectivities to SOM and/or GAL were distinctly less numerous. Non-adrenergic (i.e. TH-immunonegative) R-P neurons were NPY-, VIP-, SOM-, NOS- or GAL-IR. The present study has provided further evidence for the somatotopic and neurochemical organisation of the porcine IMG.

13.5 **DISTRIBUTION AND IMMUNOHISTOCHEMICAL CHARACTERIZATION OF PARACERVICAL NEURONS INNERVATING THE OVIDUCT IN THE PIG.**

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The present study was aimed at disclosing the distribution of paracervical neurons projecting to the ampulla and isthmus of the porcine oviduct and the pattern(s) of co-existence of tyrosine hydroxylase (TH), dopamine β-hydroxylase (DβH), neuropeptide Y (NPY), substance P (SP), calcitonin gene-related peptide (CGRP) and nitric oxide synthase (NOS) within these nerve cell bodies. The fluorescent retrograde tracer Fast Blue (FB) was applied into the wall of the ampullar (n=3) and isthmal (n=3) part of the organ in six sexually immature female pigs. After a survival period of three weeks paracervical ganglia (PCG) were collected. 10 µm-thick cryostat sections of the ganglia were examined for the presence of FB-positive (FB^+) nerve cells under the fluorescent microscope. FB^+ neurons were counted in every third section. After that sections containing FB^+ neurons were processed for double-labelling immunofluorescence according to the method of Wessendorf and Elde. 78,6% of FB^+ neurons projected to the isthmus while 21,4% of the studied population innervated the ampulla of the oviduct. Double-labelling immunofluorescence revealed the existence of following different chemically coded subpopulations of studied perikarya: $TH^+/D\beta H^+$, TH^+/NPY^+, TH^+/NOS^+, TH^+/NOS^-, SP^-/NOS^+, $SP^+/CGRP^+$. The majority of FB^+ neurons within PCG were $TH^+/D\beta H^+$. Immunohistochemical studies revealed that the porcine PCG comprised both, noradrenergic and non-noradrenergic subpopulations of FB^+ neurons. Noradrenergic neurons were located mainly in the cranial part of the ganglion while the non-noradrenergic ones were found in the middle region.

13.6 **EXPRESSION OF TYROSINE HYDROXYLASE IN THE PORCINE SYMPATHETIC GANGLIA**

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A fragment of porcine tyrosine hydroxylase cDNA was cloned with PCR. Three cDNA sequences of TH precursor from cattle (Bovtha), man (Hshth1r) and rat (Rattoha) were analyzed with ClustalW software to identify regions displaying homology in the three sequences. The primers covering the homological regions were designed. The sense primer covered nucleotides 950-963 of the Bovtha sequence and the antisense primer covered nucleotides 1352-1372 of the Bovtha sequence. The total RNA was isolated from porcine coeliac ganglion and used for reverse transcription with dT_{18} primer and avian myeloblastoma virus reverse transcriptase. The resulting cDNA was used for PCR with designed primers. The product of PCR was analyzed with agarose electrophoresis and was shown to be of the expected size (ca. 413 bp). The PCR product was isolated, subcloned into pBluescript KS vector and used for preparation of molecular probes. The probes were either single stranded DNA probes produced with assymetric PCR or RNA probes produced with *in vitro* transcription. DNA and RNA probes were labeled with digoxigenin. The inferior mesenteric ganglia were dissected out from female piglets (body weight of ca. 10 kg) perfused transcardially with 4% paraformaldehyde. 20 µm cryostat sections were used for *in situ* hybridization with digoxigenin labeled probes. The *in situ* hybridization revealed TH hybridization signal in the majority of neurons in the porcine IMG. Only scarce neurons were devoid of TH hybridization signal.

13.7 **THE PRESENCE OF AChE - POSITIVE NERVE FIBERS IN THE DEEP PINEAL GLAND AND PINEAL STALK OF ADULT ALBINO RATS**

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The presence of the cholinergic innervation in the pineal gland of different mammalian species including the rat is still the matter of controversy resulting from contradictory reports on this subject. The animals used in this study were kept in LD 12:12 (L 8.00-20.00 h). The adult rat's pineal glands of both sexes (n=34) were collected between 10.00 and 12.00 h. The examination was performed at the light microscope level, by application of the AChE-histohcemical method according to Tago et al.,(cryostat sections).The rat superficial pineal gland showed the lack of AChE-positive nerve structures however, the AChE-positive nerve fibers were found in the deep pineal gland and the pineal stalk. These results are in agreement with reports on different innervation of the particular parts of the rodent pineal gland. It is well known that the rodent deep pineal gland and the pineal stalk are the primary targets of the central innervation by the pinealopetal nerve fibers derived from the forebrain and mesencephalon via stria medullaris thalami and habenular complex. On the contrary, superficial pineal gland is mainly innervated by the peripheral, sympathetic noradrenergic nerve fibers originating from the superior cervical ganglia. The neurotransmitter of that central pineal innervation has not been elucidated yet. The presence of the AChE-positive nerve fibers in the deep pineal gland and pineal stalk may indirectly support the existence of cholinergic innervation at least of some of the rat pinealocytes present in these parts of the gland. It is possible that cholinergic neurons in the medial habenular nuclei may project to the rat deep pineal gland and pineal stalk.

13.8 **INVESTIGATIONS OF THE FUNCTIONAL CONDITIONS OF THE FIRST-ODER TRIGRMINAL NEURONES IN SEMILUNAR GANGLIA IN MAN USING IMMUNOHISTOCHEMICAL METHODS**

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The main sensory nerve the stomatognatic system is the fifth cranial trigeminal nerve. Its first neurones is localized, among others, in the semilunar-Gasser ganglia. It is mainly on the level of this neurone that the changes in the functions of the nervous part the stomatognatic system are observed.

The aim of this paper is to determine functional conditions of the first neuron localized in the semilunar-Gasser ganglia by the use of peroxidase method in immunohistochemical investigations of substance -P /SP/ and neurofilaments / NF/.

The material contains 26 human trigeminal ganglia obtained during autopsy, 5-12 h after death. Peorxidase indirect methods was used in immunohistochemical investigations. Morphometric analysis was perfomed using the computer program.

Neurones with SP/+/ reactions were localized sparely in the ganglia without any significant structural patterns. There is no definite distribution of the products in neuroplasma either. NF /+/ reaction was found in nerve cells of all size and in their fibres and was distributed uniformly in the structure of the ganglia.

SP positive immunohistochemical reaction in medium and large size cells confirms additional rile of substance -P as a transmitter of mechanical impulses.

On the basis of those investigations can be determine functional efficiency of the trigeminal neurones in semilunar ganglia.

13.9 **PURIFIED EXTRACTS FROM PREDEGENERATED PERIPHERAL NERVES ENHANCE THE REGROWTH OF OPTIC NERVE FIBRES AND SURVIVAL OF RETINAL GANGLION CELLS**

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The inability of axons to grow across damaged central nervous system (CNS) tissue is a well-known consequence of injury to the brain and spinal cord of adult mammals. Our previous studies revealed that 7-days predegenerated peripheral nerve grafts facilitated neurite outgrowth as well as survival of retinal ganglion cells. The purpose of the present paper was to examine if the extracts obtained from such peri-pheral nerves exert similar effect. Experiments were carried out on adult male Wistar C rats. Animals were assigned into 4 equal groups. In the groups 1-3, fragment of optic nerve was excised and subseque-ntly a connective tissue chamber filled with proper fraction was sutu-red into the site of excision. In the fourth, control group, optic nerve was totally transected and both ends of cut nerve were sutured one to another. Four weeks following surgery fluorescent dyes were applied: DiI into the end of implants and rhodamine B to the *corpus vitreum*. After 48 hours animals were perfused transcardially and the chambers and retinas were subjected to histological and immunohistochemical procedures. Labelled cells and growing fibres were examined using fluorescence microscope and photographed. They were counted and the results were subjected to statistical analysis. On the basis of obtained results we can state that the extracts from predegenerated nerves exert the strongest neurotrophic influence upon the injured retinal ganglion cells.

MAGNETIC RESONANCE LANDMARKS OF THE CAVERNOUS SINUS AND PARACAVERNOUS REGION. 13.10

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The purpose of the work was to evaluate magnetic resonance anatomy of the cavernous sinus. Heavily T2-weighted submillimetric sequence in sagital, coronal and axial planes was performed in 16 healthy patients. The sequence provides high contrast between fluid and other structures of the cavernous sinus. High signal intensity of the venous spaces of the cavernous sinus is a kind of a background for internal carotid artery, cranial nerves, meninges as well as bony and fibrous structures.

Different MR landmarks of the cavernous and parasellar region were introduced and demonstrated. MR images, superior to computer tomogaphy, allow a detailed assessment of the cavernous sinus anatomy. Magnetic resonance tiny anatomical structures delineation may help the neurosurgeon to trace the exact outline of the tumor and help to plan an adequate strategy if the complete resection is attempted.

13.11 **THE GUINEA PIG (CAVIA PORCELLUS) BASOLATERAL COMPLEX OF AMYGDALA: MORPHOMETRIC AND GOLGI STUDIES**

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The morphometric analysys suggests that corticomedial part of amygdaloid complex is composed of more homogenous populations of neurons, usually smaller than these encountered in the basolateral part. Golgi preparations indicate that each from examined nuclei contain one or several morphologically well defined types of neurons not observed in the basolateral part of amygdaloid complex.
1.Medial nucleus: Homogeneous neuronal population comprising large number of small, lightly stained cells The medium-size neurones were also observed but in very small proportion. Golgi preparations: small and medium-size spiny projective neurones with ovoid or elongated fusiform somata
2.Cortical nucleus: Heterogeneous neuronal population composed of large number of. pyramidal, semi-pyramidal medium-sized cells and rarely distributed small ovoid neurones. Two subdivisions can be recognized: anterior and posterior parts. Golgi preparations: two subpopulations can be ditiguished: medium-size spiny projective neurons with pyramidal or semi-pyramidal somata and small aspiny nerve cells with thin dendrites and highly arborized axons.
3.Central nucleus: Neuronal population consists of ovoid or semi-pyramidal medium sized and small cells. Golgi preparations: three subpopulations are distiguishable: medium-size and small spiny projective neurons with pyramidal or semi-pyramidal somata and small aspiny nerve cells.

A CYTOARCHITECTONIC AND GOLGI STUDY OF THE PARAMAMILLARY NUCLEI IN GUINEA PIG 13.12

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The present studies were carried out on adult brains of guinea pig. The scraps were stained for myelin and perikaryons or impregnated according to two modyfications of Golgi method. The paramamillary nuclei contain the nucleus supramamillaris (Sm) and nucleus tuberomamillaris pars posterior (Tm). In Nissl scraps majority of Sm cells are spindle-shaped and rounded, whereas - Tm cells are multipolar as well. In Golgi preparations Sm cells are mainly bipolar with perikaryons from rounded to extremely elongated, fusiform (18-50 μm in long axis). They have 2 (rarely 3), medium size or thick dendritic trunks emanating from opposite poles. These bipolar cells were: 1- weakly arborised (up to 3°) with dendrites running according to prolongation of long axis of their soma, and they almost were devoided of dendritic appendages, which usually may be observed on distal dendrites; 2 -rich arborised (up to 5°) with relatively numerous knob-like appendages, which were observed also on primary dendrites. Often, only one dendritic trunk developes pen-shaped dendritic tree (rounded cells), or one dendritic trunk gives off collaterals and turns back in opposite direction (fusiform cells). The multipolar cells were numerous in Tm. They have 3-4 various thickness dendritic trunks. Their perikaryons possess rounded, triangular or quadrangular shape and sometimes irregular surface. Dendrites of rounded perikaryons were more arborised than triangular and quadrangular cells. Only in multipolar neurons it was able to observe collaterals of axons, which usually arise from dendritic trunk.

13.13 **THE NEURONAL STRUCTURE OF THE RED NUCLEUS IN GUINEA PIG: KLÜVER-BARRERA AND GOLGI STUDIES**

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The present studies were carried out on adult brains of guinea pig. The scraps were stained for myelin and perikaryons or impregnated according to two modyfications of Golgi method. The red nucleus in guinea pig consists of main, ventral, magnocellular part (RNm) and dorsally located, parvocellular part (RNp), more variable in shape, number and location of cell groups forming this part. Generally, the RNm is composed of large and medium-sized neurons, while the RNp - of medium and small cells, however occasionally in both parts different cells were met. On the basis of Golgi scraps, neurons of these two parts of the red nucleus were characterised. The RNm neurons, have stellate, multipolar or fusiform and pear-shaped perikaryons measure from 30-65μm in long axis. They possess 2-5 thick or conically emanating dendritic trunks, which after 6-40μm divide into 2°-4° dendritic branches. These ending branches usually are evident longer than their parent dendrites and possess a few knob-like spines. Some dendrites do not divide at all. The RNp perikaryons measure from 15-30μm in long axis (majority - about 20μm). They have usually piriform or fusiform shapes and 1-3 primary dendrites. The dendritic patterns some of them were similar to neurons of RNm. In other RNp cells, only thin primary dendrites immediately arising from the perikaryon were seen. A few neurons with enlargements on their dendrites and axon, resembling growth cone, were met too. In all kinds observed neurons, both in RNm and RNp, axon emerges from soma or from dendritic trunk at the distance 12-16μm from perikaryon.

13.14 **THE DISTRIBUTION OF SUPERIOR COLLICULUS INPUTS TO ARCHITECTONIC SUBDIVISIONS WITHIN PULVINAR IN MONKEYS: IN VIVO AND IN VITRO EXPERIMENTS.**

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Recent re-examinations of the architecture of the inferior pulvinar, PI, has revealed new divisions, changing the basic proposal that three nuclei posterior, medial, and central, subdivide PI in primates. The new interpretations of pulvinar organization raise a questions about how these subdivisions relate to inputs from the superior colliculus, SC. In present study, we examined pulvinar connections with the superior colliculus in New World monkeys, 3 owl monkeys (*Aotus trivirgatus*), 3 squirrel monkeys (*Saimiri sciurreus*) and 2 marmosets (*Callithrix jacchus*). In owl and squirrel monkeys these projection systems were traced *in vivo* by anterograde transport of 2% WGA-HRP injected into SC. 2-4 days after injections the brains were perfused with 2%-paraformaldehyde, removed, and stored in 30% sucrose overnight. Next day brains were cut into 40-50 um frozen sections in the coronal plane and reacted to reveal WGA-HRP. In marmosets crystal of fluorescent tracer DiI was deposited into fixed SC block*in vitro*, under the eye guidance. The brains were kept in fixative about 2 months at 40 C, and then cut on vibratome into 50um coronal sections, which were mounted unstained for fluorescence. The resulting patterns of WGA-HRP or DiI labeled terminals were related to the 4 architectonic nuclei of the inferior pulvinar distinguished in sections processed for CO, AChE, Cat-301, and Cb-D28K for owl and squirrel monkeys or myelinated fibers for marmosets. In all species SC inputs were mostly distributed in the pulvinar and in the dorsal lateral geniculate nucleus, LGNd. Within the pulvinar SC inputs were most dense in posterior (PIp) and central medial (PIcm) nuclei. Sparser inputs were to the central lateral nucleus (PIcL), and, in some cases, locations in the lateral and medial pulvinar. Medial nucleus of PI, PIm, was not labeled. In the LGN, terminals of axons coming from SC were sparse and unevenly distributed, aggregating in patches in the koniocellular (K) layers. *...Supported by NEI Grant EY02686.*

13.15 **NEURONES IN THE CERVICAL ENLARGEMENT OF THE CAT SPINAL CORD WITH COLLATERALS ASCENDING TO THE INFERIOR CEREBELLAR PEDUNCULE AND DESCENDING TO SACRAL SEGMENTS**

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Experiments were performed on eight α-chloralose anaesthetized cats. The electrophysiological method of antidromic stimulation and recording of intracellular or extracellular action potentials was used. The main purpose of this study was to demonstrate whether neurones located in the cervical enlargement gave off collateral branches ascending to the inferior cerebellar peduncle (restiform body, RB) and descending to the sacral segments (S1/S2). The recordings were taken from 78 neurones located in the grey matter of C6/C7 segments of the spinal cord. Four groups of cells of various projections were distinguished. In 23% out of the total sample bidirectional projection to RB and S1/S2 segments was established, in 14% bidirectional projection to RB and the level of Th13 segment was revealed, while in the remaining cases only descending branches to S1/S2 (48%) or the Th13 level (15%) were found. Axonal conduction velocities ranged from 15 to 83 m/s for branches ascending to RB and from 32 to 98 m/s for descending branches. It is suggested that parallel transmission of information to supraspinal and spinal centres plays important role in the process of movement coordination.

13.16 **DISTRIBUTION OF PONTOCEREBELLAR NEURONS WITH COLLATERALS TO THE PARAMEDIAN LOBULE IN THE RABBIT**

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Most of the cerebro-cerebellar information is relayed through the pontine nuclei (PN). Although several studies provide data on branching of individual axons in the pontocerebellar link, no one has up till now proved the existence of collateralization in the ponto-paramedian lobule pathway. In this study the method of retrograde fluorescent double labeling was used in the rabbit. After surgical preparation unilateral injections of 5% Fast Blue and 2% Diamidino Yellow were made into the cortex of rostral (forelimb region) and caudal (hindlimb region) sublobules of the paramedian lobule (PML), respectively. Apart from numerous single labeled neurons a small number of cells was double labeled in the bilateral PN with a very clear contralateral preponderance. It seems that the major divergent projections to PML arise from the paramedian and dorsolateral pontine nuclei and these from the lateral and peduncular pontine nuclei are minor. It is concluded that some of pontine neurons may give off axon collaterals that reach the rostral and caudal PML simultaneously. The present findings suggest functional meaning of the projection under study in the coordination of forelimb-hindlimb activity.

13.17 **THE COMPARATIVE ASPECTS OF THE OVERLAP BETWEEN LIMBIC AND SOMATO-MOTOR ZONES IN THE CLAUSTRUM**

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Although some experiments confirmed the existence of the limbic, motor, somatosensory and visual projecting zones in the rabbit and rat claustrum, their overlap have not been studied yet. Therefore the aim of our study was to compare the overlap of the claustral projecting zones in the rabbit and rat by means of retrograde transport of fluorescent tracers.

Nine adult Wistar rats and nine New Zeland rabbits were used for this study. The animals received combined injections of Fast Blue (FB) into the limbic cortex and Diamidino Yellow (DY) into the motor, visual or somatosensory fields. Next the animals were transcardially perfused and their brains were cut frontally into 50-μm–thick sections.

In the rat FB and DY-labeled neurons were distributed throughout the whole rostrocaudal extent of the claustrum but "motor" neurons predominated in the anterodorsal part, "somatosensory" - in the central and "visual" - in the posterior part of claustrum. "Limbic" cells were located mainly in its ventromedial portion and in the higher degree intermingled with neurons of other sensoric zones of this structure. In the rabbit, not like in the rat, the motor, somatosensory and visual projecting zones were more circumscribed and overlap with their limbic projection was observed in the anterior, central and posterior parts of the claustrum, respectively.

Our findings give support to the integrative role of the claustrum as the relay structure between different cortical areas of the cerebral cortex and the limbic system.

13.18 **INTERNAL CONNECTIONS WITHIN THE CLAUSTRUM OF THE RAT**

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Claustrum is a large subcortical structure consisted of two parts: dorsal (insular claustrum) and ventral (endopiriform nucleus). According to the topography of the connections of these parts of the claustrum with the neocortex and allocortex the theory about the claustrocortical interrelations was created. However, up to now, we have no information about the internal connections between these two parts of the claustrum. Eight adult Wistar rats were used for this study. The care and treatment of the animals were in accordance with the guidelines of the local ethical committee. After perfusion the brains were fixed in 4% formalin for three to seven days. Next they were cut frontally into the 5-mm-thick slabs and then the cristal of lipophilic carbocyanine tracer (Dil) was placed into the endopiriform nucleus or insular claustrum. After one to three months incubation in the dark place at room temperature the slabs were cut frontally on the vibratom into the 50-μm-thick sections. Every wet section was studied in a fluorescent microscope (LEICA DMLS).

After application of Dil into the insular claustrum labeled fibers in the endopiriform nucleus and the insular cortex were observed. After administration of the fluorescent tracer Dil into the endopiriform nucleus the labeled fibers were present in the insular claustrum mainly in its anterosuperior part as well as in the piriform cortex. We did not observe ramification of the projecting fibers.

The presence of connections between both parts of the claustrum may support the theory about the integrative role of this structure in the brain.

13.19 **VISUAL AREAS AND THEIR INTERHEMISPHERIC CONNECTIONS IN THE OPOSSUM, *MONODELPHIS DOMESTICA***

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Organization of neocortical areas and connections in the South American short-tailed opossum (*Monodelphis domestica*) is almost unknown. The only ones, that were localized, are the primary visual and somatosensory areas. In the absence of corpus callosum, opossums' neocortical hemispheres are connected via the anterior commissar. In our earlier investigations we localized area 17 in this species. Experiments on the closely related *Dydelphis* showed existence of the visual belt (areas 18 and 19). In the present experiments we studied thalamic and interhemispheric connections of the visual cortex. Either three or four injections of different retrograde and anterograde dyes were made into the primary and presumptive secondary visual areas of one hemisphere in each animal. After 3 to 5 days of survival the animals were anaesthetized, perfused with 0.9% NaCl solution followed by 4% paraformaldehyde in 0.1M phosphate buffer. The brains were cut on a cryostat at 50 μm. Cortex of two animals was flattened and cut tangentially to the pial surface, in three other opossums coronal sections were made. All sections were mapped with the aid of the MCID system. Some of them were later stained for myelin or for cytochrome oxidase. Analyzing the pattern of thalamic connections we showed the existence of the secondary visual areas. As in eutherians, visual areas of the opossum are visuotopically connected with the homonomic visual areas of the second hemisphere.

This work was supported by KBN Grant No 4PO5A.0811.11.

13.20 **SYNAPTIC CONNECTIONS FROM FORELIMB AFFERENTS TO NEURONES LOCATED IN THE CERVICAL SPINAL CORD THAT PROJECT TO SACRAL SEGMENTS AND THE CEREBELLUM IN THE CAT**

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Neurones distributed in C7-Th1 segments of the spinal cord were electrophysiologically investigated in α-chloralose anaesthetized cats. Intracellular recordings were made from cells with branching axons that were antidromically activated from ipsilateral or/and contralateral sacral segments of the spinal cord and from the ipsilateral inferior cerebellar peduncle. Postsynaptic potentials were recorded following electrical stimulation of afferent fibres in branches of the ipsilateral forelimb nerves: median, ulnar, superficial radial and deep radial. Both excitatory and inhibitory effects from muscle and skin afferents were determined in these neurones, however the dominating action was inhibition. Polysynaptic IPSPs from group I and II muscle and high threshold afferents were revealed in the majority of cases. However, in a proportion of neurones monosynaptic EPSPs from group I and disynaptic EPSPs from group II muscle afferents could be evoked. Excitatory and inhibitory actions from skin afferents were less frequently observed. The results suggest that double direction neurones under the study integrate information from the forelimb and send it simultaneously to spinal and supraspinal centres. It is likely that they subserve the system involved in the coordination of movements of fore- and hindlimbs.

Session 14 - Poster Session: Hippocampus

14.1 ASYMMETRY OF BIDIRECTIONAL CONNECTIONS BETWEEN DORSAL HIPPOCAMPUS AND THE RHINAL CORTEX AREAS IN THE DOG (*Canis familiaris*).

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Connectivity of the hippocampus with entorhinal and perirhinal cortices were examined. Injections of the fluorescent tracers (FB, DY) and biotynylated dextran amine (BDA) were placed in the dorsal hippocampus.

Retrogradely labelled cells were found exclusively in the dorsolateral area (DLEA) and ventrolateral area (VLEA) of the entorhinal cortex. In DLEA, the majority of labelled cells were distributed in layer III, and a smaller number of cells in layer II were observed. However, in the part of DLEA adjacent to the VLEA area, the labelled cells were equally grouped in both layers. In contrast to that, in VLEA not numerous labelled cells in layer II were found. The most medial part of entorhinal cortex was free of labelled cells. The distribution of retrogradely labelled cells suggests the existence of lateromedial gradient of intensity of the entorhino-hippocampal projection.

In contrast to retrograde labelling, the dense anterogradely labelled axonal terminals were followed into both the entorhinal and perirhinal cortex. The main projection reached the internal lamina of the DLEA with continuation of labelling in the field 35 of the perirhinal cortex. Some axons passed into the neighbour parts of VLEA and to the field 36, forming moderate density of terminals. Additionally, in the external lamina of DLEA, not numerous labelled axonal terminals were observed.

Thus, the dorsal hippocampus has bidirectional projections with DLEA and lateral part of the VLEA, whereas, its projection to the perirhinal cortex is unidirectional.

Sponsored by James McDonnel Foundation grant No. 95-13.

14.2 INTRAHIPPOCAMPALLY ADMINISTERED NMDA ENHANCES VASOPRESSIN (AVP) RELEASE INTO THE MICRODIALYSATE OF THE HIPPOCAMPUS.

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Vasopressin may facilitate the consolidation of memory from short - term to long - term memory stores; it has been demonstrated in animal studies and amnesic syndromes. The hippocampus seems to be a structure in which AVP plays a physiological role in the consolidation of information.The microdialysis technique allows to study AVP concentration in the extracellular fluid of the hippocampus and in the caudate nucleus of rabbits in chronic experiments. Microdialysis probes were inserted into both structures and microdialysis was performed three times weekly in male rabbits. AVP concentration was measured in the 180-min. samples of outflowing fluid by radioimmunoassay. In all rabbits AVP concentrations were different and variable in succeeding samples in both structures. In control rabbit AVP concentration in the fluid perfusing the hippocampus (9.6±3.3 pg/sample) was significantly higher than AVP concentration in the fluid perfusing the caudate nucleus (5.2±1.1 pg/sample) (mean±SE). In experimental rabbits 56 mM K^+ fluid perfusing both structures enhanced about 3.4 fold AVP release in the caudate nucleus and about 2.5 fold in the hippocampus. NMDA in perfusing fluid (concentrations from 1 mM to 32 mM) intensified about 3.6 times AVP release in the hippocampus. Dose dependence was not observed. *Supported by Medical University of Lodz.*

14.3 COMPARISON OF THE EFFECTS OF SEROTONIN IN THE HIPPOCAMPUS AND THE FRONTAL CORTEX

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Serotonin (5-HT) has been linked to a large array of behaviors and clinical conditions and drug actions. The great diversity of 5-HT receptors and their differential distribution in different neuronal populations underlies such a broad range of functions.

Hippocampus and frontal cortex receive serotonergic innervation and neurons in both areas express diverse types of 5-HT receptors. Moreover, different 5-HT receptors are often coexpressed by the same neuron. We studied the effects of 5-HT and 5-HT receptor subtype selective agonists on electrophysiological properties of neurons in the rat hippocampal and cortical slices, using extracellular and intracellular recording. In hippocampal principal cells inhibitory effects of 5-HT predominated. They were mediated directly by 5-HT_{1A} receptors linked to the opening of K^+ channels and indirectly by 5-HT_4 and probably also 5-HT_3 receptors, via excitation of inhibitory interneurons. 5-HT increased excitability of hippocampal neurons via 5-HT_4 receptors through a reduction in the calcium-dependent K^+ conductance. In cortical neurons 5-HT evoked predominantly excitatory responses which involved actions at 5-HT_2 receptors. However, similarly like in the hippocampus, 5-HT enhanced inhibitory synaptic transmission in cortical slices. Therefore, the cellular actions of 5-HT in cortical areas involve both direct and network effects which involve diverse 5-HT receptor subtypes.

14.4 CORTICOSTERONE-INDUCED CHANGES IN HIPPOCAMPAL 5-HT RECEPTORS

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It has been suggested that functional interactions between corticosteroids and the central 5-HT system may provide a biochemical basis for hormonally induced alterations in mood in behavior. Animal studies have shown that corticosteroids can alter several elements of 5-HT neurotransmission, in part via regulation of 5-HT receptor expression. Steroid - 5-HT receptor interactions within the hippocampus have been established. Hippocampal pyramidal neurons express at least two subtypes of serotonergic receptors, 5-HT_{1A} and 5-HT_4. These receptors have opposite, inhibitory and excitatory effects, respectively, on neuronal excitability. The present study was aimed at determining the effect of repeated administration of corticosterone (10mg/kg, twice daily for 7 days) on neuronal responsiveness to 5-HT, the selective 5-HT_{1A} receptor agonist 8-OH-DPAT, and the selective 4-HT_4 receptor agonist zacopride. The experiments were performed *ex vivo*, in the CA1 area of rat hippocampus, using extracellular and intracellular recording. Treatment with corticosterone significantly decreased the inhibitory effect of 8-OH-DPAT and 5-HT and increased the excitatory effect of zacopride. It is concluded tnat repeated treatment with corticosterone shifts the function of the 5-HT system in the hippocampus towards the 5-HT_4 -receptor mediated increase in neuronal excitability.

Supported by the KBN grant 4PO5A09214

14.5 THE EFFECTS OF TEMPERATURE ON HIPPOCAMPAL THETA-LIKE ACTIVITY PARAMETERS RECORDED *IN VITRO.*

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In vitro experimental model has been successfully used in a number of investigations concerning physiology and pharmacology of rhythmical slow activity. The effect of temperature of the bathing medium on theta-like activity parameters recoreded from rat's hippocampal formation slices was estimated in the present study. Hippocampal activity was registered from CA3c area of hippocampus proper in the presence of 50 μM carbachol. Registration was performed in temperature range from 18 to 42°C. Frequency, amplitude, time duration of subsequent theta trains and intertrains intervals were measured. The highest amplitude and frequency of theta rhythm were observed in temperature range 34 - 38°C. Time duration of theta trains and intertrains intervals were relatively longer in the lower temperature (30 - 32°C). Theta activity was not observed in temperature lower than 30°C and higher than 38°C (only epileptic discharges were obeserved). Similarities between *in vitro* and *in vivo* theta activity generation are discussed.

14.6 THETA-LIKE ACTIVITY IN THE HIPPOCAMPAL FORMATION *IN VITRO:* AMPLITUDE AND PHASE PROFILE.

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Laminar profile of carbachol induced theta-like activity was studied in the hippocampal formation slices. One recording electrode was positioned in the stratum oriens of CA1 (reference). Depth profile was constructed by tracking the second recording electrode (roving) in 100 μm steps through CA1, DG and CA3C regions perpendicular to the hippocampal fissure. Three amplitude maxima were found. One was placed in the stratum oriens of CA1, the second in the stratum moleculare of the dorsal blade of dentate area, and the third one was placed just above the CA3C piramidal cells. In addition, we observed two gradual phase shifts of carbachol induced theta-like activity. The first one occurred in the stratum radiatum, and the second one in the hilus. Our findings closely resemble the type II profile observed in freely moving and urethane-anestheized rats. Neural mechanisms responsible for a production of *in vitro* and *in vivo* theta are discussed.

14.7 THE EFFECT OF POSTERIOR HYPOTHALAMIC INJECTION OF PROCAINE ON HYPOCAMPAL THETA RHYTHM IN THE CAT

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The earlier study demonstrated that PH region (posterior hypothalamus and supramammilary nuclei) comprises a critical part of ascending brainstem synchronizing pathway, responsible for a production of hippocampal (HPC) theta rhythm in rats. In addition, it was also reported that inputs ascending from the brainstem caudal to PH, contribute primary to the frequency of HPC theta and secondarily to its amplitude. In a present study we attempted to provide further support for the above mentioned hypothesis using freely moving cats. Local anaesthetic, procaine, was microinjected into PH region (20%/4μl). The inactivation of PH resulted in abolishment of the HPC theta rhythm.These data confirm a critical role played by the PH region in the generation of HPC theta rhythm in cats.

14.8 INTRASEPTAL MICROINJECTIONS OF GABA AGONISTS: EFFECTS ON CARBACHOL INDUCED HIPPOCAMPAL THETA.

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Hippocampal formation theta field activity depends on integrity of cholinergic/GABA-ergic neurons of the medial septum/vertical limb of diagonal band of Broca. Cholinergic nature of hippocampal theta rhythm was demonstrated many years ago. In the present study intrahippocampal microinjections of carbachol (cholinergic agonist) induced well-synchronized long lasting episodes of theta activity. This effect was observed at least for 30 min. The effect of intraseptal microinjections of the GABA-A and GABA-B agonists (muscimol and 3 - aminopropyl (methyl) phosphine acid = SKF-98541, respectively) on carbachol-induced theta activity was investigated in freely-moving cats. We did not observe any changes in cholinergically-induced hippocampal theta after intraseptal bilateral microinjections of GABA-A and GABA-B agonists in the first 30 to 40 min. Then, the blocking effect of GABA agonists on theta rhythm was observed. The effect of medial septal GABA-ergic system on hippocampal theta activity evoked by local cholinergical excitation is discussed.

14.9 ACOUSTIC STARTLE REFLEX IN RATS WITH RADIATION-INDUCED HIPPOCAMPAL LESION

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Fear conditioning during spatial learning depends on the hippocampus. Lesion of this structure disrupt conditioned freezing to the context but not to the explicit cues. Damage of the CA3 hippocampal area produces hyper-reactivity to sensory stimulation. Locomotor hyperactivity following hippocampal granule cell removal has also been reported. The main question of the study was: to what extend a neonatal radiation-induced hippocampal lesion leads to emotional changes in adulthood? Acoustic startle response (ASR) was studied in two groups of adult rats. The rats from the first group were exposed to neonatal x-ray irradiation. Their ASR responses were compared with those from the intact rats which formed a control group. The ASR was tested during two sessions with different illumination of acoustic chamber. During the first session the rats were tested in the darkness while during the second test was performed in the illuminated acoustic chamber. The irradiation resulted in a significant reduction of granule cells of the hippocampus (about 60%). The lesion resulted in emotional and behavioral changes evidenced by modification of the acoustic startle response. The irradiated rats exhibited significantly increased amplitude of startle response. In contrast to the light condition, the darkness context caused decline of the ASR amplitude in the control group and not significant changes in the lesioned animals. The results support the hypothesis that a damage of the hippocampus disrupt the motor inhibition.

14.10 CONDITIONED SUPPRESSION AND CONDITIONED INHIBITION IN RATS AFTER RADIATION INDUCED HIPPOCAMPAL DAMAGE.

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The effects of neonatal X-ray irradiation on learning abilities were studied in two groups of adult rats. The rats of experimental group (Group H, 14 Ss) were exposed to neonatal X-ray irradiation (600 rads) 6-18 h after birth. They showed a significant reduction of granule cells in the hippocampus (about 60%), compared with the control rats (Group C, 10 Ss). All animals underwent a conditioned emotional response (CER) training in which classical defensive conditioning was superimposed on on-going alimentary instrumental bar presses. In this training two stimuli were used: 70 dB wide-band noise as conditioned defensive stimulus (CS), and darkness as conditioned inhibitor (CI). The CS applicated alone elicited suppression of bar presses, whereas the occurrence of CI evoked the gradual weakening of suppressive effect of CS. In Group H an acquisition and stability of CER in consecutive stages of experiment were significantly stronger than in Group C. Nevertheless, a clear impairment in development of weakening effects of the CI on the CS presented in a compound (CI+CS) disappeared during retraining. These results suggest that the X-ray irradiation damage of the hippocampus attenuate the acquisition of the conditioned inhibition task but during prolonged training this deficit can be compensated.

14.11 THE EFFECTS OF HIPPOCAMPAL LESIONS ON THE REVERSAL OF PERFORMANCE IN PROBABILISTIC CHOICE.

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The purpose of this experiment was to investigate the memory processes involved in the reversal of discrimination learning and the role of hippocampus in these processes. Fourteen rats were rewarded with food for pressing bars in several forms of „two-armed bandit" situation (Davis and Staddon, 1990). The animals were submitted to a choice situation in four conditions: L (only left responses rewarded), R (only right responses were rewarded), N (extinction) and F (forced-alternation). After the training eight animals were submitted to a neurotoxic lesion in dentate gyrus of hippocampus, by intra-dentate injections of colchicine and six rats were submitted to a sham lesion. After the recovery from the surgery, the animals were retrained as in the pre-lesion trials. The pre- and post-lesion data were compared. The statistical analysis revealed no difference between the experimental and the sham groups. The post-lesion trials did not show effects of the lesion in dentate gyrus of hippocampus on reversal performance of rats submitted to choice situations. Lesion in dentate gyrus did not affect the reversal performance of rats in probabilistic choice perhaps because the task used involves an egocentric strategy that does not require hippocampal function.

Financial Support: Fapesp, Capes and CNPq.

Session 15 - Poster Session: Calcium binding proteins

15.1 METAL CATIONS DECREASE THE CALCIUM-BINDING CAPACITY OF NEURONAL CALRETININ

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Calretinin (CR) is a neuronal, Ca^{2+}-binding EF-hand protein of unknown function. EF-hand proteins act as Ca^{2+}-buffers (protecting cells from excess intracellular Ca^{2+}-influxes) and/or Ca^{2+}-sensors (forming part of Ca^{2+}-regulated pathways induced by intracellular Ca^{2+}-influxes). We have used a competitive $^{45}Ca^{2+}$-overlay technique to measure the effect of metal cations on the Ca^{2+}-binding capacity of CR. The method employs variable amounts of dot-blotted pure proteins, 1.5 μM concentrations of $^{45}Ca^{2+}$ with 10 μM concentration of competing metal cation. Visualization is by exposure to film and densitometry to measure the amount of $^{45}Ca^{2+}$ cations bound per protein molecule. It provides a fast overview of the general metal-binding properties of EF-hand proteins that might be relevant to their function(s). Our results show that Tb^{3+}, Cu^{2+} and Pb^{2+} reduce the capacity of CR to bind Ca^{2+} whereas Mg^{2+} and Cd^{2+} do not. The results of tryptophan fluorescence experiments support ≥10 μM affinities of Tb^{3+} and Cu^{2+} for CR. The ability to bind metal cations, other than Ca^{2+}, would affect both the proposed functions of CR as a Ca^{2+}-buffer (reducing the Ca^{2+} capacity) or Ca^{2+}-sensor (as an antagonist or modulator of Ca^{2+}-binding). Metal cations, such as Cu^{2+}, are linked to the same neurodegenerative diseases as CR - could CR play a protective role against Cu^{2+} and other metals? Reports that S100B, an EF-hand protein, binds Cu^{2+} and may protect against Cu^{2+}-mediated oxidation damage [Nishikowa et al., J.Biol.Chem, 272, 23037-41, 1997] and the relationship between an organotin compound and CR expression levels [Geleso et al., Exp.Neurol., 154, 645-53, 1998] suggests such a function might exist. This work was supported by a grant to PG from the State Committee for Scientific Research No 6 P04B 010 15.

15.2 DISTRIBUTION OF DIVERSE CALCIUM BINDING PROTEINS AND CELL ADHESION MOLECULES IN THE BARREL CORTEX OF MICE LACKING CELL ADHESION MOLECULES

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Adhesion molecules have been implicated to play important roles in morphogenesis, regeneration and synaptic plasticity in the brain. There are indications that these molecules are involved in events underlying cortical pattern formation e. g. the barrel field formation in the primary somatosensory cortex (*Dev. Biol.*1989, 131: 243-260).

The aim of the present study was to investigate how knockout of NCAM, L1 and tenascin R genes affect distribution of specific calcium binding proteins and cell adhesion molecules in the barrel cortex of adult mice. In these knockout mice the pattern of thalamocortical afferents innervating barrel cortex seems to differ from that in the wild type mice. We used immunocytochemistry to visualize calbindin, parvalbumin, Wisteria floribunda agglutinin (WFA) and HNK-1 in frontal sections from the brains of knockout and wild type mice. In NCAM knockout mice the modular organisation of layer IV of the barrel cortex was difficult to distinguish in Nissl stained sections. Nevertheless, the distribution of parvalbumin, calbindin, WFA and HNK-1 immunoreactivity cells and immunopositive profiles revealed clear modular (patchy) distribution in layer IV. In the other two knockouts the barrel cortex cytoarchitectonics of layer IV showed faint modular organization. The immunoreactivity of parvalbumin, calbindin, WFA and HNK-1 revealed patchy pattern of distribution within layer IV of barrel cortex. However, the barrel cortex of tenascin R knockout mouse expressed substantially less HNK-1 immunoreactivity than other knockouts and wild mice.

15.3 CO-LOCALIZATION OF THE CALCIUM BINDING PROTEINS AND RETROGRADE TRACERS IN THE RAT BASOLATERAL AMYGDALA

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The co-localization of the calcium binding proteins, calbindin-D28k or parvalbumin, and the retrograde tracer, Fluoro-Gold in the basolateral amygdala of the rat were studied using the two-laser, two color confocal approach. The study was performed in five adult rats in which the Fluoro-Gold was injected into the frontal association cortex. Then the localization of the retrogradely labeled neurons in the basolateral amygdala was compared with the localization of the calcium binding proteins positive neurons visualized by the standard immunohistochemical method with primary antibody against calbindin-D28k or parvalbumin.

The present study has shown that most of the retrogradely labeled neurons in the posterior part of the basolateral amygdala were also calbindin-positive. PV-ir endings are present around the projective neurons, while the retrogradely immunopositive labeled puncta were observed also around the inhibitory cells. It suggests that in the basolateral amygdala there is a specific loop between the projective and inhibitory neurons. The projective neurons activate the inhibitory cells that inhibit the activity of neurons sending axons out of the amygdaloid body. On the other hand the presence of the calcium binding protein in the projective neurons of the basolateral nucleus can protect the amygdalocortical loops from various types of damaging agents.

15.4 DISTRIBUTION OF A NOVEL CALCYCLIN BINDING PROTEIN (CacyBP) IN THE RAT BRAIN

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Calcyclin (S100A6) is a calcium binding protein belonging to the S-100 family. Originally the protein has been purified from Ehrlich ascites tumor (EAT) cells but also it has been found in tissues such as brain, lung, stomach and placenta. The search for calcyclin function has led us to the identification in EAT cells and in the mouse brain, of a 30 kDa protein that binds calcyclin. The protein was named calcyclin binding protein (CacyBP). A clone of CacyBP was isolated from the mouse brain cDNA library and sequenced. The full sequence of this clone showed no homology to sequences in a GenBank which indicated that CacyBP is a novel protein. Recombinant CacyBP was expressed in *E. coli* and shown to bind calcyclin in a physiological range of calcium concentration. Polyclonal antibodies against CacyBP were produced and used for the analysis of its distribution in different rat tissues. Western blots using these antibodies showed the highest expression of CacyBP in the rat brain, liver, spleen and stomach. Thus, the cellular localization of this protein in the brain by immunohistochemistry was studied. It was found that CacyBP is expressed in some neurons of the hippocampus, cerebellum and cortex. To learn more about its subcellular localization of CacyBP, the nuclear, cytosolic, mitochondrial and microsomal fractions from rat brain were prepared. The western blot using antibodies showed the highest level of CacyBP in the cytosol and moderate in the endoplasmatic reticulum. This work was supported by grant 6P04A05415 from the State Committee to Scientific Research.

