METABOLIC PUMPING IN INSECT MUSCLE AND RESTING MEMBRANE ELECTROGENESIS

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A study of the literature concerning the resting potential (RP) of insect muscle reveals a number of apparent departures from the situation found in other excitable cells. The insects, adult Colorado beetle and mealworm, and mealworm larva, chosen to this study differ much in their haemolymph ionic content (e.g. Colorado beetle's haemolymph contains only 2 $mmol \cdot l^{-1}$ of Na^+) and so they have different ionic requirements for the resting membrane potential (RMP) maintenance and the contribution of pumps to it. The present study examines the time dependence of the RP in muscles of studied insects and tries to determine the role of metabolic pumping in the genesis of this potential using of sodium pump inhibitor - ouabain and, in the case of Colorado beetle, K-H pump inhibitor - SCH 28080. Experiments were performed in situ on indirect flight muscles of adult Colorado beetle and mealworm, and on ventral longitudinal muscles of mealworm larva by "soaking" the muscles in standard (respective for each of insects studied) or modified salines for 2h, and impaling several cells in each preparation. The conventional microelectrode technique was used. The control mean values of muscle RP in adult Colorado beetle and mealworm, and mealworm larva, measured just after the equilibration time, were, respectively, 75.0, 50.6 and 38.3 mV, and only slightly decreased with time. In Colorado beetle's muscles, application of 1 $mmol \cdot l^{-1}$ ouabain caused a membrane hyperpolarization of around 6 mV after 90 and 120 min. Application of 1 $mmol \cdot l^{-1}$ SCH 28080 resulted in a slight decrease of the RP value. In the muscles of adult mealworm, ouabain caused a slight, gradual hyperpolarization of the RP (by 2.8 and 4.5 mV after 1 and 2h, respectively). Essentially identical results have been obtained on ventral muscles of mealworm larva. Thus, it seems that the skeletal muscles of studied insects do not contain an ouabain-sensitive metabolic mechanism, the functioning of which is necessary for long-term maintenance of the RMP. In insects, like Colorado beetle, with unconventional ion levels in their haemolymphs, at least the Na^+ gradient may be in the reverse direction, and the outward K^+ gradient much reduced. The operation of the classical Na^+-K^+ pump would not be suitable for these conditions, and, therefore, such pumping mechanism may not be present in these insects. If an electrogenic pump is present in muscle fibers of studied insects, its properties differ appreciably from those described for the electrogenic sodium pump which appears in many nerves and muscle cells.

Comparison of electrical activity and ionic currents in an isolated axon and in an isolated neuron.

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The comparative approach to investigation of the axonal and neuronal mechanisms underlying rythmogenesis in invertebrates has lead to an important conclusion that rythm generation is the result of complex interactions between cellular, synaptic and network properties. The aim of this study is to compare the membrane properties of insect giant axon, which propagates action potentials and insect neurosecretory cells called dorsal unpaired median (DUM) neurons involved in the modulation of skeletal and/or visceral muscles via the release of octopamine. Giant axon and DUM neuron are endowed with a specific set of ion currents that shape and define their integrative properties. For a detailed study of the biophysical and pharmacological properties of these currents, application of double oil-gap method and patch-clamp technique to giant axon and DUM neuron respectively, has proven a useful strategy. The axonal membrane properties can be described by a capacitance in parallel with three types of currents: a fast transient sodium current sensitive to tetrodotoxin, a delayed rectifier (DR) potassium current blocked by 4-aminopyridine and a leak current. They allow to generate only short evoked action potentials (0.5 ms in duration) however at high frequency (up to 300Hz). By contrast DUM neurons spontaneously generate electrical activity in the abscence of synaptic input or other external stimuli. DUM neurons can fire repetitive impulses with remarkably regular intervals. The study of ionic mechanisms underlying this spontaneous electrical activity reveals that DUM neuron soma possess voltage-dependent Na channels responsible for the depolarizing phase and background Na channels playing a role in driving the membrane potential to threshold of action potential. In addition DUM neurons also display multiple K^+ currents such as $I_{K_{Na}}$, $I_{K_{Ca}}$, $I_{K_{DR}}$ and I_{K_A} like, involved in the repolarization, afterhyperpolarization and regulation of the firing frequency. Moreover two distinct types of low voltage activated Ca^{2+} currents: a transient and a maintained current have been described. The first one is involved in the initial part of the predepolarization and the second one participates in the last two-third of this predepolarization.

Symposium 4 - Cortical sources of oscillation

DOES IN VITRO THETA-LIKE ACTIVITY REFLECT PHYSIOLOGICAL AND PHARMACOLOGICAL PROPERTIES OF EPILEPTIFORM DISCHARGES ?

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The generation of EEG theta rhythm in the mammalian limbic cortex is a prime example of rhythmic activity that involves central mechanisms of oscillations and synchrony. In 1986 we demonstrated for the first time that bath perfusion of hippocampal slices with the cholinergic agonists resulted in theta-like oscillations. The coincidence in properties of the on vivo and in vitro recorded rhythmic slow activity leads to a general conclusion that the generation of theta in both these preparations share common mechanisms.