15.5 **Distribution of calbindin-D28k and parvalbumin immunoreactive neurones in the cerebral cortex of rats neonatally treated with competitive NMDA receptor antagonist.**

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Our previous findings revealed that neonatal blockade of NMDA receptors in rats results in psychotic-like behavioural disturbances in adult life such as increased responsiveness to novelty, cognitive deficits and enhancement of aggressive behaviour. The present study was undertaken to characterise the morphologic organisation of the cerebral cortex following this treatment. As an immunohistochemical marker of cortical neurones we used antibody against calbindin-D28k (CB) and parvalbumin (PV), the calcium-binding proteins which label different populations of presumably GABAergic interneurones which have complementary distribution in the cerebral cortex.

Animals were treated with competitive antagonist of NMDA receptor CGP 40 116 during the first three weeks of life, while the immunohistochemical experiments were performed on adult rats (60 days old).

So far in animals after blockade of NMDA receptors in neonatal period we observed a reduction (c. a. 30%) in a number of calbindine-D28k immunoreactive neurones in the cingulate cortex, while no modifications in the number of parvalbumin-immunoreactive cells were detected.

This result indicate that the disruptions of glutamate transmission in the early period of life causes long-lasting changes in the cytoarchitecture of the cerebral cortex seen as a reduced population of CB-positive neurones.

Session 16 - Poster Session: Motor processing

16.1 **INFLUENCE OF 8-OH-DPAT, A SELECTIVE 5-HT$_{1A}$ RECEPTOR AGONIST, ON THE HALOPERIDOL-INDUCED MUSCLE RIGIDITY IN RATS.**

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Some anatomical and behavioural data suggest that serotonin (5-HT) may be implicated in motor functions. Although neuroleptics are believed to cause extrapyramidal symptoms by blocking the striatal dopamine receptors, it is suggested that the 5-HT-mediated transmission is also involved in these effects. However, no information is available on the role of serotonin receptors in the neuroleptic-induced muscle rigidity in rats. 8-OH-DPAT (8-hydroxy-2(di-N-propylamino) tetralin), a selective 5-HT$_{1A}$ receptor agonist, has been found to reduce the synthesis of 5-HT in the brain and to induce behavioural effects compatibile with the reduced central function of 5-HT. The aim of present study was to find out whether 8-OH-DPAT was capable of counteracting the haloperidol-induced muscle rigidity. The muscle tone was measured as mechanical resistance of the hind foot to passive movements in the ankle joint. The reflex EMG activity of the gastrocnemius and tibialis anterior muscles was simultaneously recorded. To increase the muscle tone, the animals were pretreated with haloperidol (1mg/kg). 8-OH-DPAT (0.125, 0.25 and 0.5 mg/kg), injected 1h later, caused a significant and dose-dependet decrease in both muscle resistance (MMG) and the EMG activity in examined antagonistic muscles. The obtained results suggest that 8-OH-DPAT is effective in relieving the haloperidol-induced muscle rigidity.

16.2 **SCH 58261, AN ADENOSINE A$_{2A}$ ANTAGONIST, COUNTERACTS THE RESERPINE-INDUCED MUSCLE RIGIDITY IN RATS**

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A number of studies with selective antagonists of A$_{2A}$ receptors have shown that these compounds suppress parkinsonian-like symptoms such as, catalepsy or akinesia in rodent and primate models of Parkinson's disease (PD). However, it is well known that in the course of PD - besides akinesia and tremor - there also occurs muscle rigidity. Recently we showed that blockade of adenosine A$_{2A}$ receptors by the selective antagonist SCH 58261 counteracted the muscle rigidity induced by haloperidol and potentiated the effect of a subtreshold dose of L-DOPA in that model. The aim of the present study was to find out whether SCH 58261 influenced the parkinsonian-like rigidity induced by reserpine in rats. The muscle rigidity was measured using a mechanomyographical method (MMG). Reserpine was injected in a dose of 5 mg/kg ip, and 16 hours later the rats were given α-methyl-p-tyrosine (250 mg/kg ip). Measurements started 20 h after reserpine injection and lasted 90 min. Reserpine increased the muscle resistance developed in response to both passive extension and flexion of the hind foot in the ankle joint. SCH 58261 in doses of 1 - 5 mg/kg ip did not influence per se muscle tension in rats. However, SCH 58261 injected 15 min before reserpine caused a significant decrease in the reserpine-induced muscle rigidity in rats. The present results suggest that selective antagonists of adenosine A$_{2A}$ receptors may be useful as a new approach in the treatment of Parkinson's disease.

16.3 EFFECT OF SUBSTANCE P ON A MOTOR SYNAPSE

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Substance P (SP) responses were studies on phrenic motoneurons (PM) in the *in vitro* brainstem spinal cord preparation, using the whole-cell current- and voltage-clamp technique. SP evoked significant membrane potential depolarisation of PM, a tonic activity and an increase duration of the motoneuron population discharge (PD).

The neurokinin receptor agonist NK_1 (10^{-7} and 10^{-8}M) depolarised PM. NK_2 agonist (10^{-7} and 10^{-8}M) induced marginally significant depolarisation and no effect on membrane input resistance. NK_3 receptor agonist induced significant effects when applied at 10^{-7}M. In TTX containing aCSF, only SP and NK_1 agonist remained active.

In current-clamp mode, SP significantly increased PM input resistance, decreased the rheobase current and shifted the Intensity/Frequency curves leftwards. Spike frequency adaptation and single spike configuration were not modified by SP. SP increased significantly the duration of the spontaneous action potential discharge.

AP-5 (10^{-4}M) application at the spinal cord significantly decreased the PD duration without affecting the amplitude and blocked the effect of SP.

In voltage-clamp mode, under TTX, SP produced an inward current (I_{SP}) averaging 87±50 pA. The response was not significantly reduced by extracellular application TEA, Co^{2+}, Cs^{+} or 4-AP. I_{SP} was blocked only by muscarine. When using hyperpolarising voltage stepping protocols from -30 to -60 mV after a -60 to -30 mV depolarising prepulse, no PM displayed any M-like potassium current. I_{SP} displayed no voltage sensitivity over the range -100 to 0 mV with voltage ramp protocols. It reversed near the expected K^{+} equilibrium in a $[K^{+}]_0$ 3 mM.

It is suggested that SP tunes the gain of this motor synapse by acting postsynaptically. The modulation is obtained through an effect of SP on the NMDA component of synaptic input and reduction of a leak current.

16.4 RECOVERY OF HINDLIMB LOCOMOTOR FUNCTIONS AFTER DIFFERENT PARTIAL SPINAL LESIONS IN RATS

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The hindlimb locomotor deficits were analyzed in rats subjected to spinal lesions, of different extent, in order to establish a template for further studies on the effectiveness of various reparative techniques on motor recovery after partial spinal lesions. The operated animals were divided into 3 groups: I. with complete or almost complete spinal lesions (n=4); II. with ventral funiculi or parts of them spared (n=5) and III. with a lesion confined to dorsal columns (n=1). Motor deficits were assessed by quantified observation of locomotion on a flat surface and on a horizontal ladder and by footprint analysis.

The speed and the degree of locomotor recovery depended on the extent of lesion. In group I of animals the ability to keep the hindlimbs flexed in all the joints when sitting and to perform flexions in the hip and ankle joints when the animals displaced themselves on the forelimbs, returned 2 to 3 weeks after surgery. No further improvement was observed in these animals in later postoperative periods (36 to 154 days). The animals from group II resembled those from group I in the first two postoperative weeks. However, during the 3rd and 4th weeks, the ability to support the hindquarters returned, so that the animals could walk by themselves. The hindlimb support functions successively improved and reached a relatively stable level after 6-7 weeks. However, up to the end of postoperative observation (62-119 days) the animals walked on a broader base and often defectively placed the feet. The analysis of footprints showed an almost twice increase in the support base. In animals walking relatively well on a flat surface, walking along a ladder disclosed still deficiencies (missing of rungs). When the lesion was confined to the dorsal columns, (group III) the locomotor deficits were compensated during the first 4 weeks after surgery.

In animals from group I and II, the selected antigravity muscles (mm. soleus and vastus lateralis) or the tibial nerve were stimulated with an electric current, after the animals had reached a plateau, to examine the effectiveness of this method to restore (group I) or improve (group II) the hindlimb support functions. The results obtained did not indicate a perceptible amelioration of these functions.

The results of this study show that the ventral funiculi, or even parts of them, are sufficient for the spontaneous recovery of hindlimb support functions in locomotion, although the locomotion itself displays some long-lasting deficits as evidenced by adequate behavioral tests.

16.5 EMG OF FORE- AND HINDLIMB EXTENSOR AND FLEXOR MUSCLES DURING SLOPE WALKING IN THE RAT

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The aim of our study was to characterise the fore- and hindlimb muscle activity during various task of quadrupedal locomotion of freely moving rats. Ten 4-month-old adult Wistar rats were trained to walk on a 2 m long runway placed horizontally or at various angles. During each run the EMG activity of physiological flexor and extensor muscles (biceps and triceps or extensor carpi radialis muscles and flexor digitorum profundus in forelimbs, tibialis anterior or extensor digitorum longus and soleus muscles in hindlimbs) was recorded. The stance and swing phases of each limb were recorded simultaneously using contact electrodes. During each experimental session each animal was forced to walk along the runway which was placed consecutively at various slopes (horizontally, +18° and -18°). Examination of the flexor and extensor EMG burst activity showed a significant correlation of their onsets with the onsets of swing and stance phases during locomotion in all tested experimental conditions. The most interesting finding is the significant ($p<0.0001$) increment in amplitude of stance-related EMG burst activity obtained in forelimb muscles during up-slope locomotion in comparison to down-slope locomotion. In contrast, in EMG activity of hindlimb stance-related muscles (m. soleus) this difference was not observed. However, the soleus muscle displayed the increased number of high amplitude EMG signals during up-slope locomotion. The greater changes that occurred in the activity of forelimb than hindlimb extensor muscles during up-slope walking might be due to the different role of the fore- and hindlimbs in various locomotor tasks in freely moving rats.

(Supported by KBN grant 4.PO5A.115.12, Poland)

16.6 FUNCTIONAL CHANGES IN SOLEUS MUSCLE AFTER ITS TRANSPOSITION INTO THE PLACE OF ANTAGONISTIC EXTENSOR DIGITORUM LONGUS IN THE RAT

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A functional recovery after peripheral or central nervous system injury is observed in mammals due to plasticity of neuronal connections. The aim of this study was to examine whether transposition of soleus muscle (Sol) into the flexor position will result in functional readjustment of the transposed muscle. Transposition of the Sol (with intact nerve and blood vessels) into the bed of the extensor digitorum longus muscle (EDL) was done in the left hindlimb in 7 ratlings (6 days old) and 6 adult rats.

Three months after the transposition a visual inspection of locomotor movements of these rats did not show any difference from the intact ones. To asses the functional readjustment of the transposed muscle, the EMG activities of the transposed Sol, gastrocnemius lateralis and tibialis anterior of the left hindlimb, and tibialis anterior, EDL and Sol of the contralateral hindlimb were recorded during locomotion, scratching and reflex responses to passive dorsi- and plantar-flexion of ankle. During locomotion the Sol transposed in 2 adult rats showed additional burst of EMG activity corresponding with the flexor pattern, while the EMG activity in 4 other adult rats showed locomotor pattern typical for intact animals. In all rats operated as ratlings additional burst was present during flexor activity in all muscles transposed, moreover, they could be activated both by dorsi- and plantar-flexion, when recorded 3 months after transposition.

The results showed that spinal network generating the locomotor pattern is able to readjust to the new situation of transposed soleus muscle. This readjustment is easier in half-grown animals than in adults. Nevertheless, the innate activity pattern of the transposed soleus muscle is still prevailing.

16.7 DIFFERENCES IN THE SHAPE OF UNFUSED TETANI OF FAST FATIGABLE AND FAST RESISTANT MOTOR UNITS IN THE RAT MEDIAL GASTROCNEMIUS MUSCLE

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In a majority of studies of motor units the division of these units into fast and slow has been based on the "sag" profile in unfused tetanus. The time course of the sag in unfused tetani of fast units was analyzed in the present study. Fast motor units of rat medial gastrocnemius were classified as fast fatigable (FF) or fast resistant (FR) on the basis of a fatigue index calculated during the standard fatigue test. In middle-fused tetani (fusion index 0.25-0.75), it was observed that for FF motor units the sag was shorter and occured earlier than for FR units. Moreover, in FF units, the sag was followed by potentiating tension, whereas for FR units this potentiation was weaker or even absent. A tetanus shape index, which expressed the ratio of the area of the first part of tetanus record (between the tension record and the baseline, from the beginning of tetanus up to the lowest point during the sag in force recording) to the area under the second part of tetanus (from this lowest point up to end of the recording) was introduced. For FF units, this index ranged from 0.13 to 0.47, whereas for FR units it ranged from 0.54 to 17.8 (with one exception). These results showed that the difference in unfused tetanus expressed in this tetanus shape index could be used as an accurate alternative method of dividing fast units into FF and FR groups. Moreover, the difference in sag time course in FF and FR units suggests that the metabolisms responsible for this contractile phenomenon have significantly different time courses in IIa and IIb muscle fibers, constituting FF and FR units, respectively.

ALDRIN-INDUCED STIMULATION OF LOCOMOTOR ACTIVITY AND BRAIN REGIONAL GABA 16.8

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Aldrin (2-5 mg/kg/day, p.o.) stimulates locomotor activity (LA) of rats in a dose dependent manner maximally following its treatment for 12 consecutive days. Treatment of aldrin for 30 consecutive days normalised the aldrin-induced increase in LA. Single dose of aldrin (2-5 mg/kg, p.o.) significantly increased the steady state level of γ-aminobutyric acid (GABA), activities of glutamic acid decarboxylase (GAD), and GABA-transaminase (GABA-T) and also EOS-induced GABA accumulation in cerebellum, hypothalamus and pons-medulla. The [^{3}H]-GABA binding under similar condition with aldrin was significantly reduced in all the above mentioned brain regions. Treatment for 12 consecutive days with aldrin produced more effect in those parameters in different brain regions than that observed with single dose of aldrin. It was also found that aldrin under these conditions produced greater effect in those parameters in cerebellum than that observed in hypothalamus and pons-medulla. These results, thus, suggest that aldrin-induced stimulation of LA may be correlated with the region specific inhibition of GABAergic activity. The degree of aldrin-induced effect depends on the regions of the brain and dose and duration of the aldrin treatment under its short-term condition.

(Supported by University Grants Commission, New Delhi and University of Calcutta, Calcutta, India.)

16.9 MODULATIONBY SEROTONIN (5-HT) AND NORADRENALINE (NA) OF ACTIVATION OF SPINAL INTERMEDIATE ZONE INTERNEURONES BY GROUP I AND II MUSCLE AFFERENTS.

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Modulatory actions of serotonin (5-HT) and noradrenaline (NA) on transmission from group I and II muscle afferents to spinal interneurones were tested in deeply anaesthetised cats. 5-HT and NA were applied ionophoretically close to interneurones with input from group II afferents (n = 19) or from both group I and II muscle afferents (n = 16) in intermediate zone in L4 and L5 segments. 5-HT and NA actions were assessed from responses of single interneurones to electrical stimulation of peripheral nerves. The interneurones were recorded from at the depths at which distinct components of field potentials were evoked by group I (early component) and group II (later component) muscle afferents. The responses were classified as evoked by group I afferents when they were evoked by stimuli (1.5T) which were sub-threshold for group II afferents and/or were evoked at segmental latencies (0.9-1.5 ms from group I incoming volleys) which were too short to be compatible with synaptic actions of group II afferents. The responses were classified as evoked by group II afferents when they were evoked by stimuli which were supra-threshold for group II afferents (usually >2T), as judged from the appearance of both early and late components of the field potentials evoked by these stimuli.

Responses evoked by group I muscle afferents were facilitated in all tested interneurones. They were facilitated by both NA and 5-HT. Responses evoked by group II afferents were either facilitated or depressed. 5-HT facilitated them in all interneurones co-excited by group I and II afferents and in some interneurones with more selective input from group II afferents while it depressed them in the remaining interneurones. NA depressed responses evoked from group II afferents in all interneurones. Thus in neurones co-excited by group I and II afferents NA had an opposite effect on responses evoked by group II afferents (depressed) and on responses evoked by group I afferents (facilitated).

The results of this study lead to the conclusions that:

1. Modulatory actions of monoamines serotonin (5-HT) and noradrenaline (NA) are related to the functional type of spinal interneurones.
2. These modulatory actions are related to the origin of synaptic actions to be modulated (kind of muscle afferents).
3. Modulatory actions of 5-HT and NA may either strengthen or counteract each other.

Title: PROPERTIES OF Na^+ CURRENTS IN MUSCLE AND SKIN VASOCONSTRICTOR SYMPATHETIC NEURONES IN RATS. 16.10

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The kinetic properties of Na^+ currents were compared in putative muscle (MVC) and skin (SVC) vasoconstrictor postganglionic sympathetic neurones isolated from the stellate and superior cervical ganglia, respectively. Neurones were labelled with a fluorescent tracer - Fast Blue, injected into the lumbar portion of the diaphragm (in the case of MVC) and into the ear tip (in the case of SVC). Voltage dependent Na^+ current was then isolated and recorded from labelled cells. The major findings of this study were: 1) Peak Na^+ current was larger in MVC than in SVC (3.1 nA vs. 2.0 nA; for 30 mM Na^+ in extra- and 10 mM in the intracellular solution). 2) The somata of MVC were larger than skin SVC, as indicated by the values of their membrane capacitance (21 pF vs. 12 pF). 3) Na^+ current densities were not significantly different in MVC and SVC. 4) Recovery from inactivation was slower in MVC with 90% recovery time being 97 ms for MVC and 45 ms SVC. 5) Half inactivation voltage for steady-state inactivation was larger in MVS than in SVS (–66.3 vs. –60.8, respectively). Slope factor was not significantly different. 6) Half activation voltage equalled –20.9 mV and –16.7 mV in MVC and SVC, respectively and was larger in MVC. Slope factors for activation were not significantly different.

The results show that Na^+ current kinetic properties are different in muscle and skin vasoconstrictor sympathetic neurones in rats.

Supported by KBN, grant No 4 P05A 070 14.

16.11 **MECHANOMYOGRAPHIC (ACOUSTIC) SIGNALS RECORDED DURING UNFUSED TETANI OF THE SINGLE MOTOR UNITS IN MEDIAL GASTROCNEMIUS MUSCLE OF THE RAT**

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Mechanomyographic (MMG) signals generated during evoked isometric, unfused contractions of motor units in the rat medial gastrocnemius muscle were recorded using the piezoelectric transducer in paraffin oil bath. For the three types of motor units MMG signals were analysed during their unfused tetani evoked with the stimulation at frequencies of 20, 40 and 80 Hz. Each successive contraction within the tetanus was reflected in one component in MMG. The increase in stimulation frequency induced a rise in the peak tension and increase in fusion of this tetanus but decrease in the amplitude of force oscillation in the middle part of unfused contraction. This decrease in oscillation of tension was reflected in a decrease of a "peak to peak" amplitudes of MMG signals. The correlation between the amplitude of MMG signal and the amplitude of force oscillation was found. It is concluded that the amplitude of acoustic signals during unfused contraction depends on the tetanic fusion and on the amplitude of force oscillation during tetanus.

Session 17 - Poster Session: Glia

17.1 **CYTOSKELETAL REARRANGEMENTS DURING AMMONIA INDUCED APOPTOSIS IN C6 GLIOMA CELLS**

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Ammonia toxicity can cause a number of neuropsychiatric disorders. Down–stream effects of ammonia neurotoxicity include intracellular redistribution of PKC coupled to its activation and phosphorylation of microtubular associated protein 2 (MAP-2), which are both involved in cytoskeletal polymerisation - depolymerisatin balance and cell apoptosis. The aim of the present study was to correlate ammonia (10mM ammonium chloride) – induced apoptotic changes in C6 glioma cells, shown by nuclear Hoechst 33258 staining and DNA laddering, to the cytoplasmic microtubular rearrangements, evaluated by indirect anti-Tub$\beta_{I,II}$ and anti-TyrTubα immunostaining. Condensation of early apoptotic nucleus is accompanied by redistribution of dispersed cytoplasmic microtubular network, which cumulates close to the nuclear envelope. Moreover, immunostaining of both Tub$\beta_{I,II}$ and TyrTubα disappears from the cell cytoplasm at the late stage of apoptosis, when the nucleus is fragmented into apoptotic bodies, suggesting complete depolimerization of the microtubular structures. This is consistent with earlier findings that microtubular disruption is involved in the transmission of the apoptotic signal. Preliminary results suggest, that microtubular rearrangements in C6 glioma apoptotic cells are under control of PKC mediated phosphorylation of microtubular associated proteins.
Supported by SCSR grant 4 PO5A 096 14 to J.A.

17.2 **Persistent activation of JNK signaling pathway modulates transcriptional activity of AP-1 transcription factor during cyclosporin A-induced apoptosis of glioma cells.**

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AP-1 transcription factor, known to play a role in cell proliferation and activation, is also involved in apoptosis of cells in response to stress, DNA damaging agents or lack of survival signals. To understand how AP-1 might contribute to distinct biological processes, we tested a hypothesis that changes in AP-1 composition or phosphorylation state modulate its transcriptional activity during cyclosporin A-induced apoptosis of glioma cells. The induction of AP-1 DNA binding activity composed of c-Jun, JunB, JunD and ATF-2 proteins preceded apoptosis. The compositional changes of AP-1 were associated with an elevation of c-Jun and JunB protein levels and appearance of phosphorylated c-Jun and ATF-2 at 15-40 hr posttreatment. Immunocytochemistry and staining with Hoechst 33258 revealed an accumulation of phosphorylated c-Jun protein in apoptotic cells. Since c-Jun expression and transcriptional activity is stimulated by phosphorylation at Ser-63/73 by c-Jun N-terminal kinase (JNK), we measured JNK activities. We found the prolonged induction of JNK activity in extracts from cyclosporin-treated cells that suggests an involvement of persistent JNK activation in the initiation of glioma cell apoptosis. We provided an evidence that variations in AP-1 composition and phosphorylation resulted in modification of transactivating potential towards different promoters. While collagenase AP-1/TRE-dependent transcription was downregulated during apoptosis, Fas ligand promoter became activated.

17.3 EFFECT OF ANTIDEPRESSANTS AND PROPRANOLOL ON PHOSPHOLIPASE D ACTIVITY, PHOSPHATIDYLSERINE FORMATION AND INTRACELLULAR CALCIUM RESPONSE IN GLIOMA C6 CELLS.

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In the present study we investigate the effect of imipramine, amitriptyline, mianserine (antidepressant drugs) and propranolol on phospholipase D activity, phosphatidylserine formation and changes in intracellular calcium concentration in glioma C6 cells. For measuring the phospholipase D activity, the cells were prelabelled with [1-^{14}C]palmitic acid and phospholipase D–mediated synthesis of [^{14}C]phosphatidylethanol was determined. We have found that imipramine, amitriptyline and propranolol stimulate [^{14}C]phosphatidylethanol formation at concentration of 250-1000 μM. Mianserine do not affect phospholipase D activity. The present study also shows that antidepressants inhibit TPA-mediated phospholipase D activity. This study demonstrate that in glioma C6 cells antidepressants affect phospholipase D independently of protein kinase C and are also able to block TPA-mediated phospholipase D activity. Imipramine, amitriptyline and propranolol stimulate phosphatidylserine formation at concentration as low as 25 μM. This concentration may be reached in clinical treatment. On the other hand, antidepressants and propranolol evoked depletion of intracellular calcium stores in glioma C6 cells at very high (1mM) concentration. These data show that antidepressants act not only on receptors present in the plasma membrane but may affect various metabolic processes in the cell.

CHANGES IN REACTIVE BEHAVIOURS OF MACROPHAGES AND ASTROCYTES SHOW OPPOSITE TRENDS IN THE INJURED BRAIN OF 6-DAY-OLD RAT EXPOSED TO GAMMA-IRRADIATION AT DIFFERENT STAGES OF PRENATAL DEVELOPMENT 17.4

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Pregnant Wistar rats were exposed to a single 1.0 Gy dose of gamma rays on gestational days 13, 15, 17 or 19 (E13s, E15s, E17s and E19s, respectively). A mechanical injury was made in the cerebral hemisphere of their 6 day-old male offsprings. The injured rats were injected with [^{3}H]thymidine on day 1 or 2 after injury and killed 4 hours after the injection. Brain sections were immunostained for glial fibrillary acidic protein (GFAP) or S-100beta protein or processed for BSI-B4 isolectin histochemistry, subjected to autoradiography and examined microscopically to record proliferating astrocytes and macrophages. The intensity of astrocyte proliferation in response to injury showed a gradual decrease from the level maximal in E13s to minimal in E19s. The total number of macrophages as well as number of their divisions were minimal in E13s then showed a regular increase in E15s and E17s, and reached their maximal levels in E19s. Thus, changes in the reactive behaviour of astrocytes and macrophages were regarded as being related to the stage of prenatal development when irradiation of the brain was performed. Nevertheless, trends of changes showed by the two cell types were opposite. Therefore, the recruitment and proliferation of macrophages, and the astrocyte proliferation were considered as reactive processes occurring under control of different regulatory mechanisms acting within the region of injury.

17.5 **The calcineurin inhibitor - cyclosporin A - reduces survival of reactive astrocytes from trauma and hippocampal dentate gyrus cultures**

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Cyclosporin A (CsA) is a clinically important immunosuppressive drug widely used to prevent graft rejection following organ or bone marrow transplantation. Although there are reports of serious neurologic alterations associated with the use of the drug, the precise mechanism of its action on the central nervous system still remains unknown. We have previously reported that CsA inhibits proliferation and induces apoptotic cell death of C6 glioma cells. In the present study we show that cyclosporin A, in a dose-dependent manner, affects the survival of reactive astrocytes from traumatic mature brain. We observed changes in cell and nuclear morphology typical for apoptosis. This death was accompanied with DNA fragmentation as revealed by positive TUNEL staining. The hippocampal dentate gyrus cultures containing both neurons and glial cells exposed to CsA also undergo apoptotic cell death. TUNEL-positive staining was observed only in neurons that developed pyknotic morphology after treatment with CsA. In contrary, astrocytes from mixed neuronal/glial cultures were unaffected by exposure to CsA at doses toxic for neurons. These results suggest that the clinical syndrome of cyclosporin A toxicity may, at least in part, reflect induction of cell death in the brain. As a major target of CsA is a Ca^{+2} /calmodulin dependent protein phosphatase- calcineurin, these observations raise a possibility that calcineurin is involved in the regulation of neuronal and glial cell survival.

IMMUNOCYTOCHEMICAL AND ELECTRON MICROSCOPICAL OBSERVATIONS OF ASTROCYTES IN THE PERIAQUEDUCTAL GRAY DURING THE OESTRUS CYCLE OF THE RAT 17.6

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The periaqueductal gray plays an important role in female reproduction. It contains neurones, which are under the influence of steroid hormones produced by female gonads.
The aim of the study was to investigate the influence of estrogens on the astrocytes of the periaqueductal gray during the oestrus cycle of adult (100 days) rats. The studies were carried out both under the light microscope, using immunocytochemical method for glial fibrillate acid protein (GFAP), and the electron microscope. Results: Observations under the light microscope showed in afternoon proestrus and estrus periods a significant increase in immunoreactivity for GFAP surface density of astrocytic processes, whereas it decreases in the phases of metestrus and diestrus. Studies at electron microscope level showed that in afternoon proestrus and estrus delicate astrocytic processes surround a large part of cellular bodies of the neurones in the examined brain area. In metestrus and diestrus phases a significant covering of cellular bodies by axo-somatic synapses in the number of astrocytic processes were observed. On the basis of the results obtained it is proved that astrocytes succumb naturally to reversible changes during the oestrus cycle of the female rat.

17.7 **DEVELOPMENTAL EXPRESSION OF VARIOUS ANTIGENS ON THE MICROGLIAL CELLS IN THE RAT BRAIN**

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Microglia is considered the main cellular component of immune system of the brain. Ameboid microglial cells in fetal rat brain can express: antigens of major histocompatibility complex class I (OX-18) and class II (OX-6), complement type 3 receptor (OX-42) and macrophage antigens of unknown function (ED1).

In the present study we have examined the expression of these antigens in postnatal brain. A total number of 40 brains at various ages (from P0 to P90) were studied. Care and treatment of the animals were in accordance with the guidelines for laboratory animals established by the local ethical committee. After perfusional fixation the brains were frozen and cut on the cryostat and stained using standard immunohistochemical method with following antibodies: OX-6, OX-18, OX-42 and ED1.

OX-6 and OX-18 immunopositive cells were not observed at any postnatal stage. On the birthday ED1 and OX-42 immunoreactive cells were localized in ventricular zone, corpus callosum, intermediate zone and external capsule. They were oval or round in shape. At P4, ED1 and OX-42 immunolabelled cells appeared also in anterior commissure and internal capsule; some of them possessed short processes. To the end of the second postnatal week the number of ED1 positive cells has increased but their morphology and localization have not changed significantly. After this time considerably decline of the number of ameboid ED1 positive cells and increase of the number of OX-42 positive ramified microglial cells were observed. Starting from P21 the population of microglia consisted only of OX-42 positive ramified cells that were localized in white and gray matter. In our opinion at this time final population of resting microglia is formed.

17.8 **THE GLIAL RESPONSE IN THE ACUTE LEAD-INDUCED NEUROTOXICITY IN THE RAT.**

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The lead toxicity is still a major medical problem. Although the clinical manifestations of its neurotoxicity are well documented, the subcellular mechanisms are still the open question. The purpose of the study was to assess the glutamatergic transmission in synaptosomes after acute lead exposure and to investigate whether it exerts an influence on the astroglia functions. Most studies concerning lead toxicity, including glia toxicity, was done using cell culture. Since the procedure of isolation of glial derived vesicles was developed, it become possible to asses the lead-astroglia interactions in vivo. The animal model of acute lead toxicity was used. The experimental group of rats was injected i.p. with 15 mg lead acetate per kg b. w., the control one was injected with distilled water. The uptake of glutamate was found to be lowered in lead toxicity conditions and the release in the presence of KCl was increased. The glial fractions, named glial plasmalemmal vesicles (GPV) were obtained from rat brains using Percoll gradient. The morphological examination of the fraction purity was done. The activity of the glutamine synthetase as the marker enzyme was determined. It was slightly elevated in the fraction obtained from Pb-exposed rats but the difference was statistically insignificant. Additionally the glutamate uptake to GPV was measured and was found to increase in lead-exposed rats. The early response of the glia to lead-induced impairment of glutamatergic transmission consists on the regulatory effect of the elevated amino acid concentration.

17.9 **LONG-TERM GLIAL CELLS ACTIVATION IN RAT HIPPOCAMPAL FORMATION AFTER GLOBAL TRANSIENT ISCHEMIA INDUCED BY CARDIAC ARREST**

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The late sequelae of the temporary brain ischemia revolves a great interest due both to the question of reversibility of tissue damage caused by ischemia, and the progressive nature of ischemic encephalopathy stressed by reanimatologists. We have recently found that in the first two weeks after global transient ischemia in rats induced by 10 min cardiac arrest the degeneration of the pyramidal cells in the CA1 sector of the hippocampus was accompanied by activation of astroglia producing several substances, presumably both of pro-and anti-apoptotic activity. In the present studies, we have examined neuronal and glial changes 6 months after resuscitation. Using immunocytochemical techniques we have studied the pattern of expression of NGF, its receptor TrkA and IL-1beta. In general, the neuronal degeneration was not strongly pronounced and was visible mainly in the CA1 region. The neuronal immunoreactivity of all investigated parameters in aged ischemic rats and in aged controls was much less expressed than in corresponding groups of young animals. On the other hand, persisting gliosis, especially strong in the regions of neurodegeneration, was found in ischemic rats. Several reactive astrocytes expressed NGF and IL-1beta immunoreactivity. Particularly intense was the immunoreactivity of TrkA. Interestingly, TrkA positive reactive astrocytes invaded the CA1 pyramidal cell layer.Perhaps the prolonged TrkA upregulation by reactive astrocytes could be a part of the mechanism which may enable glial cells to participate in survival-promoting processes.

Session 18 - Parallel Symposium: Cortical mechanisms of audition

18.1 INSTRUCTION-GUIDED PROBLEM SOLVING IN HUMAN AUDITORY CORTEX

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Functional Magnetic Resonance Imaging (fMRI) is the first method which offers the opportunity to map task-specific activation in the human brain with a spatial resolution adequate for a mechanistic representational view, namely by delineating functional fields in cortical systems. The differential activation of functional fields as specialized subunits of a sensory cortex has emerged in animal studies as a key constituent of stimulus processing with distributed roles. In various fMRI studies of auditory cortex (AC) we pursued the question whether functional field activation in both hemispheres in addition to specialized stimulus processing also reflects the task executed on the presented stimuli. This approach relied on the distinction of so far 4 functional areas including primary AC which were identified with fMRI.

Insights were obtained from four vastly different auditory tasks involving heterogeneous speech and non-speech acoustic stimulus material:

(1) Identification of affective content in prosodically intonated word material
(2) Recall of tone sequences (melodies) versus musical instruments after previous encoding
(3) Foreground-background decomposition of non-speech acoustic scenes (cocktail party effect)
(4) Tracking of acoustic motion in space

The unifying picture has emerged from these studies that all acoustically complex stimuli activate all 4 areas of the superior temporal gyrus of the human brain bilaterally including the planum temporale hitherto considered as primarely involved in sensory speech analysis. While there is influence of the acoustic material on the distribution of activity, the task to be executed on the material also strongly influences in which territory of AC dominant activity is seen and in which hemisphere. This suggests that „top down" principles strongly determine AC field specializations.

Stiller et al., MAGMA 5, 32-47 (1997)
Scheich et al., Eur. J. Neurosci. 10, 803-809 (1998)

18.2 SPECIFIC FEATURES OF THE CORTICAL CONNECTIONS OF THE CANINE TEMPORAL CORTEX

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The distribution of retrogradely labelled neurones (fluorochrome dyes) and anterogradely labelled terminals (BDA methods) allow us to conclude that tonotopic fields of the middle (EM) and anterior (EA) areas of the ectosylvian cortex as well as non-tonotopic posterior ectosylvian area (EP) are interconnected by short cortical axons between the neighbour areas. The prominent part of the canine neocortex situated below EP forms the posterior composite gyrus (CP). We found that predominant CP connections are restricted to the neighbour cortical areas of EP and the posterior sylvian area (SP). Therefore, it may be considered as unimodal auditory association cortex. In the sylvian gyrus the auditory information converge with the visual one, taking rise in the association visual areas of the ventral occipital cortex. Polimodal character of the sylvian projections suggests that it constitutes a higher order of association cortex. The distant projections from the auditory areas originate in EA, CP, and SP. The EA efferent connections terminate in the dorsal frontal cortex of the precentralis area. Projections of CP and SP are predominantly directed ventrally into the perirhinal cortex and the lateral amygdaloid nucleus. Moreover, the sylvian cortex constitutes two synaptic pathway reaching the dorsolateral prefrontal cortex.

According to our present knowledge it can be suggested that connections passing dorsally through area EA take part in functions of sound localisation and proper motor reactions, whereas, projections running ventrally through PC and SP are involved in evaluation of quality of auditory information.

18.3 TEMPORAL LOBE STRUCTURES AND AUDITORY MEMORY

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Several experiments were conducted on dogs, in order to determine the influence of the temporal lobe structures lesions on auditory recognition Delayed Matching-to-Sample (DMS) task with trial-unique stimuli, and on memory of place guided by auditory stimuli (spatial Delayed Responses task).

The results indicate that memory of place was impaired only after bilateral hippocampal lesions, without injury to the underlying cortical areas. Lesions to the anterior temporal lobe (including amygdala and adjacent cortical areas) as well as bilateral removal of the rhinal cortex (perirhinal and entorhinal) did not impair spatial Delayed Responses guided by acoustic stimuli. On the other hand, neither hippocampal nor rhinal cortex lesions (separated or combined) impaired performance of the auditory recognition DMS task with trial-unique stimuli. The performance of the auditory recognition task was impaired after bilateral removal of cortical auditory association areas of the temporal lobe.

The role of the auditory association areas in recognition memory has been discussed.

Sponsored by US-Polish Joint MSC II Found No. MZ/HHS-93-124, and James McDonnell Foundation grant No. 95-13.

18.4 Decoding communication sounds in primate auditory cortex

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The use of species-specific vocalisations as stimuli in auditory research seems appropriate, because they are proven communication signals in each species that must be processed by the brain. Each vocalisation can be broken down into constituent elements (or features) which are common to various auditory communication systems, including human speech. On that basis, classes of complex sounds can be established that are shared by different species, and the principles for their processing in auditory cortex can be analysed. Features common to both monkey calls and human speech are, for instance, band-passed noise (BPN) bursts and frequency-modulated (FM) sweeps. In the lateral belt areas of monkey superior temporal gyrus (STG) neurons are selective for the bandwidth and center frequency of BPN bursts and for the rate and direction of FM sweeps. Lateral belt neurons also show selectivity for the complete calls themselves, brought about by combination-sensitivity both in the spectral and temporal domain. Neurons of the same type may exist in higher areas of human auditory cortex and may be involved in the processing of speech sounds. Using complex feature elements (BPN and FM) directly in imaging studies of human auditory cortex, larger areas are activated than by pure tones, and several auditory maps beyond the primary field become apparent. However, neuroimaging techniques cannot achieve cellular resolution. For a full understanding of the neural basis of speech perception it will be mandatory, therefore, to continue a concerted effort combining imaging in humans with single-neuron studies in animal models.

Session 19 - Parallel Symposium: Newer neurochemical strategies for neurological and psychiatric disorders

19.1 INSIGHTS ON NEUROCHEMICAL MECHANISMS OF HYPERACTIVITY AND POTENTIALLY NEW THERAPEUTIC STRATEGIES

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Human hyperkinetic activity (HKA) is thought to be associated with an alteration of dopamine (DA) neurochemistry in brain. This conventional view became solidified in the 1970s largely on the basis of observed HKA in DA-lesioned animals and effectiveness of the dopaminomimetics, amphetamine (AMP) and methylphenidate (MPH), in abating HKA in humans and in animal models of HKA. However, because AMP and MPH release serotonin (5-HT) as well as DA, we investigated the potential role of 5-HT in an animal model of HKA. We found that a greater intensity of HKA was produced in rats when both DA and serotonin (5-HT) neurons were damaged at appropriate times in ontogeny. Moreover, effects similar to that of AMP are produced by *m*-chlorophenylpiperazine (*m*-CPP) and 1-phenylbiguanide (1-PG), respective 5-HT_2 and 5-HT_3 agonists; but not by the 5-HT_{1A} agonist 2-dipropylamino-8-hydroxy-1,2,3,4-tetrahydronaphthalene (8-OH-DPAT). The effect of *m*-CPP was shown to be replicated by desipramine, and was largely attenuated by the 5-HT_2 antagonist mianserin. These findings implicate 5-HT neurochemistry as potentially important therapeutic targets for treating childhood hyperactivity.

19.2 **LIGAND BINDING SITES AND SIGNAL TRABSDUCTION PATHWAYS OF THE BRAIN 5-HT_{1A} RECEPTORS**

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Activation of a specific receptor may activate different signal transduction pathways. For instance 5-HT_{1A} receptor may modulate adenyl cyclase, phospholipase C activity and K^+ channel activities as well as Ca^{2+} level 1, 2]. An important question is whether these different signal transduction pathways reflect multiple signalling mechanisms for a single receptor or reflect multiple receptor subtypes that cannot be differentiated with the available drugs. It is supposed that a single G-protein linked receptor can couple to different pathways. It is also anticipated that G-protein coupled receptors can adopt different conformations which selectively and differentially couple them to a specific second messenger [1, 2].
We synthesised several ligands of 5-HT_{1A} receptor possessing very high to very low affinity. For some of them we determined pre- and postsynaptic intrinsic activity and examined their influence on serotonergic, dopaminergic and noradrenergic neurotransmission. We also identified a pharmacophore model and binding pockets in the tri-dimensional model of the receptor. We found that ligands may bind to different binding pockets at different 5-HT_{1A} receptor sites [3-6].
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19.3 SYNERGISTIC EFFECTS OF OLANZAPINE AND OTHER ANTIPSYCHOTIC AGENTS IN COMBINATION WITH FLUOXETINE ON NOREPINEPHRINE AND DOPAMINE RELEASE IN RAT PREFRONTAL CORTEX

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A recent clinical study demonstrated that olanzapine combined with fluoxetine showed superior efficacy than either drug monotherapy in patients with treatment-resistant major depressive disorder (MDD). To understand the neurochemical mechanism underlying the clinical findings, we studied the effects of olanzapine and other antipsychotics in combination with fluoxetine on neurotransmitter release in rat prefrontal cortex (PFC) using microdialysis. Three hours after drug treatment, olanzapine (3mg/kg) increased NE and DA levels to 102±8% and 102±7% of the baseline and fluoxetine (10 mg/kg) increased NE and DA to 188±14% and 143±13%, respectively. Olanzapine followed by fluoxetine increased NE and DA to 269±24% and 349±36%, respectively. Risperidone (1 mg/kg) increased NE and DA to 144±13% and 113±7%, and when combined with fluoxetine it increased NE and DA to 221±33% and 279±40%, respectively. Neither the D2-type antipsychotic haloperidol (1 mg/kg) or the 5-HT_{2A} antagonist MDL100907 (1 mg/kg) appreciably changed the NE and DA release and their combination with fluoxetine was not different from fluoxetine alone, suggesting that the synergism is not D2 or 5-HT_{2A} receptor mediated. Therefore, olanzapine combined with fluoxetine produced a robust and persistent increase of NE and DA release in PFC, which could contribute to their increased antidepressant effect in MDD.

19.4 EXPERIMENTAL BACKGROUND FOR THE SEARCH OF NEW ANTIEPILEPTIC DRUGS

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Animal models of epilepsy, mainly the maximal electroshock and the pentylenetetrazol test, led to the discovery of conventional antiepileptic drugs - benzodiazepines, carbamazepine, ethosuximide, phenytoin, and valproate. Among new antiepileptic drugs, some of them (tiagabine, vigabatrin) were discovered by a rational strategy assuming that enhancement of GABA-ergic inhibition may produce anticonvulsant effects. Tiagabine effectively inhibits GABA reuptake and vigabatrin is a irreversible inhibitor of GABA transaminase; both drugs produce a substantial increase in synaptic GABA. Probably, these two antiepileptics do not possess other mechanisms of action. Gabapentin (a derivative of GABA) was also developed on the basis of the rational strategy, but its anticonvulsive activity seems to involve mechanisms not necessarily related to GABA-mediated inhibition. Other new antiepileptic drugs (felbamate, flunarizine, lamotrigine, topiramate) may act via blockade of voltage-dependent sodium (or calcium) channels, inhibition of glutamate-mediated excitatory events and, similarly to tiagabine or vigabatrin, potentiation of GABA-related mechanisms. At present, potential antiepileptics are being searched among glutamate antagonists, acting at ionotropic non-NMDA receptors and ligands affecting metabotropic glutamate receptors. Actually, antiepileptic drugs enhancing GABA-ergic transmission, may aggravate non-convulsive seizures (for example, encountered in absence epilepsy). Consequently, benzodiazepines and valproate have to act via other mechanisms against absences.

Session 20 - Parallel Symposium: Ultrasonic communication in rodents

20.1 **COMPARATIVE CHARACTERISTICS OF 22 AND 50 kHz TYPES OF ULTRASONIC CALLS IN ADULT RATS.**

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Adult rats emit two main patterns of ultrasonic calls: long calls with 20-32 kHz in sound frequency ("22 kHz calls"), and short calls with 35-70 kHz in sound frequency ("50 kHz calls"). The goal of the presentation is to compare behavioural, pharmacological and neurophysiological aspects of these call categories. The 22 kHz, or alarm calls, have long duration of single calls (300-3900 ms) and appear in aversive situations, when the rat anticipates pain, attack, or is endangered or threatened in other way. Emission of alarm calls is usually accompanied by locomotor inhibition. 22 kHz calls may be induced by a direct cholinergic stimulation of an elongated medial vocalization strip stretched from the mesencephalon to the forebrain. The strip receives cholinergic terminals from the ascending mesolimbic activating system from the laterodorsal tegmental nucleus. Release of acetylcholine from these terminals causes neuronal inhibition in the vocalization strip, which was postulated to trigger ultrasonic alarm calls and concomitant locomotor inhibition. The 50 kHz calls have short duration of single calls (20-200 ms) and appear in affiliative social situations, non-agonistic encounters, and when the rat enters familiar home cage. 50 kHz calls may be induced, at least from some regions of the vocalization strip by a direct glutamatergic stimulation, and the calls are increased in number by amphetamine. Glutamate increases neuronal firing in the vocalization strip and causes behavioural activation. Supported by NSERC of Canada.

20.2 Brain mechanism of ultrasonic communication in hamsters.

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The sexual behavior of female hamsters includes consummatory and appetitive elements. While lordosis is the definitive consummatory response, hamsters use high-frequency sounds to initiate and structure mating. Evidence supporting this view of hamster ultrasounds includes their dependence on hormones and males, along with their impact on vocalization, approach and lordosis.

Analyses of the neural mechanisms of sexual behavior have focused on lordosis, which seems to depend on competing systems based in the ventromedial hypothalamus (VMN) and lateral septum (LS) or medial preoptic area (POA). The depression of lordosis by VMN lesions suggests a system that facilitates behavior when active. Conversely, the facilitation of lordosis by damage to the LS or POA suggests a system that normally inhibits behavior. The outputs of these systems seem to be integrated with each other and tactile inputs within the midbrain periaqueductal gray (PAG), prior to the elaboration of responses within the brainstem and spinal cord.

To what extent does this model extend to other sexual responses, such as the ultrasounds used by hamsters for courtship? To answer this question, we have observed the responses of female hamsters to lesions of the LS, PAG, POA or VMN. These studies reveal several of the brain areas controlling ultrasound production. They also show similarities and differences in the brain mechanisms for ultrasound production and lordosis. For example, both lordosis and ultrasonic calling are facilitated by damage to the LS or POA. However, the magnitude of this effect is much greater for ultrasound production than lordosis. Similarly, PAG lesions have clear disruptive effects on vocalization but inconsistent effects on lordosis. Conversely, VMN lesions have lasting disruptive effects on lordosis, while the ultrasound rates of lesioned animals eventually rebound to levels higher than those seen preoperatively. These differences emphasize the extent to which distinct mechanisms are required to control and explain distinct sexual responses.

20.3 **PRECONTACT VOCALIZATIONS IN 50 kHz AS A MEASURE OF SEXUAL LEARNING IN MALE RATS.**

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It has been postulated that ultrasonic vocalizations in 50 kHz band (very short duration 3-15 msec. calls, emitted by male and female while looking for a sexual partner, courtship and copulation) pays a communicative role in sexual behavior of rats. In the presented experiments ultrasonic vocalizations in 50 kHz band emitted by male rats during 5 min period before introduction of female (PAV, precontact alone vocalizations) was analyzed as a parameter describing an acquisition of a sexual experience. Subject to an investigation were changes in the main sexual parameters, changes of PAV, role of contact with anestrous and estrous female, as well as conditioning to odor and background cues. Mount and intromission latencies decreased after first copulatory session. Contrary to that ejaculation latencies changed only during fourth session. Number of PAV was gradually increasing during first three sessions. Contacts with anestrous, estrous but with closed vagina, and estrous with open vagina female lead to an increase in number of PAV but within a different time course. Most effectively PAV was influenced by contact with estrous female. In extinction sessions the exposition to background cues resulted in a linear decrease in number of PAV during ten subsequent sessions. After exposition to odor cues no linear tendency was detected although a number of PAV was lower than during last copulatory session.

Our results suggest that in course of an acquisition of a sexual experience, PAV reflexes learning process and depends on rewarding value of socio-sexual contact.

20.4 OLFACTION AND ULTRASONIC COMMUNICATION IN RODENTS; EVOLUTIONARY ADVANTAGE

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Rodents, being the object of attacks of predators, have developed intraspecific communication which can be understood only by members of own species. This is based on interaction between specific pheromones and ultrasonic calls. In adults ultrasonic vocalization can be a part of courtship or aggerssive behaviour in unisexual groups. Rodent species differ in their ability to vocalization, e.g. in pine voles and collared lemmings both sexes emit ultrasounds. In mice or bank voles only males produce high frequency calls and their vocalization is stimulated by female pheromones. Vomeronasal organ is involved in transmission of female chemosignals. Cold stress is a factor stimulating ultrasonic vocalization in new-born rodents. Neonatals modify ultrasonic calls in response to olfactory cues. Bank vole pups identify odour of own nest, and newborn laboratory mice are able to discriminate between odour of different genotypes or social status of conspecifics. An important question is whether olfactory and ultrasonic interaction has any ecological significance. A large part of rodent activity takes place in a limited area such as burrow or a nest located underground. Deposited chemosignals are present even if the absence of an animal and in these environmental conditins ultrasonic calls can serve for intraspecific communication. This work was supported by grant from PB 4919/PO4/98/15.

Session 21 - Parallel Symposium: Anatomical and functional heterogeneity of the amygdalar complex

21.1 **ANATOMICAL AND FUNCTIONAL HETEROGENEITY OF THE AMYGDALOID COMPLEX. Asla Pitkänen. A.I.Virtanen Institute for Molecular Sciences. University of Kuopio, Kuopio, Finland.**

The amygdaloid complex is composed of more than ten nuclei each of which have unique cytoarchitectonic, chemoarchitectonic and connectional characteristis. In line with anatomic heterogeneity, the amygdaloid complex has been shown to be involved in a number of functions that include memory, attention, interpretation of emotional significance of sensory stimuli, perception of body movements and generation of emotional aspects of dreams. I will focus on three major aspects of the amygdaloid complex. (1) The partitioning of the amygdala into nuclei and nuclear divisions and on intra-amygdaloid connectivity. There are three levels of connectivity in the amygdala: intradivisional, intranuclear and internuclear. Information entering one nucleus is distributed into other amygdaloid areas via parallel internuclear pathways. Many of the internuclear connections are reciprocal. Many of the intra-amygdaloid pathways converge in selective amygdaloid nuclei, particularly, in the central nucleus and the amygdalohippocampal area. (2) Cortical and subcortical inputs enter the selective divisions of the amygdaloid complex which are not necessarily the same amygdaloid areas which give origin to the reciprocal connections. (3) In pathological conditions, like in temporal lobe epilepsy, the amygdaloid damage may be nucleus or subdivision specific which correlates with the impairment in functions mediated by the amygdala.

21.2 **DEFENSIVE CONDITIONING-RELATED FUNCTIONAL GETEROGENEITY AMONG NUCLEI OF RAT AMYGDALA.**

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Amygdala is a complex forebrain structure proposed to play a pivotal role in fear conditioning circuitry. c-Fos immunomapping was applied to investigate functional activation of particular amygdalar nuclei following the two-way active avoidance training. Injection of AP5 into the basolateral amygdala nucleus was used to investigate the role of NMDA receptor in this process. The training procedure resulted in an increase of c-Fos expression within cortical, medial, lateral, and basolateral, but not central, nuclei. The expression in the cortical nucleus correlated negatively with grooming behavior, whereas c-Fos immunolabeling of other three nuclei could be associated with the number of intertrial responses. On the other hand, no correlation was observed between c-Fos upregulation and measures of instrumental learning related to avoidance or escape behaviour. However, injection of AP5 resulted in the dramatic increase of escape latency and deterioration of avoidance reaction learning. Blocking of NMDA receptor produced also specific alteration of c-Fos expression in amygdalar nuclei. The results obtained with c-Fos mapping of various regions of rat amygdala, combined with a fine dissection of behavioral repertory, imply that there are specific functional links between particular parts of the structure and distinctive behaviors that reflect various emotional states of the animal.

21.3 **THE ROLE OF THE AMYGDALA IN CONDITIONED AND UNCONDITIONED STIMULI PROCESSING**

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The amygdala is considered to be one of the most significant brain structures involved in mechanisms of fear conditioning, emotional memory, and reactivity to pain and stress evoked events. It is apparently a part of the brain stimulus-analyzing system, which shares in regulation of the autonomic and endocrine reactions, but first of all it integrates the internal milieu with external signals. The structure receives a wide range of sensory inputs from the thalamus, hippocampus, as well as several cortical and brainstem areas, and it participates in emotional evaluation of these information. The aim of our research was to investigate the role of different amygdaloid nuclei in mechanisms of cognitive appraisal of different modality cues that acquire conditioned properties, and to explain their function in evaluation of unconditioned nociceptive stimuli. Various experimental procedures were performed in cats and rats. They included escape and/or avoidance training, exploration of pain reactivity and post-stress analgesia after partial amygdaloid lesions The results indicated that the lateral and baso-lateral nuclei play significant role in the analysis of subtle signaling and reflexogenic attributes of acoustic and visual primary cues during instrumental avoidance conditioning. No changes of pain and stress evoked reactivity were observed after the basolateral lesions. The function of the central and medial nuclei is mostly related to evaluation of emotional validity of pain and stress evoking unconditioned stimuli. The lesions of these structures caused handicapped instrumental escape responding and severely disturbed an opioid form of post-stress analgesia.

21.4 **AUTOBIOGRAPHICAL MEMORY ACTIVATES THE RIGHT AMYGDALA AND TEMPORO-FRONTAL LINK – A PET STUDY**

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The neuroanatomical correlates of self-experienced versus self-created, fictitious ("false") emotionally laden information was tested. Both kinds of information belong to Tulving's episodic memory system. Autobiographical memories constitute that part of the episodic memory system that is composed of significant life events, usually of the distant past. ^{15}O-positron emission tomography was used to study the neural networks engaged in retrieving autobiographical and fictitious information of closely similar content. The principally activated brain regions overlapped considerably and constituted temporal and inferior prefrontal regions and the cerebellum. Most interestingly, activations of the right amygdala and the right uncinate fascicle - interconnnecting prefrontal and temporopolar areas – were found when subtracting fictitious from autobiographical retrieval, suggesting that it is the emotional flavor which distinguishes the retrieval of true from that of "false" memories, and that autobiographical memory retrieval requires an interaction between prefrontal and temporal cortices.

Session 22 - Parallel Symposium: Neurogenetics

22.1 NATURE V. NURTURE IN THE TRANSMISSIBLE SPONGIFORM ENCEPHALOPATHIES

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There has been much controversy as to the cause of the transmissible spongiform encephalopathies (TSEs). For many years their pattern of infectivity suggested a slow virus, slow because the incubation period could be several years. However, the infective agent could not be detected by electron microscopy and was resistant to treatments which inactivated all viruses. No nucleic acid was found in preparations containing the purified agent. No immune response followed infection. In 1982, evidence was obtained to suggest that the infectivity was due to a protein particle, the prion protein (PrP), which could exist in two isoforms a normal PrP present in all tissues, and a PrP resistant to enzyme degradation which accumulates in nerve cells and is the cause of the fatal neurodegenerative disease. It is not known what causes the change from normal to resistant PrP, but the prion hypothesis proposes that a post-translational modification is responsible for a conformational change in the PrP molecule and that this sets off a chain reaction leading to the conversion of normal PrP into resistant PrP. It is possible that the conformational change could be induced by chemical treatments or environmental factors, but this has not yet been shown, nor have preparations of purified PrP been shown to cause disease in experimental animals.

Gerstmann-Straüsser-Scheinker disease is a familial TSE due to mutations in the prion protein gene on chromosome 19, sporadic Creutzfeld-Jacob Disease (CJD) is a TSE possibly due to somatic mutation of the prion gene, Kuru is a TSE due to cannibalism, iatrogenic CJD has resulted from parental injection of human growth hormone prepared from pooled cadaveric pituitaries and variant CJD is a new TSE linked to eating beef contaminated with bovine spongiform encephalopathy. TSEs are unconventional in that they can be caused either by gene mutation or infection and in that the infective agent is not a microorganism. These facts must be considered both in the differential diagnosis of a neurodegenerative disease and in the investigation of a TSE epidemic.

22.2 ANALOGIES AND HOMOLOGIES – GENETIC CONTROL OF THE NERVOUS SYSTEM IN MAN AND LOWER ORGANISMS

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In the past ten years it has become apparent that all living organisms share far more of their genetic and developmental processes than anybody had previously imagined. Almost every human gene has an exact counterpart in the mouse, but even much more distantly related organisms such as the zebrafish, the *Drosophila* fruit fly, the nematode worm *Caenorhabditis elegans* and even yeast use many of the same genes and developmental pathways as humans.

Differences in anatomy do not necessarily mean that different genes are involved. The human eye and the *Drosophila* compound eye are controlled by the same master gene, *PAX6*. The *minibrain* gene is required for brain development in *Drosophila* and is probably involved in Down syndrome. The presenilin genes that can cause early onset Alzheimer disease have counterparts in the nematode worm. Thus research into development and functioning of the simple nervous systems of lower organisms can provide insights into development and function of the human nervous system. As complete genome sequences become available for a number of organisms (the human sequence will be mostly finished by 2002), the pace of discovery will increase.

22.3 GENETICS OF THE SPINAL MUSCULAR ATROPHY

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The proximal childhood spinal muscular atrophy (SMA) is an autosomal recessive condition with overal incidence of all three forms – I, II and III – of 1/6000 to 1/10.000.

The locus of SMA was assigned to chromosomal region 5q11.2-13.3 in which in 1995 two candidate genes were identified, namely the genes of neuronal apoptosis inhibitory protein (NAIP) and survival motor neuron (SMN). According to the accumulated evidence mutations (most of them deletions) present in telomeric copy of the SMN gene are the major determinant of the SMA phenotype.95% of SMA patients affected with SMA have a deletion of exons 7 and 8 of SMNt.

Until recently the cause of variebility of clinical picture was not clear since the deletions of SMN gene have been detected not only in severe but also in milder types of SMA. However more sophisticated recent molecular analyses provided evidence that milder types II and III are not due to deletions but to gene-conversion events in which SMNt is replaced by SMNc.The product of the gene – SMN protein was found in novel nuclear structure, called „gems" and it is known that SMN protein interacts with RNA binding proteins. There is evidence that in severe form of SMA motoneurons of the spine lack SMN protein.Spectacular progress in molecular analysis of SMA has already been made.However, there are still several important problems to be solved, such as: the role of SMN protein in pathogenesis of SMA as well as the cause of incomplete penetrance and gender influence in type III of the disease.

22.4 GENETICS OF ALZHEIMER DISEASE

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Alzheimer disease (AD) is a neurodegenerative disorder associated with cognitive dysfunction. In patients brains extracellular β amyloid deposits in the form of senile plaques and neuronal fibrillary tangles are present. Three genes causing familial early onset(EOAD), which is a small part of AD, have been identified: coding presenilin1 and 2, and β amyloid precursor protein. The great majority of AD is multifactorial and has a late onset(LOAD). Apolipoprotein E (ApoE) is a polymorphic protein playing an important role in the nervous system coded by three alleles 2, 3 and 4. A strong association between the ε4 allele and LOAD prevalence as well as the age of onset of EOAD. Down syndrome patients early in their life (in their forties or even thirties) develop AD due to the dosage effect of β amyloid precursor protein. There is a possibility that particular apoE alleles modify progression of the disease. On the other hand ε4 allele is associated with higher plasma levels of atherogenic lipids which could also contribute to the vascular brain pathology.

Session 23 - Oral communications - part 1

23.1 THE ROLE OF GLIAL-PIAL BARRIER LESIONS AND IMPAIRED VASCULARISATION IN CORTICAL CONVOLUTIONS ANOMALIES

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The abnormal patterns of cerebral convolutions range from severe, presenting nearly lissencephalic convexity, less advanced seen in polymicrogyric changes to anomalies restricted to tertiary gyri and sulci. Lesions within glial-pial barrier found in examined cases with malformations were confirmed by immunohistochemical investigations. The abnormal development of vessels penetrating the cortex from meningeal plexus and their disturbed course coexisted often with glial-pial barrier lesions. All observed changes have to be considered as inducing necrotic and reparative processes and disturbing the last phase of neuronal migration. They may also play an evident role in disturbances of final cortical layering and folding with obliteration of interconvolutional sulci.

23.2 REGIONALIZATION OF THE CEPHALIC NEURAL TUBE IN STAGED HUMAN EMBRYOS

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Establishment of developmental compartments in the vertebrate nervous system is based on studies of Drosophila embryo. Regionalization in the neural tube is known to be regulated by genes that display restricted expression temporally and spatially. In human embryos during stage 9 (25 days) three main parts of the future brain are present: prosencephalon, mesencephalon and rhombencephalon. At this stage brain shows 6 primary neuromeres. During stage 10 and 11 (28 and 29 days) secondary neuromeres can be distinguished. The highest number of neuromeres is visible in embryos during stages 13 and 14 (32 and 33 days). In these embryos 16 neuromeres are present. They are following: one telencephalic (T), four diencephalic (D1, D2, Par. and Syn.), two mesencephalic (M1, M2), one isthmic (Isth.) and eight rhombencephalic (Rh. 1 to Rh. 8). In embryos of stage 13 within diencephalon two divisions appear: synencephalon and parencephalon. Such numbers of neuromeres persists to stage 17 (41 days). In later developmental stages due to appearance of the longitudinal zones within particular parts of the brain, which impose transversally oriented neuromeres, the neuromeric pattern within the neural tube progressively disappears.

23.3 UPREGULATION OF D1-RECEPTORS IN THE LIMBIC SYSTEM AFTER BRIEF MATERNAL SEPARATION IS SUPPRESSED BY MATERNAL VOCALIZATIONS

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Experience during early phases of life, in particular traumatic emotional experience such as maternal separation, appears to be important in shaping an individual's responsiveness and behavioral strategies at later stages of life. We speculate that such traumatic events interfere with the structural and functional maturation of limbic brain circuits. To test this hypothesis we investigated i) changes in the density of dopaminergic D1-receptors after repeated brief maternal separation and exposure to a novel environment and ii) the influence of a learned, positively associated emotional vocal signal, the maternal call, on D1 receptor regulation. Exploratory activity was tested in a) the "classical" open field test, i.e. without presentation of any stimuli, and b) "enriched" open field, during which maternal calls were presented to the pups. D1 receptor density was analyzed in different subregions of the medial prefrontal cortex, hippocampus and amygdala using the ligand [^{3}H]SCH23390. Our results show i) that repeated brief maternal separation and exposure to an unfamiliar environment induces an upregulation of D1 receptor density in the hippocampal CA1 region, amygdalar nuclei and the medial prefrontal cortex, but not in CA3 of hippocampus, and ii) that the presentation of maternal calls during maternal separation counterregulates this effect. The acoustic presence of the mother reduced the exploratory activity during open field tests and it significantly suppressed D1 receptor upregulation the medial prefrontal cortex, but not in the other brain areas. These results indicate that vocal communication, which is an essential part for the establishment and maintenance of the infant-mother attachment, is used to provide the newborn with emotional input that reduces stress during exposure to an unfamiliar environment. Furthermore, the mere acoustic presence of the mother is sufficient to regulate experience-induced changes of D1-receptor densities.

Supported by grants LSA 1865A/0025 and 2517A/0086H.

23.4 REGULATION OF CELLULAR GROWTH AND DIFFERENTIATION IN THE NERVOUS SYSTEM - A NEW INTRACELLULAR-INTEGRATIVE PATHWAY.

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The genesis of cells, their differentiation, and growth are fundamental processes underlying the development and remodeling of the nervous system (NS). These developmental programs are controlled by a plethora of signals from cell surface or extracellular matrix molecules, and by soluble cytokines, neurotransmitters, or hormones. How are those signals integrated to trigger specific multi-gene program rather than chaotically activate or inhibit gene activities, remains unknown. Studies from our laboratory have shown a novel integrative signal transduction mechanism in which "growth factor" (FGF-2) and its high affinity receptor (FGFR1) translocate directly from the cytoplasm into the cell nucleus under the control of cell-cell contact, soluble cytokines, and trans-synaptic or hormonal signals. In the nucleus these proteins interact with the nuclear matrix and transcriptional factors to regulate the cell cycle, differentiation, and survival. We will discuss: (a) the evidence that FGF-2 and FGFR1 integrate cell contact, cytokine, trans-synaptic, and hormonal signals, transmit the integrated signal by translocating from the cytoplasm to the nucleus, and regulate the cell cycle, differentiation, and survival; (b) molecular/transcriptional mechanisms through which diverse external and cytoplasmic signals are integrated by the intracrine FGF-2/FGFR1 pathway; (c) molecular mechanisms through which nuclear FGF-2/FGFR1 control multi-gene programs for cellular proliferation, growth, and differentiation; and (d) disruption of intracrine FGF-2/FGFR1 signaling in neoplastic transformation.

23.5 EFFECT OF BODY TEMPERATURE ON PLASMA PH AND IRON LEVEL IN NEWBORN RATS EXPOSED TO ANOXIA

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Hypoxia-induced free iron release in the red cells is often observed in human newborns. The released iron is absorbed and deposited in the brain. The deposits are involved in late-onset neuronal damage. Both oxidative stress and a deficit of antioxidants are likely to induce extremely delayed, iron-catalysed free radical formation, culminating in disorders such as Alzheimer and Parkinson diseases. Because a moderate decrease in cerebral temperature (by 2-3°C) provides a considerable neuroprotection (among others due to blocking free radical formation), newborn mammals, which show similar decrease in body temperature, could be protected against iron-induced neurotoxicity. However, there is a common belief that neonatal hypothermia leads to acidosis, which is an immediate threat to the newborn. Therefore, the aim of the present study was to check effects of body temperature on plasma pH and iron levels in newborn rats exposed to anoxia for 25 minutes. Their body temperature was clamped, both during and after anoxia, at a level typical of neonates (32°C), healthy adults (37°C) or febrile adults (39°C). Blood samples for the analyses were collected from carotid artery at 0, 10, 20, and 30 minutes following the anoxia. Control blood samples were obtained from neonates breathing atmospheric air. Neither pH nor plasma iron levels were influenced by anoxia in neonates having their normal body temperature of 32°C. On the other hand, hyperferremia and acidosis developed in remaining two groups of rats and tended to be proportional to their body temperatures. In conclusion, reduced body temperature is likely to protect newborn rats against delayed neurotoxic effects of anoxia.

23.6 CHANGES IN PHOSPHORYLATION OF TAU PROTEIN IN GERBIL BRAIN CORTEX DURING AND AFTER ISCHEMIA

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Tau is a microtubule-associated protein. Its hyperphosphorylated form is a prominent component of the neurofibrillary tangles in Alzheimer's disease. The neurons in layers 3, 5, and 6 of the cerebral cortex are known to be vulnerable to ischemic insults. The aim of this study was to establish changes in the phosphorylation of tau protein in the gerbil brain cortex after 5 min forebrain ischemia, induced by bilateral common carotid artery occlusion, resulting in 87.8% decrease in cortical blood flow. The insult was followed by recovery for 20 min, 2 h, 24 h, 3 and 7 days. Proteins of the cortical homogenates were separated by electrophoresis and analysed by Western blotting using antibodies against phosphorylated (12E8 and PHF-1) and non-phosphorylated (Tau 14 and Tau 46) tau epitopes. In control animals, three polypeptides 63, 65 and 68 kD of tau were immunoreactive with Tau 14 and Tau 46 antibodies. The 63 kD polypeptide was also immunoreactive with 12E8 antibody and the 68 kD polypeptide with PHF-1. Ischemia resulted in a significant decrease of the PHF-1 immunoreactivity, which persisted during 20 min and 2 h of recovery, and increased above control level 3 and 7 days later. In contrast, ischemia resulted in an increase of the 12E8 immunoreactivity, which returned to control levels during recovery. The results suggest that the two phosphorylated sites are regulated by a different mechanism and may play different roles in the microtubule assembly and dynamics. These sites may also define various pools of vulnerable neurons in the brain.

23.7 PROPERTIES OF CALCIUM CURRENTS IN CARDIAC POSTGANGLIONIC SYMPATHETIC NEURONES.

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The aim of the study was to investigate the kinetic properties and identify the subtypes of Ca^{2+} currents in the cardiac postganglionic sympathetic neurones of rats. Neurones were labelled with a fluorescent tracer - Fast-Blue, injected into the pericardial cavity. Voltage-dependent Ca^{2+} currents were recorded from dispersed stellate ganglion cells that showed Fast Blue labelling. Only high threshold voltage-dependent Ca^{2+} currents were found in the somata of cardiac sympathetic neurones. Their maximum amplitude, mean cell capacitance and current density were respectively: 0.67 nA, 19.3 pF and 36.4 pA/pF (n=21). The maximum Ca^{2+} conductance was 51.3 nS (n=14). Half activation voltage equalled +11.0 mV and the slope factor for conductance 11.1 (n=14). As tested with a 10 second pre-pulse, the Ca^{2+} current began to inactivate at –80 mV. Half inactivation voltage and slope factor for steady-state inactivation were –36.6 mV and 14.1 (n=9), respectively. Saturating concentration of L channel blocker (nifedipine), N channel blocker (ω-conotoxin-GVIA), P/Q channel blocker (ω-Agatoxin-IVA) and N/P/Q channel blocker (ω-conotoxin-MVIIC) reduced the total Ca^{2+} current by 26.8% (n=7), 57.1% (n=12), 25.9% (n=6) and 69.4% (n=6), respectively. These results show that the somata of cardiac postganglionic cardiac sympathetic neurones contain significant populations of N, L and P/Q high threshold Ca^{2+} channels.

Supported by KBN grant No. 4P05A.0137.08 and 4.P05A.070.14

23.8 THE ROLE OF HYPOTHALAMUS IN THE MODULATION OF PAIN.

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The traditional view that noxious stimuli activate the hypothalamus via multineuronal circuits has been recently changed. Anatomical evidence demonstrated that the hypothalamus and other areas of the limbic system (implicated in the affective components of pain) are direct recipients of the input from the dorsal horn neurons. Information about innocuous and noxious, both cutaneous (skin) and visceral (intestines, reproductive system) stimuli was proved physiologically to be directly processed in these areas.
Hypothalamic lesion reduce intractable pain in patients and increase the pain threshold in experimental animals. The hypothalamus also affects other processes related to pain, for example, stress-induced analgesia, affective states and immune functions. In depressed patients, pain and alterations in immune function often coexist. The hypothalamus may play important role in the integration of these processes through abundant direct neural connections and neuroendocrine outflow. The hypothalamic-pituitary-adrenal (HPA) axis is widely considered to be the organizing structure for defensive responses to intense injury or chronic stress. Both hypothalamic, pituitary and adrenal lesions induce changes in nociception and inflammatory reactions. Such lesions may cause also both structural and functional changes in the nociceptive and immune systems. The results of recent studies on the spinohypothalamic tract neurons will be presented and their integrative role in HPA axis activation will be discussed.

Session 24 - Plenary Lectures

24.1 CLAUSTROCORTICAL NEURONAL LOOP AND ITS RELATION TO CORTICAL FUNCTIONS.

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Claustrum is the comparatively large brain structure, which connections and function had been unknown. Due to investigations performed with various degeneration and axonal transport methods it has been found in last decades that claustrum is intensively connected with various areas of the cortex.
These connections form the claustrocortical neuronal loop similar in many respects similar to that connecting thalamus with the cortex.
The descending limb of the claustrocortical loop is formed by neurons of the 6. cortical layer, the ascending – by large and medium-sized glutaminergic cells of the claustrum. GABAergic claustral neurons seem to be exclusively the interneurons with short axons.
Neurons localized in various parts of the claustrum project to particular areas of the cortex. Due to that, they may influence specific functional systems of the brain. Especially, the relationship of the claustrum to the visual system seems to be very close. It has been found, that similarly to the lateral geniculate body and visual cortex, there is retinotopical organization of visual neurons in the claustrum. The experimental lesions of the posterior part of the claustrum change deeply the function of cortical visual neurons, what may be of importance in brain pathology.

24.2 NEURAL RECOGNITION MOLECULES AND SYNAPTIC PLASTICITY

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Recognition molecules at the cell surface and in the extracellular matrix have been shown to be important not only during ontogenetic development, but also in regeneration and synaptic plasticity in the adult nervous system. Neural adhesion molecules of the immunoglobulin superfamily, such as L1 and NCAM have been shown to be instrumental in modifying synaptic efficacy and formation and/or stabilization of synapses. We have evidence that NCAM is involved in stabilization of synapses after long term potentiation in vitro and in acquisition and storage of learned tasks. Furthermore, recent experiments highlight the possibility that recognition molecules and their associated carbohydrates, such as the HNK-1 carbohydrate shared by many recognition molecules in the nervous system, regulate basal synaptic transmission and long term potentiation via a direct or indirect interaction with neurotransmitter receptors.

Session 25 - Poster Session: Neural plasticity

25.1 NMDA RECEPTOR SUBUNIT NR2B IN MOUSE SOMATOSENSORY CORTEX AFTER BEHAVIORAL TRAINING.

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NMDA receptor channel mediates a large fraction of excitatory synaptic transmission in the cerebral cortex. The receptor-channel complex contains NR1 subunit and variable number of NR2 subunits. The proportion of NR2A and NR2B subunits underlies the functional heterogeneity of NMDA receptor channel. We characterized the expression of NR2B mRNA in mouse somatosensory barrel cortex by *in situ* hybridization after classical conditioning in which stimulation of a row of vibrissae was paired with a tail shock. Three days of conditioning produce changes in representation of the stimulated row of vibrissal in the somatosensory barrel cortex. ^{35}S-labelled oligodeoxynucleotides were used as sense probes for hybridization to 10 µm brain sections.No significant alterations in NR2B mRNA expression were found in somatosensory cortex after 3 days of training. The hybridization signal was the strongest in cortical layer VI and I/II. In the region of the barrel field also layer IV contained focal regions of heavier labeling, corresponding to the centers of the barrels. It appears that in the adult cerebral cortex representational plasticity, which, as we found previously (Jablonska et al. 1999), depends upon NMDA receptor, is not accompanied by changes of NR2B expression at the transcription level.
The study was supported by Howard Hughes Medical Institute grant to M.K.

25.2 PLASMA MEMBRANE Ca^{2+} ATPase 1 AND 4 mRNA EXPRESSION IN RESPONSE TO KAINATE TREATMENT

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Kainate, which mimics glutamate toxicity, causes either immediate cell death by continued depolarization or its delayed onset due to intracellular accumulation of toxic levels of Ca^{2+}. Increase in Ca^{2+} results in the formation of free radicals, which may alter the functioning of Plasma Membrane Ca^{2+} ATPase (PMCA). Therefore, this primary calcium handling system of the cell membrane may play a possible role in neuronal degeneration. We have analyzed the distribution of mRNAs of two Ca^{2+}-dependent Membrane ATPases in the rat hippocampus. The effect of kainate (injected at10 mg/kg) was studied by in situ hybridization. Adult Wistar male rats were sacrificed at the following times post kainate injection: 1 hr, 6 h, 24 h, 72 h. In situ hybridization was performed using specific gene probes for PMCA 1 and PMCA 4. The expression pattern of PMCA 1 mRNA in the control and kainate-treated rat hippocampus was analyzed and is found to be in accordance with previously documented data. PMCA 1 is the most abundant of the four ATPases and is expressed significantly in the pyramidal cell layer of CA1-CA3 as well as the granule layer of the dentate gyrus. Following kainate injections, the expression of PMCA 1 mRNA remained unchanged during hours 1-4. By 12 h there was a decrease in CA1 and CA3, which continued through 48 h and 72 h post-kainate. The presence of PMCA 4 mRNA in the brain was not investigated previously. Our results in control animals localize this mRNA mostly to the piriform cortex, habenula, the choroid plexus, and especially the CA1 and CA3 hippocampal regions and the dentate gyrus. Following kainate insult, a noticable increase in PMCA 4 mRNA expression was noted in the dentate gyrus at the 1h time point. A significant decrease in the CA1 and, to a lesser degree, in CA3 was observed at 24h following kainate insult. These results may imply kainate evoked regulation of PMCA 4 gene expression, which, in effect, may suggest a role for PMCA 4 protein in functional response to kainate stimulation.

25.3 A ROLE FOR THE RYANODINE-SENSITIVE INTRACELLULAR CALCIUM STORE IN LONG-TERM MEMORY FORMATION IN CHICK

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Training chicks on a one-trial passive avoidance task results in an enhanced increase in NMDA- and AMPA-stimulated elevation in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) in synaptoneurosomes isolated from left and right IMHV of chick brain 10 min and 3-6 h post training, respectively. Here we report results determining the source of these increase in $[Ca^{2+}]_i$. Chicks were trained on the aversive substance, methylanthranilate (M), or water (W), or did not experience the training procedure (Q). Synaptoneurosomes, prepared from the IMHVs of chicks from each group, were stimulated with 0.5 mM NMDA or AMPA in the presence of 100 nM dantrolene, an inhibitor of Ca^{2+} release from the ryanodine-sensitive intracellular Ca^{2+} store. At 10 min after training, when the enhanced NMDA-stimulated increase in $[Ca^{2+}]_i$ occurred in M birds, dantrolene completely abolished the enhanced response to NMDA, reducing the increase to that observed in W and Q chicks. At 6h after training, the time when the enhanced increase in $[Ca^{2+}]_i$ with AMPA occurred, dantrolene had no effect in the enhanced increase observed in M chicks. Therefore, this intracellular store is not involved in the enhanced AMPA-stimulated increase in $[Ca^{2+}]_i$ observed 6h after training, but is the source of the enhanced increase in $[Ca^{2+}]_i$ evoked by NMDA 10 min after training.
The effect of injecting 2.5 µl 20 mM dantrolene (50 nmol per IMHV) 3 or 30 min before, or 30 min or 3h after passive avoidance training, was determined at 30 min, 3h or 6h after training. Those chicks injected 30 min before or after training, and tested at 3h after training, exhibited amnesia for the task. Chicks injected at times earlier or later from the time of training did not exhibit amnesia. Therefore, inhibition of Ca^{2+} release from the ryanodine-sensitive intracellular store, around the time of training, affects performance in the task, and coincides with the time that dantrolene inhibits the enhanced increase in $[Ca^{2+}]_i$ stimulated by NMDA observed in synaptoneurosomes.

THE INVOLVEMENT OF MESOLIMBIC SYSTEM IN MEMORY ENHANCING EFFECT OF CCK-8US AND CAERULEIN IN RATS. 25.4

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The involvement of dopaminergic projection to the central amygdala and dorsal hippocampus in the facilitatory effect of cholecystokinin unsulfated octapeptide (CCK-8US) and caerulein (CER) on memory motivated affectively was investigated in male rats. CCK-8US and CER were given sc. at the doses of 10 µg/kg and 0.5 µg/kg, respectively, immediately after a single learning trial in a passive avoidance situation, ten days after bilateral 6-OHDA lesions to the central amygdala or to the dorsal hipopocampus. In order to protect noradrenergic neurones against destruction by neurotoxin, thirty min before surgery rats were pre-treated intraperitoneally with 25 mg/kg of desmethylimipramine, an inhibitor of noradrenaline uptake. Bilateral 6-OHDA lesions to the central amygdala totally abolished and to the dorsal hippocampus significantly attenuated the facilitatory effect of CCK-8US and CER on retention of passive avoidance behaviour evaluated 24 h after the learning trial. Neither destruction of dopaminergic endings in each structure, nor application of CCK-8US and CER changed the spontaneous psychomotor activity of rats estimated in an "open field" test. These results may indicate that in the facilitatory effect of CCK-8US and CER on memory motivated affectively is involved the dopaminergic projection from ventral tegmental area to the central amygdala and dorsal hippocampus.

25.5 ETHANOL INVOLVEMENT ON SOCIAL MEMORY IN ADULT RATS WITH DISTURBED CIRCADIAN CYCLE.

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It is known that administration of ethanol [ETOH] to rats during pre- and postnatal period results an impairment in the social memory after the animals have reached the adult age (Kelly and Tran, 1997). The aim of this study was to evaluate the effect of chronic ETOH (3 months) treatment on social recognition task performance in adult rats. *Materials&Method:* Preferring ETOH [PRF], non-preferring [NPF] and control [KN]-(ETOH naive) male Wistar rats kept on a 24/0 h dark cycle or control animals with normal diurnal cycle-[KD]-(12/12 h night/day) were investigated using social recognition paradigm (Thor and Holloway, 1982, Danzer *et al.*, 1987). The two different interexposure intervals (30 or 120 min) allowed to measure the different memory conditions: short-term recognition (STR) and long-term recognition (LTR), respectively (Griffin and Taylor, 1995). *Results:* It was found that chronic ETOH treatment both in PRF and NPF groups led to better fulfilment of the STR tasks compared to KN rats. This effect appeared specific, since no change in this condition was noticed when an unknown juvenile rat was exposed to the adult rat during second contact. However using LTR procedure it was observed that PRF and NPF rats performed these tasks worse in comparison to KN animals. Moreover no differences between KN and KD groups in social memory STR and LTR paradigms were noticed. *Conclusion:* Our results confirm the hypothesis that in some conditions probably when toxic effects of ETOH are not observed yet ETOH may express a positive influence especially on short-term memory of adult rats what corresponds with data sometimes postulated by the others in humans (Dufouil *et al.*, 1997).

THE EFFECT OF A SIX WEEKS PHYSICAL TRAINING ON ASSOCIATIVE LEARNING IN RATS 25.6

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Various effects of physical exercise have been extensively studied. However, its effects on the higher functions of the central nervous system remain largely unexplored. In this study we investigated the influence of chronic exercise (six weeks daily treadmill training with increasing running time up to 60 min at 20 $m \times min^{-1}$ per day) on the associative learning in 17 male Wistar rats. During the course of the training these animals, along with 18 control not trained, but appropriately handled rats, were taught how to find food in a delayed nonmatching to sample (DNMS) setting.

The trained rats learned DNMS slower and had overall percentage of correct choices significantly ($p<0.01$) less than these in the control group. However, when only those rats which finally reached the criterion of 80% correct choices in two consecutive sessions were compared, no significant differences between the trained and not trained animals were found.

These results show that the long-term physical training may differently influence rats' cognitive processes depending on their congenital abilities.

25.7 MOCLOBEMIDE ENHANCES LEARNING AND MEMORY OF AVERSIVE STIMULI IN RATS

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Moclobemide is reversible and selective inhibitor of monoamine oxidase A with antidepressant activity. In this study we examined, in male Wistar rats (180-220 g), effects of a single and multiple (14 days) intragastrical doses of 20 mg×kg^{-1} of moclobemide on the acquisition of conditioned avoidance responses (CARs), retrieval of passive avoidance, recognition memory, allocentric (Morris maze) and egocentric (T-maze) spatial memory as well as psychomotor activity (open field) and anxiolytic effects (elevated 'plus' maze) of the drug.

Moclobemide, given for 14 days, increased rate of the CARs acquisition, retrieval of passive avoidance, and had some anxiolytic properties as shown in the 'plus' maze. The drug, however, did not influence rats' psychomotor activity tested in the open field and their ability to discriminate between familiar and unfamiliar objects. Single dose of moclobemide was ineffective in all the tests employed. Both, single and repeated treatment with moclobemide influenced neither allocentric nor egocentric spatial memory.

It is concluded that moclobemide, given chronically, enhances learning and memory motivated aversively.

* We thank Hoffman La-Roche for the sample of moclobemide.

25.8 POSSIBILITIES AND RESTRICTIONS OF COMPUTER BASED VIDEO TRACKING SYSTEM IN THE ANALYSIS OF EMOTIONAL BEHAVIOR

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Advance in computer technology makes possible using computer based video tracking systems for automated acquisition and analysis of behavioral data in many commonly used tests. Automated system, in contrast to manual data acquisition method, is time-saving, unbiased and allows to eliminate errors resulting from human observer's fatigue or inter-rater differences. The aim of this work is to show on actual data the advantages and disadvantages of using commercial video tracking system EthoVision® to the analysis of rat's emotional behavior. The experiment was performed in order to assess the emotional profile of each subject within the group of young adult rats (n=20). EthoVision® system was used to collect and analyze data from three various tests: Open Field, Elevated Plus Maze and Social Interaction. The main advantage of the system is that it allows to measure factors that are hard to obtain during manual data acquisition like velocity, distance between two rats, distance to various points of interest. Moreover, there is a possibility of changing the parameters of measured factors (i.e. zones numbers and sizes) even after the registration, what eliminates the necessity of preliminary tests. In Social Interaction Test EthoVision cannot recognize the character of social contacts, but it can measure duration, time pattern and exact distance between two rats. To obtain full ethogram therefore, the manual ratings of videotapes is required. Recognition of behavioral events e.g. fight or mounting is much easier in combination with EthoVision, because the time frame is already determined by the system. Presented results show, that automated data acquisition method is an efficient, reliable and convenient tool for the analysis of most aspects of emotional behavior.

25.9 NR2A SUBUNIT OF NMDA RECEPTOR IN MOUSE SOMATOSENSORY BARREL CORTEX DURING LEARNING – INDUCED CHANGES OF CORTICAL BODY MAPS.

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The NMDA receptor plays a crucial role in plastic changes of the nervous system. The receptor subunits are encoded by two gene families, NR1 and NR2A-D. The properties of the receptor are regulated by changing the relative expression of the subunits The present study examined the expression of NR2A subunit mRNA in the somatosensory barrel cortex of mice after behavioral training in which stimulation of vibrissae was paired with a tail shock. We have previously found that such treatment induces plastic changes in cortical representations of the stimulated vibrissae. Brain sections from trained animals were used for *in situ* hybridization to NR2A mRNA. ^{35}S oligodeoxynucleotides were used as sense probes. In brain sections from cerebral hemispheres contralateral to the side of the muzzle stimulated during the training, an tendency for increase of the hybridization signal was found in layer IV of the barrel cortex. The expression of NR2A was higher over the barrels representing vibrissae stimulated during the training than over adjacent, non-stimulated barrels in the same hemisphere. The results indicated that plastic changes of cortical body maps are linked to changes in composition of NMDA receptor complex.