However, one more issue should be addressed. The known ability of CCH induced epileptiform activity (when administered in an appropriate concentration) would suggest that theta-like activity also has an epileptiform component. The theoretical implication of this suggestion would be that theta-like activity reflects the physiological and pharmacological properties of epileptiform discharges. The experiments we have been conducting for the last 10 years on slice preparations suggest that the in vitro induced theta-like activity does not reflect the physiological and pharmacological properties of epileptiform activity. Since it was much more in common with the naturally occuring theta then with epilepsy, we have adapted the term "theta-like" activity.

Oscillatory brain dynamics in auditory and visual system

C. Pantev, Muenster

Not received

Transient and Steady State Responses of Somatosensory and Auditory Cortex in Humans

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"Transient responses" are responses evoked by stimuli presented at long interstimulus intervals such that the brain returns to its initial state before the next stimulus occurs. If the stimulus is presented at shorter intervals so that responses to successive stimuli overlap in time, the procedure is referred to as a "steady state procedure" and the response as a "steady state response" (Regan, 1989). We have adopted steady state procedures for imaging sensory representations in experiments on cortical plasticity in humans, because these procedures afford rapid acquisition of data and concentrate signal energy at known repetition rates. Present findings suggest that sensory representations or "maps" derived from steady state procedures differ from maps based on transient methods. The relation of steady state and transient responses is discussed.

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Neuromagnetic Sensorimotor Rhythms

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The human cerebral cortex generates a variety of rhythmic oscillations detectable directly from the cortex or the scalp. The recent development of whole scalp neuromagnetometers has made non-invasive studies of oscillatory brain activity feasible in awake human subjects. In this presentation, results of studies will be surveyed which investigated the human magnetic mu rhythm originating close to the primary somatosensory hand area and consisting of dominant frequency components near 10 Hz and 20 Hz. Sources of the 20 Hz component cluster anterior to those of the 10 Hz component suggesting a dominant contribution of the precentral motor cortex. 10 Hz and 20 Hz rhythms exhibit a characteristic modulation after electric median nerve stimulation, with a suppression immediately after the stimuli and a strong rebound above the prestimulus level within 500 ms afterwards. This rebound is left-hemisphere dominant and differentially affected by various motor tasks and passive tactile stimulation of the hands. Interestingly, the 20 Hz component is also modified when the subject imagines the performance of a motor task, such as exploratory finger movements, indicating that the primary motor cortex is active in motor imagery. In contrast, simultaneously recorded evoked responses in the somatosensory cortex are not affected by motor imagery. Obviously, rhythmic sensorimotor activity and stimulus-locked evoked responses reflect different aspects of signal processing in the sensorimotor cortex.

ATTENTION RELATED OSCILLATORY ACTIVITY WITHIN SENSORY SYSTEMS

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Oscillatory signals of three frequencies (alpha, beta, gamma) are exceptional in the sense that they are the least damped when transmitted through the sensory channels. The functional significance of alpha band is usually discussed in relation to its role in gating the sensory information during sleep/arousal transition mechanisms; whereas the gamma band might provide coactivation frequencies between cells from different brain areas during feature integration processes. The third band at the beta frequencies (15-30 Hz), has been commonly neglected with respect to its possible physiological functions.

We have adopted two different experimental paradigms to study the activity of the sensory systems during active behavior. During the classical conditioning paradigm we have found that the ERPs evoked by vibrissae stimulation in the barrel cortex of the rat increased in amplitude with first reinforcement. This change was due to increased power of alpha, beta and gamma components of Fourier-transformed ERPs, possibly indicating that the novel situation activated the vibrissa-barrel sensory channel. We also observed that local field activity recorded from many sites in lateral geniculate nucleus and primary visual cortex of cats attending to visual stimuli during differentiation task contained an enhanced amount of power within the beta band as compared to activity observed during auditory or erroneously ended visual trials. This activity comprised of short bursts of oscillations. They were found to correlate in time with gamma-frequency bursting.

We propose that attention preactivates the specific functional connections within sensory systems with the use of the beta frequency carrier. In consequence it would provide a necessary background activation to allow binding of the required cell assemblies with gamma synchronized oscillations.

Nonlinear Oscillations in Cortical Structures

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Neural mass activity produces irregular time series such as those measured in EEG and MEG (magnetoencephalography). If we do not view EEG and MEG as a stochastic signal, e.g., as the noise of the brain's engine (or an autoregressive process), but, at least partially, as a measure of lawful and deterministic regulatory processes of neuronal assemblies, we can apply the measures from the theory of nonlinear dynamical systems (chaos theory) to characterize and quantify the brain mechanisms at work. Even if the theoretical premises cannot be fulfilled, we may use the theory of chaos to define and quantify complexity as it appears in a given time series. Here, we show some examples of pure "chaotic" behaviors extracted in the MEG and EEG experiments on epilepsy and normal spontaneous activity by applying local Lyapunov exponent in the running window technique. This method allows also to detect sudden changes of the dynamics in the measured time series. Those changes are biological analogs to critical transitions in a course of physics. They confirm the nonstationary character of EEG/MEG and, in addition, hold promise to be used as the predictors of epileptic seizures. Using the multichannel techniques of EEG and MEG increases also spatial information about the localization of oscillatory centers. The loss of the signal stability is also observed in the interictal states. Supposing that the interictal oscillators are localized in the same way as the ictal ones leave us to expect, in case of focal epilepsy, that positions of oscillatory centers correspond to the position of epileptic foci. For cases of epilepsy in which the focus is difficult to localize there is a chance to indicate the side of the functional distortion, what may be of diagnostic value.