The study was supported by Howard Hughes Medical Institute grant to M.K.

25.10 EFFECT OF ANGIOTENSIN CONVERTING ENZYME INHIBITORS ON MEMORY IN PATIENTS WITH HYPERTENSION

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The aim of the study was to explore the effects of captopril, enalapril and trandolapril on learning and memory in patients undergoing standard antihypertensive therapy with these drugs.

Twenty eight subjects (12 men and 16 women, mean age 55,2± 2.15 years), with mild to moderate hypertension (according to the WHO criteria), and the mean dissease duration of 3 years, were included. There were 3 experimental groups: 1) 8 patients (59.8 ± 2.36 years old) received captopril (34± 2.7 mg× day^{-1}) during 2.1 ± 0.77 years; 2) 13 patients (52.7± 1.73 years old) received enalapril (15.5 ± 1.98 mg× day^{-1}) during 1.6± 0.56 years and 3) 7 patients (53± 2.36 years old) received trandolapril (2 ± 2.7 mg× day^{-1}) during 1 ± 0.77 years. Sixteen normotensive, healthy control subjects (6 men and 10 women), 52.7±1.99 years old taking no medication were tested in parallel to the above treated subjects. Rey Auditory Verbal Learning Test (AVLT) and Choynowski Memory Scale (CMS) were run in each subject. There was no significant differences in comulative (over 5 trials) recall of 15 words in AVLT between patients receiving captopril, enalapril, trandolapril and the controls. Although, the treated subjects tended to be worse than the untreated ones on the recall test they performed on CMS as well as controls. We conclude that the therapy with ACEIs does not change verbal learning in hypertensive patients but it may slightly weaken recall.

25.11 **SPREAD OF ACTIVATION AFTER SINGLE AND REPETITIVE STIMULATION OF THE MESOCORTICOLIMBIC SYSTEM: A C-FOS STUDY**

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The mesocorticolimbic system regulates various adaptive behaviors including exploratory locomotion and ingestive reactions, it is also engaged in self-stimulation and drug-seeking behavior. This system undergoes dynamic changes after its repetitive activation as it was shown by neuropharmacological methods. In the present work we studied a spread of activation throughout the brain as measured by c-fos expression, in conditions of a single and repetitive stimulation of the mesocorticolimbic system by natural stimuli (exposure to a new environment) and electrical stimulation of the midbrain ventral tegmental area (VTA). After 1h exposure of rats to a new environment which evoked robust exploratory reaction fos-immunoreactive nuclei were found in numerous structures of both hemispheres, mainly prefrontal, cingulate and pyriform cortex, in a group of nonspecific and limbic thalamic nuclei and in the lateral septum, lateral hypothalamus and n. accumbens shell. After electrical stimulation of VTA which also evoked exploratory behavior the picture of c-fos expression was similar except the hormonally-active hypothalamic nuclei which were heavily stained in the stimulated rats. Repetitive exposure of rats to both the environmental stimuli and electrical VTA stimulation resulted in a decrease in c-fos expression in all brain structures except those belonging to the mesocorticolimbic system. The results obtained show dynamic changes in the brain circuitry associated with appetitive behaviors.

Session 26 - Poster Session: Rhytmic activities and sensory systems

26.1 **CIRCADIAN RHYTHMS IN LOCOMOTOR ACTIVITY AND IN THE VISUAL SYSTEM OF THE BLOWFLY**

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Just as in other animals, insects exhibit circadian rhythms in their behaviour and in the sensitivity to light of their visual system. In the fly's visual system circadian rhythms have been detected not only in the retina but also in the first visual neuropile (lamina). In this neuropile two populations of first-order interneurons, L1 & L2, which receive direct visual input from the retina, exhibit daily size changes. In the housefly their axons swell during the day and shrink at night, whereas in the fruit fly the largest axon size occurs at the beginning of night. The differences between these two species may depend on the different activity rhythms in the two flies. To study this we examined both rhythms, locomotor activity and L1/L2 size changes, in the blowfly *Calliphora vicina*. For this, locomotor activity of flies was monitored in a day/night cycle (LD 12:12) as well as in constant darkness (DD). The flies were then fixed and L1 & L2's axon cross-sectional areas measured planimetrically from semithin plastic sections. Flies were prepared during the day and night in LD and in the fly's active and rest periods in DD. In LD, L1 & L2 were both larger during the day than at night and the larger sizes correlated with locomotor activity of the flies. Moreover, arrhythmic flies showing a high activity level had larger cells than flies with a low level of activity. In DD, both cells were larger in the middle of rest period, however, rather than in the middle of active one, showing that both circadian rhythms became reversed in-phase after one week of DD. The results obtained indicate the existence of a correlation between both circadian rhythms, lamina and locomotor, and that these operate in-phase in LD, with larger cell sizes during the period of the flies' activity.

Supported by grants to E.P. (HHMI75195-543102) and to B.C. (KBN6PO4C01916)

26.2 **CIRCADIAN CLOCK IN THE BRAIN CONTROLS CIRCADIAN RHYTHMS IN THE FLY'S OPTIC LOBE**

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The fly's visual system shows many circadian rhythms detected in the retina photoreceptors and also in the first visual neuropile, the lamina. In the lamina, two interneurons, monopolar cells L1 and L2 change sizes during the day and night, swelling by day and shrinking by night. This rhythm is circadian, robust for L2 but weak for L1, because is also maintained under constant darkness (DD) and continous light (LL) conditions. The rhythms in L1, L2 may be generated in the circadian clock located in the central brain where behavioural circadian rhythms origin or in the lamina itself. To test this we have examined changes in cross-sectional area of L1, L2 axons in the housefly (*Musca domestica*) under day/night (LD12:12), DD and LL within the optic lobe severed 24 hrs earlier from the rest of brain. Next the fly's optic lobes were fixed for EM and the cross-sectional area of axons was measured from sections using computer based morphometric system. The obtained results showed that after lesion the rhythms in L1, L2 size changes were maintained only in LD but disapeared in DD and LL, however, in LD they were weaker than in control. It indicates that circadian rhythms in the fly's lamina are generated outside the optic lobe but daily changes of light in LD are able to induce a weak exogenous rhythm in L1, L2 size changes.

Supported to E.P. by HHMI 75195-543102.

26.3 RHYTHMIC OSCILLATION IN THE RAT INTERGENICULATE LEAFLET NEURONS AFTER LIGHT –OFF

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The intergeniculate leaflet (IGL) is a subdivision of the thalamic lateral geniculate complex and is an important component of the mammalian circadian timekeeping system. The IGL integrate photic and nonphotic information to modify the suprachiasmatic nucleus (SCN) activation. The aim of the present study was to explain whether the light manipulation modulated the activity of neurons. We recorded multiple unit neuronal activity (MUA) from dorsal and ventral part of lateral geniculate nuclei and from IGL in anaesthetized rats. In all subdivision of lateral geniculate complex we observed spontaneous firing rates of cells but only in IGL after light ON the neurons activity was significant increase. But just after light OFF, the responses in IGL stabilized and exhibited surprisingly a highly regular oscillatory patterning. However, this effect was only observed in the area of the anatomical localization of the IGL. The results show that light is a most important stimulus for IGL neurons but by oneself can't evoked rhythmically oscillation in the rat IGL neurons. To evoked such rhythmically modulation, the IGL need additional nonphotic inputs. According to the others investigation we suggested that light -OFF influences on the IGL activity like nonphotic inputs.

ACKNOWLEDGEMENT: This work was supported be the Committee for Scientific Investigations, Grant no PB418/PO4/96/10.

26.4 DIFFERENCES IN INFORMATION FLOW FOR HIGH FREQUENCY EEG ACTIVITY WITHIN LIMBIC-MOTOR CIRCUITRY IN FREELY MOVING RAT

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High frequency rhythms (30-100 Hz) in EEG accompany various behavioural states (i.e. various emotional/motivational conditions). Our interest was aimed at functioning of limbic-motor interactions involved in emotional behaviour. Thus we analysed an information flow among the n. accumbens (a nodal point between the motivational and executory systems), its main inputs (basolateral amygdala, ventral subiculum), and its output (subpallidal area). The EEG signals were recorded during locomotion in various behavioural states (emotional/motivational). The directed transfer function (DTF) was used to determine a direction and intensity of information flow among the structures in frequency domain. Three frequency bands were analysed (32.0-35.4 Hz, 67.7-72.2 Hz, 78.2-87.2 Hz). The intensity of information flow among studied structures was compared between various experimental situations. Information flows in low emotional level tasks did not differ from motionless situations. In situations when emotions were induced by additional stimuli during a locomotor task (locomotion along horizontal ladder or along wide runway with a ringing bell stimulus) only the interaction between n. accumbens and subpallidal area was different when compared with the locomotion at low emotional level. In situations when emotional/motivational background was different (alimentary or sexual) the interactions among all investigated structures significantly changed (i.e., between limbic structures and n. accumbens, between n. accumbens and subpallidum, and between limbic structures and subpallidum). During maze exploration the intensity of majority of the information flows was lower, when compared with the low emotional locomotion. Comparison of the DTF values calculated in the band of 67.7–72.2 Hz for various situations showed that they did not differ among the locomotion periods, the distinctions appeared only when non locomotion periods were compared.

Summarising, one can say that in high frequency ranges of EEG the differences between emotional states are reflected by changes in pattern of information flow among limbic-motor structures.

26.5 INVOLVEMENT OF GABA-ERGIC TRANSMISSION IN THE PEDUNCULOPONTINE TEGMENTAL NUCLEUS IN GENERATION OF HIPPOCAMPAL THETA RHYTM.

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The pedunculopontine tegmental nucleus (PPTg) particularly its cholinergic neurons were shown to participate in the brainstem control of the hippocampal theta rhythm. In our previous experiments we found that temporal inactivation of the pedunculopontine tegmental nucleus (PPTg) by means of direct procaine injections completely blocked the sensory-elecited hippocampal theta rhythm. In the present work we studied a possible involvement of GABA-ergic transmission in PPTg in the regulation of the hippocampal theta rhythm.The experiment was done on urethane anaesthetized male Wistar rats implanted with bilateral hippocampal recording electrodes in the stratum moleculare of the dorsal blade of the dentate gyrus and with an injection cannula unilaterally in the region of PPTg. Theta rhythm was elicited by tail-pinch before and after unilateral microinjection of $GABA_A$ receptors antagonist (bicuculline 50ng/0.5μg) and agonist (muscimol 100ng/0,5μg) directly into PPTg. Spectral analysis was performed on the 5-s epochs of hippocampal EEG during tail-pinch using a fast Fourier transform (FFT). Bicuculline enhanced sensory-elicitable theta which was expressed as an increase in the mean FFT values for peak magnitude by about 60% and an increase in the mean FFT values for peak frequency by 0.8 Hz. Muscimol attenuated theta rhythm which was manifested as a reduction of the mean FFT values for peak magnitude by about 50% and reduction of the mean FFT values for peak frequency by 2.8 Hz. The results indicate that PPTg GABA-ergic transmission is involved in the regulation of hippocampal theta rhythm.

26.6 MULTIMODAL EVOKED POTENTIALS IN HUNTINGTON'S DISEASE PATIENTS.

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Huntington's disease (HD) is a progressive, autosomal dominant neurodegenerative disorder resulting from selective neuronal loss primarily involving the striatum, other basal ganglia, cerebral cortex and cerebellum. 20 patients aged from 14 to 56 with DNA confirmed HD with CAG repeats ranging from 40 to 85 were examined clinically and by neurophysiological methods. The aim of our study was to evaluate functional integrity of somatosensory (SEPs), visual (VEPs) and auditory (BAEPs) pathways as well as their cortical generators in correlation with EEG and MRI findings.

Alteration of SEPs shape and diminution of amplitude of early cortical potentials was obtained in 80% of median and 65% of tibial stimulated nerves. Prolonged spinal and/or central conduction time (CCT) was found in 38% and 50% of patients respectively. Similarly, the P100 amplitude of VEP was decreased in 50% of examined cases, whereas the P100 latency was prolon-ged in four patients only. Absolute and interwave latencies and amplitudes of BAEPs were normal while the interwave amplitude differences of I and V waves were abnormal in 50% of patients. EEG revealed low amplitude background activity in 65% of patients with diffused slowing in 6 cases. MRI showed cortical atrophy in 64% of HD patients, basal ganglia hyperintensity in T2 and marked atrophy in 81% of cases. We concluded, that diminution of the amplitude of EEG, and of early components of SEPs and VEPs are probably due to cortical atrophy and functional alteration of volley transmission via thalamus and other basal ganglia. Prolonged spinal conduction time and CCT in HD patients could be associated with involvement of spinal cord sensory pathways.

26.7 AUDITORY SKILLS DEVELOPMENT IN A PATIENT PROVIDED WITH AUDITORY BRAINSTEM IMPLANT

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The development of hearing perception in a patient provided with an auditory brainstem implant (ABI) was assessed over 12 months after the surgery. Electrophysiological, psychoacoustic and speech perception tests were periodically carried out. Responses to electrostimulation by individual implant electrodes were measured to evaluate the functions of levels of auditory sensation vs. stimulus intensity. Dynamics of threshold levels and maximum comfort levels, as well as pitch specific sensations was determined. In the presented paper, the results of psychoacoustic tests are compared with those of electrophysiological measurements. The so far achieved results indicate a good stability of implant electrodes operation accompanied by a significant improvement of patient's open speech recognition abilities. The results prove that the ABI helps to develop communication skills and substantially improves the quality of life.

26.8 **COINCIDENCE OF OSCILLATORY ACTIVITY WITHIN BETA AND GAMMA RANGE IN THE CORTICO-THALAMIC SYSTEM OF CATS ATTENDING TO VISUAL STIMULI.**

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We have previously shown[1] that fast Fourier transforms (FFTs) of the local field potentials (LFPs) recorded in the lateral geniculate nucleus (LGN) and primary visual cortex (VCx) of cats attending to visual stimuli display peak in beta (16-24 Hz) frequency band of higher amplitude as compared to the FFT peak calculated during auditory attentive situation. In the present study we attempted to find the possible coincidence between such beta activity and the gamma oscillations which were proposed to play crucial role in the feature binding hypothesis. The LFPs, registered on the videotape during the experiment, were filtered off-line within 16-24 Hz and 30-45 Hz frequency ranges. The envelopes of both beta and gamma activities recorded at the given site were then obtained and the normalized temporal crosscorrelation between them calculated. The significant positive correlation was found in those LGN and VCx sites, which corresponded to the central representation of the visual field. The correlation function peaked within 50 ms from the center of the histogram. The correlation values obtained during the visually attentive trials were significantly stronger, as compared to those calculated from the data recorded in auditory trials.

These findings support the hypothesis[1] stated previously that beta activity provides the necessary excitatory background for appearance of oscillations in gamma band.

1. M. Bekisz and A. Wróbel, Acta Neurobiol. Exp. (1993), 53: 175-182.

26.9 **CUTANEOUS REFLEXES IN CHRONIC SPINAL RATS**

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One of the prerequisites of successful stimulation of the recovery processes following spinal cord injury is to reduce secondary effects of the damage in isolated part of the spinal network, peripheral nerves and muscles. Little attention has been paid so far to introducing cutaneous stimulation technique to achieve that purpose. Our aim is to find out whether cutaneous stimulation, activating various muscle synergies, might be used as an effective therapeutic technique in chronic spinal animals. The reflexogenic cutaneous fields of the hindlimbs have been explored in 4 rats spinalized at low thoracic segments. Skin areas were tested with a blunt probe of 0.5 mm in diameter once a week during 5 postsurgery months. Light indentation of the glabrous or hairy skin or slow motion of the probe along hairs elicited movements of the limbs more regularly than other stimuli. Chronic spinal rats were hypersensitive to non noxious mechanical stimulation of the hind limb during the whole testing period. Tactile or light pressure stimuli elicited vigorous, withdrawal phasic reactions of the tested limb or both limbs, followed occasionally by clonic shaking movements. Even deflection of a single hair was sufficient to induce the reaction. The strongest and repetitive reactions were elicited by stimuli applied to the areas around the ankle joint and metatarsus on the dorsal, lateral and palmar aspects of the paw. Weaker and less stable reactions were evoked from the medial side of the limb. Our data indicate that in the long time span after spinalization specific tactile stimuli powerfully activate complex motor reactions creating good tool for rehabilitation.

This work was supported by State Committee for Scientific Research: 1305/P05/98/14

26.10 NONSPECIFIC INFLUENCE OF ACETYLCHOLINE ON THE ACTIVITY WITHIN SOMATOSENSORY CORTEX

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We have previously shown (Musial et al. 1998, Neuroreport 9:2627-2631) that potentials evoked (EP) in the barrel cortex by whisker stimulation consist of two components reflecting activity of the supra- and infragranular pyramidal cells, correspondingly. In nonanaesthetized rats the well habituated EPs were dominated by first component whereas introduction of the reinforcing stimuli (US) enhanced the second component and its amplitude became larger than the amplitude of the first component (Wrobel et al. 1998, Exp. Brain Res. 123:117-123).

To check the role of cholinergic pathway in these effects we have stimulated electrically nucleus basalis magnocellularis (NBM) in urethane anaesthetized rats. As expected, NBM stimulation resulted in overall increase of cortical EP's amplitude indicating that activities of both supra- and infragranular pyramidal cell groups were enhanced.

No reversal of the amplitudes of the two basic components of the responses was, however, observed. These results suggest that acetylcholine is not directly responsible for specific changes observed in cortical activity during conditioning, despite its known role in increasing the general cortical excitability.

26.11 **PROCESSING OF NEUTRAL STIMULI IN THE SITUATION OF SUCCESS AND FAILURE.**

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The effects of emotional states of 'being successful' vs 'being unsuccessful' were studied by means of P300 component of event-related potentials (ERPs). Subjects were instructed to reduce P300 amplitude using feedback. Feedback was random but relative probability of different signals created the situations of 'being successful' or 'being unsuccessful'. Potentials, evoked by light stimuli in a standard 'dd - ball' procedure, were recorded from Fz, Cz and Pz scalp sites. The amplitudes of P300 components were reduced in 'unsuccessful' trials whereas in 'successful' trials they did not differ significantly from responses recorded without the feedback. There were no significant differences in peak latencies. These findings indicate that tonic emotional states affect the processing of neutral stimuli and that late components of ERPs can be useful in the analysis of these alterations. The results also indicate the asymmetry between the effects of positive and negative emotional states. Manipulated feedback is suggested as an useful model in the studies of emotions. Data can also facilitate the interpretation of the real feedback effects.

26.12 THE INFLUENCE OF ATTENTION ON NEUROPHYSIOLOGICAL RESPONSES TO SPOKEN SENTENCES

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The unique feature of language is its temporal dimension. The functional MRI and other visualising methods, despite their excellent spatial resolution, cannot provide the information about how the processes involved in language understanding and expressing occur in time. Recording of electrical activity of the brain during presentation of language material is a natural tool to explore dynamic processes of language.
The authors present the components of the event related potentials sensitive to semantic and syntactic incongruities of language material. Preliminary results of authors own investigation on the influence of subject's attention on the parameters of the N400 potential waveform are included.

26.13 **AUDITORY RECOGNITION TRAINING IN MONKEYS: INFLUENCE OF EXPERIMENTAL DESIGN**

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Three rhesus monkeys were trained in auditory Delayed Matching-to-Sample (DMS) task (Kowalska, *Acta Neurobiol Exp 57:345*). Training of sound-guided responses toward activated speaker (stages **A & B**) was followed by recognition tasks (stages **C & D**). Despite of 1180-1740 trials, none of the monkeys could learn simplest sound-guided directional responses (**A**). With modified apparatus, but with the same procedure, the monkeys were able to learn directional responses quickly (120-260 trials on **A & B**, to criterion 90% in 100 trials). One monkey learned the DMS task to criterion 85% in 100 trials after 960 trials in **C & D**. The other two monkeys perform on 70-74% after 2200 trials of **C & D**. We showed that the introduced modifications of the design improved significantly learning of auditory guided tasks. Thus, our monkey learned auditory recognition task faster than in other comparable experiments have been achieved.

	before the modification	**after the modification**
Manipulanda	movable speakers	touchpads on speakers
Reward	a raisin found under the displaced loudspeaker	a Skittle candy delivered from a food dispenser to a metal bowl
Wooden screen	lowered only during ITIs to separate monkey from speakers	raised during whole session
View inside	one-way transparent window	CCTV camera
Acoustics	reverberant	partially dampened

Supported by McDonnell grant no. 95-13.

26.14 **NATURE OF TONIC INFLUENCE OF FRONTAL COTREX ON LOCUS COERULEUS NEURONS**

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The functional influence of the frontal cortex (FC) on the noradrenergic nucleus locus coeruleus (LC) is still unclear. Though, it has been proved in early research works that frontal cortex controls activity of locus coeruleus neurons in a tonic way (S.J.Sara et al., 1995 and G.Aston-Jones et al.1998), rapports about the direction of this influence (inhibition or excitation) are contradictory. To elucidate this subject, we examined firing rates of locus coeruleus neurons after inactivating frontal cortex activity of urethane anesthetized rats.

The FC was inactivated by local infusion of lidocaine (2% solution, 180nl, 300nl or 500nl at 1nl/s rate) while extracellularly recording multi unit activity of locus coeruleus neurons. FC activity was monitored with two electrodes placed 300μm and 900μm from infusion site so exact moment and extent of neuronal shut down could be established. After total inactivation of FC activity, there was sustained increase in LC firing rates in 4 out of 10 examined animals (40%) while LC cells did not change their activity in 6 out of 10 animals (60%). In no case did frontal cortex shut down inactivate locus coeruleus neurons activity.

These results indicate that the frontal cortex provides relatively weak inhibitory influence on locus coeruleus neurons.

26.15 **EFFECTS OF BRAIN REINFORCING SYSTEMS ON THALAMIC NOCICEPTIVE EVOKED POTENTIALS IN DEVELOPING RABBITS: NEUROCHEMICAL ANALYSIS**

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The aim of the present study was to examine the role of opioids in the realization of antinociceptive effects caused by brain rewarding or aversive stimulation in 20-40-, 41-60-day-old and adult rabbits. Reward or aversive sites in the hypothalamus or adjacent brain areas were identified respectively by their association with self-stimulation or with escape behaviors. The effects of these sites stimulation were studied on evoked potentials (EPs) in the nonspecific nociceptive thalamic nucleus to peripheral noxious stimulation in anesthetized rabbits before and after injection of naloxone (N) and every 10 minutes thereafter for 1hr. Depending on the emotional value of brain site, its stimulation caused complete or incomplete inhibition of EPs. In the former case, N attenuated inhibitory effect in all age groups under rewarding stimulation, whereas under aversive stimulation N caused similar attenuation only in 20-40-day-old and 3-5-month old rabbits. In the case of an incomplete inhibition of nociceptive EPs, N either potentiated the antinociceptive effect of reinforcing brain stimulation or did not exert any influence on it. The effect of N manifested itself more strongly under the stimulation of reward system than of aversive one and was most pronounced in 20-40-day-old rabbits. Results show that opioids are involved in the antinociceptive effects elicited by brain reinforcing stimulation, at least in part. The role of endogenous opiate system attenuates with age.

This research was supported by a grant from RFFI (N 96-15-97793).

26.16 **ONTOGENETIC PECULIARITIES OF ANTINOCICEPTIVE EFFECTS OF BRAIN REWARDING AND AVERSIVE STIMULATION IN RABBITS**

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The effect of brain rewarding or aversive stimulation in the medial forebrain bundle on the evoked potentials (EPs) of the thalamic centromedian-parafascicular complex (CM-Pf) were investigated in 20-40-, 41-60-day-old and adult rabbits. Brain reinforcing systems were preliminarily determined by intracranial self-stimulation (ICSS) or escape behaviors. Both rewarding and aversive stimulation inhibited EPs to peripheral noxious stimulation. A positive correlation between the ICSS intensity and the inhibitory effects of rewarding stimulation on EPs was found in 20-40-day-old and adult rabbits. The strongest effect of rewarding stimulation occurred in the youngest age group, at a current intensity of 60-90% in relation to the intensity, which was theshold for ICSS. This effect significantly declined in 41-60-day-old (100-175%) and was enhanced again in adult rabbits (80-110%). Aversive stimulation inhibited EPs in all age groups at the lower current intensity if stimulation of these areas in behavioral tests caused the active-, but not the passive-defensive responses. In all rabbits the duration of recovery period of EPs after termination of rewarding stimulation was positively correlated with ICSS intensity. After the termination of aversive stimulation it was longer if the aversive sites were associated with the passive, but not with the active patterns of defensive behavior. The data obtained are among the first to show that every period of ontogenesis is characterized by definite peculiarities of the inhibitory influences of rewarding and aversive stimulation on nociceptive EPs in the CM-Pf.

Session 27 - Poster Session: Neurochemistry and neuropharmacology

27.1 **3,5-DHPG IS INVOLVED IN BEHAVIORAL ACTIVITY OF GABA-B RECEPTORS**

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Metabotropic glutamate receptors class I (I mGluRs) play an important role in learning tasks in hippocampal-dependent forms of memory. The some types of learning and memory are affected by the activation of pre- and postsynaptic GABA-B receptors. Literature date indicates similarities and interaction between I mGluRs and GABA-B receptors. The aim of present study was to investigate the influence of 3,5-DHPG on behavioral activity of GABA-B receptors, which activity were modulated by baclofen and phaclofen.

The drugs (agonists: I mGluRs - 3,5-dihydroxyphenylglycine (3,5-DHPG), GABA-B receptors - baclofen; antagonist: GABA-B receptor - phaclofen) action were studied in two behavioral tests: the passive avoidance situation and the open field test. The results of this study indicated that 3,5-DHPG (0.01,1.0 nmol i.c.v.). Facilitated the consolidation process in passive avoidance test and did not affects spontaneous locomotor activity. Neither baclofen (0.25 mg/kg ip.) nor phaclofen (0.5 μg i.c.v) influenced consolidation in passive avoidance situation and locomotor activity in the open field. Significantly prolonged consolidation was observed only when 3,5-DHPG and baclofen were given together. Coadministration of 3,5-DHPG, baclofen orphaclofen did not change the locomotor activity. The main conclusion is that 3,5-DHPG as I mGluRs agonist modulates the effect of baclofen, selective GABA-B receptor agonist, on consolidation in the passive avoidance situation.

27.2 **GLUCOSE UPTAKE IN THE BRAIN OF RATS AFTER ADMINISTRATION OF QUINPIROLE AND 7-OH-DPAT, TWO CENTRAL DOPAMINE (DA) RECEPTORS AGONISTS**

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It has been demonstrated that changes in glucose utilization in the central nervous system affects dopaminergic neurotransmission. Therefore the DA D_2/D_3 receptors agonist quinpirole and the 7-OH-DPAT, the DA D_3 receptors agonist were injected (390.9 nmol/kg IP) to male adult Wistar rats 30 minutes before 6-^{3}H-D-glucose 0.05 μCi/g IP body weight. Then half of rats were scarified 15 minutes and other half 4 hours later. In the second part, newborn male rats were treated daily, for the first 11 days of their lives, with both DA agonist 195,4 nmol/kg IP (for priming of the DA receptors), and when they reached adult age were injected with 6-^{3}H-D-glucose and then scarified as above. In both studies brain segments (striatum, cortex, hypothalamus, pons) were separated, dissolved and radioactivity counted in scintillation counter. Our results demonstrate that quinpirole increased glucose uptake in the brain after single injection and ontogenic treatment. Decrease in glucose uptake was observed in some part of the brain after single injection of 7-OH-DPAT, but not in primed animals.

Supported by the Silesian Medical University: NN-4-30/98.

27.3 **NORADRENERGIC CIRCUITRY IN MAJOR DEPRESSION**

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Converging evidence from years of intensive research has implicated that a disorder of brain noradrenaline (NE) occurs in major depression (MD). Our studies concentrate on the protein chemistry of the principal source of NE in the brain, the locus coeruleus (LC) and its projection area, the amygdala, using postmortem brain tissue from psychiatrically characterized major depressives and carefully matched control subjects. The distribution of NE transporters, tyrosine hydroxylase (TH) and α_2 - adrenoceptors along the rostro-caudal extent of the LC was uneven and was paralleled by a similar uneven distribution of neuromelanin-containing NE cells in both groups of subjects. Levels of TH were robustly higher in the LC of subjects diagnosed with major depression, in 10 out of 13 major depression - control pairs. In marked contrast to TH, the specific binding of [^{3}H]nisoxetine to the NE transporter in the LC was significantly lower in majordepression. Binding of [^{125}I]-PIC to α_2 - adrenoceptors was moderately higher in subjects with major depression as compared to psychiatrically normal controls. Our data reveal changes in the levels of NE proteins in the LC in major depression that are similar to changes produced by NE depletion in rats. The studies in the core nuclei of amygdala of the same matching subject groups are in progress and the data will be presented at the Meeting. The present study provides further evidence that disregulation of NE neuronal circuits is at least one aspect of the pathophysiology of major depression, and is due to decreased synaptic NE availability.

27.4 **REACTIVITY OF THE CENTRAL DOPAMINE (DA) RECEPTORS IN RATS PRENATALLY EXPOSURED TO SELENIUM**

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Several trace elements play important roles in the normal functions of the brain. Selenium (Se) is one of these and is known to exist as selenocysteine in several proteins such as glutatione peroxidase (Gpx), selenoprotein P and others. Most of these selenoproteins act as enzymes catalyzing the degradation of various peroxides and level of Se in the brain may be very important factor in etiology of some neurodegenerative disorders. On the other hand it is known, that central nervous system (CNS) appears to be a vulnerable target for Se toxicity. Excessive Se intake lead to its accumulation in CNS (in synaptic vesicles, microsomes, myelin and mitochondrial fractions of various brain's region) and afterwards caused lesions in several nuclei of the brain and in the reticular formation. Clinically manifestation of this excessive intake are various neurological and psychiatric disturbances. Literature data suggested that free form of Se can modulated neurotransmitters systems of CNS including dopaminergic system.
Wistar rats were allowed to drink water with Selenium (Se) 1 or 5 ppm ad libitum throughout pregnancy. Control rats received tap water only. Behavior of 2 months old male offsprings was investigated by several psychopharmacological methods. Oral activity, yawning, catalepsy, stereotypy and others were recorded following respective central DA receptors agonists and antagonists administration.
The results indicate that Se applied during pregnancy produce moderate functional changes in the brain of offsprings.
Supported by Silesian Medical University: NN-5-062/98.

27.5 A COMPARISON OF PSYCHOTOMIMETIC EFFECTS OF MK-801 AND CGP 40116 IN BEHAVIOURAL PARADIGMS

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The aim of the study was first, to compare psychotomimetic effects of a non-competitive (MK-801) and a competitive (CGP 40116) NMDA receptor antagonist and second, to evaluate whether competitive NMDA receptor antagonist is able to attenuate psychotomimetic effects induced by MK-801. We have found that MK-801 (0.4 mg/kg) when given alone, produced strong locomotor hyperactivity and disrupted process of sensorimotor gating, while CGP 40116 (1.25, 2.5 and 5 mg/kg) was ineffective in both models of psychosis. Moreover, it was found that locomotor stimulatory effect of MK-801 and the MK-801-evoked disruption of the process of sensorimotor gating were both antagonised by prior administration of CGP 40116. In the delayed alternation test, both CGP 40116 (2.5 and 5 mg/kg) and MK-801 (0.05 - 0.4 mg/kg) increased the number of errors revealing their detrimental effect on spatial working memory and selective attention. The presented results indicate that both classes of the NMDA receptor antagonists may produce qualitatively different behavioural effects, as evidenced by the experiments with locomotor activity and prepulse inhibition of the acoustic startle response. Moreover, the competitive NMDA receptor antagonists may attenuate psychotomimetic effects related to the non-competitive blockade of that receptor. Taking into account the above described effects, the potential therapeutic properties of CGP 40116 and other competitive NMDA receptor antagonists should be put forward carefully since in the range of doses effective against MK-801, CGP 40116 may impair cognitive functions in experimental animals.

27.6 **THE ROLE OF CORTICOSTERONE IN BEHAVIORAL SENSITIZATION TO COCAINE IN RATS**

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Behavioral sensitization to psychostimulants, e.g. cocaine (COC) has been implicated in the development of addictive behaviour. This phenomenon is mediated mainly by activation of the mesocorticolimbic dopamine system, however several studies suggest a potential role for corticosteroids. To examine the role of corticosterone (CORT) in the COC-induced acute and sensitized locomotor effects, male Wistar rats (250-280 g) were injected repeatedly (5 days) with vehicle (VEH)+VEH, VEH+COC (10 mg/kg, i.p), CORT (10 mg/kg, sc; -60 min)+VEH or CORT+COC. After a drug 2-day withdrawal period (on day 8) all animals were challenged with VEH+VEH, and on day 10 – with VEH+COC (10 mg/kg). Locomotor activity was recorded on days 1, 5, 8 and 10 for 1 hr; measurements started immediately after the last injection. CORT, given acutely or repeatedly with COC during the induction phase of sensitization, did not affect the COC-induced hyperactivity. Challenge dose of COC enhanced the locomotor effects of rats repeatedly treated with VEH+COC (by 50%) and CORT+COC (by 166%). Repeated CORT treatment failed, however, to sensitize rats to the challenge dose of COC. Post-cocaine adrenalectomy did not influence the expression of sensitization. Our findings indicate that (**1**) CORT given during induction phase potentiated the expression of COC-induced sensitization; (**2**) there is no "cross-sensitization" between CORT and COC; (**3**) endogenous CORT is not required for the expression of the behavioral sensitization once established.

27.7 INTERACTION OF CHOSEN H_1 ANTAGONISTS WITH MORPHINE IN IRRADIATED RATS.

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The results of the simultaneous usage of H_1 antagonists with morphine (MOR) and radiotherapy in the treatment of neoplastic pain are not well recognised and obtained data are frequently contradictory (Bakhtiarian *et al.*, 1998, Bartolini *et al.*, 1998). The aim of this study was to assess chosen H_1 antagonists effect on MOR analgesic activity and central-opioid receptor density in previously irradiated rats. *Materials&Methods:* Male Wistar rats irradiated with X-rays (5.2 Gy) were single (1x) or multiple (5x) pretreated with antazoline (80 mg/kg, *im*)-(ANT), astemizole (1.5 mg/kg, *ip*)-(AST), cetirizine (1.5 mg/kg *im*)-(CET) or clemastine (1.0 mg/kg, *im*)-(CLE) and MOR (7.5 mg/kg, *sc*) analgesic effects was measured using Nilsen method in chosen periods. After 60 min of morphine administration the analysis of opioid receptors density in brain synaptosoms was performed using [^{3}H]naloxone according to method of Mitchell. *Results:* It was found that 1x treatment with H_1 antagonists did not alter MOR response, while 5x ANT, CET or CLE led to decrease of analgesic activity of MOR. Multiple AST administration had no effect on MOR-induced analgesia. These results correlated with the lowering of obtained B_{max} values after 5x ANT, CET and CLE administration, however for AST a such relationship was not found. *Conclusion:* Generally subchronic treatment with H_1 antagonists lead to decrease of morphine analgesic activity and density of opioid receptors in irradiated rats, but for understanding the details of mechanism of this relationship further investigations are needed.

INVOLVEMENT OF PROTEIN KINASE A IN THE MECHANISM OF ANTIDEPRESSANT ACTION. 27.8

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Protein phosphorylation modulates the expression of specific function of various proteins and represents a major biochemical mechanism by which cells integrate extracellular signals and maintain their homeostasis, cellular functions and survival. The phosphorylation of substrate proteins by protein kinases play a key role in signal transduction and function of neurons. Protein kinases have been associated with several physiological and pathological states including depression. It has been observed that antidepressant drugs decreased the activity of protein kinase C in soluble and particulate fractions from rat brain cerebral cortex and hippocampus. Our previous results showed that repeated imipramine administration significantly decreased CaM-KII activity in the soluble fraction of the rat hippocampus; a prolonged ECS produced an identical effect. It has also been observed that a chronic treatment of rats with antidepressants reduces ciclic AMP-dependent protein kinase activity in soluble fraction whereas increases its activity in particulate fraction from rat brain frontal cortex.
Our results indicate that chronic imipramine administration causes an increase of PKA activity in the rat hippocampal particulate fraction. The similar but lower effect was observed for chronic electroconvulsive treatment. We have also found that repeated ECS treatment produces an increase of PKA activity in the soluble fraction from rat hippocampus.
We have also observed the antidepressant-like effect in the forced swimming test after the i.v.t. administration of PKA activators (dibutyryl-cAMP, 8-Br-cAMP) and PKA inhibitor (Rp-cAMPS).
Our results suggest that the activity of cAMP-dependent protein kinase is modified by antidepressants.

27.9 ACUTE AND CHRONIC EFFECTS OF MORPHINE ON CREB REGULATION IN Neuro2a MOR1A CELLS.

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Increasing evidence supports an important role for the transcription factor CREB in mediating the opioid-induced changes of the cAMP pathway. We investigated effects of morphine administration on CREB regulation in Neuro2a MOR1A cells expressing μ-opioid receptors.
Morphine produced an increase of phosphorylated CREB (pCREB), which peaked after 30 min and after 1 h of incubation declined below basal level. This effect of morphine was reversed by naloxone. Prolonged (3 days) exposure to morphine caused only a slight increase in the pCREB level in Neuro2a MOR1A cells and appeared to be connected with the upregulation of CREB protein level. The drug withdrawal elicited a rapid (after 30 min) increase in pCREB, which returned to the basal level after 4 h.
An analysis of nuclear protein binding to the consensus cAMP/Ca^{2+}-responsive element (CRE) using an electrophoretic mobility shift assay revealed that acute and chronic morphine increased binding to the CRE element and this effect was further potentiated during withdrawal. Consequently, we investigated the effect of morphine on CRE-directed gene transcription. Cells were transfected with a DNA construct in which the expression of a reporter gene was controlled by four consecutive CREs. Morphine after 1 day of treatment inhibited the basal expression of the reporter gene.
Our results reveal a dual (inhibitory and stimulatory) action of morphine on the transcription factor CREB and indicate that the CREB is likely to contribute to long-lasting consequences of the exposure to opioids.

This study was supported by KBN grant Nr.4.P05A.017.13

THE INFLUENCE OF OLANZAPINE ON COGNITIVE FUNCTIONS AND CATALEPSY IN RATS AFTER SINGLE AND CHRONIC ADMINISTRATION 27.10

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Olanzapine is a second generation of antipsychotic agents, with 5-HT_{2A}, D_1, D_2 and D_4 antagonistic and cholinergic properties. The anxiolytic and memory improving activity of olanzapine was postulated mostly on the basis of its clinical effects. From the preclinical and the clinical findings it was hypothesized that olanzapine might have lower propensity to produce extrapyramidal symptoms (EPS), than haloperidol. In this study the anxiolytic effect of olanzapine and its effects on memory and catalepsy (indicative of extrapyramidal side effects) was investigated. Olanzapine 0.5 mg/kg was administered to male Wistar rats i.p. 25 min before the tests. In chronic experiments olanzapine was administered to rats for 21 days. It was found that olanzapine had anxiolytic activity in doses causing no sedative effect, after prolonged administration no signs of tolerance appeared. Olanzapine administered once and over 7 days does not improve memory in maze test, however after 14 and 21 days of treatment the memory was improved. Olanzapine in dose 0.5 mg/kg induced a slight degree of catalepsy, only after single administration, but no signs of catalepsy was observed after 7 and 14 days of treatment. SUMMARIZING, it can be stated that olanzapine has an anxiolytic and memory improving effects in rats. This results suggest, that olanzapine has behavioural pharmacologic properties associated with potent antipsychotic activity and minimal extrapyramidal effects.

27.11 **MODIFICATION OF AVOIDANCE BEHAVIOR IN RATS BY CHRONIC ADMINISTRATION OF DOPAMINERGIC AGONIST AND ANTAGONISTS.**

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This study aimed investigation of the effects of D-amphetamine (in doses: 0.5, 1.0, and 2.0 mg/kg) and two selective D1 and D2 dopamine receptor blockers: SCH-23390 (0.025 mg/kg) and haloperidol (0.05 mg/kg), on two-way shuttle avoidance in adult Möll-Wistar rats. In the course of the experiment, none of the applied drugs influenced probability of avoidance execution. In contrast to this, all the drugs significantly effeted avoidance latencies. As revealed by the analysis of cumulative response latency distributions, the major effect of chronic amphetamine administration was a dose dependent increase in the freguency of short-latency avoidances (latencies<2sec) to compare with unchanged long-latency avoidance responses. D1 and D2 dopamine receptor blockers showed differential effect on the avoidance execution. Haloperidol caused significant reduction in the frequency of short-latency avoidances. Conversely, rats receiving SCH-23390 did not differ from the control subjects in the frequency of short-latency responses, but showed lower probability of avoidances emitted toward the end of the 5-sec CS-US interval. Under concomitant administration of amphetamine and dopamine receptor blockers, no compensatory increase in the frequency of short-latency avoidances was observed in the haloperidol group. On the contrary, in the SCH-23390 group, when SCH-2339 was applied together with amphetamine, there was noted a dramatic increase, much above the control level, in the frequency of short-latency avoidances. These results argue for the different nature of short- and long-latency avoidance responses and suggest involvement of DAD2 receptors in the process of response initiation. Interestingly, higher dose of SCH-23390 (0.050 mg/kg) resulted in a profound behavioral break-down but only when it was administered together with amphetamine. The latter observation seems to indicate that behavioral output of dopaminergic transmission may depend more on the balance of D1/D2 DA receptors than on the independent modulation of a particular receptor system.

27.12 **EFFECT OF REPEATED ADMINISTRATION OF VENLAFAXINE ON THE LEVEL OF mRNA CODING FOR DOPAMINE D_1 AND D_2 RECEPTORS IN RAT BRAIN**

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Venlafaxine (VEN) is a representative of a new class of antidepressants (SNRIs) which selectively inhibit the neuronal reuptake of noradrenaline and 5-hydroxytryptamine but - in contrary to classical tricyclics - show no affinity for neurotransmitter receptors.

In the present study we wanted to check whether VEN given repeatedly (10 mg/kg, perorally, twice daily, 14 days) was able to induce biochemical changes, similar to those found by us previously following tricyclic antidepressants administration, in the dopaminergic system (i.e. decrease in the responsiveness of dopamine D_1 and an increase in the responsiveness of dopamine D_2 receptors). The experiment was performed on male Wistar rats. Binding to rat brain dopamine D_1 and D_2 receptors was assessed using [^{3}H]-SCH 23390 and [^{3}H]-spiperone respectively. Levels of mRNA coding for dopamine D_1 and D_2 receptors were measured in the striatum and nucleus accumbens using an in situ hybridization method.

The obtained results indicate that, repeated VEN affects neither the binding (B_{max} and K_D) of [^{3}H]-SCH 23390 to dopamine D_1 receptors and of [^{3}H]-spiperone to dopamine D_2 receptors in the limbic forebrain and striatum, nor the level of mRNA coding for dopamine D_1 receptors in the core and shell parts of the nucleus accumbens and in the striatum. The level of mRNA coding for dopamine D_2 receptors was increased in all the brain regions studied, especially in the shell part of the nucleus accumbens.

The present results indicate that the level of mRNA coding for dopamine D_2 but not D_1 receptors is regulated by repeated administration of VEN. Changes dopamine D_2 receptors seem to be important to the mechanism of antidepressant action of VEN.

27.13 **EFFECT OF ACUTE TREATMENT WITH ANTIDEPRESSANT DRUGS ON THE REACTIVITY OF D_3 DOPAMINERGIC AND β-ADRENERGIC RECEPTORS IN THE RAT BRAIN.**

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Clinical efficacy of antidepressant drugs (ADs) is achieved by the repeated treatment. Therefore, in animal studies designed to define the biochemical changes which may be important for the mechanism of action of ADs, the se drugs are administered for at least two weeks. A decrease in the number of β-adrenergic receptors in the rat brain after repeated treatment with ADs (the so-called β-down regulation) was one of the first demonstrations of alterations at the biochemical level. The hypoactivity induced by salbutamol (10 mg/kg), a β-adrenergic receptor agonist, is reversed by repeated treatment with ADs, which correlates well with „β-down regulation". An interesting finding of the present study is that a similar effect, i.e.: reversal of the salbutamol-induced hypoactivity, was also observed after acute treatment with ADs, followed by 13 drug-free days. We also found adaptive changes after repeated treatment of rats with ADs in another neurotransmitter receptor system, i.e. at the level of dopaminergic D_3 receptors. A low dose of (±)7-OH-DPAT (0.05 mg/kg), a dopamine D_3 receptor agonist, induced locomotor hypoactivity in rats. AD given repeatedly attenuated that effect, which indicates the subsensitivity of dopamine D_3 presynaptic receptors. Comparable attenuation of the (±)7-OH-DPAT-induced locomotor hypoactivity was also observed in a group of rats subjected to acute treatment with ADs, followed by 13 drug-free days. No such effects at the level of β-adrenergic receptors or dopaminergic D_3 receptors were observed 2 or 72 h after acute treatment with ADs.

Since adaptive changes at the level of β-adrenergic or dopaminergic D_3 receptors were observed not only after repeated but also after acute treatment with ADs, if the single administration of the drug was followed by 13 drug-free days, it is concluded that the most important factor necessary to trigger such an effect is time rather than daily drug administration.

27.14 **SIMILAR ACTION OF THE ECS AND IMIPRAMINE CHRONIC TREATMENTS ON SUBTYPES OF THE α_1 ADRENOCEPTOR**

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Imipramine (IMI) belongs to tricyclic antidepressants whose pharmacological action is based on inhibition of noradrenaline and serotonin reuptake. Electroconvulsive treatment is regarded as the most efficient therapeutic means. Either treatment given chronically is known to induce an increase of the total α_1-adrenergic receptors binding. The aim of our study was to compare the effect of chronic treatment with IMI with those induced by electroconvulsive shock (ECS) on the mRNA expression of α_{1A} and α_{1B} subtypes (α_{1A}-AR, α_{1B}-AR) of the adrenergic receptor in various brain structures of the Wistar rat. Rats were treated with IMI (10mg/kg ip, 2x daily) or ECS (150mA, 250ms, daily) for 14 consecutive days. 24h after the last injection the animals were decapitated; total RNA was isolated and analyzed using a *Northern blot* hybridization procedure with specific cDNA probes radiolabelled with [α^{32}P]-dCTP. The α_{1A}-AR mRNA expression was assessed in the cortex and hippocampus while the α_{1B}-AR expression - in the cortex and thalamus. We found that both chronic IMI and ECS increased the expression of α_{1A}-AR mRNA in rat cortex, but not in the hippocampus. Neither treatment affected the α_{1B}-AR mRNA expression in the brain structures studied. We postulate that the increase in of the total density of α_1-adrenergic receptors observed in binding experiments after either chronic IMI or ECS, is due to the enhanced expression of the α_{1A}-AR mRNA.

27.15 THE INFLUENCE OF ADENOSINE RECEPTORS AGONISTS AND ANTAGONISTS ON THE AMPHETAMINE INDUCED STEREOTYPY IN RATS

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Many experimental data indicate the existence of a marked and antagonistic interaction between adenosine and dopamine in the brain. Striatal adenosine A2A and dopamine D2 receptors are mainly localized in the striatopallidal GABA-ergic neurons. Adenosine A1 receptors are widely distributed in the brain, and they are also present in the striatum and are co-localized with dopamine D1 receptors in the striatonigral- striatoendopeduncular neurons.

In the present experiments, we investigate the involvement of adenosine A1 and A2 receptors in the amphetamine-induced sterotypy in rats. Sterotyped behaviour was induced in rats by s.c. injection of amphetamine 2mg/kg.

Adenosine A2A receptor agonist - CGS 21680 (1 and 2 mg/kg i.p.) and A2/A1 agonist - NECA (0.05 and 0.1 mg/kg i.p.) dose-dependently reduced amphetamine-induced stereotypy. CPA - adenosine A1 receptor agonist was also able to decrease this amphetamine effects.

Nonselective antagonists of adenosine receptors (caffeine and theophylline - 20mg/kg i.p.) enhanced the stereotyped activity of amphetamine. Similar interaction was observed after i.p. injection of A2 receptor antagonist - DMPX (3 and 6 mg/kg i.p.) plus amphetamine (administered at the dose of 2 mg/kg,and at the treshold dose of 0.5 mg/kg). A1 receptor antagonist - CPT (1 and 3 mg/kg i.p.) did not influence amphetamine sterotypy.

These results agree with the hypothesis of an antagonistic modulation of dopamine receptors by adenosine receptors in the brain.

27.16 **ROLE OF 5-HT REUPTAKE INHIBITION AND 5-HT_{1A} RECEPTORS IN THE DISCRIMINATIVE STIMULUS EFFECTS OF FENFLURAMINE**

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Fenfluramine (FEN) is an amphetamine congener which, until recently, was prescribed as an appetite suppressant. Although structurally similar to amphetamine, the subjective effects of FEN differ and studies of the stimulus properties of the drug suggest that enhanced serotonin (5-HT) transmission mediates this behavioral effect. Release of 5-HT from nerve terminals occurs via a mechanism which is Ca^{2+}-dependent and sensitive to presynaptic autoreceptor activation, as well as a Ca^{2+}-independent process sensitive to uptake blockers. The purpose of the present study was to assess the role of 5-HT reuptake inhibition and 5-HT_{1A} receptors in the stimulus effects of FEN. Male Sprague-Dawley rats were trained to discriminate FEN (2 mg/kg, i.p.; 20 min pretreatment interval) from saline (i.p.) in a two-choice, water-reinforced FR 20 drug discrimination paradigm. In substitution tests, the selective 5-HT reuptake inhibitor fluvoxamine (FLX; 20 mg/kg, i.p.; 60 min), the 5-HT_{1A} agonist 8-OH-DPAT (0.04 and 0.4 mg/kg, i.p.; 25 min) and the 5-HT_{1A} antagonist WAY 100635 (1 mg/kg, sc; 45 min) elicited 45.2, 19.6, 12.1 and 14.5% drug-lever responding, respectively. In combination experiments, FLX (5-20 mg/kg) attenuated the FEN-induced discrimination, to 40% drug-appropriate responding. Pretreatment with low doses of 8-OH-DPAT (0.01 and 0.04 mg/kg) attenuated the stimulus effects of FEN; a complete blockade was observed after pretreatment with 0.01 mg/kg of 8-OH-DPAT. The antagonistic efficacy of 8-OH-DPAT was lost at higher doses (0.1 and 0.4 mg/kg). WAY 100635 (0.25-1 mg/kg) given in combination with the submaximal dose of FEN (1 mg/kg) produced a significant enhancement of the discriminability of FEN (to 89% drug-lever responding). These results support an involvement of both impulse-dependent (a role for somatodendritic 5-HT_{1A} autoreceptors) and impulse-independent (a role for 5-HT reuptake transporter) mechanisms in the stimulus effects of FEN.
Supported by NIDA DA 06511 and DA 00260 (KAC).

27.17 CLINICALLY AVAILABLE NMDA ANTAGONIST, MEMANTINE ATTENUATES ANALGESIC MORPHINE TOLERANCE IN A MOUSE TAIL FLICK TEST.

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Converging lines of evidence indicate that *N*-methyl-D-aspartate (NMDA) receptor antagonists attenuate the development of morphine tolerance in rodent antinociception assays. This study extends these findings to the effects of clinically available NMDA receptor antagonist, memantine (MEM). Mice were tested for analgesia using the tail-flick apparatus. Radiant heat was focused on the lower third of the tail. Movement of the tail activated a photocell, turning off both the light and a reaction timer. Male Albino Swiss mice were used in this experiment. When tested alone, morphine produced analgesic effect with ED_{50} ~ 3.1 mg/kg. During the development of tolerance period (6 subsequent days), mice received 10 mg/kg s.c. b.i.d. morphine injections. This treatment shifted to the right the cumulative dose-response to ~ 14.7 mg/kg (5.8 fold), as indicated on day 8 of the experiment. MEM (2.5, 5 and 10 mg/kg) co-administration, 30 min before morphine during the development of tolerance period, inhibited the right-shift of morphine cumulative dose-response curve. MEM at doses of 2.5, 5 and 10 mg/kg pretreatment resulted in ED_{50} values of 10.8, 4.8 and 2 mg/kg, respectively, resulting in fold changes of 3.4, 1.0 and 0.9, respectively.

These data indicate that low affinity, clinically available NMDA receptor antagonist, memantine may be used to inhibit the development of morphine tolerance.

27.18 **MODULATION OF THE NMDA RECEPTOR INFLUENCES THE COCAINE-INDUCED PLACE PREFERENCE IN RATS**

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Many drugs that are abused by humans e.g. morphine, cocaine and amphetamine have been shown to induce the conditioned place preference (CPP) in rodents. There is a hypothesis that these drugs facilitate dopaminergic transmission in mesolimbic and mesocortical neurons. Probably dopamine (DA) release is controlled by glutamatergic mechanisms and NMDA/glycine receptors are involved in this interaction.

In our study, we have examined the influence of memantine, a clinically used, uncompetitive NMDA receptor antagonist and ACPC, a partial agonist of the strychnine-insensitive glycine site on the reinforcing properties of cocaine (5 mg/kg) measured in the CPP paradigm in Wistar rats. After determination of initial preferences, animals were conditioned with cocaine for 3 conditioning trials. Pairing of memantine (7.5 mg/kg) or ACPC (50 mg/kg) with each injection of cocaine prevented the acquisition of cocaine-induced place preference. Memantine but not ACPC, given in a single injection on the test day, attenuated the expression of cocaine-induced CPP. Neither memantine nor ACPC given alone produced any effects in the CPP paradigm.

The results of the present study suggest the involvement of the NMDA/glycine receptors in the rewarding effects of cocaine. As the action of memantine was very significant we can speculate that this NMDA receptor antagonist, having no abuse potential, might be a promising candidate for the treatment of cocaine dependence.

27.19 **THE ACTIVITY OF CORTICAL LYSOSOMAL ENZYMES AFTER LONG-TERM APPLICATION OF ANTAGONISTS OF H2 RECEPTOR**

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The ranitidine (R) is one of the most common used H2 blockers on market. The aim of the study was to evaluate secretion of lysosomal enzymes in rat's cerebral cortex after long-term application of therapeutic doses of ranitidine. Methods: The experiment was carried out on Wistar rats, which received ranitidine in intraperitoneal injection (i.p.), twice a day for six weeks. The administration was in two doses: R1- 0.714 mg/kg B.W. and R2- 7.14 mg/kg B.W. There was one control group which received i.p. corresponding volume of isotonic salt solution. After 6 weeks the animals were killed. The cerebral cortex was taken for biochemical investigations. Activities of the free fractions of lysosomal enzymes (lipase, beta-D-galactosidase, sulphatase, acide phosphatase, N-acetyl-beta-D-glucosaminidase, cathepsin B, D and L) were examined in cortex homogenate. The ANOVA test was employed in the verification of the result. Result: The activity of lysosomal enzymes was not statistically different ($p > 0.05$). Conclusion: The long-term application of ranitidine do not change the activity of free fraction of cortical lysosomal enzymes.

27.20 **OPIOID ANTAGONISTS AND CAPTOPRIL DID NOT ATTENUATE HIGH VOLUNTARY ALCOHOL CONSUMPTION INDUCED BY LIVER CIRRHOSIS**

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Long-term self-administered thioacetamide (TAA, 0.03%) was used to render rats cirrhotic. The role of the opiate and/or renin/angiotensin systems in the development of high voluntary alcohol intake was examined in these animals with liver dysfunction. TAA rats selected for the study had liver insufficiency: plasma bilirubin levels were 3-4 times higher, prothrombin time >50% longer and they drank voluntarily 7-13 times more alcohol than untreated controls (7.41±0.47 vs 0.57±0.37 g EtOH/kg b.w.). Prior to therapy rats underwent a 3 day control "free choice" test. During the control test and throughout therapy animals were kept in metabolic cages and had free access to fluids (H_2O and 10% EtOH) and food. Consumption of fluids and feed and urine output were recorded daily, except when studying the effects of 5 days Naloxone and Naltrexone therapy when they were monitored 2 h, 4 h, 6 h and 24 h after the treatment. Drugs, Naloxone (10 mg/kg/day, 5 or 10 days), Naltrexone 10 mg/kg (5 days) or Captopril 20 mg/kg (10 days) were given s.c., at the beginning of dark phase of 24 h cycle. Controls received saline. None of the drugs affected voluntary alcohol intake, suggesting that neither opioids nor renin/angiotensin are implicated in pathomechanism of liver damage related excessive alcohol drinking. Although the overall 24 hour fluid consumption was not affected by opioid receptor antagonists, more detailed recordings showed that alcohol, water and thus total fluid intake by TAA and control rats were significantly less 2 h-6 h after Naloxone or Naltrexone administration.

27.21 **EFFECT OF L-ARGININE AND NITRO-L-ARGININE METHYL ESTER (L-NAME) ON AMPHETAMINE AND APOMORPHINE INDUCED STEREOTYPED BEHAVIOR AND DOPAC RELEASE IN THE STRIATUM OF RATS**

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Nitric oxide (NO), a novel intracellular messenger of mammalian brain, affects a variety of physiological and pathological functions. Previously, we showed that NO modulates central dopamine (DA) D_1 and D_3 receptor reactivity to agonists (SKF 38393 and quinpirole, respectively) in rats. In the current study we examined the possible role of NO in amphetamine- (AMPH-) and apomorphine- (APO-) induced stereotyped behavior and release of dihydroxyphenylacetic acid (DOPAC) from neostriatum of adult Wistar rats. Using the rating scale of Costall and Naylor, L-NAME (25 mg/kg IP) but not L-arginine (L-ARG; 300 mg/kg IP) was found to attenuate AMPH (4 or 10 mg/kg IP) induced stereotyped behavior of male and female rats. Neither L-NAME nor L-ARG modified the APO stereotypy score or when given alone. Using differential pulse voltammetry in the neostriatum, L-NAME but not L-ARG prevented the reduction in DOPAC in chloral hydrate anesthetized male rats acutely treated with AMPH (4 mg/kg IP). Findings indicate that NO is essential in mediating effects of AMPH on DA release and in mediating AMPH-induced behaviors.

Supported by the Silesian Medical University, grant NN-4-30/98 and Fogarty International Science Exchange Program with Poland (RMK).

27.22 **COMPARISON DOPAC RELEASE IN THE STRIATUM AND SOME BEHAVIORAL EFFECTS OF QUINPIROLE AND 7-OH-DPAT, THE DOPAMINE D_2/D_3 RECEPTORS AGONISTS IN RATS**

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This study was undertaken to determine if repeated treatments of rats with a low dose (195 nmol/kg IP) of quinpirole or 7-OH-DPAT, administered in early postnatal ontogeny (birth to 11 days after birth), can produce long-term sensitization to these agonists in adulthood. From dose-effect curves (49-1584 nmol/kg IP) it was found that quinpirole but not 7-OH-DPAT produced a 2-fold increase in yawning, vs. non-primed rats, at 8 weeks after birth. Using differential pulse voltammetry in chloral hydrate anesthetized rats, we found that DOPAC release in neostriatum was diminished by acute treatment with quinpirole (195 nmol/kg IP) but not 7-OH-DPAT (195 nmol/kg IP). These findings indicate that ontogentic treatments with quinpirole but not 7-OH-DPAT produce long-term sensitization of the target receptors, and there is no cross-sensitization between examined DA agonists. Beside, the quinpirole but not 7-OH-DPAT modulate dopamine metabolism in the striatum of rats.

Supported by Silesian Medical University: NN-1-31/99 and Fogarty International Science Exchange Program with Poland (RMK).

27.23 Changes in the content of neuroactive amino acids in cerebral cortical microdialysates of awake and ketamine-anesthetized rats in the asymptomatic stage of thioacetamide-induced hepatic encephalopathy (HE).

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The content of Asp, Glu and Tau was measured in cerebral cortical microdialysates of control rats and rats with toxic liver damage induced with thioacetamide (TAA), at a stage without overt HE symptoms. A significant above control increase of all three amino acids was noted in TAA-treated rats when microdialysis was carried out in awake animals .This result confirms earlier observations in striatal microdialysates derived from TAA-treated rats under throtane anesthesia and supports the notion of ongoing disturbances of excitatory transmission and their possible modulation by Tau in this apparently asymptomatic (subclinical) stage of HE. Ketamine (KET) anesthesia produced an increase of Glu, Asp content, but not of Tau content in the microdialysates of control rats, in keeping with the NMDA autoreceptor-antagonizing, Glu and Asp- release blocking action of KET. KET anesthesia abolished the TAA-induced increase of Glu and Asp content, but not that of the Tau content. The effects of TAA treatment and KET anesthesia were nonadditive, indicating that the underlying mechanisms of accumulation of the amino acids overlap only partly.

Supported by Merz&Co, GmbH&Co, Frankfurt, Germany.

MECHANISM OF α–KETOISOCAPROATE ACCUMULATION IN RAT CEREBRAL CORTICAL NEURONS 27.24

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Glutamate, the main excitatory neurotransmitter of the central nervous system, is produced in neurons from glutamine synthesized exclusively in glial cells. The balance of nitrogen is regulated by „trafficking" of leucine, taken up by astrocytes, and the product of leucine transamination, the α-ketoisocaproic acid (KIC), being transported to neurons. The mechanism of [^{14}C]KIC accumulation, followed in the presence of aminooxyacetate (an inhibitor of transaminases), was studied in the cerebral cortex cells isolated from the adult rat brain. Several steps of purification (filtration, dextran gradient centrifugation) resulted in a preparation without detectable glial fibrillary acidic protein, being enriched in neuron-specific enolase. Accumulation of KIC was not affected by Na^+ replacement, its initial velocity was observed to be higher upon lowering of external pH. The detected inhibition by α-cyano-4(OH)cinnamate pointed to an involvement of one of MonoCarboxylate Transporters (MCT). Accumulation of KIC was inhibited by lactate, the effect of pyruvate was detected to be much weaker. Other branched-chain α-ketoacids (ketoisovalerate, keto-methylvalerate), as well as β-hydroxybutyrate and valproate decreased the transport of KIC by 30, 60 and 80%, respectively. The observed characteristics of KIC accumulation in the cortical neurons indicate an involvement of one of the MCT transporters, distinct, however from the known MCT1, MCT2, MCT3. An involvement of another isoform of MCT in the process of KIC accumulation in neurons has been postulated.

27.25 **OCTOPAMINERGIC NEUROMODULATORY NEURON BACKGROUND Na^+ CHANNELS SENSITIVE TO SCORPION α-TOXINS.**

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Voltage-dependent Na channels are responsible for the depolarizing phase of the action potentials (AP). They are known as the main target for scorpion α toxins which slow down the mechanism of inactivation, prolongate channel open time and induce short bursting activity without any change of channel conductance. In autoactive cockroach neurosecretory DUM neurones, application of TTX induces a hyperpolarization. We found that this TTX-induced hyperpolarization results from an inhibition of a background sodium conductance. Activity of the background Na (bNa) channels (37pS conductance) is observed as single openings at steady-state potential of -50mV. Voltage-dependence of channel open probability (Po) is bell-shaped with a maximum at DUM neurone resting potential (-50mV). At potential more negative than -75mV channel activity is still observed but appears in bursts of increased activity separated by silence periods. Similar activity is induced at -50mV by application of scorpion α -toxins (LqhαIT, 10^{-8}M; AaHII, 10^{-6}M). Open time is unchanged and voltage-dependence of Po remains bell-shaped but Po is 25 times greater compared to control. Change from beating into bursting bNa channel activity affects spontaneous firing of DUM neurons: beating pattern is transformed into activity consisting of bursts separated by silent periods during which the membrane potential is hyperpolarized. Alteration of firing pattern is not accompanied by any change in AP waveforms, which indicates that bNa channels represent a new target for α toxins.

B. Lapied *et al.*, 1999 (in press).

INFLUENCE OF β CYFLUTHRIN ON THE CHOSEN BEHAVIOURAL PARAMETERS IN FROG 27.26

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The mechanism of pesticide action varies, and by these ways there are different forms of toxification. It is known that pesticides from the pyrethroid groups modify the action of sodium channels and by this way influence organism's excitability. Disturbances of this process are the main cause of insect death. Most environments are permanentlly or seasonally poisoned with various pesticides. They act not only upon insects but on other animals, including human too. These pesticides (action and detoxication) are very important because they are applied in large quantities and areas; and they are one of the causes of fall in population of frog from year by year. Therefore, recognition of their action on chosen physiological and behavioral parameters in frog were the aim of studies. The experiments were carried out on ten *Rana temporaria.* Each individual was tested in 5 groups: control and next subjected to pesticide (instant effect) and in 3 independent groups after exposure to a pesticide in successive time sectors repeatedly after a few days, to observe the influence of time on a return to the physiological norm (which is evidence of detoxification of this pesticide). For these studies were used 2,5% β cyfluthrin in very little dose (did not cause lethality) and after a lapse of time frog returned to norm. Frog's movement activity was studied by an ultrasound method and a choice of ambient temperature in a thermal gradient, all parameters were analysed by computer. The examined pesticide caused changes of behaviour of frog; influence of time on return to the physiological norm was observed; dose used was within the physiological efficiency limit because the changes were reversible and not lethal.

27.27 **CORRELATED INHIBITION OF GLUTAMINE UPTAKE AND PHOSPHATE-ACTIVATED GLUTAMINASE ACTIVITY IN RAT BRAIN MITOCHONDRIA BY NATURAL AND SYNTHETIC TRANSPORT INHIBITORS.**

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The study investigated the effects of a spectrum of natural amino acids, and their natural, and synthetic (common and newly designed) analogues on Gln uptake and phosphate-activated glutaminase (PAG) activity in nonsynaptic mitochondria of the rat brain. Among the natural amino acids, His and different large neutral (long chain and aromatic) amino acids turned out to exert a strong inhibitory effect on the transport. Among the synthetic analogues, a newly synthesized compound, (2'-nitryl -biphenyl)alanine (MRC01), inhibited Gln transport with a potency higher than any other compound examined, and with relative amino acid specificity. The transport was not inhibited by most of the short-chain and basic amino acids among those, Tau, Pro and Lys. His, MRC01 and one other strong transport inhibitor, Hcys, all inhibited the PAG activity, while no inhibitory effect on the enzyme was noted with Tau, Pro or Lys. The results indicate that Gln uptake to rat brain mitochondria may limit the metabolic rate of the amino acid.

Supported by the State Committee for Scientific Research (grant no. 4P05 096 14).

27.28 **REGULATION OF PROTEIN KINASE C (PKC) ACTIVATION IN NEUROBLASTOMA NB-2a CELLS**

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Accumulation of palmitoylcarnitine in neuroblastoma NB-2a cells was observed to inhibit proliferation with a concomitant promotion of differentiation of cultured mouse neuroblastoma NB-2a cells. The activity of phorbol ester stimulated PKC activity measured in permeabilized NB-2a cells was observed to be inhibited by palmitoylcarnitine in a concentration-dependent way [Nałęcz et al., 1997, Acta Neurobiol. Exp.,57, 263-274]. A possibility of a specific effect of palmitoylcarnitine on the particular isoforms of PKC was studied with specific polyclonal antibodies, directed against unique peptides. In particular, the distribution of PKC isoform localization was estimated by Western blot technique, after separation of the cytosolic and membrane fractions. In case of PKC β, δ and ε, palmitoylcarnitine seems to cause retardation of high molecular weight forms in the cytosol with a concomitant decrease of the 40-50 kDa form in the membrane fraction. Due to the polymorfizm of high molecular weight forms, an effect of palmitoylcarnitine on the phosphorylation status of PKC has been postulated. In case of PKC α, a slight stimulation of proteolysis upon exposure to palmitoylcarnitine was detected. On the contrary to the effect of these isoforms, a significant increase of the 53 kDa form of the PKC γ was detected in the membrane fraction in the presence of palmitoylcarnitine. These observations point to an isoform-specific interaction between PKC and palmitoylcarnitine, thus excluding an unspecific effect of hydrophobicity.

27.29 **THE EFFECT OF PESTICIDES ON THE MUSCLE RESTING POTENTIAL AND BEHAVIOUR OF INSECTS**

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It has been known that ion channels of cell membrane are the major target sites of action of certain insecticides. Ion channels are ubiquitous and play crucial roles in generation of resting and action potentials and transmitters releasing. Disfunction of ion channels can threfore result in drastic changes in function of organism at both cellular and behavioural levels. The aim of the present study was to examine the effect of pyrethroid insecticide (BULLDOCK 025 EC) on the muscle resting potential and thermal behaviour of adult *Periplaneta americana* L. Experiments were performed in situ on dorsoventral muscles. The conventional microelectrode technique was used. Both in the control, untreated group of insects, and in the experimental groups RP was measured in standard physiological saline. The insect's behavior was observed in thermal gradient during 24 hours. The pesticide was given in the form as a drop on the upper surface of the abdomen in doeses LD_{50}. RP of the control group was about -68mV. The RP measured at 5 min and 24 hours after application of Bulldock was significantly lower than control values. However RP measured of insects which after application of pesticide stayed 24 h in thermal gradient was similar to that observed in the control ones. The insects after application of pesticide prefered ambient temperature about 10°C higher than the insect of control group. It probably accelerated their metabolism and in this way faster degradated inscticide. The depolarization observed in our experiments is probably caused by modification of action of sodium channels by synthetic pyrethroides.

27.30 MODIFICATION OF $GABA_A$ RECEPTOR GATING BY CHLORPROMAZINE REVEALS SYNAPTIC GABA TIME COURSE

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The effect of chlorpromazine (CPZ) was studied on the GABAergic miniature inhibitory synaptic currents (mIPSCs) in neurons, using patch-clamp technique. In control conditions, mIPSCs were characterised by a biphasic decay (τ_{fast}=10.9±2.2ms, A_{fast}=60±6%, τ_{slow}=60.3±6.7ms, A_{slow}=100%-A_{fast}). CPZ caused a dose-dependent decrease in mIPSCs amplitude (at 30 μM CPZ, 31% decrease) and an acceleration of the decaying phase (at 30μM CPZ, τ_{fast}=7.1±0.8 ms, A_{fast}=67±4%, τ_{slow}=27.2±5.8ms). In order to explore the mechanism underlying CPZ action, the ultrafast perfusion system was used (solution exchange time - ca. 100 μs) to evoke GABA responses in the non-equilibrium conditions similar to those in synapse. Currents induced by 1 mM GABA applied for 2 ms to excised patches, mimicked well mIPSCs. Applications of GABA in the presence of various CPZ concentrations reproduced the effect of this drug on mIPSCs making it possible to determine the underlying mechanism. We found that CPZ affected mIPSCs by decreasing binding and by increasing unbinding rate of GABA to $GABA_A$ receptors. The knowledge of mechanism of CPZ action and the comparison between the time course of control mIPSCs and that of mIPSCs affected by CPZ allowed to numerically deconvolve the synaptic GABA time course. We estimated that the peak synaptic GABA was ≥ 3mM and the time constant of agonist clearance was ca. 100 μs. This result seems particularly important as the time course of synaptic agonist is a key factor in determining both kinetics and pharmacological modulation of synaptic currents.

27.31 PACAP-EVOKED ACTIVATION OF cAMP SYNTHESIS IN AVIAN BRAIN

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Pituitary adenylate cyclase-activating polypeptide (PACAP) is a recently discovered neuropeptide that in tissues occurs in two forms designated $PACAP_{38}$ (a predominating form) and $PACAP_{27}$; both stimulate cAMP production in the central nervous system (CNS) of mammals, amphibians and fishes. Till now nothing is known about its biological activity in avians. To fill this gap the present work was aimed at studying PACAP action on cAMP formation in the hypothalamus (HTh) and cerebral cortex (CCx) of four birds, ie. chick, duck, goose and turkey.

Using brains of 2-3-week-old chicks, we observed a strong, concentration (0.0001-1 μM)-dependent stimulatory action of $PACAP_{38}$ and $PACAP_{27}$ in HTh and CCx with respective E_{max} values reached at 0.1 μM: ≈ 800-900 and 500-600% of control. There was no difference in potency between the two PACAP forms. In contrast, vasoactive intestinal peptide (VIP; 0.01-3 μM) appeared to be nearly 1,000-times less potent than PACAP, producing at 3 μM significant increases in cAMP formation by 104 and 92% (above control) in HTh and CCx, respectively. An emerging rank-order of potency: $PACAP_{38} \approx PACAP_{27} \approx$ VIP suggests that receptors mediating increases in cAMP production in the tested brain regions of chick represent the PAC_1-subtype. Further studies were performed on adult avians (duck, goose and turkey). In duck and goose (both PACAP forms), and in turkey (only $PACAP_{27}$ was tested), the peptides nicely stimulated cAMP production in HTh and CCx in a concentration-dependent manner; however their effects were weaker than those in chicks. Generally, the observed responses were larger in HTh than in CCx; the duck tissues appeared to be more sensitive to PACAP than those of goose and turkey.

Conclusions: The presented here data showed for the first time that PACAP is capable of potently stimulating cAMP production in the avian central nervous system. At least in chicks the PACAP-evoked cAMP effects are mediated through the PAC_1-type receptor.

27.32 EFFECTS OF NEAR-ULTRAVIOLET LIGHT (UV-A) ON MELATONIN BIOSYNTHESIS IN THE VERTEBRATE PINEAL GLAND AND RETINA

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Pineal gland and retina of various vertebrates synthesize melatonin (MEL) in a circadian rhythm generated by an endogenous pacemaker. Visible light is a predominant environmental factor controlling MEL production. The aim of this study was to analyze the effects of UV-A light (λ_{max}=365 nm) on the nocturnal activity of serotonin N-acetyl-transferase (NAT, a key regulatory enzyme in MEL synthesis) and MEL content in the pineal glands of chick and rat and the retina of chick. Acute exposure of dark-adapted animals to UV-A light produced a marked decrease of NAT activity and melatonin content. The magnitude of these changes was dependent on exposure time, species and tissue (with the following rank-order of sensitivity: rat pineal gland >> chick retina ≥ chick pineal gland). The suppressive action of UV-A light on MEL synthesis in the chick retina and pineal gland involves catecholamines and is mediated by D_4-dopamine and α_2-adrenergic receptors, respectively. A short UV-A pulse (1 min - rats, 5 min - chicks) imposed on animals during the first half of the night produced a small decline of NAT activity that gradually deepened during the first 40 min of treatment with constant darkness. After that, the enzyme activity began to rise reaching control values by 2 hrs (chicks) or 3 hrs (rats). The present results provide evidence that UV-A light is a powerful signal capable of controlling MEL biosynthesis in the chick pineal gland and retina, and the rat pineal gland. *Supported by the KBN grant No. 4 PO5A 07412*

27.33 BLOOD PLATELETS AS A MODEL FOR CHANGES OF ACETYL-COA METABOLISM IN DIABETIC BRAIN.

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Type 3, insulin independent, glucose nonsuppressible transporter is known to supply glucose into platelets and neurones. Hence, diabetic hyperglycaemia is likely to bring about similar changes in metabolism of glucose-derived acetyl-CoA in both types of cells. Respective enzymological and metabolic parameters in human blood platelets from diabetic subjects (n=20) and in brains from rats with 10 day streptozotocin diabetes (STZ, n=8) were compared, to substantiate this hypothesis. Fructosamine levels in sera of diabetic people and rats were 60% and 38% higher than in healthy controls, respectively. In diabetic human platelets activities of hexokinase (HX), pyruvate dehydrogenase (PDH), ATP-citrate lyase (ACL) were found to be 27, 37 and 49%, respectively higher than in controls. Acetyl-CoA levels and malonyl dialdehyde synthesis in diabetic platelets were 60 and 50% higher than in controls. STZ diabetes in rats lasting 20 days caused 30% decrease of HX activity in hippocampus (HP) and cortex and 30% increase in striatum and hypothalamus (HT). Diabetes increased PDH activity by 24, 83 and 91% in HP, cerebellum and HT. On the other hand, ACL activity rose by 39, 69 and 91% in HP, cortex and HT, respectively. Acetyl-CoA content and acetylcholine synthesis in nerve terminals of STZ forebrains were 30 and 51% higher than in controls, respectively. These data indicate that diabetes leads to increased level of glucose-derived acetyl-CoA both in platelets and in brain yielding excessive thromboxane A2 and ACh synthesis, respectively. Hence, in diabetes changes in platelet acetyl-CoA metabolism may correspond to those taking place in the brain. Supported by KBN grant 4 PO5A 086 15.

27.34 EFFECT OF *ANTISENSE* OLIGODEOXYNUCLEOTIDES (ODNs) COMPLEMENTARY TO mRNA ENCODING DOPAMINERGIC D_2 RECEPTOR, AND THE *RANDOM* SEQUENCE, ADMINISTERED BILATERALLY INTO THE NUCLEUS ACCUMBENS OF THE RAT.

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The selectivity of the widely used drugs acting at the level of dopamine receptors should be reconsidered, especially in light of the recent identification of new subtypes of these receptors. The *antisense* oligonucleotides (ODNs), complementary to the specific mRNA sequences encoding dopamine receptors, can be used as alternative. In the present study we used *antisense* ODN complementary to D_2 receptor. The *random* ODN was used as a control. The ODNs were administered bilaterally into the nucleus accumbens septi (NAS) of the rat in a dose of 0.25nmol/0.5μl twice daily for 3 days. Administration of *random* but not *antisense* ODN resulted in the decrease of the binding of [^{3}H]raclopride (D_2/D_3 receptor antagonist), but not of [^{3}H]quinpirole (D_2/D_3 agonist with the preference for D_3 receptors). Other effects of *random* ODN administration were also observed at the biochemical level, i.e.: significant decrease in the level of mRNA encoding not only dopamine D_2 receptor, but also proenkephalin and dopamine D_1 receptor in the NAS. On the other hand, both *antisense* as well as *random* ODNs induced similar alterations in the behaviour of experimental animals, namely the reversal of D-amphetamine-induced locomotor hyperactivity and the enhancement of overall locomotor activity - which was inhibited by (±)7-OH-DPAT (D_3 receptor agonist), but only in *random* ODN-treated animals.

Significance of these perplexing results will be discussed in the context of the literature data concerning the non-specific effects of ODNs administered into the rat brain.

27.35 β-ADRENERGIC RECEPTORS IN THE DUCK BRAIN

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Besides their role in synaptic communication, β-adrenergic receptors are suggested to be involved in processes of neuronal plasticity, both in mammals and birds. Detailed analysis of β-adrenergic receptors in chick and pigeon brain revealed that β_2-adrenergic receptors are the main subtype in these tissues. The aim of this study was to characterize β-adrenergic receptors in the cerebral cortex (CCx) of ducks using in vitro binding of [^{3}H]dihydroalprenolol ([^{3}H]DHA), a β-adrenergic receptor antagonist. The specific binding of [^{3}H]DHA to membrane preparations of CCx was found to be stable, saturable, reversible and of high affinity. Scatchard analysis revealed that [^{3}H]DHA binds to a single class of sites with high affinity (K_D in the range of 0.7-0.9 nM). The number of binding sites in the CCx of 1 month old ducks (B_{max} = 61.5 fmol/mg protein) was significantly lower than the receptor number found in the tissue of 7 month old animals (B_{max} = 120 fmol/mg protein). Competition studies revealed the following relative rank-order of potency of compounds to inhibit the specific [^{3}H]DHA binding: ICI-118,551 > S-propranolol >DL-propranolol >> betaxolol >>> yohimbine, WB-4101, ketanserine. A potent and a weak action of ICI-118,551 (a highly selective β_2-adrenergic receptor antagonist) and betaxolol (a selective β_1-adrenergic receptor antagonist), respectively, indicates the [^{3}H]DHA binding sites in the CCx of ducks represent β_2 subtype of adrenergic receptors.

27.36 DISTRIBUTION OF TrkB RECEPTORS IN MOTOR-SENSORY CORTICAL AREAS IN THE RAT

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Beneficial effects of neurotrophins on recovery processes in the damaged nervous system are exerted via high affinity Trk receptors. To study the effects of BDNF on the recovery processes following spinal cord transection in adult animals requires detailed information on the distribution of BDNF signal transducing Trk B receptors not only at the site of the lesion but also at the source of affected descending projections. As a first step in our study on the effects of BDNF on the recovery processes in the spinal rats we tested a distribution of Trk B receptors in the motor-sensory cortical areas, the source of the cortico-spinal descending system.

Adult Wistar rats were transcardially perfused with 2% paraformaldehyde and 0.2% parabenzoquinone in 0.1M PBS. Immunocytochemistry was performed on cryostat, free-floating sections (50 μm), with polyclonal rabbit anti-TrkB antibody (1:200, Santa Cruz, sc-12) using ABC (Vector) detection system with DAB as a chromogen. To establish nonspecific staining the antibody was preincubated with control peptide. The procedure eliminated completely the staining. Data were analyzed under light microscope by two independent observers. Arbitrary three rank scale was used to estimate the strength of the reaction. The highest rank [3] was given to the reaction of the septal neurons which were used as a reference on a given slice. In the motor-sensory cortical areas the strongest reaction [on average rank 2] was observed in the pyramidal neurons of layer V and at the outer border of layer VI. The effect was consistent throughout tested material. These results will be compared with a distribution of TrkB receptors in the chronic spinal rats.

This work was supported by State Committee for Scientific Research: 1305/P05/98/14 and 1030/P05/96/11.

27.37 LOCALIZATION OF 5-HT$_{1A}$ RECEPTOR IN THE BRAIN OF THE OPOSSUM, *MONODELPHIS DOMESTICA*

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Serotonin (5-HT) is one of the main modulatory neurotransmitters present in all mammals. Presence of serotoninergic neurons in one of the species of marsupials, the South American short-tailed opossum (*Monodelphis domestica*) has been shown recently (Luque et al., 1998). We tried to find if serotonin mediates its influences via the same receptors in all mammals, including marsupials. As a first effort, we examined the distribution of the 5-HT$_{1A}$ receptor in opossum, using antibody against the rat's 5-HT$_{1A}$ receptor. Adult animals were perfused with 0.9% NaCl solution followed by 4% paraformaldehyde in 0.1M phosphate buffer. The brains were cut either coronally or horizontally. The immunohistochemistry showed specific staining in many structures. The highest density of receptor labeling was found in the hippocampus (fields CA1, CA3), piriform cortex and insular cortex. Equally high labeling was found in the brainstem serotoninergic structures, such as the dorsal and median raphe nuclei. Very dense labeling was also present in the pedunculopontine nuclei, where perikarya, axon hillocks and initial segments, as well as dendrites were labeled. Moderate level of labeling has been seen in the parietal cortex and low level of labelling in the occipital cortex, especially in its medial part, containing visual cortex. Low level of labeling has been shown in the nuclei of thalamus and hypothalamus. Our results show that distribution and density of the 5-HT$_{1A}$ receptors in the opossum is very similar to that described in the rat, and therefore the expression of this important receptor follows a general mammalian pattern.

This work was supported by KBN Grant No 4PO5A.0811.11 .

27.38 Mpα2 SUBUNIT OF AN INSECT NICOTINIC ACETYLCHOLINE RECEPTOR - EXPRESSION AND FUNCTIONAL CHARACTERISATION

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A cDNA encoding putative nicotinic acetylcholine receptor subunit Mpα2, isolated from the nervous system of a peach-potato aphid *Myzus persicae*, was expressed in *Xenopus* oocytes in order to study its functional properties. Expression of functional receptors was assessed by two electrode voltage clamp measurement of membrane current. Application of 10 μM nicotine or 100 μM ACh (+ 1 μM atropine) to an oocyte injected at least 5 days previously with Mpα2 cDNA only rarely produced a detectable membrane current. The maximal response noted was a smooth inward membrane current never exceeding 10 nA, when held voltage-clamped at -60 mV. No desensitization was detected upon application of either agonist. Interestingly, the response to nicotine in Mpα2 cDNA injected oocytes was insensitive to α Bgt (0.1 μM). Co-injection of Mpα2 and chick β2 subunit cDNA, to boost expression, resulted in some 2.5-fold (up to 35 nA) increase in the amplitude of the induced current generated by nicotine. The current was dose-dependent and only partially blocked by 0.1 μM α Bgt. In addition, it was activated over a large voltage range and showed a reversal potential of 1 ± 4 mV. Preliminary results suggested that the currents were not sensitive to 10 μM bicuculine but could partially be blocked by 10 μM pirenzepine.

27.39 **NMDA-INDUCED MITOCHONDRIAL PERMEABILITY TRANSITION IN RABBIT BRAIN: *IN VIVO* AND *IN VITRO* STUDY**

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Acute excitotoxicity, with swelling of neurons and intracellular organelles, has been identified with necrotic neuronal damage. However, the mitochondrial permeability transition (MPT), dependent upon opening of cyclosporin A (CsA)-sensitive megachannels, resulting in swelling of mitochondria and release of proapoptotic cytochrome C, is supposed to trigger apoptosis. The aim of this study was to assess if N-methyl-D-aspartate (NMDA)-induced swelling of neuronal mitochondria in the rabbit hippocampus *in vivo* is CsA sensitive. NMDA (1 mM) was applied for 20 min to the hippocampus in a control, or 5 μM CsA-containing dialysis medium *via* transhippocampal microdialysis probes permeable for macromolecules. Changes in extracellular Ca^{2+} concentration were monitored. Ultrastructural changes in pyramidal neurons in the CA1 sector of the hippocampus, in the vicinity of microdialysis probes, were examined 30 min after NMDA application. In separate *in vitro* experiments, Ca^{2+}-induced swelling of isolated rabbit brain mitochondria was studied spectrophotometrically. Application of NMDA to the hippocampus *in vivo*, as well as incubation of isolated rabbit brain mitochondria with Ca^{2+} in supraphysiological concentrations, induced swelling of mitochondria, which was almost completely prevented by CsA. Thus, NMDA-induced swelling of mitochondria in the hippocampal neurons, which is sensitive to CsA, may be attributed to the MPT caused by activation of megachannels. Its role in the process of excitotoxic neuronal death will be discussed.