Symposium 5 - Molecular and morphological aspects of neural apoptosis

TRKB SIGNALING IS REQUIRED FOR POSTNATAL SURVIVAL OF NEURONS IN THE CENTRAL NERVOUS SYSTEM.

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Newborn mice carrying targeted mutations in genes encoding neurotrophins or their signaling Trk receptors display severe deficits in the peripheral nervous system (PNS). These deficits are mainly due to an increase in the rate of cell death during embryonic development. However, no apparent deficits were dectected in the central nervous system (CNS) of these animals. In contrast to sensory neurons, the development of the CNS neurons continues during the first two weeks of life. *trk*B (–/–) mutant mice with a predominant C57Bl/6 genetic background have an increase in their life span compared to the early generations of animals. In this study, we show that *trk*B (–/–) mice have a significant increase in cell death in different regions of the brain after the first postnatal week. This cell death, that is more severe in the oldest surviving *trk*B (–/–) mutant mice, is apoptotic in nature. The most affected region in the brain is the dentate gyrus of the hippocampus, although, significant increase in pyknotic cells was also detected in cortical layers (II-III and V-VI). In these regions, some of the pyknotic cells are labeled with different calcium-binding proteins. Furthermore, the survival of hippocampal neurons and motor neurons after axotomy lesions were decreased in mice lacking TrkB tyrosine kinase receptors. These results suggest that neurotrophin signaling through TrkB receptors is essential for late postnatal survival of CNS neurons. Moreover, TrkB signaling appears to protect CNS neurons from axotomy-induced cell death.

Molecular mechanism of the apoptotic cell death - focus on the involvement of transcription factors

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Cyclosporin A (CsA) is a powerful immunosuppresant that prevents activation of some transcription factors, in particular NFAT, involved in lymphokine gene expression. NFAT is a complex of CsA-sensitive component, NFATp, and the AP-1 transcription factor composed of Fos and Jun family proteins. Some neurological complications and cytotoxic effects of CsA therapy have been reported but precise mechanism of CsA action is unknown. To get insight into possible mechanism of CsA cytotoxic effect, we investigated the effects of CsA (Sandimun, Sandoz) on rat C6 glioma cells. We have found that CsA exerts antiproliferative effect on glioma cells and induces cell death which showed all features typical of apoptosis. Cells treated with 75 mg/ml CsA exhibited cell body shrinkage and chromatin condensation followed by fragmentation; death was accompanied by the appearance of DNA "ladder" upon gel electrophoresis; apoptotic changes were abrogated by the cycloheximide, a inhibitor of protein synthesis. Using Electrophoretic Mobility Shift Assay we studied DNA binding activities of AP-1 and NFAT transcription factors during CsA-induced apoptosis. Besides the elevation of the AP-1 DNA binding activity beginning at 6 hr after CsA addition, we observed remarkable changes in the composition of AP-1 complex in dying cells (24 hr after CsA treatment). These changes were manifested by the appearance of JunB and JunD proteins in the complex as well as the increase of c-Jun protein. Our findings suggest that both increase and differences in AP-1 composition may be responsible for specific role of this factor in the regulation of apoptosis. The NFAT DNA binding activity which was present in proliferating C6 glioma cells, decreased to undetectable level as early as 6 hr after CsA-treatment. It raises a possibility that NFAT is the primary target for Csa in glioma cells and the mechanism of CsA action in glial cells can be similar to that operating in lymphoid cells.

EXCITOTOXICITY OF KAINIC ACID AND QUINOLINIC ACID: ANIMAL AND TISSUE CULTURE STUDIES.

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Kainic acid (KA) and quinolinic acid (QUIN), exo- and endogenous excitatory amino acids of both neuroexcitatory and neurotoxic properties, have been widely applied in experimental neuropathology. The aim of the present studies was to evaluate the dynamics and pattern of ultrastructural changes induced by these neurotoxic compounds in animal models of Huntington's disease or epilepsy and in organotypic or dissociated cell cultures derived from selected regions of rodent brains. Both *in vivo* and *in vitro*, typical excitotoxic changes consisted of progressive degeneration of large striatal neurons and pyramidal hippocampal cells and axon-sparing, postsynaptic lesions accompanied by extensive fibrillar gliosis. Data from *in vitro* experiments indicated that glial fibrillary changes should be considered as a primary response of astroglia to excitotoxins, independent of neuronal damage. Delayed QUIN-induced neuronal and glial pathology has been evaluated in long-lasting experiments *in vivo*. A tissue culture model allowed to study the direct effect of various concentration of KA or QUIN applied at different stages of tissue maturation and cell differentiation. An appropriately modified culture environment was used to demonstrate the neuroprotective effect of calcium channel blockers: nimodipine, verapamil and divalent cations: zinc, magnesium against QUIN neurotoxicity. QUIN-induced mitochondrial calcium overload and its prevention by nimodipine, was visualized by oxalate-pyroantimonate cytochemical technique for electron microscopy. The results support the important role of calcium entry in the development of delayed excitotoxic neuronal damages.