27.40 **THE ACTIVITY OF CONSTITUTIVE NITRIC OXIDE SYNTHASE IN PREFRONTAL CORTEX OF RATS AS AN EXPONENT OF EMOTIONAL STATE BEFORE DEATH.**

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15 s and 10 min of short-term aversive sensory stimulation of rats resulted in the significant increase in consitutive nitric oxide synthase activity in prefrontal cortex as measured by the [^{3}H]-cytrulline formation method. A role of nitric oxide as a neurotransmitter in the forming of emotional and cognitive processes was underlined. Thus the possibile relation between the stimulation applied and the observed increase in enzymatic activity was suggested.

27.41 **THE REACTIVITY OF THE CEREBELLUM TO ALARM PHEROMONES ARGUED TO ITS INVOLVEMENT IN EMOTIONAL RESPONSE TO UNCONDITIONED AVERSIVE STIMULI.**

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The cerebellum plays a significant role not only in motor but also in cognitive and emotional functions. The reaction of the cerebellum to the different stimuli causing central emotional response was investigated in various models. The authors evaluated changes in the glutamate concentration in the post-mortem cerebella of rats exposed to the odor produced by stressed conspecifics. The animals were exposed to this olfactory stimulus individually and in pairs. Statistically significant differences were obtained in favor of exposed animals as well as of single versus paired. The possible involvement of the cerebellum in the central fear response caused by stress-odor was discussed.

27.42 **EFFECT OF L-CARNITINE ON ACTIVITIES OF ACETYL-COA METABOLIZING ENZYMES IN SN56 CHOLINERGIC SEPTAL HYBRID CELLS.**

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L-carnitine (CAR) is known to improve function of cholinergic neurones and protect them against different neurotoxic inputs. It exerts these effects presumably through the increase of acetyl-CoA provision to cells mitochondrial and cytosolic compartments. The aim of this work was to find out whether CAR may cause appropriate changes in expression of enzymes of acetyl-CoA metabolism in cultured clonal cells derived from septal cholinergic neurons. Three day culture with 1 mM CAR caused 31 and 34% increases of pyruvate dehydrogenase (PDH) and carnitine acetyltransferase activities. On the other hand, the highest 47 and 70% activation of ATP-citrate lyase (ACL) and choline acetyltransferase (ChAT) activities was observed at 0.1 mM CAR in the culture medium, respectively. Increase of CAR concentration to 1.0 mM resulted in gradual decrease ACL and ChAT activities to the control values. For acetylcholinesterase highest 61% rise of was found at 0.25 mM CAR, whereas 1.0 mM CAR suppressed enzyme activity back to the control values. These data suggest that CAR may increase viability of cholinergic neurones through the correlated increase of the expression of PDH and ACL that increase of acetyl-CoA provision in mitochondria and cytoplasm for energy and acetylcholine synthesis, respectively. Supported by KBN project 4 05A 044 12.

27.43 **ROLE OF p75 RECEPTORS IN REGULATION OF ACETYL-COA METABOLISM IN SN56 CHOLINERGIC CELL LINE.**

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Nerve growth factor (NGF) through its high affinity trkA receptors exerts protective and differentiating influences on cholinergic neurones. The role of low affinity p75 receptors remains obscure. In this work trkA negative, p75 positive SN56 cells were used to reveal possible role of the latter in modification their phenotype. Increasing concentrations of NGF in culture medium from 1 to 100 and 500 ng/ml caused gradual suppression of pyruvate dehydrogenase (PDH) activity to 67, 52 and 17% of control values, respectively. Maximal suppression of ATP–citrate lyase (ACL) by such treatment did not exceed 30%. On the other hand, NGF in these concentrations did not affect choline acetyltransferase (ChAT), carnitine acetyltransferase, acetylcholinesterase and lactate dehydrogenase activities. SN56 cell differentiation with 1mM dbcAMP or 0.001 mM retinoic acid (RA) caused 110 and 145% increase of ChAT activity in the cells, respectively. RA had no effect while cAMP caused 30% suppression of PDH and 37% increase of ACL activities, respectively. Effects of RA and cAMP were additive. In RA and RA+cAMP differentiated cells, NGF (100 ng/ml) decreased PDH and ACL activities like in nondifferentiated cells. In addition, slight 21% decrease of ChAT activity was found. On the contrary, in cAMP treated cells no significant NGF effects were observed. These data indicate that p75 receptor-dependent effects of NGF on septal cholinergic neurones are likely to be mediated by protein kinase A pathways. Suppression of PDH activity and decrease of acetyl-CoA production, caused by activation of p75 receptors may explain increased vulnerability of some NGF-treated neurones to various neurotoxic stimuli. Supported by KBN project No 4 P05A 04412.

27.44 **COGNITIVE CHANGES ASSOCIATED WITH ESTROGEN REPLACEMENT IN A RAT MODEL OF MENOPAUSE.**

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Ovarian steroid deficiency associated with menopause has been linked to cognitive impairment in normal aging and in Alzheimer's disease. Some of these effects are atteneuated by hormone replacement therapy, suggesting a potential casual relationship between endocrine decline and cognitive aging. In females, a major change that takes place with age is a mid-life alteration in the circulating levels of ovarian hormones, which coincides with changes in cognitive function. There is no substantial decline in cognitive function immediately after menopause or following an oophorectomy in women. Our results indicate that the onset of cognitive deficit following the removal of the source of circulating ovarian hormones (surgical ovariectomy, OVX) is postponed for a longer period of time in young females than in middle-aged females. Furthermore, we have found that estrogen replacement prevents the impairment in memory in both age-groups: young and middle-aged. In contrast to younger age-groups, the surgical removal of ovaries in old female rats did not produce additional cognitive decline other than the one already present in the old females with intact ovaries. In aged females, estrogen replacement was not effective in improving memory. This data provides an important insight into how the cessation of cyclic estrogen production might compromise memory and suggests that the susceptibility to estrogen-dependence changes with age and endocrine status.

Supported by NIA RO1-AG15947

Session 28 - Poster Session: Peptides

28.1 **EFFECT OF BUSPIRONE ON NEUROPEPTIDE Y (NPY) CONTENT IN RAT BRAIN**

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To determine whether the NPY system is involved in the action of buspirone (BUSP), we estimated the effect of BUSP treatment on NPY content in naive rats. BUSP was administered i.p. (1.5 or 5 mg/kg twice daily, 8.00 and 18.00) for 1, 7 or 21 days (acute, subchronic and chronic dosage). NPY levels were measured by RIA in the amygdala (AM), nucleus accumbens (NAC), hypothalamus (HP) and frontal cortex (FC) isolated 24 h after the last dose. Acute lower-dose BUSP treatment decreased only AM NPY content. NPY levels in NAC were decreased, but increased in FC after both subchronic doses of BUSP. In chronically treated rats AM and HP NPY levels increased after the lower and higher doses of BUSP respectively, while NPY levels in NAC were decreased after the lower dose of BUSP. In addition, we examined NPY content in rats trained in the passive avoidance test (an animal anxiety model). NPY levels were estimated in the same brain structures isolated 6 h after the test trial. In the rats with anxiety-related behavior NPY levels increased in AM, HP and NAC but decreased in FC. BUSP decreased anxiety in a dose-depend manner in these rats. The effect of anxiety on NPY content was attenuated by both doses of BUSP in AM and HP, and by the higher dose of BUSP in NAC and FC. These results are first to show that BUSP alters NPY content in the rat brain. They also confirm the previous findings that the NPY system contribute to anxiety and they suggest that this system might be involved in the antianxiety action of BUSP.

28.2 **cAMP FORMATION IN CHICK BRAIN: EFFECT OF PITUITARY ADENYLATE CYCLASE-ACTIVATING POLYPEPTIDE (PACAP), HISTAMINE AND FORSKOLIN**

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PACAP occurs in two forms know as $PACAP_{38}$ and $PACAP_{27}$; both stimulate cAMP production in the central nervous system (CNS) of mammals, amphibians and fishes. Recently we extended an array of PACAP-sensitive animals to avians. This work was aimed at studying PACAP action on cAMP formation in the chick hypothalamus (HTh) and cerebral cortex (CCx) alone and in combination with forskolin - a direct activator of adenylyl cyclase, and histamine - a potent stimulator of cAMP generation in the chick CNS.

$PACAP_{27}$ and $PACAP_{38}$ showed similar potency in stimulating cAMP formation in chick brain (EC_{50} values ≈ 24-28 nM); they produced maximal effects at 0.1 μM in HTh and CCx of 900-1000 and 500-600% of control. For the interaction with histamine (HA; 1-10 μM) and forskolin (FOR; 0.3 μM) we used only $PACAP_{38}$, at low concentration range of 0.001-0.1 μM. $PACAP_{38}$ stimulated cAMP formation producing increases (in % of control) at 0.001-0.03 μM of 138.6-637.0 in HTh, and at 0.001-0.1 μM of 100.0-399.6% in CCx. HA increased cAMP formation upto 230.0±30.1 at 1 μM in HTh and 317.9±14.2 at 10 μM in CCx. The respective values for 0.3 μM FOR were: 193.7±30.3 and 278.3±15.5. In the presence of FOR, the effects of PACAP were enhanced in both HTh and CCx; showing an additive or synergistic type of interaction. However, combination of PACAP with HA showed no positive interaction, and the effects observed were typical for only one of the tested substances.

Conclusions: *1.* PACAP, HA and FOR at concentrations used significantly stimulated cAMP production in the chick brain; *2.* FOR enhanced the ability of PACAP to stimulate cAMP synthesis, *3.* Although both PACAP and HA are potent stimulators of cAMP production, given together they curiously interact showing the effect of only one substance; 4. The obtained data support a neuromodulator role of PACAP in the avian CNS.

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28.3 VASOACTIVE INTESTINAL PEPTIDE (VIP)-SENSITIVE cAMP-GENERATING SYSTEM IN RAT CEREBRAL CORTEX: INTERACTION WITH NEUROTRANSMITTERS

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VIP is an established neuromodulator that evokes diverse biological effects, most of which being linked to its stimulatory action on cAMP production. In this study we analyzed interactions between VIP and some neurotransmitter systems on cAMP production in rat cerebral cortex (CCx). VIP concentration-dependently stimulated cAMP synthesis in CCx slices, producing increases by 57, 226 and 433 % (above control) at 0.1, 0.3 and 1 μM, respectively. NA at 10 and 100 μM increased cAMP production by 26 and 107 %, respectively; yet, added to VIP it potentiated the peptide action: VIP 0.1 μM - 53%; VIP+NA 10 μM - 183%, VIP+NA 100 μM - 310% (above basal value). A similar interaction has been observed between VIP and 10 and 100 μM adrenaline. The actions of both adrenergic transmitters were mediated via α_1-adrenergic receptor as the effects of a combination of VIP+NA (or VIP+adrenaline) were prevented by 1 μM prazosin and not by propranolol, selective antagonists of α_1- and β-adrenoceptor, respectively. Furthermore, a selective α_1-agonist phenylephrine (10 and 50 μM) mimicked the effect of NA or adrenaline. The stimulatory action of 0.3 μM VIP on cAMP production was not modified by dopamine (0.1 mM), histamine, serotonin or muscarinic drug carbachol (each at 0.1 and 1 mM). Conclusions: (1) in rat CCX the cAMP generating system is under control of positively interacting VIP-ergic and α_1-adrenoceptor-derived signals; (2) the VIP-stimulated cAMP does not seem to be modulated by signals resulting from activation of muscarinic cholinergic receptor, as well as receptors for dopamine, histamine, and serotonin.

28.4 EFFECT OF RESERPINE OR 6-OHDA ON NPY AND CRF EXPRESSION AND SYNTHESIS IN RAT AMYGDALA

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Some studies indicate that peptide expression in neurons of certain brain structures may be regulated by classic neurotransmitters, especially monoamines. In the present study a possibility of such regulation in the limbic nucleus, amygdala was investigated. Monoamine depletion by reserpine, or specific dopaminergic (DA) lesion by 6-OHDA were performed in male Wistar rats. Brains were taken out 24 h after reserpine (10 mg/kg,ip) or 10 days after unilateral 6-OHDA intramesencephalic injection (8μg/4μl), and were processed by immunohistochemical or in situ hybridization methods to study neuropeptide Y (NPY) and corticoliberin (CRF) containing neurons in the amygdala. NPY and CRF-immunoreactive (IR) neurons were counted in the amygdala sections under microscope, and the mRNA expression was measured as optical density units in autoradiograms. It was found that reserpine increased the number of NPY-IR neurons in the amygdala. CRF-IR showed an insignificant tendency to decrease. 6-OHDA induced an increase in NPY-IR, but a decrease in CRF-IR in the lesioned amygdala. At the same time the mRNA expression of both peptides increased after 6-OHDA.

The results indicate that DA may be an important modulator of NPY and CRF expression and synthesis in amygdala neurons. Levels of the peptides seem to be differently regulated, as impairment of DA transmission increases NPY but usually decreses CRF-IR. The synthesis of both peptides is enhanced after DA lesion. Discrepancy between the increased synthesis and the decreased level of CRF after 6-OHDA suggests its intense release from amygdala neurons.

The study was supported by the KBN grant no.4 P05A 101 09.

28.5 INVOLVEMENT OF ENDOGENOUS OPIOID SYSTEMS IN NEUROPATHIC PAIN.

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Mechanizms underlying the neuropathic pain which develops after peripheral nerve injury are not thoroughly understood. A number of several findings suggest that endogenous opioid systems may play a role in neurpathic pain.

The experiments were carried out on male Wistar rats. Effects of i.th. administration of the specific κ receptor agonist U50,488H and antagonist norBNI we were studied in a neuropathic pain model. In our behavioral study, a tail flick test, a cold water tail flick test and a cold water allodynia test were used. We also examined the expresion of PDYN and PENK mRNA 2, 4, 10 and 18 days after nerve crushing or ligation.

In our study we observed an ipsilateral increase in PDYN mRNA in laminae I-IV in the lumbar spinal cord after sciatic nerve injury. The level of PENK mRNA on ipsilateral side was the same as on the colateral side. Our behavioral results indicate that allodynia in an animal model of neuropathic pain can be relieved by continuous systemic administration of a kappa opioid receptor agonist, whereas an antagonist of the kappa opioid receptor potentiates the allodynic effect caused by nerve injury.

The present study shows that the prodynorphin system and the kappa opioid receptor are capable of controlling the development of processes induced by nerve injury.

Supported by the KBN grant 4 PO5A 093 15

28.6 CHANGES IN THE OPIOID SYSTEMS AND CRF IN THE AMYGDALA IN VARIOUS MODELS OF DRUG DEPENDENCE

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Recent evidence suggests that the amygdala represents an anatomical substrate for the action of drugs of abuse. In particular, the intraamygdalar peptidergic system has been implicated in rewarding responses and chronic effects of drugs of abuse.

Changes in the amygdalar expression of the opioid precursors proenkephalin (PENK) and prodynorphin (PDYN), and of the corticotropin-releasing hormone (CRF) were examined by a semi-quantitative *in situ* hybridization histochemistry in rats and mice subjected to chronic morphine and cocaine.

Single morphine and cocaine administration increased the level of PDYN mRNA in the central nucleus of rat amygdala, while chronic administration of morphine and cocaine had no influence on the level of PDYN mRNA. Further, morphine withdrawal increased the level of PDYN mRNA in that structure in both mice and rats. The CRF mRNA level visibly increased after single morphine and cocaine administration, as well as upon withdrawal of those drugs. In contrast, drugs of abuse had little, if any, effect on PENK mRNA; on the other hand, chronic cocaine had a tendency to increase the PENK mRNA level.

The above findings point to crucial importance of amygdalar PDYN and CRF neurons in processing withdrawal signals. Increases in the PDYN and CRF gene expression in the central nucleus of the amygdala after morphine and cocaine suggest a rise in the biosynthesis and, possibly, in the further release of specific peptides. The adaptive changes in peptide systems may play some role in the neurochemical mechanism of drug addiction.

Supported by grant no. 4 P05A 060 16

28.7 NOCICEPTIN/ORPHANIN FQ - MORE THAN ONE PEPTIDE

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Metabolism of nociceptin/orphanin FQ(OFQ/N) was studied in the spinal cord of rats. The heptadecapeptide was efficiently cleaved by a neutral serine endopeptidase, thus releasing the major metabolite, $OFQ/N_{(1-11)}$, further truncated to the final product, $OFQ/N_{(1-6)}$. Biological activity of these fragments was tested in vivo, after intracerebroventricular and intrathecal injections. Hexapeptide exhibited a bi-phasic effect, causing antinociception up to 10 min after injection, followed by a hyperalgesia. The analgesic effect was blocked by naloxone and hyperalgesia was inhibited by NMDA - and NMDA/glycine site antagonists. Similar effect was observed when $OFQ/N_{(1-11)}$ was applied. The results indicate that shorter nociceptin fragments still possess their biological activity though possibly acting via receptors other than ORL1.

28.8 CHARACTERIZATION OF ANTINOCICEPTIVE EFFECTS OF AA 501, A NOVEL OPIOID AGONIST AND NEUROKININ ANTAGONIST

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Many physiological and pharmacological studies have indicated that substance P and opioids are functionally antagonistic in the mediation of nociception and antinociception in vivo. We have previously demonstrated that biphalin, a novel opioid tetrapeptide dimer (Tyr-D-Ala-Gly-Phe-NH-)2, given intrathecally with a peptide substance P antagonist produces potent spinal antinociception in rats (Misterek et al., 1994). AA 501, a novel compound, binds both to opioid and substance P receptors. Because this novel peptide construct consists of the opioid sequence Tyr-D-Ala-Gly-Phe- covalently linked through a hydrazide bridge to a Z-Trp (N-a-Carbobenzoxy-Trp) moiety, and this moiety has putative neurokinin 1 (NK-1) receptor blocking effects, we have now evaluated the activity of AA 501. After Animal Research Committee approval, adult male Sprague-Dawley rats (225-250 g) were implanted with chronic indwelling IT catheters with tips at the T13-L1 spinal level. Animals had one week to recover from surgery, during which they were habituated daily to the laboratory environment and analgesic testing apparatus. For evaluation of antinociceptive properties of AA 501 we used tail-flick test and formalin test. For measurement of thermal antinociception, a tail-flick apparatus was utilized (baseline latency approximately 3 sec, cutoff 10 sec). Responses were expressed as % maximum possible effect (%MPE): [%MPE=(post-treatment latency-baseline latency)/(cut off time-baseline latency) x100]. In the formalin test 50 microl of 5% formalin solution was injected s.c. into the dorsal surface of the rat hind paw. Pain behavior was quantified by periodically counting the occurrence of spontaneous flinching/shaking of the injected paw. AA501 was administered IT 15 minutes before or 9 min after formalin injection.

Results. IT administration of AA501 produced dose-dependent antinociception in tail-flick and formalin tests. The time course of antinociceptive responses to lower doses of AA501 (0.25 and 0.5 µg) peaked at approximately 50% MPE at 15-30 min and declined to 20-30% MPE at 60 min. Intermediate doses (1 and 4 µg) produced an antinociceptive effect that reached 60-80% MPE at 5-45 min. Higher doses (8 and 25 µg) produced a maximal antinociceptive response (100% MPE) of long duration (30-75 min). Antinociceptive effects of AA501 were reversed by 10 min pretreatment with naloxone IT 10 µg and diminished by SP IT 150 ng. AA501 administered IT in dose 1µg 15 min before formalin injection into the rat hind paw completely blocked the biphasic response to formalin. AA501 administered 9 min after formalin injection also blocked occurrence of the second phase of the response to formalin.

We have previously shown that a hybrid peptide containing both beta-casomorphin-like and substance P-like structural characteristics possessed an antinociceptive effect in the mouse hot plate test after intrathecal administration (Lipkowski et al.,1994). AA501 was designed to concurrently activate opioid receptors and block NK-1 receptors. The antinociceptive effects of IT AA501 in the tail-flick and formalin tests are highly significant and dose-dependent, suggesting its value as a potential therapeutic agent or useful tool for study on pain modulation.

28.9 UNIQUE ANALGESIC PROPERTIES OF A CHIMERIC PEPTIDE, ESP7, WHICH POSSESSES OPIOID AND SUBSTANCE P MOIETIES

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Tolerance, addiction, and side effects to classical pain medications such as opioids are frequent concerns in the clinical setting. Recently, the co-administration of several medications with different pharmacological profiles has gained popularity as a means of overcoming these concerns. Novel single molecules that possess multiple analgesic targets may offer the advantage of weaker side effects and delayed tolerance and overcome the need to dose with separate medications. Previous research suggests that Substance P can slow the development of tolerance to the analgesic effects of morphine (1). As a result, in the present study we tested ESP7, a novel compound, which was designed to both opioid and Substance P receptors.

After Animal Research Committee approval, adult male Sprague-Dawley rats (200-250g) were implanted with chronic indwelling IT catheters (tips at T13-L1). Rats with neurological impairment were not used. Animals had 3-4 days to recover from surgery during which time they were habituated daily to the laboratory environment and analgesic testing apparatus. For measurement of the thermal antinociceptive properties of ESP7, the tail flick test was employed. Rats were placed in the tail flick apparatus, a light source was directed at the distal two-thirds of their tail and the latency to remove the tail was recorded (baseline latency approximately 3 sec, cutoff 10 sec). Three measurements were made at each pre- and post-treatment time point. Responses were expressed as % maximum possible effects (%MPE): % MPE = (post-treatment latency - baseline latency)/cut off time - baseline latency) X 100.

We have previously shown that a hybrid peptide containing both beta-casomorphin-like and substance P-like structural characteristics possessed an antinociceptive effect in the mouse hot plate test after intrathecal administration (2). The preliminary data on ESP7 are very promising. ESP7 at graded doses produces antinociceptive responses in the rat tail flick test after daily repeated intrathecal administration. Naltrexone blocks the effects of ESP7, indicating that the analgesia is opioid in nature. Intriguingly, no significant tolerance develops to ESP7 over a five-day period. The novel peptide may have potential therapeutic value for the treatment of acute and chronic pain.

Acknowledgements:

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[Department of Anesthesia funds, NIDA Grant 04128 and The Richard Saltonstall Charitable Foundation]

Session 29 - Poster Session: Neuropathology

29.1 The isolated case of Huntington's disease – confirmed de novo mutation in the IT15 gene.

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Huntington's disease (HD) is an inherited neurodegenerative disorder. The gene responsible for HD (IT15) contains a $(CAG)_n$ repeats track, which is expanded on HD chromosomes. The normal alleles of the gene have from 6 to 35 repeats. The HD patients have more than 37 repeats (38-180).The range of 30-35 CAG repeats is not pathogenic but this is a CAG range from which the new mutation could arise and it is called "intermediate allels" (IAs). It behaves as a 'premutation' – patients with IAs are not at risk of developing the disease, but there is a small risk that their children may eventually manifest the symptoms.

We present the family with a sporadic case of HD, caused by expansion of IA IT15 allel to the fully mutated stage during the spermatogenesis. The described case meets the criteria allowing us to diagnose it as *de novo* mutation in IT15 gene.
Both parents of the patient live beyond the expected age of onset and they carry the non-pathogenic HD allels (father is a carrier of the IA). The paternity in this family has also been molecularly confirmed.
We have found evidence of the IA-giving rise to new mutation for the first time in the material of 200 molecularly analysed HD families.

29.2 Characterization of the CAG repeats expansion in families with molecularly confirmed Spinocerebellar ataxia type 1.

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Spinocerebellar ataxia type 1 (SCA1) is a dominantly inherited neurodegenerative disorder which occurred with frequency less than 1/100 000, characterized by selective neuronal loss in cerebellum, brain stem and spinocerebellar tracks.

The genetic basis of SCA1 is *dynamic mutation* – expansion of unstable $(CAG)_n$ repeats in translated region of the gene SCA1. This repeats are highly polymorphic in the population and range from 6 to 39 on normal chromosomes, while the SCA1 alleles contain 40-81 repeats.

We report the characteristic of $(CAG)_n$ repeats distribution in 12 patients from 6 Polish families with clinically diagnosed and molecularly confirmed SCA1.

29.3 DOES A DEFECT IN RETROGRADE TRANSPORT OF NGF PROVOKES PROGRESSIVE DYSFUNCTION OF CHOLINERGIC NEURONS IN AGING BRAIN?

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Nerve growth factor NGF is essential for supporting the cholinergic neurons of the basal forebrain (BF). According to the neurotrophic hypothesis, NGF is synthesized by target cells of BF neuronal projection, binds to NGF receptors located on nerve terminals, and is retrogradely transported to neurons of BF. Perturbations in NGF transport may be the reason of cholinergic neuronal degeneration and cognitive dysfunction in aging brain and in clinical dementias of Alzheimer's type. To test directly whether axonal transport is impaired in senile brain, repeated injections of fluorescent tracers (fluorogold, nuclear yellow, and true blue) of retrograde transport were applied into the functional areas of cerebral cortex in young (4 mo-old) and aged (24 mo-old) rats. We found a corresponding disruption in the connectivity between the cortical areas and the BF. However, in the same animals there were no differences in average density of retrogradely labeled cells in some others brain regions, e.g. thalamic areas, striatum, brain stem. Furthermore, by using small injections of BDA into the BF we observed a lower density of BF terminals in the cortical layers of aged rats. The biochemical results, using staining for AChE, ChAT and p75NGF receptor, showed that BF neuronal degeneration in aged rats is the most consistent chemical phenotypic loss which correlates with the severity of cholinergic innervation in cortex.

Given these results, it seems that in the aged brain cholinergic functions are actively dependent on retrogradely derived NGF. Nonetheless, the general failure of axonal transport does not seem to be the major reason of reduction in target-derived trophic support. May be a defect in NGF receptors production or in autophosphorylation of NGF/receptor complex are responsible for the cholinergic deterioration in aging brain.

29.4 CYCLOOXYGENASE-2 AND AMYLOID BETA PROTEIN IN THE BRAINS OF RESUSCITATED INDIVIDUALS

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Cyclooxygenase-2 (COX-2) is a key enzyme in conversion of arachidonic acid to prostanoids. The enzyme is highly inducible in response to inflammatory stimuli. Recently, seizures and focal brain ischemia were reported to induce COX-2 mRNA and protein. The aim of our study was to examine expression of COX-2 in the brains of individuals, who survived global brain ischemia caused by cardiac arrest but died in few hours, days or weeks after resuscitation. Age of the patients ranged from 45 to 78 years. Samples of autopsy brains were fixed in formalin and embedded in paraffin. Specific antibodies were used to detect COX-2 and amyloid beta protein (Aβ), which had been previously considered as a risk factor for aging neurons in stress. The results show, that already few hours after resuscitation numerous nerve cells were Aβ immunopositive. Expression of COX-2 in neurons was observed during few days after resuscitation. The increasing number of activated glial cells were COX-2 immunopositive as well as macrophages in areas of gradually developing necrosis. During few weeks after resuscitation in brains with amyloid deposits, necrotic changes occupied large areas of parenchyma. In those brains amyloid plaques were resoluted and evacuated by macrophages. Our results provide evidence, that following brain ischemia COX-2 participates in metabolism of the affected neurons and activated glial cells. Necrotic changes and inflammatory reactions are more advanced in brain areas with amyloid deposits.

29.5 **DISCRIMINANT ANALYSIS OF THE PATHOLOGICAL CHANGES IN THE TEMPORAL LOBE IN ALZHEIMER'S DISEASE**

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The present study aimed to identify possible structural abnormalities in the anterior temporal lobe in Alzheimer's disease (SDAT) using MRI. We measured volumes of the structures of the temporal lobe e.g. the hippocampus, amygdaloid body, lateral ventricle, superior temporal gyrus, basolateral region as well as the parahippocampal gyrus in 29 SDAT subjects and in 14 age-matched controls.
In control, but not in SDAT patients, the right hippocampus, superior temporal gyrus and lateral ventricle were larger than the left ones in contrast to the left amygdaloid body, which was larger than the right. In SDAT the normalized volume of the left amygdaloid body and basolateral region decreased significantly (about 33% and 27%, respectively). Discriminant analysis showed that using the normalized volume of above-mentioned structures (basolateral region on both and amygdaloid body on the left side) one is able to classify the cases with sensitivity and specificity as large as 1.0 and 0.97, respectively.

29.6 VASCULARIZATION OF THE HUMAN TEMPORAL CORTEX IN AGING AND DEMENTIA - MORPHOMETRICAL AND IMMUNOHISTOCHEMICAL STUDY.

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Brain function in most situations is dependent on cerebral perfusion. Metabolic studies suggest that the pathogenesis of AD may be coupled with impaired vascular delivery of nutrients to the brain. A reduction of glucose and oxygen delivery to the brain can result in cholinergic deficit and formation of b-amyloid and neurofibrillary tangles. Taking these data into consideration, we decided to estimate the vascularization of temporal lobe. Postmortem material from 19 demented and 27 age matched control subjects were analyzed. Demented subjects were diagnozed as Alzheimer's type (AD), vascular(VaD) and mixed (MD) dementia. Vessels were visualized by means immunohistochemistry using monoclonal antibody directed to epithelial cell marker (HaM 56, DAKO M632). The morphometrical analysis was performed using the Quantimet 570 Image Analysis System. The area fraction and the density of vessels were estimated in gray and white matter. Statistical analysis was performed using SPSS program for Windows.

29.7 **ENZYMATIC PROPERTIES OF BETA-AMYLOID**

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Alzheimer Disease (AD) is a heterogenous disorder with a variety of molecular pathologies converging predominantly on abnormal amyloid deposition (as well as neurofibrillary tangles) particularly in the brain. Beta-amyloid aggregation into senile plaques is one of the pathologic hallmarks of AD. Beta-amyloid is generated by a proteolytic cleavage of a large membrane protein, amyloid precursor protein (APP). We have observed a new property of beta-amyloid. The amyloid 1-42 fragment possesses a serine protease and esterase activity, *in vitro*. Three independent methods were used to test the enzymatic properties of beta-amyloid. While esterase activity involves imidazole catalysis, protease activity is consistent with participation of serine peptidase triad: catalytic Ser, His and Glu (or Asp). The contribution of several sub-amyloid fragments (12-28, 40-1, 31-35, 25-35, 35-25), denaturation, chemical modification and free radical scavengers were investigated. Although the amino acid triad is a necessary requirement for the observed protease reactivity, it is not sufficient since the secondary structure of the protein significantly contributes to the proteolytic activity. The ability of beta- amyloid to cleave peptide or ester bonds could be thus responsible for either inactivation of other proteins and/or APP proteolysis itself. This property may by responsible for early pathogenesis of AD since there is emerging evidence that non-plaque amyloid is elevated in Alzheimer patients.

This study was supported in part by the Polish Committee for Scientific Research, Project No 4PO5A11412.

29.8 **β-AMYLOID INTERACTION WITH IL1β IN VITRO.**

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Cerebral deposition of β-amyloid protein is a pathological feature of Alzheimer's disease. Different lines of evidence suggests that the amyloid 1-40 fragment possesses protease and esterase activity and is able to generate reactive oxygen species, *in vitro*. The ability of β- amyloid to cleave peptide or ester bonds could be responsible for inactivation of other proteins or changing their properties. IL-1β molecule - proinflammatory cytokine is implicated in many processes in the pathogenesis of AD. The aim of the study was to put more light into the question whether β- amyloid can directly change properties of IL-1β molecule. We have observed that preincubation of IL-1β - 17kD protein with amyloid fragment 1-40 decreases IL-1β amount as recognized by ELISA in comparison to preincubation of IL-1β with non- active amyloid fragment 40-1 and to control without amyloid. Preincubation of amyloid fragment 1-40 with monoclonal anti-IL-1β antibody (MAB601, R&D Systems) has no or only slight effect on IL-1β level, suggesting action of amyloid on IL-1 molecule rather than on the antibody. This results may add to understanding of the mechanism of β- amyloid toxicity and support the hypothesis of importance of nonplaque β- amyloid in pathogenesis of AD in early stages of the disease.

This study was supported in part by the Polish Committee for Scientific Research, Project No 4PO5A11412.

29.9 β-AMYLOID LOAD IN TEMPORAL CORTEX OF PATIENTS FULFILLING CERAD CRITERIA FOR PROBABLE AND DEFINITE ALZHEIMER'S DISEASE

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The density of senile plaques, the major hallmark of Alzheimer's disease (AD), is used as an indicator of the severity of dementia – also in CERAD classification. Recent findings however have underlined the significance of β-amyloid in the etiology of AD. Consequently the precise estimation of the amount of β-amyloid as well as the influence of different clinical factors is of major interest. The study included 51 patients with clinical diagnosis of definite AD. Neuropathological investigation revealed that 13 cases fulfilled CERAD criteria for probable, and 38 for definite AD. β-amyloid load was visualized in the temporal cortex employing immunohistochemical staining using monoclonal mouse anti-human β-amyloid antibody. The total area and the number of β-amyloid deposits were estimated morphometrically by means of Quantimet 570. Along our results the highest β-amyloid load in the temporal cortex would be expected in a female patient, rather young, with presenile onset and long duration of the disease, having two ApoE ε4 alleles and a relative suffering from the same disease.

29.10 THE INFLUENCE OF ALL-TRANS RETINOIC ACID ON THE GROWTH AND APOPTOSIS IN HUMAN MENINGIOMA CELLS IN VITRO

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The influence of the all-trans retinoic acid (ATRA) on the growth of human meningioma cells was evaluated in vitro. We have incubated the passaged meningioma cells, seeded from five tumors, grown on the modified Eagle`s medium, with ATRA at the concentrations of 10^{-6}, 10^{-7}, 10^{-8} M, and compared the number of the cells to the control culture. In three cultures incubated with ATRA we have evaluated the apoptosis by means of agarose gel electrophoresis. In result we have noticed an intense DNA degradation with the ladder of oligonucleosomal DNA in the 10^{-6} M ATRA treated cultures. The 10^{-6} M ATRA incubated cultures were significantly less numerous than the control culture.

29.11 DIFFERENT COMPOSITION OF PSD AFTER CEREBRAL ISCHEMIA

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A cerebral ischemia causes changes in synaptic transmission and consequently in neurone function. Considering postsynaptic densities (PSD) as a functionally active zone in chemical synapses we evaluated the influence of global ischemia on its protein constituents. Ischemia induced changes in assembly and functions of protein kinases (CamKII and PKC), calpains and novel 85kDa/RING3 kinase. These changes were manifested in 2-fold increase in the yield of PSD in preparations from the ischemic cortex. In addition, under the EM examination, ischemic PSD looked differently than the control ones. All of the structures were thick and straight, when the control PSD were thin and often curved. Immunochemical experiments have shown, that both Ca^{2+}-dependent kinases (CamKII and cPKC: α,β,γ) were translocated to PSD very rapidly during ischemia. We observed simultaneous translocation of Ca^{2+}-independent forms of PKC: δ and ε. Control PSD contains 13% and 14% of total α, β PKC and less than 5% of ε and δ isotypes. Brain specific γ PKC was found in PSD in almost 40% of its homogenate reactivity. Postischemic, massive increase of all cPKC (almost 10 times in case of α and β and 3 times for γ) was observed in PSD. In case of δ and ε PKC, the amount were 6 and 12 times higher comparing with control, respectively. Among brain-specific forms of calpain (μ and m), only 76kDa subunit of μ-calpain was down-regulated during ischemia. This was coupled with decline of the amount of fodrin, only calpain substrate that has been shown to be a calpain target *in vivo*. These data provide evidences, that short term ischemia can modify signal transduction processes due to molecular changes in PSD structure and thereby function of this structure.

29.12 IMPACT OF FOCAL CEREBRAL ISCHEMIA/ REPERFUSION ON THE MICROCIRCULATION IN THE CEREBRAL CORTEX, SELECTIVE VASCULAR PROTECTION WITH FREE RADICAL SCAVENGERS.

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Nitric oxide (NO) and prostacyclin (PGI_2) produced locally in blood vessels are two important mediators of flow-dependent vasodilatation in cerebral circulation. They also mediate vasodilatation to many vasoactive agents which loss their flow increasing effects when the release of NO and/or PGI_2 is impaired. The aim of present study was to find out: 1) whether focal ischemia/reperfusion affects NO and PGI_2 – dependent regulation of cerebrocortical microcirculation, and 2) whether scavenging of superoxide radical which has been reported to protect the brain against ischemia/reperfusion injury has also beneficial effect on the regulation of cerebral microcirculation under these conditions. The experiments were performed on 52 anesthetized and mechanically ventilated male Wistar rats. Cerebrocortical microflow (LDF) was continuously monitored using laser-Doppler probe. Focal cerebral ischemia was induced for 30 minutes and was followed by reperfusion. Following experimental groups were studied: 1) sham rats, 2) rats with ischemia/reperfusion, 3) rats with ischemia/reperfusion pretreated with polyethylene glycol-conjugated superoxide dismutase (PEG-SOD). The response of LDF to either L-NAME (NO inhibitor), or indomethacin (prostaglandins inhibitor), acetylcholine (ACh,) or CO_2 was tested in these groups. Regulation of LDF during reperfusion was disturbed. There was no response to indomethacin suggesting loss of basal, prostacyclin – dependent vasodilatation. Normal vasodilatatory responses to ACh and CO_2 were also abolished. Pretreatment with PEG-SOD did not affect severity of ischemia or time course of reperfusion. It did not restore the response of LDF to indomethacin but resulted in preservation of its response to ACh and CO_2. Our results demonstrate impairment of NO- and PGI_2-dependent regulation of microcirculation during reperfusion following focal cerebral ischemia. They also suggest that reported beneficial effect of PEG-SOD on the survival of neurons in vivo may be due, in part, to the protection of the vascular wall by this compound.

29.13 **ALTERATIONS OF DOPAMINE TRANSPORT AND DOPAMINE D2 RECEPTOR BINDING IN RAT BRAIN AS A CONSEQUENCES OF TOTAL CEREBRAL ISCHEMIA CAUSED BY CARDIAC ARREST**

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One of the acknowledged targets of ischemia is synaptic transmission in brain. Up to know several investigations have studied the same aspects of neurotransmission in brain under ischemic conditions in different experimental models of complete or partial cerebral ischemia. However, none of these models accurately reflects the human condition of global cerebral ischemia resulting from acute, temporary cardiac arrest. Cardiac arrest model in rat, proposed by Karpachev et al. is most controllable among several previously tested ischemia models and cardiac arrest is currently an important affliction of humans. Our present studies that have been performed using this model, have been designated to focus on the question of whether or not the dopamine transport, and dopamine D2 receptor binding observed in long-term post resuscitation, differs from that observed in the early phases of this pathological process. The effects of 10 min global ischemia were measured immediately and after 15 min, 1 h, 7 days post resuscitation stages. The results of these studies have shown: the uptake and release of dopamine in synaptosomes after 10 min of global ischemia, decreased by about 25% and 20% respectively comparing to control. This effect was enlarged to 30% after 10 min ischemia and 15 min resuscitation. These changes developed after 1 h of recirculation. The uptake and release of dopamine normalized almost completely after 7 days post clinical death. Global ischemia and conditions of post-cardiac arrest also affected the dopamine receptor by increase of KD immediately, after 10 min ischemia and by increase its affinity (reduced KD) in all times of resuscitation (60% after 7 days). Simultaneously we observed decreased density of receptor (Bmax) by about 16% after 1h resuscitation and by about 70% after 7 days post clinical death. Results show that the global ischemia and recirculation after cardiac arrest, may lead to disturbances in the CNS by interfering with the transport and receptor binding of dopamine which is involved in the regulation of a variety of functions including locomotor activity, emotion and neuroendocrine secretion.

29.14 ULTRASTRUCTURAL STUDIES ON THE NERVOUS SYSTEM IN STREPTOZOTOCINE (STZ) - INDUCED DIABETES AND EXPERIMENTAL CEREBRAL ISCHEMIA IN THE RAT

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Diabetes is often complicated by stroke. We examined the nervous system ultrastructure in Wistar rats with experimental stroke at the 9th week of STZ-induced diabetes. Diabetes resulted in swelling of mitochondria and Golgi complex, presence of numerous lysosomes in neurons and dark neurons in different brain structures. Swelling, and sometimes apoptosis of endothelial cells, thickening of basal membrane in blood vessels, perivascular microglia and phagocytes were observed. Advanced degenerative changes were noted in sciatic nerve.

Additional bilateral common carotic artery (CCA) ligation and injection of 0,05ml of air into the left CCA lead to swelling of perivascular astrocytic processes, proliferation of microglia and presence of numerous phagocytes. Degenerative changes of neurons were observed mainly in hippocampus (CA1). In putamen and pallidum, hemorrhagic foci were found. It seems that diabetes produces both - neuropathy and encephalopathy as a result of alterations in cellular respiratory activity and metabolism and overproduction of free radicals. Hence, antioxidants should be considered in diabetic patients' treatment.

29.15 **THE ACTIVITY OF LYSOSOMAL ENZYMES IN MOTOR CORTEX OF RABBITS DURING EXPERIMENTAL DIABETES**

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The experiment was carried out on 44 male rabbits of New Zealand breed, including 11of them selected as the control group. With regard to the remaining animals, diabetes was caused by an intravenous administration of solution of alloxan (Sigma Chemical Comp., St.Louis, MO, USA) in the physiological saline at a dose of 10 mg/kg of body weight. On day 7 the glucose level in the blood was measured by a glucometer to confirm the presence of diabetes. From this day time of disease was counted. The rabbits were sacrificed after 21, 42,90 and 180 days. The motor cortex was taken for biochemical investigations. Activities of the free fractions of lysosomal enzymes; lipase, acid phosphatase, sulphatase, beta-D-galactosidase, N-acetyl-beta-D-glucosaminidase, cathepsin B, D and L were examined in homogenate. Results: In the course of the experiment some changes of the selected enzymes were observed. After the first stage of the disease (21 days) activity of acid phosphatase, lipase and cathepsin D and L decreased as compared to the initial values determined on the healthy animals. The tendency towards diminishing the level of activity of these enzymes (except lipase) remained the same toll the 42nd day of disease. Then the level of the activity started to increase and reached the highest value on the 180th day of illness for lipase, cathepsin D and L, and on the 90th day for acid phosphatase. The activity of the free fractions of the remaining enzymes gradually increased till it reached the maximum value on the 42nd day of illness.

29.16 **EFFECT OF EXPERIMENTAL ENCEPHALOMYELITIS ON THE α SUBUNIT OF THE Gs PROTEIN IN THE CENTRAL NERVOUS SYSTEM**

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$G_{s\alpha}$, a GTP-binding protein, abounds in the CNS and transduces the activation of adenylyl cyclase (AC) following receptor stimulation. Two known splice variants of $G_{s\alpha}$ differ in alternative use of exon 3 (29bp), and their different affinity to AC is postulated. The aim of our study was to assess the influence of chronic experimentally induced encephalomyelitis (ECE) on $G_{s\alpha}$ in various brain structures of SJL mice. Total RNAs were isolated from the cortex, hippocampus and hypothalamus, reversely transcribed to DNA, and amplified using a PCR technique. The products were analyzed by size using a poliacrylamide gel electrophoresis on the laser fluorescence automatic system. We obtained three fragments of $G_{s\alpha}$-cDNA: 270bp, 378bp, 442bp. The 270bp fragment was the most abundant cDNA (86% of the total cDNA). The relative amount of the 378 bp product (378bp/270bp) was lower in ECE (0.05) than in normal mice (0.11). A comparison cDNA fragments with the published data (Sullivan, 1986) indicates that the 270 bp fragment represents a small variant of $G_{s\alpha}$. It is not clear yet whether the two other fragments are coding variants of $G_{s\alpha-l}$, or rather immature nuclear transcripts. Our experiments indicate that in addition to changes induced in the immune system, ECE may affect signal transduction pathways coupled to Gs/AC in the cerebral cortex.