Tissue Inhibitor of Metalloproteases-1 (TIMP-1) is Differentially Induced in Neurons and Astrocytes Following Seizures and Cerebral Ischemia: Evidence for Developmental-, Immediate Early Gene- and Lesion-Response

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The Tissue Inhibitor of Metalloproteases-1 (TIMP-1) is a 28.5 kDa secreted glycoprotein. Although considered a multifunctional molecule that possesses growth promoting properties and mitogenic activity, TIMP-1 is mostly recognized as an inhibitor of matrix metalloproteases (MMPs). MMPs constitute a family of zinc-binding and Ca^{++}-dependent endopeptidases. The excessive cleavage of the extracellular matrix (ECM) associated with an imbalance of the MMPs/TIMPs ratio has been correlated with the invasive potential of brain tumor cells, and with the histopathogenesis of inflammatory-related diseases. Nedivi et al. (1993) isolated TIMP-1 in the CNS as a candidate plasticity gene in kainate (KA)-treated rats. We postulate that controlled proteolysis is crucial in the development and plasticity of the CNS, whereas excessive proteolysis contributes to various neuropathologies. We investigated *in vivo* the expression of the tissue inhibitor of metalloproteases-1 (TIMP-1) in the rat CNS following KA-induced excitotoxic seizures and cerebral ischemia. *In situ* hybridization revealed that TIMP-1 mRNA is rapidly induced in most regions of the adult forebrain, following patterns of neuronal hyperactivity. Neuronal activity appears to be necessary but not sufficient to trigger TIMP-1 induction, since it is not observed in seizing 10-day old pups. Following seizures, the rapid induction of TIMP-1 is not prevented by inhibition of protein synthesis, suggesting that TIMP-1 is induced in neurons as an immediate early gene. The initial neuronal upregulation is followed by enhanced expression in astrocytes, as assessed by double labeling experiments. In the hippocampus, rapid increases in mRNA are followed by delayed increases in TIMP-1 immunoreactivity in the perisomatic and dendro-axonic areas, suggesting secretion of the protein. Three days after KA treatment, strong immunoreactivity is found in astrocytes and in the cell bodies and dendro-axonic projections of resistant neurons such as the dentate granule cells. Taken together, the results suggest that TIMP-1 may be instrumental for neurons and astrocytes in coupling early cellular events triggered by seizures with the regulation of long-lasting changes involved in tissue reorganization and/or neuroprotection.

Neurodegeneration-associated expression of cathepsin D.
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Cathepsin D (CatD) is major lysosomal aspartic protease. a protease, CatD can be involved in cell destruction mechani including those activated in neurotoxic insults. In this study expression of CatD was determined in two neurotoxic condi in rat brain. Kainic acid (KA), agonist of glutamate receptor evokes in rats seizures followed by massive neurodegenerati mainly in the structures associated with limbic system. MK- is an antagonist of NMDA receptor. Administration of MK- to rats in doses exceeding 1 mg/kg causes degeneration of fe percent of neurons in retrosplenial cortex. Increased CatD expression was observed in both KA- and MK-801- evoked neurodegeneration. This increase was evident at both mRNA protein levels as determined by northern blotting and immunohistochemistry. In both conditions the increase of mRNA level was well established 24 hours after the treatmer and reached maximal values 72 hours after the treatment. Immunohistochemistry for CatD revealed intense staining observed mainly in cells presenting features of degenerating neurons. This localization was further confirmed by compa the patterns of CatD and GFAP immunoreactivities. In orde study the mechanisms of neuronal death activated by KA ar MK-801, DNA fragmentation was studied using agarose gel electrophoresis and *in situ* detection techniques. KA was fou to evoke apoptotic, ladder-like fragmentation of DNA in the structures affected by the toxin. Degenerating cells appearin retrosplenial cortex in response to MK-801 were shown to contain no DNA, as revealed by DNaseI pretreatment of bra sections and subsequent TUNEL staining for fragmented DI This result suggests rapid degradation of cellular DNA in th course of MK-801-evoked neurodegeneration. Thus, increa expression of CatD seems to be a common element of degenerative cascades triggered by different compounds an differentially engaging DNA fragmentation.

Symposium 6 - Neurobiology of addiction

IS THERE A COMMON MECHANISM OF DRUGS OF ABUSE?

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Despite extensive studies, the neurochemical basis of drug addiction is only partly understood. A number of data suggest that the interaction of endogenous opioids and the dopaminergic mesolimbic system is critically involved in the neurochemical mechanism of tolerance and dependence on drugs of abuse belonging to different groups of pharmaceutics. Recently evidence has been accumulated that NMDA receptors and nitric oxide are also implicated in the effects of drugs of abuse, since the increased level of nitric oxide is a consequence of the NMDA receptor activation. In fact, blockade of the NMDA receptor and inhibition of nitric oxide synthase lead to a delay in developing tolerance and counteracting some behavioural symptoms of withdrawal.

We found that chronic administration of different drugs of abuse (morphine, psychostimulants, ethanol) increased the synthetic activity of prodynorphinergic neurons, especially during their late withdrawal. That effect was antagonized by pretreatment with L-NAME, a nitric oxide synthase inhibitor. On the other hand, kappa opioid receptors were down-regulated as a result of the increased release of prodynorphin-derived peptides. We also observed down-regulation of NMDA receptors and upregulation of mu opioid receptors in certain brain structures. The obtained data indicate that a prolonged exposure to drugs of abuse results in an enhanced activity of the prodynorphin system, and that the NMDA/NO pathway may modulate this adaptive neuronal response.

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Opioids and Addictive Processes

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The great progress made in opioid research during the last two decades offers new perspectives for the understanding (and possible treatment) not only for opioid addiction but also of addictive processes in general.