29.17 **EXPRESSION OF THE NERVE GROWTH FACTOR, INTERLEUKIN-1BETA AND NF-kappaB TRANSCRIPTION FACTOR IN NEURONAL AND GLIAL CELLS OF THE RAT SPINAL CORD DURING EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS**

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Changes of NGF presence in brain nonneuronal cells in response to experimental allergic encephalomyelities (EAE) in Lewis rats have been recently reported (Micera et al., Exp. Neurol. 1998, 154, 41). In the present study we have investigated the expression of NGF and the two crucially coupled with its synthesis substances: interleukin-1beta (IL-1beta) and the transcription factor NF-kappa B, in the lumbar spinal cord of EAE Lewis rats at 14th day postimmunization. In control, CFA (complete Freund's adjuvant) injected rats immunoreactivity of all three parameters was found in motoneurons an in scattered glial cells, displaying morphological characteristic of oligodendrocytes. In addition, some astrocytes, both in gray and white matter showed IL-1beta and NF-kappa B immunoreactivity. A striking similarity of the response of all three parameters to the disease has been found. In all EAE rats, their motoneuronal immunoreactivity has been considerably diminished, and on the other hand, a strong upregulation of the immunoreactivities has been observed in glial cells: oligodendrocytes and in astroglia (especially strong for IL-1beta). These data support the existance of a functional link between the local NGF system and EAE. Moreover, the data point to interactions between different cell populations in EAE. It is hypothesized that the enhanced production, by glial cells, of NGF and substances coupled to its production, compensate for the diminished synthesis of this neurotrophin in motoneurons.

29.18 **AMYLOID BETA PEPTIDES EVOKE ALTERATION OF CHOLINERGIC PHOSPHOINOSITIDE SIGNALS, LIPID PEROXIDATION AND PARP ACTIVITY IN BRAIN**

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Deposition of fibrillar ß-amyloid peptide has been implicated in the pathomechanism of neuronal degeneration and cellular death in Alzheimer's disease. In this project the effect of aggregated Aβ (1-40) on phosphoinositide-specific phospholipase C (PLC) was investigated in synaptic plasma membranes (SPM) and cytosol prepared from brain cortex of adult rats. Moreover, the role of Aβ (1-40) in activation of lipid peroxidation was evaluated. The activity of PLC acting on phosphatidylinositol (PI) and phosphatidylinositol-4,5-bisphosphate (PIP_2) was determined using exogenous labeled substrates. The subcellular fractions were the source of enzyme(s). The radioactivity of lipid messengers derived from [^{14}C]arachidonylo-PI degradation was determined. The aggregated form of Aβ (1-40) inhibited Ca^{2+} regulated PI and PIP_2 degradation by SPM and cytosolic enzymes. This Aβ decreased the level of arachidonic acid exclusively through the effect on the cytosolic enzyme(s). Moreover, Aβ (1-40)significantly decreased muscarinic cholinergic receptor dependent, G-protein regulated PIP_2-PLC in SPM. Aggregated, neurotoxic fragment, Aβ (25-35) mimicked the effect of full-length Aβ peptide. Amyloid β (1-40) enhanced the level of conjugated double bonds in membrane lipids and also the level of malondialdehyde indicating activation of free radical stimulated membrane peroxidation. Moreover, the effect of Aβ on poly-(ADP-ribose)polymerase (PARP) activity in nuclear fraction from different regions of adult and aged brain was determined. In aged brain significantly lower PARP activity was observed in hippocampus with no change in cerebellum and brain cortex. Aβ (25-35) exlusively in higher concentration (25-50 μM) enhanced PARP activity in brain. Effect of Aβ (25-35) on PARP activity was additionaly investigated in PC-12 cells in culture. Our study indicated that deposition of aggregated Aβ may be responsible for the alteration of phosphoinositides signalling, peroxidation processes and PARP activity in Alzheimer's disease. Pharmacological agents preventing amyloid β fibrilisation and aggregation may offer new terapeutic approaches in Alzheimer's disease.

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30.1 **INVOLVEMENT OF ERK/SAPK MEMBERS OF MITOGEN ACTIVATED PROTEIN KINASE FAMILY IN PKC-INHIBITION TRIGGERED APOPTOSIS IN N2A CELL LINE.**

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Neuroblastoma N2a cells respond with apoptosis to specific inhibition of protein kinase C (PKC) by low concentration of staurosporine (10nM) or Go6796 (50nM). The apoptotic phenotype of dying cells, estimated routinely by the Hoechst 33258 staining, was confirmed by DNA laddering, TUNEL and cJun/AP1 immunochemistry. It has been found that fraction of cells undergoing apoptosis after PKC inhibition increased markedly under concomitant lowering of serum in culture medium. We have used an inhibitor of MEK activation (PD98059) to clarify possible involvement of ERK-mediated signal transduction pathway to the observed apoptotic effect. Interestingly, while PD98059 treatment of N2a cells clearly reduced ERK phosphorylation and activity to about 20% of control, it neither influenced cells viability nor modified the proapoptotic effect of PKC inhibitors. In contrast, simultaneous application of both apoptotic inductors (STS treatment and serum deprivation) to N2a cultures, in addition to ERK inhibition, significantly stimulated SAPK phosphorylation/activity and increased contribution of already phosphorylated c-Jun to the enhanced AP1 dimmer formation. These results confirm that dynamic balance between ERK/SAPK pathways activity is decisive for apoptotic program and that SAPK is activated by adverse signals leading to cell death.
Supported by SCSR 6 P04A 01014 grant.

30.2 INFLUENCE OF THE HOT NOCICEPTIVE STIMULUS ON THE BLOOD FLOW IN THE SCIATIC NERVE IN THE RAT.

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The aim of the study was to investigate the influence of hot nociceptive stimulus applied on the rat's foot on the area innervated by sensory branches of the sciatic nerve, on the nerve blood flow (NBF).
Experiments were performed on 12 Wistar male rats 300-350 g b.w. Animals were anesthetized with chloral hydrate, paralized with pancuronium bromidum and artficially ventilated. Sciatic nerve was exposed at the lengh of 18 mm proximally to its ramification into fibular and tibial nerves.
After removing muscular fascia covering the nerve, NBF was measured with laser flow probe of 1,2 mm diameter placed over sciatic nerve trunk. Flow probe was mount in movement–stabilizing holder. Standarized thermode (2 mm ^{2}) was placed on the plantar surface of the foot. After increasing temperature up to 100°C we observed significant increased NBF ranging from 20 to 40 % ($p<0,001$)
We conclude that stimulation of the nociceptive fibers leads to functional hyperemia in the sciatic nerve.

30.3 CIRCADIAN RHYTHM OF SPERM RELEASE IN MALE COTTON LEAFWORM - *SPODOPTERA LITTORALIS*; TEMPORAL DISTRIBUTION OF PER PROTEIN IN MALE REPRODUCTIVE SYSTEM.

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Sperm release from testis into the vas deferens in male *Spodoptera littoralis* is controlling by circadian clock located in reproductive system. Such rhythms are self-sustained, persist in contrast darkness with close to a 24-h period *in vivo* and in isolated reproductive tracts cultured *in vitro*. Sperm release is restricted to 6-hour gate each day in insects kept under light/dark conditions. The first release of eupyrene sperm bundles was observed 4 hours before light off and is the result of complex interactions between spermatozoons and terminal epithelial cells separating testis follicles from the vas deferens. Moreover the daily rhythm of sperm release is disrupted by continuous light.

It was found that sperm release is synchronized to rhythm of PER protein appearance in different regions of male reproductive system. These results suggest cells of vas deferences to be circadian clock because PER protein level oscillates in these ones.

30.4 THE ROLE OF MOUNTS AND INTROMISSIONS IN TRIGGERING OF THE EJACULATION IN MALE RATS.

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The role of mounts and intromissions in triggering of the ejaculation was investigated in 8 male rats. The normal estrous female (NF) and the estrous female with the vagina covered by adhesive tape (CF) were used. The sessions consisted of two phases - Phase A and Phase B. During Phase A of the experimental sessions the male rats copulated with NF or with CF until 4 intromissions or 4 mount bouts were achieved respectively. During Phase B the male copulated alternately with NF or CF. The number of intromissions displayed in phase B did not depend on the character of the copulatory behavior in Phase A (mounts or intromissions). At least 2 intromissions had to be performed before ejaculation. The ejaculation in 91.6% of cases occurred during the copulation with CF.

The results of this study indicate that: (i) the mounts and the intromissions exert the similar influence on the ejaculatory potential, (ii) the storage of the kinesthetic information from intromission is essential for triggering of the ejaculation, and (iii) the stimulation of the genital receptors localized on the glans of penis plays crucial role in ejaculatory secretory reflex (the emission of the semen).

30.5 TEMPERATURE AND JHA-DEPENDENT CHANGES IN THE BRAIN AND PROTHORACIC GLANDS OF *GALLERIA MELLONELLA* LARVAE.

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The development of *Galleria mellonella* larvae of the last instar is arrested by lower temperature of 18°C for several months described as facultative larval diapause. Similar results were obtained by juvenile hormone anlogue (JHA-fenoxycarb) treatment. The aim of this study was to find out what kind of changes occur in the brain neurosecretory cells and in the prothoracic glands during prolong larval development.

It was found that during larval diapause there is an accumulation of neurosecretion in different cells, among them in PTTH-producing cells as well. Similar results were obtained after fenoxycarb treatment.

During larval diapause a gradual programmed cell death in the prothoracic glands was observed and TUNEL labeling was used to study apoptosis-dependent degeneration during natural diapause as well as during prolongation of larval development of JHA-treated larvae. The same methods were used in order to study a programmed cell death after transferring diapausing larvae to optimal temperature of 30°C.

30.6 BRAIN REGIONAL ADENOSINERGIC ACTIVITY: WITHDRWAL EFFECT OF THEOPHYLLINE.

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Withdrawal of theophylline(Th),following development of tolerance to Th [10 mg/kg/day p.o.for 16 consecutive days] reduced the locomotor activity (LA) with time. The maximum reduction (54 %) of LA was observed at 48hr of Th withdrawal and then gradually became normal with time.Under this condition, measuring the brain regional (a) adenylate cyclase (ADC) activity by the estimation of c-AMP and (b) adenosine deaminase (ADA) activity by measuring the adenosine level, it was observed that the withdrawal of Th following the development of tolerance to Th significantly reduced the ADC activity in cerebral cortex (74%),cerebellum(89%), corpus striatum (81%), hypothalamus (85%), hippocampus (65%) and in pons-medulla (76%) as well as the ADA activity in cerebral cortex (27%), cerebellum (48%), corpus striatum (59%), hypothalamus (52%), hippocampus (20%), and pons-medulla (70%).These results,thus,suggest that the withdrawal of Th following its tolerance stimulates central adenosinergic activity and may reduce LA under similar condition.

(Supported by University Grants Commission ,New Delhi and University of Calcutta, India.)

30.7 **BLOCKADE OF ADENOSINE A_{2A} RECEPTORS DECREASES HALOPERIDOL-INDUCED MUSCLE RIGIDITY IN RATS**

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It has recently been suggested that blockade of adenosine A_{2A} receptors may be beneficial to the treatment of Parkinson's disease (PD). It is well known that in the course of PD - besides akinesia and tremor - there also occurs muscle rigidity. Hence the aim of the present study was to determine whether the blockade of A_{2A} adenosine receptors by the selective antagonist SCH 58261 influenced the muscle rigidity induced by haloperidol. Haloperidol in a dose of 0.5 mg/kg (ip) induced muscle rigidity measured mechanomyographically as an increased muscle resistance developed in response to passive extension and flexion of the hind foot in the ankle joint. SCH 58261 in doses of 0.1 - 5 mg/kg ip decreased dose-dependently the haloperidol-induced muscle rigidity. Moreover, administration of L-DOPA (50-100 mg/kg ip) diminished the muscle rigidity evoked by haloperidol. When SCH 58261 (0.1 mg) was injected together with L-DOPA (50 mg), in doses which per se did not affect the haloperidol-induced muscle rigidity, a clear potentiating effect was observed. The present results show that the blockade of adenosine A_{2A} receptors by SCH 58261 produces a strong antiparkinsonian-like effect per se, and also potentiates the effect of low doses of L-DOPA. These results suggest that selective antagonists of adenosine A_{2A} receptors may be useful as a new approach in the treatment of Parkinson's disease.

30.8 **BLOOD-BRAIN BARRIER CONTROLS THE LEVEL OF CARNITINE IN BRAIN**

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Carnitine (4-trimethylamino-3-hydroxybutyric acid) accumulates in brain, although to much lower extent than in peripheral tissues. This compound was observed to accumulate in neurons and, in the presence of choline, to stimulate acetylcholine synthesis. Brain microcapillary endothelial cells accumulate carnitine and this process was found to be specifically inhibited by butyrobetaine, a natural precursor of carnitine synthesis. In the present study, a mechanism of a possible carnitine release was investigated in an *in vitro* system, with cultured rat brain endothelial cells (RBE4). Upon removal of carnitine (after 1 h loading) the cells are capable to release about 50% of this compound. The process was unaffected by sodium ions replacement. It was, however, strongly stimulated upon protein modification with mersalyl, an nonpermeable SH group reagent. Neither glycoprotein P (no effect of vincristine) nor system L for amino acids (no effect of leucine, alanine, 2-aminobicyclo(2,2,1)heptane-2-carboxylic acid) was involved in the efflux of carnitine. Choline stimulated the release of carnitine only after longer times, what, in parallel with no influence of hemicholinium-3, a specific inhibitor of choline transporter, would point to an indirect effect on the further metabolism. The only compound found to inhibit the efflux of carnitine was butyrobetaine, a carnitine precursor. These observations would lead to the conclusion that the same transport system is involved in the uptake and efflux of carnitine by brain endothelial cells, which are capable of releasing this compound in case of lowering its content in the external medium.

30.9 PATTERN OF ETHANOL and SWEET SUBSTANCES INTAKE in HIGH and LOW VOLUNTARY ALCOHOL DRINKING RATS

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The dopamine is an important transmitter for reward and reinforcement produced by drinking. Data from literature have been shown that common neurochemical system may mediate positive reinforcing properties of sweets and drug of abuse. Numerous studies have been demonstrated an association between saccharine and alcohol intake in rats and mice.

New lines of rats have been selectively outbred for 7 years and 17 generations in our laboartory for high and low ethanol intake (WHP, Warsaw High Preferring and WLP, Warsaw Low Preferring). The present study was aimed to assess drinking of 2.5, 5.0, 10.0 and 30% sucrose solutions and 0.001, 0.01, 0.1% saccharine solutions between WHP and WLP rats. Intake of sucrose and saccharine solutions was significantly higher in WHP rats than WLP rats (except for the lowest 2.5% sucrose concentration). Also, WHP rats consume much more 0.1% saccharine solution than WLP animals. Further, in concurent availabilty of highly palatable 10% sucrose solution, ethanol intake dramatically declined in the WHP rats. The measurement of temporal pattern of ethanol drinking both in lines of rats, over a 24 h period has shown that maximal ethanol consumption occured during the natural dark phase (19.00 - 07.00 hours), consistent in with the nocturnal habits of rats.

Our results suggest that higher consumption of sweet solutions may be important biological factor to understand the mechanism of ethanol action and our results confirmed those reported in genetically selected HAD and sP rats.

30.10 **THE EFFECTS OF ETHANOL ON THE SYNAPTIC CONTACTS OF THE ORGANOTYPIC CULTURE OF THE RAT CEREBRAL CORTEX.**

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Ethanol has a clear teratogenic effect during organogenesis, but relatively little is known about the mechanism of morphological alterations in postnatal development of neocortex. In the present study we examined how ethanol exposure affects the synaptic morphology of cerebral cortex culture. The effects of different concentrations (0.5%, 1%; w/v) of ethanol were investigated in organotipic culture of 1-day-old rats cerebral cortex. Ethanol was added to the medium from 1 to 20 days of development in vitro. A quantitative morphometric analysis of synaptic parameters revealed significant between-groups differences. Prolonged ethanol exposure produced an increase in the area and perimeter of presynaptic terminals and in the length of the postsynaptic thickening. These parameters were significantly increased after the exposure of 1% ethanol as compared with 0.5% ethanol or control groups. The percentages of both perforated and multiple synaptic contacts were significantly higher in the cultures grown with 0.5% ethanol. No difference was found in the relative number of vesicles adjacent to the synaptic apposition. These results show that the ethanol exposure applied during the period of synaptogenesis produces the hypertrophy of axon terminals and the lengthening of the postsynaptic thickening and may change normal interneuronal relationships.

30.11 **REGULATION OF CALRETININ GENE TRANSCRIPTION IN THE BRAIN**

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Calretinin (CR) is a member of the EF-hand family of calcium binding proteins, mainly expressed in some brain neurons. The function of CR is unknown. We showed that CR does not buffer Ca^{2+}-overload induced by ionomycin, ADP and thapsigargin (Billing-Marczak et al., BBA 1449, 169-177, 1999). These, and our earlier data, suggest that CR acts as a calcium sensor rather than as a calcium buffer. To further elucidate CR's role we checked the effect of increased calcium ion concentrations on the CR gene promoter activity. No activation of transcription from CR's promoter was observed, suggesting a calcium independent regulation of the CR gene. We showed that a 1.5 kb CR promoter and its 5' deletion mutants drove the expression of a luciferase reporter construct in primary cultures of rat brain and in PC 12 cells (Winsky et al., Soc. Neurosci. Abstr., 23, 1997). In the present work, we found that a reporter construct containing a -115/+54 bp piece supports luciferase expression in glioma C6 and Hep-2 cells and we confirmed its activity in cultured neurons. Gel mobility shift assays identified two complexes between the -115/+54 bp piece of the CR gene promoter and proteins present in nuclear brain extract. One of the complexes was specifically competed by -115/-30 bp, but not by -92/+54 bp. Thus, the region -115/-92 seems to contain a binding site for the transcription factor(s). A theoretical analysis using MatInspector showed that this region contains sites for IK1 and IK2. Further studies, using affinity column chromatography with a -115/-92 piece attached to the resin, will help to isolate and identify the factor(s) responsible for CR expression in the brain. We are supported by a Polish-American Maria Skłodowska-Curie Joint Fund II grant, nr PAN/NIH-97-311.

30.12 PURIFICATION OF HEPARIN-BINDING PROTEINS FROM RAT BRAIN

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Heparin-binding protein active specially during first steps of morphogenesis. The protein-carbohydrate interaction may establish as hetero- and homophilic cell recognition system to provide signal transduction. The idea of our work was to study heparin-binding activity in different protein fraction of rat brain during postnatal development and to isolate the heparin-binding protein. (HBA) was detected by enzyme-linked carbohydrate-binding assay developed in our laboratory. Briefly, the heparin-binding activity was measured in protein samples immobilized on the surface of microtiter plates using a heparin-horseradish peroxidase conjugate at pH 6.0 and was expressed as ng of bound heparin per mg of total protein. The high level of the heparin-binding activity was shown in fraction containing membrane-associated protein of rat at postnatal days 0-5. Such activity quickly decreased and was minimal after postnatal day 5. Membrane-associated protein fraction of 3-5 p.d. rats was used for purification of heparin-binding protein due to chromatography on heparin-sepharose. The 0,5 M NaCl eluents were collected and concentrated by ultrafiltration. The eluent contained only low molecular mass heparin-binding protein (under 20 kD)

30.13 **DETERMINATION IN VIVO OF FREE UNIONIZED AND IONIZED FRACTIONS AND BOUND FRACTION OF CARBAMAZEPINE AND ITS TWO METABOLITES IN SERUM AND BRAIN TISSUE OF THE RAT.**

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Therapeutic monitoring of antiepileptic drugs is of special importance because the same close of the drug could result in different concentractions in the blood of various patients and therefore could elicit pharmacological activity. Protein - bound drug does not elicit pharmacological activity. In majority of clinical studies the whole concentration of the drug in blood was determined and free fraction, which is of therapeutic importance, was neglected. The aim of this study is to elaborate a new SPE/HPLC method for determination of free unionized and ionized fractions and burned fraction of carbamazepineand its two metabolites: carbamazepine 10,11 - epoxide and carbamazepine 10,11 - trans - diol. CBZ was administered intragastrically or intraperitoneally in a dose of 50 mg/kg. Blood and brain tissue samples were taken 0,5; 1,5 and 3h after the drug administration. Free fractions were determined using method of extraction in fluid - solid state system (SPE) with sorbent bakerbund wcx. Tested substances were eluated in two stages: first stage - water for HPLC (unionized function); second stage - 95 % MeOH + 5 % 2M H_3PO_4 (ionized function).

1.It was determined in vivo that the route of administration of carbamazepine (per os or intraperitoneally) could influence the level of free ionized and unionized fractions as well as of bound fraction in serum and brain tissue.

2.SPE/HPLC method could be useful for the determination of free fractions of other groups of drugs when adequate sorbents and eluating substances are used. Supported by KBN 148-103/C-T00/96

30.14 **POST-TRANSLATIONAL MODIFICATION OF CLN3 PROTEIN AND ITS POSSIBLE FUNCTIONAL IMPLICATION**

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The CLN3 gene associated with Batten disease and encoding a novel protein of a predicted 438 amino acids was cloned in 1995 by the International Batten Disease Consortium. The function of CLN3 protein remains unknown. Computer-based analysis predicted that CLN3 may contain several post-translational modifications. Thus, to study the post-translational modification of CLN3 protein, we have expressed a full-length CLN3 protein as a C-terminal fusion with green fluorescent protein of the jelly-fish *Aequerea Victoria* in Chinese hamster ovary cell line. Previously, we have shown that CLN3 is a glycosylated protein from lysosomal compartment, and now, by using *in vivo* labeling with ^{32}P, detection with anti-phosphoamino acid antibodies, and phosphoamino acid analysis, we demonstrate that CLN3 is a phosphorylated protein. We demonstrate that CLN3 protein does not undergo mannose 6-phosphate modification and that it is a membrane protein. Furthermore, we show that the level of CLN3 protein phosphorylation may be modulated by several protein kinases and phosphatases activators or inhibitors.

30.15 DOES THE SEVORANE INFLUENCE THE INTRACRANIAL PRESSURE (ICP) IN THE RABBITS WITH EXPERIMENTAL INTRACEREBRAL HEMORRHAGE ?

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Sevorane is one of the newest inhalational anaesthetic agent. From clinical point of view the question whether *sevorane* influences in different manner on the intracranial pressure (*ICP*) in the conditions of the intracranial pathology is essential. The aim of the study was to examine the effects of various doses of *sevorane* (*1 MAC, 2 MAC, 3 MAC*) on the *ICP*, the mean arterial blood pressure *(MABP),* and the heart rate *(HR)* in the rabbits with experimental intracerebral hemorrhage *(ICH).* Experiments were performed on 6 adult rabbits, 3,5-4,0 kg weight. Care and treatment of the animals were in accordance with guidelines of *Local Ethical Commitee*. The animals were under general anesthesia. A microcapillar was introduced sterotaxically into the striatum and the intracerebral pressure injection 2 ml of blood was then performed through a 5' period of time. Following 15 minutes from the end of *ICH* induction *sevorane* was given in stepwise (15 min each) increasing concentrations: *1 MAC, 2 MAC, 3 MAC.* The continuous monitoring of *ICP, MABP,* and *HR* was performed. From 35 minutes of observation significant increase of *ICP* was seen ($p<0,01$). *MABP* and *HR* were significantly decreased comparing with the initial value from 20 min and 25 min, respectively. Our observations suggest, that the *sevorane* does not influence the *ICP* and systemic circulation in course of the intracranial pathology, in the concentrations which does not exceed *1 MAC.*

30.16 INFLUENCE OF THE EXPERIMENTAL INTRAVENTRICULAR HEMORRHAGE ON THE INTRACRANIAL PRESSURE AND HEMODYNAMIC CHANGES IN THE RABBIT.

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Investigation of the pathophysiology of the intracerebral hemorrhage should lead to improved treatment strategies. The aim of the study was to examine the effects of predetermined volumes of intracerebral haematoma on intracranial pressure *(ICP)*, mean arterial blood pressure *(MABP)*, and the heart rate *(HR)* in the rabbits, during three hours after hemorrhage. Experiments were performed on the adult rabbits (3,5-4,0 kg weight). Care and treatment of the animals were in accordance with guidelines of *Local Ethical Commitee*. The animals were under general anesthesia. The continuous monitoring of *ICP, MABP* and *HR* was performed. A microcapillar was introduced sterotaxically into the striatum and intracerebral pressure injection of 1 ml (group I) and 2 ml (group II) of blood was then performed through a 5' period of time. Monitoring parameters were recorded every minute during intracerebral blood infusion, than every five minutes for three hours. The mean volume of intraparenchymal part of haematoma in group I was 78 ± 16 µl and $4,97 \pm 3,13$ µl in group II, respectively. Additionally, in all cases of group II the evidence of intraventricular hemorrhage was observed. From the first minute (in group I) and the third minute (in group II) to the end of intracerebral blood infusion a consecutive significant increase of *ICP* was seen and remained elevated till the end of observation. *MABP* and *HR* increased significantly from the fourth minute in group I and the first minute in group II. Average values of *ICP*, *MABP* and *HR* in group II were significantly higher than that in group I. Our data suggest, that the changes of *ICP* remain the most sensitive parameter during the early course of intracerebral hemorrhage of small volume and sudden and great increase of *ICP*, *MABP* and *HR* can draw suspicion of the intraventricular hemorrhage.

30.17 THE EFFECT OF SEVOFLURANE ON THE INTRACRANIAL PRESSURE (ICP) AND CARDIOVASCULAR SYSTEM IN THE RABBIT

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Sevoflurane is one of the newest volatile anesthetics. The aim of this study was to compare the effects of sevoflurane in steadily rising and stepwise rising concentrations on the intracranial pressure (ICP) and parameters of cardiovascular function in the rabbit. Experiments were performed in 13 adult rabbits, which were randomly allocated into two groups. The project of this study received approval of the Local Ethical Committee. The animals of group I (n=8), were administered sevoflurane in increasing concentrations of 1 MAC, 2 MAC and 3 MAC each for a period of 15 minutes. Animals of group II received sevoflurane in constant concentration of 3 MAC over a period of 45 minutes. The ICP, the mean arterial blood pressure (MABP), the heart rate (HR) and the end-tidal CO_2 concentration ($ETCO_2$), were monitored. The mean values of $ETCO_2$ were not significantly different in both groups and remained stable in the whole period of observation. Statistically significant rise in the ICP was observed in 30th minute in group I, while in group II it appeared already in 10th minute. In both groups readings of ICP remained significantly elevated until the end of experiment, comparing to the resting level. Changes in the ICP revealed positive correlation with the end-tidal concentration of sevoflurane both in group I ($p<0,05$; $r=0,82$) and in group II ($p<0,05$; $r=0,69$). Decrease in the MABP was found in both groups, the pressure reached significantly lower values in 30th minute in group I and in 5th minute in group II, and continued to fall until the end of the experiment. The decrease in the HR was also observed in both groups, reaching significantly lower values in 25th minute in group I and in 5th minute in group II. In conclusion, it should be stressed that sevoflurane in doses not exceeding 1 MAC shows no significant effect on the ICP and parameters of cardiovascular function. Regardless of the administration regime, sevoflurane in doses of 3 MAC induces increase in ICP, which does not exceed the upper limit of the normal range. In concentrations exceeding 1 MAC, degree of sevoflurane's depressive effect on the systemic circulation is proportional to the administered dose.

30.18 IN VITRO AND IN VIVO GENE TRANSFER INTO NEURAL CELLS USING HSV1 AND POLYETHYLENE IMINE (PEI).

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Herpes simplex virus (HSV-1) has ability to establish life-long latent infections within neurons and, therefore, may be considered as a vector for delivering biological relevant peptides to the nervous system. While HSV exhibits a neuronal specificity for establishing a latent infection, during acute infections, HSV has the ability to infect a wide range of cell types. This coupled with the ability to make replication deficient viruses (ICP4-) opens up the potential for other therapeutic applications. Previously we have demonstrated that a fusion between the HSV latency-associated transcript promoter (LAT) and the Moloney murine leukemia virus LTR (LAT/LTR) was capable of long-term expression in peripheral neurons *in vivo*. In this study, we perform a quantitatively evaluate the ability of a non-replicating HSV vector to express ß-gal as a reporter in a number of representative cell lines *in vitro* as well as *in vivo* in the rat CNS. Cell lines and animals were infected with either an ICP4- vector (control) or an ICP4- vector with the LAT/LTR-ß-gal cassette. Cell lines examined included normal human astrocytes and human glioblastoma lines. The cell line studies demonstrated that HSV vector could efficiently transduce the majority of the cells *in vitro* when infected at multiplicities of 1-5 pfu. A quantitative comparison of ß-gal expression and the duration of expression elicited by this vector in these cells demonstrated that ß-galactosidase was only expressed abundantly in a small fraction of cells, and that levels of expression were highest in the human astrocytes. In vivo the HSV-1 ICP4- inoculated into the rat hippocamus or substantia nigra rendered a strong expression of the ß-gal reporter. Extracellular injections of plasmid DNA complexed with PEI also resulted in an efficient reporter expression both in vitro and in vivo. These data demonstrate that HSV and chemical vector PEI have potential for use as vectors for delivering genes into the CNS, but consideration must be given to the promoter utilized for the individual cell system being targeted.

30.19 **NO MODULATES MEDULLARY RESPIRATORY RHYTHM**

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We studied the role of NO in the regulation of medullary respiratory rhythm. Experiments were performed on neonatal rats under deep ether anaesthesia. The effects of NO-synthase (NOS) inhibitor (L-NAME), NO scavenger (hemoglobine), and L-arginine, on the respiratory activity of medullo-spinal preparations (SIMSP) were investigated. Inspiratory discharges (ID) were recorded from the phrenic nerve of SIMSP superfused with Krebs solution saturated with 95% O_2 and 5% CO_2. Hypoxia was modelled by 3-min-long superfusion of SIMSP with the solution saturated with 95% N_2 and 5% CO_2. Modified NADPH-diaphorase (NADPH-d) histochemistry method was used to localize the medullary NOS-containing neurons. NO enhanced the amplitude but decreased the frequency of ID both in norma and hypoxia. It was found that endogenous NO mediates the mechanism of tonic inhibitory control of the ID frequency located in the rostral ventrolateral medulla (VLM), appears to be involved in generation of the basic activity by the more caudal structures of VLM. NADPH-d positive neurones were present in all nuclei of both dorsal and ventral respiratory group of neurons. The stained cells were density packed in the rostral VLM - in the region of lateral paragigantocellular reticular nucleus. We found that NO is an essential central neuromodulator of medullary respiratory rhythm in neonates.

ARTIFICIAL BRAIN BUILDING 30.20

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Despite the progress in neuroscience, for decades the barrier of computational power prevented scientists from thinking seriously about large-scale neural models. Recently it was shown that, when combining such techniques as artificial neural networks, genetic algorithms, and Cellular Automata, the barriers stop exist. The international CAM-Brain Project coordinated at the ATR, Kyoto aims to build a neural mega-network containing billion neurons by the year 2001 and use it to control behaviors of a robot kitten. The basic element of the structure is so-called CoDi module consisting of 24×24×24 3D Cellular Automata cells in which a network of up to 1152 neurons can grow and work. Using the Korkin Machine—a special purpose supercomputer being built in the framework of the CAM-Brain Project—one can evolve desired CoDi modules employing a genetic algorithm, as well as run systems of up to 32784 ready CoDi modules. Hence, brain-builders have presently to their disposal a machinery for simulating a brain-like structures consisting of over 37 million neurons in real time. Based on our own experience with evolving CoDi modules, and taking into account that the development of the Korkin Machine is virtually limitless, we argue that the CoDi technique opens the gate to artificial brains containing tens, or hundreds, or even thousands of billions of neurons

30.21 **INTERFACE CULTURES OF ISOLATED BARREL CORTEX FROM RAT SOMATOSENSORY CORTEX.**

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We attempted to grow interface cultures from 6 days old rat somatosensory cortex. The slices of cortex were placed directly on semiporous membranes, and medium was added to the bottom of the culture dish. For ultrastructural analysis, cultures grown on the DV filters were used because they could be more easily transferred through the various steps than cultures grown directly on the insert biopore membrane. These stationary cultures were infiltrated by medium on one side and accessible to oxygen from the other side. The best results were obtained from the cultures restricted only to the barrel field region. Larger cultures containing two neighbouring regions, for instance SmI and SmII or SmI and thalamus; degenerated after 24h in culture conditions. The cultures from SmI were maintained for 14 days from postnatal day 6. Cytochrome oxydase activity was used as a functional marker of the barrels and synaptic contacts could be observed under EM. After 3 weeks in culture, necrosis of the central region of the tissue become apparent. The applicability of these cultures can be suitable for understanding properties of thalamocorical synapses following loss of connections from neighboring regions. The study was supported by The Royal Society Postdoctoral Fellowship to E.S.

EFFECTS OF ANXIOLYTIC AND ANXIOGENIC DRUGS ON, VOCALIZATION AND HEART RATE REACTIONS IN FEAR CONDITIONING PARADIGM. 30.22

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The aim of the experiment was to evaluate the effects of anxiolytic and anxiogenic drugs on selected physiological (heart rate – HR) and behavioral (vocalization – USV) reactions in fear conditioning procedure in partially restrained rats. Electric shock (3mA, 100ms) applied to the tail was used as US. 5s conditioned stimulus (CS) which preceded immediately the US and served as the *signal of danger* was expected to evoke a phasic fear. Conditioned inhibitor (CI) overlapped the last 3s of 5s CS. It predicted an omission of US, and serving as the *signal of safety* was expected to evoke a relief state. The following drugs were used: a) anxiolytics - buspirone and diazepam, b) anxiogenics - FG7142 and pentylenetetrazole. Diazepam increased baseline (pre-CS) HR and reduced conditioned HR reactions (bradycardia to CS and tachycardia to CS+CI). Buspirone decreased baseline HR and increased HR reaction to CS. Anxiogenic FG7142 decreased pre-CS HR without affecting the conditioned HR reactions. Pentylenetetrazole did influence neither HR reactions nor pre-CS HR. The effects of used drugs on conditioned HR reactions and on baseline (pre-CS) HR depended rather on the specific types of engaged receptors (BDZ or 5-HT_{1A}), then on the general profile of drug action (anxiolytic or anxiogenic). The profile of drugs' action was better seen in their influence on rats' vocalization (mainly ultrasonic „22-kHz" band). The CS suppressed ultrasonic vocalization (USV) evoked by the aversive experimental context (restraint, electric shock) while CI induced partial reappearance of USV. Buspirone and diazepam suppressed both: baseline (pre-CS) and CI induced USV, while PTZ acted in opposite direction, increasing USV. FG7142 mainly suppressed CI induced USV. Concluding, HR although often used, as a physiological correlate of defense reaction can not be trusted as an index of chemically induced emotional states. USV, however, compared to HR, provided better correlation with the profile of used drugs.

30.23 **AGONISTS OF OCTOPAMINERGIC RECEPTORS MODYFY BIOELECTRICAL ACTIVITY OF NEUROSECRETORY DUM NEURONES IN CNS OF *PERIPLANETA AMERICANA*.**

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DUM neurones in insects CNS release octopamine, an agent playing a role of neuromodulator, neurotransmitter and neurohormon. Such a neurosecretory function of DUM neurones depends probably on activity of an endogenous pacemaker which could be regulated by excitation of various cell receptors. Our study concentrates on pharmacological properties of octopamine receptors (OA-R), identified in DUM neurones of terminal abdominal ganglion of *P. americana*. Experiments were performed *in situ*, using intracellular microelecrode technique. The aim of our work was a comparison of effects of five different OA-R agonists, at a dose 10^{-5} mol l^{-1} , on bioelectrical activity of DUM neurones. Application of four of them induced:1. temporary hyperpolarization of neuronal membrane; 2. a reversible block of spontaneous activity of neurones. These two effects were well correlated and dose dependent. The efficiency of agonists was estimated from amplitude of hyperpolarization and duration of absence of spontaneous action potentials (AP) and the general rank order was established: tolazoline >naphazoline >synephrine >octopamine. The effect of clonidine was different from described above because a biphasic response was observed: a transient (mean 9,6 s) decrease (by about 54%) of frequency of action potentials followed by a prolonged acceleration (by about two times), comparing with control period. It is interesting, that the clonidine-induced increase of AP frequency was often acompanied by cell membrane hyperpolaryzation. Further studies are necessary to determine the mechanism of action of above-mentioned agonists.

DIET AND ADRENAL MEDULLA ACTIVITY 30.24

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It is well known that diet changing nutritional state of the organism can influence the activity of sympathetic nervous system, but hardly any informations about adrenal medulla activity in different diet conditions are available. The present study was performed in order to estimate if adrenal medulla activity measured as catecholamine (CA) content depends on the kind of diet. Our experiment was carried out on albino Wistar rats divided into six groups: I-control rats fed ad libitum with standard laboratory chow, II-rats fasted 24h, III-standard fed rats injected with 2-DG (glucose analouge disturbing intracellulary glucose influx), IV-rats fed only fat (lard, 10g/day), V-normal fed rats received 2-mercaptoacetate (2-MA-compound which is known to inhibit β-oxidation of fatty acids) and VI-fat fed rats injected with 2-MA. Short fasting and 2-DG injection didnt't influence adrenal adrenaline (A) content, which was 72.93±6.53μg/2ADR in fed rats, 71.18±6.59μg/2ADR in fasted ones and 76.27±13.09μg/2ADR in 2-DG injected animals, but in all other groups there was a significant decrease in adrenal A level (IV-31.86±3.20μg/2ADR, V-37.71±5.52μg/2ADR, VI-41.67±2.85μg/2ADR; $p<0.001$). In fat fed rats 2-MA injection significantly ($p<0.05$) increased A content. We observed that fasting caused significant increase in noradrenaline (NA) adrenal level from 3.18±0.46 μg/2ADR in fed to 5.53±0.73μg/2ADR in fasted rats. In 2-DG injected rats adrenal NA level remained unchanged and was 2.57±0.51μg/2ADR. In normal fed rats 2-MA injection didn't influence adrenal NA content, which was 2.97±0.39μg/2ADR. In fat fed rats both injected and non-injected with 2-MA, there is a significant decrease ($p<0.02$) in adrenal content of this amine to 1.35±0.45μg/2ADR and 1.23±0.54μg/2ADR, respectively.

30.25 **THERMOREGULATORY BEHAVIOUR, HETEROTHERMIA AND MUSCLE RESTING POTENTIAL OF BEES**

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Honey bee (*Apis mellifera* L.) is classified as a heterotherm species. Because of behavioural and physiological mechanisms of temperature regulation honey bees are able to forage over a 30°C range of air temperature (7-46°C). Body (thoracic) temperature influences many physiological parameters of insect flight muscles, including duration and amplitude of action potential, power output and others. Thermogenesis in these muscles may also be disturbed. Therefore, the aims of the present study were: 1) to determine circadian changes of selected ambient temperature in honey bee workers placed in a thermal gradient system, and 2) to check effect of temperature (within the range selected by honey bees) on resting membrane potential of the flight muscles *in situ*. The conventional microelectrode technique was used. Temperatures of the muscle preparation were kept at: 20°C, 37°C, 40°C. The highest mean prefered ambient temperature of 37°C was recorded during the day (around 1 pm), the lowest (26°C), however, accured at night (around 4 am). Upper circadian temperature of 37°C coincides with values reported as necessary to instantaneous take off in honey bees (otherwise they have to use preflight warm-up). Under such thermal conditions the average value of RP was −36,6±0,7 mV; at 20°C (about 10°C below the temperature limit of starting the flight) RP decreased to −30,5±0,8 mV, which is significantly less than in poikilothermic insects such as cockroaches; at 40°C (temperature of active bees) RP increased to −49,1±1,0 mV. Altogether, the present data are related to the unique social biology of the honey bee.

REDUCED BODY TEMPERATURE AS A DEFENCE AGAINST ANOXIC BRAIN DAMAGE IN NEWBORN RATS 30.26

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Hypoxia is one of the strongest neurotoxic factors. It inevitably accompanies parturition in mammals (it hurts 20-30% premature human newborns). Survival in anoxic mammals is determined by an ability of their brainstem to generate gasping respiratory efforts. Because a moderate decrease in cerebral temperature (by 2-3°C) provides a considerable neuroprotection, reduced body temperature at birth could be a physiological adaptation to avoid insults. Therefore, the aim of the present study was: 1) to compare developmental changes of body temperature in control, cold-(13°C), and warm-reared (35°C) rats, and 2) to record gasping in newborn rats exposed to anoxia for 25 minutes while their body temperature was clamped at a level typical of the neonates (32°C), healthy adults (37°C) or febrile adults (39°C). To record the developmental changes single animals 2-, 30-, 75-, 105-, and 210-day-old were quickly taken from their nest, using a soft cotton glove, and their rectal temperature was measured within 1-min-period, with a miniature thermocouple. Neonatal rectal temperature in warm-reared, control and cold-reared rats was lower than in adults by 2.5°C , 5°C, and 5.5°C, respectively. It was significantly lower than at the later developmental stages. Neonates having their normal body temperature of 32°C were not hurt by anoxia, as judged from an extremely long quiescent period of gasping. In rats having body temperature clamped at 37 and 39°C, however, a critical accelerated gasping developed and terminal apnea started, respectively. In conclusion, reduced body temperature protects neonates against anoxic death.

Session 31 - Parallel Symposium: Psychotropic drugs and signal transduction cascades

31.1 EFFECTS OF ANTIDEPRESSANTS - A FOCUS ON THE α_1 ADRENERGIC RECEPTOR SYSTEM

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Norepinephrine (NE), acting through its specific metabotropic receptors, utilizes at least two intracellular second messenger systems: cAMP/PKA and inositol trisphosphate/diacylglicerol/PKC. In addition, NE may affect the cytoplasmic calcium level, acting through G-protein on ion channels. Antidepressant drugs (AD) augment the synaptic availability of the primary signals of NE and/or serotonin and can induce changes at various levels of the aminergic intracellular transduction cascades. Many of the AD possess antagonistic properties toward α_1-adrenergic receptors (AR). Nevertheless, chronic treatment with these drugs may enhance the effectiveness of α_1-AR system. This happens either by an increase in the density of the receptor and its mRNA expression or by an enhancement of receptors responsiveness. The latter reflect the increased inositol phosphate (IP) response and/or a counter regulation of PKC/α_1-AR negative feedback. Since PKC mediates the potentiation of cAMP generation, the effect of AD on cAMP response can be changed as well. In addition, the AD effects on the α_1-AR responsiveness can be modulated by nifedipine, the L-type calcium channel (LCC) blocker. Recently, the involvement of α_{1A} subtype of AR in the LCC regulation was postulated. Our data shows that some AD increase the α_{1A}-AR density and its mRNA, while others additionally affect the α_{1B}-AR which is coupled to phosphoinositides cascade.