The study of motivational effects of opioids revealed μ- and δ-agonists as positive reinforcers in animal models (e.g. place-conditioning), whereas κ-agonists were found to be aversive. There is much evidence that the dopaminergic mesolimbic reward system mediates these effects: μ- and δ-agonists increase, κ-agonists decrease dopamine release in the Nucleus accumbens. Obviously, the same mesolimbic pathway is modulated by two opposing tonically active endogenous opioid systems.

The mesolimbic reward pathway mediates also motivational effects of other drugs of abuse. There is increasing evidence that opioid mechanisms are involved in such effects of psycho-stimulants, e.g. in the sensitization for the rewarding effects of cocaine. The addictive properties of alcohol seem to involve endogenous opioids too, explaining the promising effects of opioid antagonists to prevent relapse in alcoholics.

DISTRIBUTION AND REGULATION OF μ-OPIOID RECEPTORS.

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The rat μ opioid receptor exists in two isoforms termed rMOR1 and rMOR1B which are generated by an alternate splicing event at the C-terminus. When stably transfected into CHO-K1 cells both μ opioid receptor isoforms show similar affinities to opioid compounds and were equally effective in mediating inhibition of forskolin-induced cAMP formation by agonists. In addition, both μ receptor variants mediate increases in intracellular calcium concentrations and inositol triphosphate formation in response to similar concentrations of opioid agonists. In constrast, transfected cells expressing rMOR1B were much more resistant to desensitization after prolonged exposure to agonists than cells expressing rMOR1. The region of the C-terminus in which rMOR1 differs from rMOR1B contains one potential phosphorylation site (thr 394). Point mutation of thr 394 to an alanine or truncation of the C-terminus of rMOR1 resulted in a slower desensitization kinetic of rMOR1, similar to that of rMOR1B. This indicates that phosphorylation of the C-terminus of rMOR1 plays an important role in receptor desensitization.

Using specific antisera MOR1B-like immunoreactivity could only be localized in the plexiform layer of the olfactory bulb, whereas MOR1-like immunoreactivity showed the known distribution in many regions of the brain and spinal cord of rats and mice. Interestingly, in D1 dopamine receptor deficient mice the patchy distribution of MOR1 in the striatum and nucleus accumbens could not be observed in contrast to the wild type animals. In addition, repetitive application of morphine resulted in a gradual increase in the locomotor activity in the wild type mice, but did not affect the locomotion of the wild-type animals. These results provide futher evidence for the important role of striatal D1 receptors in the action of μ-opioid agonists.

The effects of acamprosate on opiate dependence/addiction

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Acamprosate prevents relapse in weaned alcoholics, however, its efficacy in relapse prevention in other drug abusers has not been examined so far. It is suggested that there exist similarities in the neurobiological mechanisms underlying alcohol and opiate addiction. Therefore, we were interested in the question whether acamprosate also interferes with opiate dependence processes.

The action of acamprosate was studied on morphine-induced behavioural sensitization procedures (locomotor activity and conditioned place preference). Acamprosate (200 mg/kg) completely abolished the expression of morphine-induced behavioural sensitization, however, it did not influence the locomotor effects of an acute morphine injection in drug-naive animals. In addition microdialysis data revealed that augmented dopamine release in the nucleus accumbens following intermittent context-dependent injections of morphine is also abolished by acamprosate pretreatment. In contrast, preliminary data indicate that acamprosate neither affected intravenous heroin self-administration during the maintenance phase nor influenced reinstatement of drug-seeking induced by a heroin priming injection or footshock during the relapse phase. Furthermore acamprosate was tested in a model of conditioned opiate withdrawal: rats were trained to lever-press for food reinforcement on a FR10. The animals were then implanted with morphine pellets and naloxone was given in the operant chambers paired with a smell stimulus. On the test day rats were exposed only to the conditioned stimulus associated with the state of withdrawal. The conditioned stimulus acquired significant behaviour-disruptive properties (e.g. a strong decrease in lever-presses), however, acamprosate did not prevent this conditioned response. In conclusion, although acamprosate prevents the expression of morphine-induced sensitization it does not interfere with reinforcing effects of intravenous heroin, relapse to heroin-seeking and conditioned opiate withdrawal.

REDUCTION OF ALCOHOL INTAKE IN ALCOHOL-PREFERRING RATS BY TACHYKININ NK-3 RECEPTOR AGONISTS: A SEARCH FOR THE SITE OF ACTION.

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Intracerebroventricular (ICV) injections of tachykinin (TK) NK-3 receptor agonists reduce alcohol intake in alcohol-preferring rats. The NK-3 receptor agonist senktide reduces ethanol intake also by subcutaneous injection, but doses about 1000 times higher than those effective by ICV injection are required, suggesting a central site of action. The present study evaluated the sensitivity of several brain sites to the effect of the NK-3 receptor agonist [$Asp^{5,6}$,$MePhe^{8}$]substance P(5-11), also referred to as NH_2-senktide (NH_2-SENK) on alcohol intake in genetically selected alcohol-preferring rats, which were bred in our Department for 22 generations from Sardinian alcohol-preferring rats of the 13th generation.

Water and food sated rats were offered 10% ethanol 2 hr/day, at the beginning of the dark phase of an inverse light-dark cycle. Injections into the medial amygdala, CA1 region of the hippocampus, nucleus accumbens and ventral tegmental area at doses up to 50 ng/site were devoid of effect. Administration into the bed nucleus of the stria terminalis inhibited ethanol intake at 5-25 ng/site, but the same doses inhibited also food intake in food deprived rats.

Bilateral injections of NH_2-SENK in the nucleus basalis magnocellularis (NBM) reduced alcohol intake, the threshold dose being 0.5 ng/site, while ICV injection of NH_2-SENK, 10 ng/rat, did not inhibit alcohol intake. On the other hand, NH_2-SENK, 25 ng/NBM, did not modify water or food intake in water deprived rats. Injection in the NBM of the TK NK-3 receptor antagonists, R-820 or S-18451, 1000 ng/site, significantly reduced the effect of NH_2-SENK 5 ng/site. The selective TK NK-1 receptor agonist [Sar^{9},$Met(O_2)^{11}$]substance P inhibited alcohol intake only at 25 ng/site; but the same dose induced marked grooming and inhibited water intake in water deprived rats.

Present results confirm that TK NK-3, but not NK-1, receptor agonists selectively inhibit alcohol intake in alcohol-preferring rats and suggest that the NBM is a site of action for their effect. Injection of the TK substance P in the NBM evokes conditioned place preference in rats, and increases extracellular levels of dopamine and 5-hydroxyindoleacetic acid in the nucleus accumbens. These findings raise the hypothesis that stimulation of TKergic mechanisms in the NBM might, at least in part, substitute for the effects of ethanol.

REDUCTION OF ALCOHOL INTAKE BY TACHYKININ NK-3 RECEPTOR AGONISTS IN ALCOHOL-PREFERRING RATS: A SEARCH FOR THE MECHANISM OF ACTION.

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Injections of tachykinin (TK) NK-3 receptor agonists in the nucleus basalis magnocellularis (NBM) reduce alcohol intake in rats. The present study was aimed at investigating the mechanism of the action on alcohol intake of the NK-3 receptor agonist NH_2-SENK, in rats selectively bred for high ethanol preference. **1)** Taste reactivity. Since NK-3 receptor agonists influence taste reactivity to NaCl solutions, the present study evaluated whether they may modify also taste reactivity to ethanol. Rats were injected in the NBM with either isotonic saline (IS; controls) or 5 ng/site of NH_2-SENK. They received intraoral infusion (0.8 ml/60 s) of water or ethanol solutions (10, 20, 40 or 60 %) 5 min later. Oral and general motor reactions were recorded. Rats injected with NH_2-SENK responded to all the ethanol concentrations with pronounced ingestive reactions, similarly as controls. **2)** Ethanol-induced conditioned taste aversion (CTA). A CTA paradigm was used to investigate whether NK-3 receptor agonists may influence the aversive effects of ethanol. After a 20 min/day drinking session of a solution containing 0.125% saccharin and 3% sucrose, rats were treated with 5 ng/NBM of NH_2-SENK or IS, and then injected intraperitoneally (IP) with IS or with ethanol (1 g/kg). IP ethanol produced a marked CTA that was not modified by NH_2-SENK. The NK-3 receptor agonist *per se* did not evoke CTA. **3)** Ethanol metabolism. To evaluate whether central NK-3 receptors may affect ethanol metabolism, blood alcohol concentration (BAC) was measured in animals injected into the lateral ventricle either with IS (controls) or with 125 ng/rat of NH_2-SENK, 5 min before intragastric administration of ethanol (0.7 g/kg). Blood samples were collected 15, 30 and 60 min following ethanol administration. In rats injected with NH_2-SENK BAC were not different from those of controls. Present results show that NH_2-SENK does not modify ethanol taste, aversive effects or metabolism. Studies aimed at investigating the role of NK-3 receptors in the reinforcing properties of ethanol are in progress.

CANNABINOIDS ACTIVATE MESOLIMBIC DOPAMINE NEURONS BY AN ACTION ON CB1 RECEPTORS

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The present study was designed to determine if cannabinoids share with other drugs of abuse the ability to activate mesolimbic dopaminergic transmission .
To this aim, the effects of the prototypical cannabinoid, Δ^9tetrahydrocannabinol (THC), the two highly potent synthetic cannabinoids WIN 55,212-2 and CP 55,940, and the putative endogenous cannabinoid anandamide on the spontaneous discharge rate of antidromically identified meso-accumbens dopamine (A10-DA) neurons were studied both in non-anesthetized and chloral hydrate anesthetized rats.
The i.v. administration of THC (0.25 - 1.0 mg/kg), WIN 55,212-2 (0.0625 -1.0 mg/kg) and CP 55,940 (0.25 - 1.0 mg/kg) produced a dose-dependent increase in the spontaneous firing of A10-DA neurons both in non-anesthetized and anesthetized preparation with a maximal percent increase of 95, 148 and 130 in non-anesthetized and 36, 85 and 35 respectively in anesthetized rats.
In contrast, anandamide up to a dose of 10 mg/kg failed to modify A10-DA neuronal activity.
The stimulant response to cannabinoids was suppressed by the i.v. administration of the specific cannabinoid antagonist SR 141716A but not by naloxone indicating a cannabinoid-receptor mediated effect. The finding that THC shares with other drugs of abuse the ability to facilitate mesolimbic DA transmission suggests that this effect may be relevant to the addictive properties of marijuana. On the other hand the lack of effect of anandamide on A10-DA neurons suggests the possibility to develop therapeutically useful cannabinoids devoid of addictive properties. Collectively, the present results indicate that exogenous cannabinoids stimulate mesolimbic dopaminergic neurotransmission by an action at CB1 receptors.
In turn, the dopaminergic firing stimulant properties of cannabinoids here reported are reminiscent of those already described for other more harmful substances such as opiates and ethanol. Consequently the present results bear not only a scientific value but important political implications.