31.2 HOW DO ANTIDEPRESSANTS INFLUENCE SIGNAL TRANSDUCTION CASCADES BEYOND AMINERGIC RECEPTORS?

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Antidepressants (ADs) affect signal transduction via changes in the convergence of signals at the level of protein kinases (3^{rd} messengers) which in turn control gene expression via phosphorylation of transcription factors (4^{th} messengers). Dependent on the eukaryotic cell type, this convergence can be synergistic or antagonistic. Since it is the phosphorylation status of transcription factors that determines gene activation and/or repression, ADs which activate both PKA and PKC would be expected to be more potent in regulating the final common link in the transduction cascade. Results obtained on CREB-P in human fibroblasts now support this view. ADs change the mRNA steady state levels of transcription factors and their translocation from the cytoplasm to the nucleus. Since PKA-mediated phosphorylation represents a highly efficient kinetic amplification mechanism, small changes in the activity of PKA will have profound effects on the net signal transduction. The demonstration that the coupling of hormonal stimulation and transcription via CREB is rate limited by the nuclear entry of PKA together with the reported nuclear translocation of PKA in the cortex of rats after chronic treatment with ADs support the notion that the delayed therapeutic action of ADs is the consequence of changes in programs of gene expression. These changes occur via 2 fundamentally different mechanisms: (a) via agonist-receptor mediated signal transduction cascades and (b) by a mechanism that is independent of agonist – receptor interactions. Since the modularity of transcription factor function allows the production of both activators and repressors from the same gene, it remains to be determined whether, for example, splicing events constitute novel targets for intracellular signal transduction cascades.

31.3 Regulation of signal transduction and gene expression by mood stabilizing drugs

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recent interest on downstream targets such as transcription factors and relevant target genes. Increased G-Mood stabilizing drugs such as lithium and the anticonvulsants, carbamazepine and valproate have multiple targets in neuronal signal transduction pathways and these targets are altered in tissues from patients with bipolar disorder(BD). The pathways include the G-protein coupled cAMP signaling pathway, the phosphoinositide generated second messenger system and intracellular calcium. Our lab has focussed on the first of these pathways using pharmacological approaches and postmortem brain studies with more protein coupled cAMP signaling has been found in cerebral cortex of subjects with BD which may be blunted by treatment with lithium. Phosphorylation of CREB (cAMP regulatory element binding protein) and its DNA binding is decreased in cultured cells after chronic lithium treatment and is localized to specific cerebral cortical and hippocampal regions in rat brain. Postmortem brain tissue from subjects with BD demonstrate regulation of CREB levels in subjects treated with mood stabilizers at the time of death. Regulation of downstream targets of cAMP signaling by chronic treatment with mood stabilizers suggests that changes in gene expression occur after treatment with these drugs. Differential display PCR and cDNA expression array were used to identify genes regulated by lithium and valproate in rat cerebral cortex and rat C6 glioma cells. We found that chronic treatment with lithium may increase CNPase II, nitrogen permease regulator 2, c-jun, M-ras, and presenilin-1 gene expression, which suggest novel targets for lithium may be relevant to its mechanism of action. Chronic treatment with valproate increased both mRNA and protein levels of 78-kilodalton glucose-regulated protein (GRP78) which possesses molecular chaperone activity and protect cells from the deleterious effects of damaged proteins. These data suggest that regulation of specific targets in signal transduction pathway and their downstream targets may be important in understanding the long term prophylactic effects of these drugs in patients with BD.

31.4 NEUROLEPTICS: FROM THE RECEPTORS TO NUCLEUS

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It is generally believed that antipsychotic and extrapyramidal actions of typical and atypical neuroleptics may be sufficiently explained by their disruptive effects of dopaminergic neurotransmission. However, the action of neuroleptics is not only superficial, but given either in a single dose or chronically they induce profound changes at the level of intracellular signal cascade and genome. The most common effect of various neuroleptics is the induction of immediate early genes. Various classes of neuroleptics may vary as to the area in which the changes are produces. Both classical and atypical antipsychotic drugs induce Fos immunoreactivity in the nucleus accumbens. The fact that the distribution pattern of early gene induction in other areas differs among atypical and classical neuroleptics is used for speculations on the targets for antipsychotic vs. extrapyramidal action. Immediate early genes, such as zif/268 and junB and junD, are also induced by neuroleptics. Of other genes whose expression is induced by neuroleptics those for neurotensin, a putative endogenous neuroleptic may be of significance. Neuroleptic treatment results also in changes in expression of genes coding for various subtypes of dopamine receptors. While an increase for D2 receptor is generally agreed upon, the data concerning the changes in the D3 receptor gene are controversial. In addition to genes coding for dopamine receptors, also genes coding for glutamate receptors, both NMDA and metabotropic, are affected. The changes produced by neuroleptics given in a single dose may differ from changes resulting from chronic administration and from withdrawal from chronic neuroleptics

Session 32 - Parallel Symposium: What the insect's brain can tell the vertebrate brain?

32.1 TRANSDUCTION OF PHOTIC STIMULI TO AN INSECT CIRCADIAN CLOCK

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In many organisms, an endogenous, self-sustaining clock mechanism maintains rhythms of about 24 hours to control a wide variety of biological processes. In order to function as a clock, this endogenous mechanism must be entrained to the daily light and temperature cycles of the external environment. However, only a few components are known that connect the endogenous oscillator to the environment, among them the plant cryptochromes as photoactive pigments that may function as entrainment of the clock by blue-light as well for animals.

The nature of photoreceptors and the ways in which environmental signals are resetting the clock will be the main subject of this review. In the blow fly (*Calliphora vicina*) circadian locomotor activity rhythms and their entrainment to light/dark cycle continued after optic tract severance or lobectomy. Extraretinal photoreceptors have been discovered in the brain of the blow fly using S-antigen antibody. Immunohistochemistry demonstrated that photic stimuli have an inductive effect on c-*fos* expression but only at times when light was capable of phase-shifting circadian locomotor activity rhythms of this insect. This might suggest that the c-*fos* gene plays a role in the transduction of photic signals for circadian entrainment. In further experiments it shown that serotonin (5-HT) might be involved in modulation of photic signal transduction in extraretinal photoreceptors of this insect.

32.2 AGONIST-SPECIFIC COUPLING OF G-PROTEIN COUPLED RECEPTORS TO MULTIPLE SECOND MESSENGER SYSTEMS.

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A novel form of intercellular signalling whereby structurally related agonists can bias the coupling of a single G-protein coupled receptor to different intracellular second messenger pathways has recently been described.[1,2,3] It is proposed that the different agonists can induce different conformational changes in the structure of the receptor which favour its interactions with different G-proteins. It is not clear if agonist binding induces the receptor to adopt novel conformations or if the binding stabilizes the receptor in one of a series of conformations which it is able to adopt spontaneously.[4]

Agonist-specific coupling has been demonstrated for some cloned insect receptors including the cloned *Drosophila* octopamine/tyramine receptor, when expressed in CHO cells[2] and a cloned *Drosophila* D1-like dopamine receptor, when expressed in *Xenopus* oocytes.[5] However, the phenomenon is not confined to insect receptors and can also be demonstrated to occur in a range of vertebrate receptors. The functional implications of this novel form of intracellular signalling by G-protein coupled receptors will be discussed.

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32.3 ARTHROPOD SENSORY SYNAPSES: STRUCTURE, DISTRIBUTION, AND NUMERICAL REGULATION

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Many arthropod synaptic contacts resemble triad and dyad synapses in the vertebrate retina. Assembly at these so-called multiple-contact synapses is specialised to incorporate >1 postsynaptic element opposite each presynaptic active zone, thereby constituting richly interconnected divergent and convergent networks. In the lamina neuropil behind the fly's compound eye, photoreceptor terminal input synapses -- counterparts of vertebrate retinal triads, have four postsynaptic elements, and are thus tetrads. Each is of relatively fixed size, with a fine structure, including a synaptic ribbon, comparable with that of retinal synapses. The number and combination of postsynaptic dendrites within each tetrad is highly determinate, and contact number is readily quantifiable. Tetrads are distributed non-randomly over the surface of the receptor terminal, tending to even-dispersion, with the placement of each site respecting a minimum spacing from its neighbours. This is remarkable given that: a) tetrads form during ontogeny by stochastic excursions of dendrites establishing chance contacts with target sites over the receptor terminal; and b) that the same cells in the adult show dynamic changes if they are appropriately perturbed. For example, functional reversals, such as when dark-adapted flies are exposed to a light pulse of as little as 2 min duration, provoke a burst of rapid synaptogenesis. This is associated with the appearance of new, small tetrads having a closer average spacing to their neighbours than larger ones. The extent that tetrad formation is activity-dependent is hard to evaluate by dark-rearing, because the receptor transmitter, histamine, is released in the dark. In the steady state, the rate of vesicle shedding (from depletion through non-replenishment under endocytotic blockade in the temperature-sensitive mutant *shibire*) is 2.6 quanta tetrad^{-1} sec^{-1}. At least some tetrad sites form however in mutant flies lacking either the synthetic enzyme for histamine, histidine decarboxylase, or normal light-evoked transmitter release.

Supported by NIH grant EY03592 and by the Killam Trust.

32.4 INSECT CHANNELS AND VERTEBRATE NEUROPHYSIOLOGY

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Insects present a diverse and rich source of material for biomedical research. *Drosophila, Periplaneta, Locusta* etc. are mostly used in neurophysiological studies. The presence of giant axons in *Periplaneta americana* allowed us to demonstrate, for the first time, that 4-aminopyridine (4-AP) is a specific blocker of K^+ channels; later use of 4-AP contributed to the demonstration of K^+ channel diversity. A synthetic form of saxitoxin, firstly tested on the cockroach axon, has provided the first chemically synthesized probe suitable for investigating the molecular pharmacology of Na^+ channels in cell membranes. Using *Drosophila* flight muscle, the biophysical properties of different K^+ currents have been established and using so common mutant flies, genetic characterization of channels made great strides. The development of patch-clamp got feasible experimentation on very small vertebrate cultured cells or brain slices. Today, molecular biology is directly applied to vertebrate proteins. However, identified neurons and circuits of insects, participate to our knowledge. For example, on short-term cultured cockroach Dorsal Unpaired Median (DUM) neurons, an increase of the Na^+ current revealed that a neosynthesis of Na^+ channel proteins is induced by axotomy and deafferentation. Because of an unexpected similarity in electrophysiological properties between octopaminergic DUM neurons and vertebrate dopaminergic neurons (DN) of *substantia nigra compacta*, this insect model is promizing to further understand the complex cellular physiology of DN and their pathology. Last but not least, considering insect and vertebrate whole populations, extended use of neurotoxic insecticides and the subsequent development of resistances, will inform us on short as well as long term consequences of the presence of such agents on man and his environment.

Session 33 - Parallel Symposium: Extracellular matrix molecules

33.1 FUNCTIONAL ROLES OF NEURAL CELL ADHESION MOLECULE IN MEMORY CONSOLIDATION

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There is now considerable evidence for neural cell adhesion molecule (NCAM) involvement in the temporal cascade of events that leads to synapse connectivity change in memory consolidation. In the 3-4h period following passive avoidance or water maze learning, we have found cell-cell interactions to be weakened by NCAM internalisation and degraded in a ubiquitin-dependent manner. At the 6-8h post-training time, NCAM re-expression becomes necessary for the transient increase in synapse formation observed in the mid-molecular layer of the hippocampal dentate gyrus. The elimination of these transient synapses is initiated in the 12h post-training period and coincides with an increased frequency of dentate infragranular neurons expressing NCAM bearing α2,8 linked polysialic acid (PSA) homopolymers. This is mediated by activity-dependent inhibition of PKCδ, a suppresser of polysialyltransferase activity. As the dendrites of these polysialylated infragranular neurons extend into the mid-molecular layer and their frequency declines with age, NCAM PSA may influence synapse selection and/or elimination in the elaboration of adult learning repertoires. For example, maintaining animals in a complex environment, or treating them chronically with tacrine or piracetam-related nootropics, attenuates decline of polysialylated neuron frequency and results in a more robust acquisition and consolidation of learning paradigms. Thus, the functional role of NCAM in memory consolidation would appear to be the physical regulation of intrinsic synapse strength and, by a polysialylation event in late development, an extrinsic influence on those to be retained.
Supported by Enterprise Ireland, the Health Research Board of Ireland, and EU Biotech and FAIR Programme Grants.

33.2 THE ROLE OF CELL ADHESION MOLECULES IN MEMORY FORMATION

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Cell adhesion is believed to be critical for the formation of neuronal connections during development and the long-lasting synaptic remodelling that occurs during learning and memory formation. In recent years a consensus has emerged that one of the most significant steps in achieving this remodelling is the synthesis, in the hours following a learning experience, of a family of glycoproteins which become incorporated into synaptic plasma membranes. The glycoprotein family includes a number of cell adhesion molecules (CAMs) whose extracellular domains can bind homophilically and/or heterophilically, thus holding the synaptic junction in a transmissional configuration.

The specificity of cell-cell adhesion must result from the integration of a number of different adhesion systems. Over the past 10 years there has been a remarkable convergence of evidence pointing to a key role for cell adhesion molecules such as NCAM and L1 in this sequence Amongst the CAM family is the amyloid precursor protein (βAPP). The high degree of evolutionary conservation of the extracellular and cytoplasmic domains of βAPP, its abundance in neurons and glia, its tightly regulated differential expression during development and ageing at both tissue and cellular levels, as well as its role in Alzheimer's disease suggest important functions of βAPP in brain tissue. The role of βAPP, as well as NCAM and L1, proteins that belongs to the family of cell adhesion molecules associated with specialised cell junctions will be discussed in the light of their role in the biochemical cascade leading to memory consolidation.

33.3 KAINATE-EVOKED CHANGES IN DYSTROPHIN mRNA LEVELS IN THE RAT HIPPOCAMPUS

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Dystrophin and dystroglycan are expressed in specific brain areas including the cortex and the hippocampus, and in neurons dystrophin has been localized to postsynaptic densities. However, the role of the dystrophin complex in neurons remains unknown. We have examined the effect of neuronal activation and neurotoxicity induced by kainate and pentylenetetrazole administration on dystrophin and dystroglycan expression in the rat brain. Kainate injection resulted in a transient but dramatic decrease in dystrophin transcript levels in the dentate gyrus granule cells, neurons not affected by kainate neurotoxicity, six hours after injection. There was also a strong, concomitant increase in dystrophin mRNA levels in the CA3 subfield. At 24-72 hours after kainate injection, the dystrophin transcript in the dentate granule cells returned to control levels while it decreased in the CA subfields, coinciding with the neurodegeneration in these areas. Comparable results were obtained with pan-dystrophin probes and probes specific to the G-dystrophin (Dp71) that predominates in the dentate gyrus. In contrast, kainate insult had no effect on the dystroglycan mRNA levels in the dentate gyrus. For comparison, seizures which are not associated with progressive neurodegeneration were induced by pentylenetetrazole: in this case the dystrophin and dystroglycan mRNA levels remained unchanged in all areas of the hippocampal formation. Since activation of glutamate receptors is thought to be involved in some forms of synaptic plasticity in the adult hippocampus, our data indicate that the dystrophin gene behaves as a candidate plasticity-related gene responding to glutamate.

33.4 MATRIX METALLOPROTEINASES AND THEIR TISSUE INHIBITOR IN BRAIN DEVELOPMENT AND PLASTICITY

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Matrix metalloproteinases (MMPs) are known to be responsible for tightly regulated degradation of extracellular matrix proteins. It is well documented that MMPs activity is critical for remodeling of peripheral tissues via controlling the cell shape, migration, adhesion, differentiation and signaling. However, their physiological role in the brain development and functioning remains unknown. As a prerequisite to functional studies we analyzed expression and activity of two MMP family members: MMP-2, MMP-9 and their natural inhibitor TIMP-1 (tissue inhibitor of matrix metalloproteinases) during selected developmental events as well as after stimulation of adult brain. The fate of MMPs and TIMP was followed at the level of mRNA (by in situ hybridization), protein (immunochemistry), enzymatic activity (gel zymography with gelatin as a substrate) as well as transcriptional activation in *timp-1-LacZ* transgenic mice. We found that within brain tissue both MMPs forms can be either soluble or ECM bound. Immunocytochemistry with anti-MMP-2 and -9 antibodies revealed localization of the proteins near cell bodies of granule and pyramidal neurons as well in glial cells. In situ hybridization with cDNA probes showed *mmp-9* and *timp-1* mRNAs in pyramidal and granule cells layers of the hippocampus suggesting neuronal localization of the messages. Glutamate receptors agonist kainate induced expression of *mmp-9* as well as *timp-1* within hours, indicating that the genes response was driven by neuronal activation. The increase in *mmp-9* mRNA and protein levels were followed by the enzyme activation at later time points (24 hours after stimulation). MMP-2 activation was observed from 3rd day on after kainate treatment at the time of massive tissue remodeling, suggesting that this enzyme may fulfill other then MMP-9 functions. In an aim to decipher whether the TIMP-1 expression is driven by physiological, sensory input we monitored *timp-1-LacZ* activity as well as *timp-1* mRNA in dark reared mice exposed to light as well as after monocular deprivation during the critical period of visual cortex formation. The contralateral (driven by the open eye) but not ipsilateral hemisphere clearly showed elevated *timp-1-lacZ* gene expression throughout the visual cortex. During critical period of visual cortex formation appropriate neuronal connections under the control of visual stimulation are established. This process involves synapses formation and elimination. MMP-9 but not MMP-2 activity and TIMP-1 transcription were enhanced starting from the time of eyes opening that trigger maturation of the cortex. In conclusion, our results suggests that driven by synaptic activity MMPs/TIMP system can be involved in neuronal plasticity during development and adulthood and points out possibility of important role of extracellular matrix in such phenomena.

Session 34 - Parallel Symposium: Molecular aspects of development and pathology of immature CNS

34.1 NEURONAL CEROID LIPOFUSCINOSIS, COMMON POLYGENETIC DISEASES

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The neuronal ceroid lipofuscinoses (NCLs) are a group of progressive, hereditary, neurodegenerative disorders that result in blindness, seizures, and progressive motor and cognitive deterioration. Classification of the NCLs into three major forms - infantile (INCL/CLN1), classical late-infantile (LINCL/CLN2), and juvenile (JNCL/CLN3) - was based in the past on age-at-onset, clinical symptomatology, and characteristics of storage material on electron microscopy (granular osmiophilic deposits, or GRODs; or curvilinear or fingerprint profiles, respectively). Presently, these three major forms of NCLs are known to be caused by mutations in the genes coding the following proteins: palmitoyl protein thioesterase (CLN1), pepstatin-insensitive carboxypeptidase (CLN2), and CLN3 protein (CLN3), respectively. Genetic defects underlying two other late-infantile variants of NCL (CLN5, CLN6), and an adult form of NCL (CLN4) remain to be further characterized. At present, 19 different mutations have been found in the CLN1 gene, 21 in the CLN2 gene, and 23 in the CLN3 gene. Some of these mutations may cause a different phenotype than that observed in the classical or typical form. Thus, recent progress in the molecular biology of NCLs prompted reevaluation of the clinicopathological diagnostic criteria of NCLs.

EXPRESSION OF LEPTIN RECEPTORS IN DEVELOPMENT AND PATHOLOGY OF IMMATURE BRAIN 34.2

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Leptin is considered to be a hormone implicated in the maintenance of energy balance as a satiety factor. In human, leptin is a polypeptyde of 167 amino acids, encoded by OB gene, which is located on chromosome 7q 31. Recently, it has been shown that leptin is produced by adipose tissue, gastric epithelium and placenta. After synthesis, leptin is secreted in a pulsatile fashion into the blood stream. The metabolic effects of leptin are achieved by its interaction with specific leptin receptors. These receptors belong to the class I cytokine receptor family. Multiple splice variants of leptin receptors are produced from the same OB-R gene. Leptin crosses the blood-brain barrier via a saturable transport system. Although in the CNS, the hypothalamus is a critical target for the satiety effect of leptin, leptin receptors are present in other brain regions as well. During human brain development, increasing expression of leptin receptor protein appears initially on choroid plexus and ependyma, later, on migrating neurons. In brains with congenital malformations, strong expression of leptin receptors was observed on neurons located in heterotopias.
Presence of leptin receptors in CNS as well as in numerous peripheral tissues suggests that the role of leptin is more complicated then being a circulating satiety factor.

34.3 THE DOPAMINE D_3 RECEPTOR : DEVELOPMENTAL AND PSYCHIATRIC IMPLICATIONS

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The dopamine D_3 receptor is one of the D_2-like receptors, its peculiar properties being related to its restricted expression in the ventral striatum of the adult brain and its regulatory mechanisms. Prenatally the expression pattern of the D_3 receptor mRNA within the rat brain also differs from that of other dopamine receptor subtypes. Starting from E14 and until birth, it is exclusively expressed in neuroepithelia, namely within the proliferative ventricular zones of the forebrain, whereas D_1 and D_2 receptors start to be expressed in migrating neuroblastes and differentiating striatal neurons well before birth. Appearance of the D_3 receptor mRNA in differentiating neurons of the ventral striatum becomes detectable during the first postnatal week, i.e. at a time when these neurons starts to be innervated by dopaminergic afferents. This pattern could reflect the role of an anterograde neurotrophic factor released by dopaminergic neurons in the regulation of D_3 receptor expression as shown in a different situation, i.e. 6-hydroxydopamine-induced lesions (Levesque et al., Proc. Natl. Acad. Sci. USA, 1992, 89: 8155). The same mechanism might be involved in the levodopa-induced overexpression of the D_3 receptor in hemiparkinsonian rats, a process accounting for the behavioral sensitization developing to this drug (Bordet et al., Proc. Natl. Acad. Sci. USA, 1997, 94: 3363). It could also account for D_3 receptor overexpression in the ventral striatum of schizophrenic patients or subjects died after a cocaine overdose. These observations raise hypothesis about the role of the D_3 receptor in schizophrenia and drug abuse and its involvement in therapies of these psychiatric diseases.

HISTAMINERGIC MECHANISMS IN BRAIN DEVELOPMENT, INJURY AND REPAIR 34.4

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We have used experimental approaches to evaluate the developmental significance of histamine in the brain. In zebrafish brain, fluorescence confocal imaging revealed two types of histamine-containing neurons. The rostral large ones sent prominent projections to rostral directions, whereas the small caudal neurons sent also caudal projections. These cells were the only ones in the zebrafish brain which displayed HDC mRNA. They made contacts with other monoaminergic neurons, which suggest that they may regulate other transmitters. To test if histaminergic neurons are activated in excitotoxic damage, rats were treated with a single dose of kainic acid s.c. H1 receptor expression increased significantly in the dentate gyrus, olfactory tubercle and striatum, reaching a peak between 3 and 24 hours. In the piriform cortex, CA3 field of the hippocampus and amygdala, H1 receptor expression decreased significantly within 12 hours, reaching a minimum after 2 days. This decline in H1 receptor expression was followed by a significant increase in density of histaminergic nerve fibers and histamine content. Within 6 months, all changes returned to control level. The results suggest that the histaminergic system in limbic seizures undergoes transient plastic changes, which may include receptor up- and downregulation in a region-specific manner and growth of histaminergic fibers into damaged areas.

Session 35 - Parallel Symposium: Mechanisms of the axon outgrowth regulation in the spinal cord

35.1 PERIPHERAL NERVE GRAFTS INFLUENCE SURVIVAL OF INJURED SPINAL MOTONEURONES

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In neonatal rats motoneurones die rapidly when their axons are damaged. The possibility that unlike in adults in the neonatal rat the distal stump of the cut nerve is unable to provide a conduit for the axons to reach their target was tested. Motoneurones to tibialis anterior and extensor digitorum longus muscles of newborn rats were prelabelled with DiY. Three days later the sciatic nerve was cut. In one group of 5 rats the proximal end of the cut nerve was connected to a segment of a nerve taken from a 4 week old animal and in another group to a segment of a nerve taken from a 3 day old rat. The number of surviving motoneurones was then assessed 1 week later. In animals that received a neonatal graft only 27±3.3% of motoneurones survived, while in those that received an adult graft almost all motoneurones survived. Thus the adult graft prevented motoneurone death. The molecular basis of the protective effect of the adult graft may be due to Schwann cells in cut adult but not neonatal nerves expressing ß1 integrins. Accordingly we modified the ß1 integrin activity in the adult graft by treating it with the cyclic RGD peptide FR1. Following this treatment the adult graft lost its ability to protect motoneurones from dying. In contrast when the neonatal nerve graft was induced to upregulate ß integrin activity by treatment with phorbol myristrate acetate, it acquired the ability to rescue a large proportion of motoneurones destined to die. Thus the expression of integrins in the peripheral nerve stump into which axons extend their growth cones seems to influence the survival of the cell body.

35.2 NGF OVER-EXPRESSION IN THE SPINAL WHITE MATTER OF TRANSGENIC MICE LEADS TO ECTOPIC SENSORY FIBRE INNERVATION AND HYPERALGESIA

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A transgenic mouse has been developed which over-expressed nerve growth factor (NGF) during postnatal development under the control of a myelin basic protein promoter. The over-expression occurred in myelinating oligodendrocytes located in the white matter of the CNS, from the date of birth to the age of 2 months. These animals displayed ectopic networks of substance P (SP) containing sensory fibres in the white matter in several locations in the CNS, including the white matter of the spinal cord, from postnatal day 5 onwards. The ectopic SP-immunoreactive (IR) fibres occurred in bundles and persisted indefinitely after the age of 4 months, although NGF levels had returned to normal. At the ultrastructural level, the ectopic SP-IR fibres established numerous synapses in the white matter of the spinal cord, preferentially on dendrites that expressed the SP receptor. Interestingly, no differences from control were detected in the SP innervation of the superficial dorsal horn. In behavioural studies, the transgenic mice displayed hyperalgesia, which was reversed by SP receptor and NMDA receptor antagonists. These results indicate that the ectopic fibres were functional and, despite their ectopic location, still preferentially innervated neurons expressing the SP receptor.

(Funded by the Canadian MRC)

35.3 NEUROTROPHINS COMBINED WITH PHYSICOTHERAPY: A NEW APPROACH TO TREAT SPINAL CORD INJURY

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There is little axonal growth following central nervous system injury in adult mammals. Partial recovery after incomplete spinal cord injury has been achieved in adults after application of peripheral nerve grafts or treatment with antibodies (IN-1) to neutralise the myelin-associated neurite outgrowth inhibiting proteins. Both interventions, aimed to supply axons with permissive environment, resulted in regeneration and compensatory anatomical plasticity accompanied by functional improvement. Although the axonal growth achieved with these methods seemed to be quite limited, combining these treatments with neurotrophic factors (that support the viability and function of neurones) significantly enhanced the promotion of axonal growth. Physical exercise was also shown to cause marked functional recovery following spinal cord injury but its mechanism remains unclear. Enhancement of neuronal activity due to exercise, and tight relationship between neurotransmitter release and neurotrophic activity, might be responsible for beneficial effects of physicotherapy. Experiments showing an elevation of neurotrophin gene expression in the nervous system by the exercise are in line with such interpretation. Physicotherapy may lead to an increase of: (1) the availability of local trophic support to damaged neurones and (2) neuronal responsiveness to trophic factors by upregulation of their receptors. Based on these assumptions we propose to treat spinal cord injury combining exogenous trophic factors with physical training. As a first step, experiments designed to test the possibility whether extensive training leads to an activity-dependent regulation of endogenous neurotrophin levels and their high affinity Trk receptors within main descending systems of the spinal cord will be described.

This work was supported by State Committee for Scientific Research: 1305/P05/98.

35.4 THE USE OF BEHAVIORAL METHODS TO PREDICT THE TRANSPLANT-INDUCED SPINAL CORD PLASTICITY

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The functional recovery following central nervous system damage is known to be dependent on various mechanisms of neuronal plasticity. Recently, great progress has been made in identifying trophic agents and molecular biological and biochemical approaches, which are known to have potential for facilitating neuronal regeneration. However, still no sufficient progress in analyzing functional recovery itself has been made. In our laboratory, the mechanisms underlying the recovery of motor function are widely investigated. In this study, our attention was focused on investigation of various behavioral methods which can be used to assess the level of recovery of motor function after spinal cord injury. Transplantation of embryonic neural tissue of the raphe nucleus region into the spinal cord below the place of total transection, was used as a method to increase the amount of motor function recovery occurring after total spinal cord transection in rats. The results of several behavioral tests (e.g. postural reflexes, overground and treadmill-induced locomotion, air-stepping or paw-shaking responses) demonstrated that the rats with total spinal cord transection which had received the graft of embryonic tissue, are more responsive to various stimuli (e.g. tactile or proprioceptive) in comparison to control animals with total spinal cord transection only. This was particularly obtained in improved locomotor patterns as well as long lasting episodes of spontaneous air-stepping. The improvement of motor function has been confirmed by an alteration in the timing, duration and variability of EMG activity of hindlimb muscles. Our study demonstrated that the behavioral methods which confirmed the greater recovery of motor function in adult rats after complete spinal cord transection, which had received a transplant of embryonic tissue, can be successfully used to evaluate the effectiveness of various processes of neuronal plasticity which are responsible for recovery of motor function. (Supported by KBN grant 4.P05A.085.14, Poland)

Session 36 - Oral communications - part 2

36.1 EARLY DEPRIVATION OF ORAL FOOD STIMULATION IMPAIRS FOOD-REWARDED CONDITIONING IN KITTENS

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Previous studies have shown that cats submitted to early visual deprivation are not impaired in acquisition a simple food-reward instrumental response to a visual cue and only slightly impaired in its acute extinction (1). In the present study the same conditioning training was given to cats submitted to early deprivation of food taste stimulation and thus of food-reward.

The kittens to be deprived were fed by stomach tube and the control kittens via a bottle during the first 75 days of their life. The deprived kittens approached the food reward in the training apparatus less willingly than the control kittens, and were dramatically impaired in both, acquisition and extinction tests.

We conclude that the value of food reward as well as the importance of its absence are severely lowered in cats deprived of food taste. A comparison with the previous data shows that early food taste deprivation appears to be more harmful for visual-food conditioning than is visual deprivation.

Supported from the State Committee for Scientific Research 0397/PO4/99/16.

1. Żernicki, B. 1991. Brain Res. Rev. 16: 1-3.

36.2 COMPARISON OF THE CONFLICT INHERENT IN THE ESCAPE AND AVOIDANCE TWO-WAY SHUTTLE BOX PROCEDURES IN RATS

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Two groups of rats, 15 subject each, were trained for 5 sessions in either signaled avoidance or unsignaled escape. In each procedure the shock trials were terminated either by short latency direct escape or long-latency staying response involving freezing, running or jumping on the charged grid. Avoidance responses performed during the 1st session determined perfect retention of direct escape responses and precluded reappearance of the conflict evidenced by staying responses. In good avoidance learners the predictive stimulus (CS) was a signal for motor action consisting of postural preparatory response and subsequent avoidance response, whereas in poor avoidance learners the CS acted as a signal of subsequent shock. Good escape learners acquired a complex instrumental response consisting of postural preparatory response toward the end of the intertrial interval and subsequent direct escape response. Poor escape learners showed deficient retention of preparatory responses and a rise of staying responses at the beginning of next sessions. Preparatory responses resulted in decrease of escape latency in each subgroup of rats. Well established active avoidance, similarly as escape responses performed with short latencies are anticipatory actions. Good learners in both procedures employed an active coping strategy in response to stress inherent to the two-way shuttle box situation, whereas a passive coping strategy has been observed in bad learners.

36.3 PHOTIC EFFECTS ON 5-METHOXYTRYPTOPHOL AND MELATONIN RHYTHMS IN CHICK PINEAL GLAND

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Pineal glands of vertebrates synthesize a number of 5-methoxyindoles, including melatonin (MEL) and 5-methoxytryptophol (5-ML). MEL is produced by the pineal gland in a daily or circadian rhythm (with high levels during the night) generated by an endogenous clock and synchronized by environmental light. In contrast to MEL, little is known about regulation of 5-ML synthesis. The aim of this work was to analyze the effects of light on 5-ML content in the chick pineal gland and to compare them with the light action on the pineal MEL level. In the pineal glands of chicks 5-ML and MEL concentrations fluctuate in a rhythmic manner. These rhythms are circadian in nature and have opposite phases. Acute exposure of chicks to white light at night increased the pineal level of 5-ML and decreased the tissue content of MEL. A 6-hr pulse of light applied early in the subjective night caused a delay in the phase of the circadian rhythms of both 5-ML and MEL compared to untreated controls. When the 6-hr pulse of light was given during the late subjective night it produced a phase advance of the circadian rhythms of 5-ML and MEL. The phase advancing effects of light on the circadian rhythms of 5-ML and MEL in the chick pineal gland were more pronounced than the phase delaying effects. This is the first evidence that light is capable of phase shifting the 5-ML rhythm in a manner similar to its action on the MEL rhythm.

Supported by the KBN grant No. 4 PO5A 074 12.

36.4 NEUROPROTECTIVE ROLE OF L-CARNITINE IN A MODEL OF MITOCHONDRIAL DYSFUNCTION

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A mitochondrial toxin, 3-nitropropionic acid (3-NPA), induces acute encephalopathy and late onset dystonia. The mechanism of 3-NPA-induced neurotoxicity has been shown to involve excitotoxicity and oxidative stress. L-carnitine may prevent mitochondrial dysfunction via enhancement of energy metabolism in neurons and glial cells. In the present study, the response of brain antioxidant enzymes to 3-NPA exposure with and without L-carnitine was investigated. Male rats were injected s.c. with 3-NPA alone (30 mg/kg) or 3-NPA and either a low (50 mg/kg) or high (100 mg/kg) dose of L-carnitine 60 min prior to 3-NPA. Control rats received either 0.1 M phosphate buffer or L-carnitine. Rats were sacrificed 90 min after 3-NPA treatment. Catalase, and superoxide dismutase (Mn-SOD and CuZn-SOD) activities significantly increased in hippocampus, caudate nucleus, and frontal cortex after treatment with 3-NPA alone. Pretreatment with L-carnitine abolished these changes. These data suggest that L-carnitine pretreatment attenuates 3-NPA-induced oxidative stress.

36.5 NALTREXONE AND CAPTOPRIL DO NOT MODIFY HIGH PREFERENCE FOR ALCOHOL EXPRESSED BY PORTOCAVAL SHUNTED RATS

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Rats with chronic liver failure caused by portocaval anastomosis (PCA) exhibit a higher preference for alcohol than sham operated pairs. The underlying mechanisms are unknown. The contribution of opioid and renin/angiotensin system was tested as non-specific opiate receptor antagonists as well as angiotensin converting enzyme inhibitors are reported to reduce voluntary alcohol intake in several mammals.

An end-to-side portocaval anstomosis was performed on male Wistar rats under ether anaesthesia (Lee & Fisher,1961). Controls were sham operated. Rats were used 8-12 mo following surgery. Before therapy they underwent 3 days control "free choice" test. During the control test and throughout therapy rats were kept in metabolic cages and had free access to fluids (H_2O and 10% ethyl alcohol) and food. Drugs, Naltrexone 10mg/kg or Captopril 20 mg/kg were given s.c., at the beginning of dark phase of 24 h cycle for 5 consecutive days. As confirmed by control test PCA rats consumed twice as much fluid and feed as SHAM rats per 24 h. Alcohol accounted for 66% of total fluid intake in PCA rats but only for 23% in Sham ones. Urine output was also greater for PCA rats. In both groups, Naltrexone therapy reduced total fluid consumption and urine excretion but had no effect on alcohol intake. Reduced H_2O consumption accounted for decreased total fluid intake. Captopril treatment did not affect any of the measured parameters. The results imply that the opioid or renin/angiotensin system are not involved in abnormal alcohol preference evoked by PCA.

36.6 EVALUATION OF CHRONIC BILATERAL CCAO AND TRANSIENT MCAO IN RATS AS POTENTIAL MODELS FOR STUDYING NEUROPROTECTIVE COMPOUNDS.

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It has been demonstrated that chronic mild hypoperfusion induced by a permanent occlusion of bilateral common carotid arteries (CCAo) in rats caused progressive and/or long-lasting cognition deficits resembling these in human dementia. We traced the effects of CCAo in SD rats for 16 months. The animals were periodically tested in 8-arm radial maze to assess their spatial working (WM) and reference memory (RM). Our results showed that ischemic rats performed the test significantly worse ($p<0.05$ versus control) over the whole experimental period but the initially observed deficits seemed to be small. Further statistical analysis revealed worsening of the performance in CCA group 16 months post insult. We conclude that bilateral CCAo might be a good model of progressive changes that may occur in humans as a result of chronic vascular disease and ageing. As a second model we chose the transient middle cerebral artery occlusion (MCAo). Two different classes of compounds were tested: an antioxidant α-phenyl-N-tert-butyl nitrone (PBN) reported to be protective against ischemic brain damage and a novel non-competitive NMDA receptor channel blocker MRZ 2/579. Transient focal ischemia of various duration times (30 min., 75 min., 2 h) was produced in SD rats as described by Zea Longa et al. (1989). Studied compounds were applicated as i.p. injections or intravenous infusion. Histological evaluation of brains 3-7 days post MCAo and simple behavioural testing revealed that in this model significant neuronal damage and motor deficits are easily detectable. In our experimental paradigms PBN failed to present significant beneficial effects but MRZ 2/579 offered protection of cortical areas post 75 min. MCAo when administered as a continuous 6 h i.v. infusion.

36.7 CALCIUM-INDUCED LONG-LASTING POTENTIATION OF SYNAPTIC RESPONSES IN MOTOR CORTEX

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The induction of activity-dependent modifications in synaptic efficacy depends usually on the rise of Ca^{2+} level in the cytoplasm of activated neurons. In the present study we investigated whether a transient elevation of extracellular Ca^{2+} concentration results in the induction of long-term synaptic changes in the motor cortex.

Field potentials evoked in horizontal pathways, contained within layer II/III and layer V of the motor cortex, were recorded from adult rat brain slices *in vitro*. After establishing a stable baseline in standard artificial cerebrospinal fluid (ACSF; 2 mM $[Ca^{2+}]_o$), slices were exposed for 10 minutes to a modified ACSF containing 5 mM Ca^{2+}. This procedure typically resulted in a long-lasting increase of responses evoked both in layer V and in layer II/III. In contrast to high-frequency stimulation-induced LTP, the effect of elevated $[Ca^{2+}]_o$ developed gradually over approx. 30 minutes and the amplitude of layer V response increased by 38±10% while that of layer II/III response increased by 20±10%. It is concluded that both superficial and deep layers of the motor cortex demonstrate a significant potential for calcium-induced synaptic modification.

Support: HHMI-0045; BW/IZ/24/98

36.8 DEMONSTRATION OF FUNCTIONAL DIFFERENCES BETWEEN MODERATE AND HIGH AFFINITY NMDA RECEPTOR CHANNEL BLOCKERS IN HIPPOCAMPAL SLICES.

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It has been suggested that moderate affinity NMDA receptor channel blockers better differentiate between pathology and physiology in CNS. To address these questions we investigated effects of memantine and (+)MK-801 (moderate and high affinity channel blockers respectively) on the induction of LTP and hypoxia/ hypoglycaemia-induced damage in CA1 of hippocampal slices *in vitro*. Memantine and (+)MK-801 blocked the induction of LTP, and both compounds were able to block hypoxia/hypoglycaemia-induced depression of EPSP amplitude. Obtained relative therapeutic indices (TI) for memantine and (+)MK-801- 0.8 and 0.24 respectively show that moderate affinity channel blocker exhibits a better therapeutic profile than its tested counterpart. In further experiments we compared the ability of memantine and (+)MK-801 to counteract deficits in the induction of LTP following reduction of Mg^{2+} in hippocampal slices - model of increased „synaptic noise". Decreasing Mg^{2+} from 1 mM to 10 μM for 60 min. enhanced baseline fEPSP slopes (87.2 ± 10.6 % above control) and abolished LTP. Long pre-incubations with memantine (1 μM) - concentration found relevant in clinic - restored the induction of LTP without changing the enhancement of baseline fEPSP slopes. Memantine (10 μM) fully restored the induction of LTP and also decreased the enhancement of baseline. In contrast, although (+)MK-801 (0.01, 0.1 and 1 μM) caused a concentration-dependent reduction in the low Mg^{2+}-induced enhancement of baseline fEPSP slopes, none of tested concentrations was able to restore LTP. In conclusion, clinically-relevant doses of memantine could produce symptomatological improvements whereas (+)MK-801 is likely to have negative effects only.

Session 37 - Plenary Lectures

37.1 ROLE OF ASTROCYTES IN GLUTAMATE HOMEOSTASIS DURING PHYSIOLOGICAL AND PATHOPHYSIOLOGICAL CONDITIONS

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Glutamate has two important roles in the brain as metabolite and excitatory neurotransmitter. During adverse conditins it can, however, act as a potent neurotoxin and aberrations in the physiologic processes governing glutamate homeostasis will lead to widespread neuronal degeneration. A physiologic extracellular glutamate concentration is maintained by fine tuning of its release, uptake and metabolism. Astroglial cells have been shown to play an extremely important role in this scenario acting as the main site for uptake and metabolism of glutamate. The enzymatic machinery required for this function appears to be highly regulated in astrocytes and the actities of transporters and enzymes are dependent upon environmental cues including neuronal signalling. These aspects (1,2,3) will be discussed in detail.

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37.2 NEUROPSYCHOLOGY OF LANGUAGE IN NORM AND PATHOLOGY

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One of the fundamental attributes of our environment is its pattering in time. Precise timing is essential for many aspects of human activities, also for language functions. Experimental studies on temporal constraints of cognition have provided key insights into the neuropsychological basis of speech perception and production. In a series of experiments, using different paradigms, we assessed time perception in two temporal ranges: around 30 ms (a high frequency level) and around 3 s (a low frequency level). The former seems to be related to duration of single units of language – phonemes, the latter to phrases i.e., strings of words in the fluent speech. We studied patients with acquired anterior or posterior lesions in the right or left hemisphere (with or without aphasia syndromes) and healthy adults or children aged from 6 to 14 years. In clinical studies, a prolongation of temporal perception of order (30-ms level) was associated with left posterior lesions and Wernicke's aphasia, whereas information processing deficits on the low frequency level were rather related to anterior lesions either bilateral or left hemispheric (Brocka's aphasia), depending on the applied experimental paradigm. On the other hand, in developmental studies on children without neurological deficits we found significant alterations on the 3-s range, which were associated with perceptual learning and cognitive development. It is postulated that the functional reorganisation of the prefrontal cortex is responsible for this developmental effect. The dynamic changes on the low frequency level of information processing during ontogenesis seem to build up a temporal platform for human behaviour.

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