Plenary lectures

Melatonin counteracts necrosis and apoptosis.

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Introduction: Melotonin is metabolised in the retina and has been implicated in a variety of functions. The present studies were undertaken to see if melatonin counteracts ischaemic insults.

Methods: Experiments were carried out on rat cortical cultures, human retinal pigment epithelial cells and the intact rabbit retina.

Results: Glutamate and oxygen deprivation stimulated the "release" of LDH from rat cortical cultures. Inclusion of MK-801 (2μM) or melatonin (100μM) in the medium almost completely attenuated the effects of glutamate or oxygen deprivation. Ischaemia was delivered to the rat retina by elevation of intraocular pressure. After a defined reperfusion time there was a reduction of the b-wave of the electroretinogram, changes in the distribution of various antigens and an alteration in the retinal morphology. These changes were clearly reduced when the animals were treated with melatonin throughout ischaemia/reperfusion.

Experimental ischaemia (oxygen and glucose deprivation) resulted in 80% of the retinal pigment epithelial cell's nuclei staining positively for DNA fragmentation by the TUNEL procedure. The cells also appeared to shrink, suggesting the process of apoptosis. Inclusion of 10% foetal calf serum or 100μM melatonin attenuated the apoptosis with the cells appearing healthy and not staining positively by the TUNEL method. Inclusion of MK-801 or luzindole (100μM) did not counteract the experimentally-induced effect of apoptosis to RPE cells.

Conclusions: The combined studies show that melatonin can experimentally counteract the effect of insults that lead to destruction of cells by apoptosis and necrosis. The possibility of elevating the retinal levels of melatonin to protect against insults such as glaucoma, which lead to damage of retinal neurones, must be considered.

FUNCTIONS OF OPIOIDS IN THE BRAIN

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Since primary identification and final cloning of opioid peptide prohormones and receptors, opioid systems have deserved utmost attention towards understanding their functional roles in the CNS. Three independent neuronal opioid systems and multiple receptors have been identified. These systems are presumed to be involved in several CNS physiological functions. Further, they have been implicated in the pathophysiology of stress, pain, seizures, drug addiction and brain injury. It is not clear, however, how the endogenous opioid systems contribute to brain pathophysiology, and how they adapt themselves and compensate for pathological stimuli? Recent molecular, biochemical and pharmacological studies into regulation of the opioid gene expression, their transcription factors and associated neurotransmitters in pathological states permitted us to better understand the role of these opioids in the brain. The obtained data suggest that opioid systems are not functionally homogenous and are likely to influence various brain functions reciprocally or in opposing ways. Evidence has been accumulated that enkephalinergic and endorphinergic systems seem to reply to acute neuronal stimulation and participate in the process of neuroadaptation, while dynorphinergic neurons react to prolonged or repeated stimuli and may contribute to the development of CNS pathology.

Oral communications II - Neurochemistry and neuropharmacology

Role of NAAG in neurotransmission. B. Wroblewska, S.E. Sullivan, T. Bzdega, and J.H. Neale., Georgetown University, Dept. Biology, Washington D.C., U.S.A.
N-acetylaspartylglutamate (NAAG) is a dipeptide present in a very high concentrations in the mammalian brain. NAAG meets most of the traditional criteria for a neurotransmitter, however the receptor for NAAG, and the role of this neuropeptide in the brain functions are not known. We have shown recently that NAAG decreases forskolin-stimulated cAMP formation and activates group II metabotropic glutamate receptor(s) in cultured cerebellar granule and glial cells. Our data obtained from the cells expressing cloned mGluRs indicate that NAAG selectively activates mGluR3 and not mGluR1a, 2, 4, 5, and 6. Using chimeric molecules (mGluR2-mGluR1a and mGluR3-mGluR1a constructs) we were able to show that NAAG interacts with the N-terminus of mGluR3 receptor, but not highly homologous mGluR2. These data suggested that NAAG, as an agonist of metabotropic glutamate receptor (mGluR3) may participate in the neurotoxic or neuroprotective mechanisms in the brain. Using cultured cortical cells (in collaboration with Drs. Bruno and Nicoletti, Univ. of Catania) we were able to show that NAAG protects neuronal cells from NMDA-induced neurotoxicity, and this protective effects are mediated through the glial cells. We have also recently cloned, sequenced and expressed the full length rat hippocampal cDNA encoding for NAAG peptidase - an enzyme which cleaves NAAG to N-acetylaspartate and glutamate. The RT-PCR screening indicates the presence of the message encoding NAAG peptidase in rat brain, kidneys and spinal cord.

INVOLVEMENT OF BRAIN DOPAMINE IN CEREBRAL ISCHEMIA AND NEURONAL DAMAGE IN RAT HEATSTROKE
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The pathophysiology of heatstroke has been extensively studied by many investigators. Its CNS syndromes include coma, delirium and confusion. When exposed animals to a high ambient temperature, the moment at which mean arterial pressure began to decrease from its peak level was taken as onset of heatstroke. Heatstroke was accompanied by hyperthermia, cerebral ischemia, neuronal damage, increased brain levels of dopamine, arterial hypotension, and increased plasma levels of interleukin-1 (IL-1). Depletion of brain dopamine produced by intracerebral injection of 6-hydroxydopamine was able to attenuate cerebral ischemia, neuronal damage and arterial hypotension and to result in extension of survival in rat heatstroke. Also, systemic administration of IL-1 receptor antagonist was able to attenuate cerebral ischemia, neuronal damage and arterial hypotension by reducing brain dopamine levels. The data indicate that amrked accumulation of brain dopamine resulting from increased plasma levels of IL-1 may be an important mechanism signaling cerebral ischemia, neuronal damage and arterial hypotension in heatstroke.

INFECTION AS A STRESSOR: THE ROLE OF CYTOKINES
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Stress is associated with an activation of peripheral and central catecholaminergic systems and the hypothalamo-pituitary-adrenocortical (HPA) axis. In the brain, stress is associated with activations of noradrenaline (NA) and CRF. Animal studies suggest activation of dopaminergic and serotonin (5-HT) systems, as well as elevations of free tryptophan (Trp). It has been shown that infections and other treatments known to activate the immune system (e.g., endotoxin) cause activation of the HPA axis, as well as NA, Trp and 5-HT in the brain, and some sympathoadrenal activation. Thus by physiological criteria these treatments are stressful. There are also parallels between the behavioral consequences of stress and infection: hypomotility, hypophagia, decreased exploration and libido and increased sleep time. Injection of cytokines, such as interleukin-1 (IL-1), IL-6 and tumor necrosis factor α (TNFα) secreted early in immune responses, activate the HPA axis. IL-1 activates NA systems, and IL-1 and IL-6 increase brain Trp and activate 5-HT, but TNFα induces no effects on NA or 5-HT. IL-1 can elicit the whole spectrum of infection-related responses, including fever and the behavioral responses. Thus these cytokines may be mediators of the responses to infections and immune challenges. Cytokine antagonists, such as the IL-1-receptor antagonist (IL-1ra) have failed to demonstrate that IL-1 is the predominant mediator of the HPA, neurochemical and behavioral responses to LPS, although some attenuations of IL-1ra on the HPA response and on ingestive behavior are observed. A monoclonal antibody to IL-6 partially attenuated the HPA responses to LPS and IL-1. Antibodies to TNFα were inneffective. These results suggest that cytokines may participate in the physiological and behavioral responses to infections, but that other factors are also involved.

Effect of tachykinins on the central respiratory activity: an in vitro study on the newborn rat

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Tachykinins are a family of neuromodulators among which substance P (SP) was the first to be evidenced. In the 1980s, neurokinin A and neurokinin B were also isolated. Three types of receptors (NK_1, NK_2 and NK_3) mediate the effects of tachykinins. SP displays a higher affinity for tachykinin NK_1 receptor. Several results have suggested that substance P might be involved in the respiratory regulation in mammals.

The aim of the present work was to investigate the effects of tachykinins, particularly SP, on the activity of the respiratory rhythm generator in the newborn rat and to determine the type of the tachykinin receptors involved.

Experiments were performed using newborn rat (0-3 days old) brainstem-spinal cord preparation which was placed in a chamber permanently perfused with artificial cerebro-spinal fluid (aCSF). Substance P and pharmacological agents acting as tachykinin receptor agonists or antagonists were dissolved in the aCSF and applied by superfusion at several concentration (10^{-10} - 10^{-6}M). Substance P and tachykinin NK_1 and NK_3 agonists induced a concentration-dependent increase in the respiratory frequency (10^{-9} - 10^{-7}M), whereas the respiratory frequency was only slightly affected by tachykinin NK_2 receptor agonist. Pre-treatments with tachykinin NK_1 receptor antagonists reduced the substance P-induced increase in the respiratory frequency but the tachykinin NK_2 receptor antagonists had no effect. The increase in the respiratory frequency induced by the tachykinin NK_3 receptor agonist was not affected by a pre-treatment with tachykinin NK_1 and NK_2 receptor agonists.

The results indicate that substance P may exert potent facilitatory effects on the respiratory rhythm generator and that tachykinin NK_1 and NK_3 receptors may be involved in the control of the respiratory frequency.

NIFEDIPINE MODULATES SOME CHANGES INDUCED BY ANTIDEPRESSANTS IN ADRENERGIC TRANSMISSION AT THE SECOND MESSENGERS LEVEL

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Antidepressant therapies produce long-lasting adaptive changes in the brain, possibly associated with intracellular Ca^{2+}. Here we investigated the effect of Ca^{2+}-channel blockade (CCB) on the changes induced by chronic antidepressant treatments: imipramine (IMI) and electroconvulsive shock (ECS), in the responses of second messenger systems linked with α_1- and β-adrenoceptors (AR) and the protein kinase C (PKC)-induced potentialization of the β-AR response in the brain cortex. Wistar rats received ECS (150 mA, 250 ms) for 10 days or IMI (10 mg/kg, i.p., b.i.d.) for 21 days, each shock or injection being preceded 15 min earlier by solvent or the Ca^{2+}-channel blocker nifedipine (NIF), 5 mg/kg. 24 h after the last treatment the rats were killed and inositol phosphates (IP) response to norepinephrine (NE) (an α_1-AR response) and cyclic AMP (cAMP) response to NE and isoproterenol (a β-AR response) in the presence and absence of a PKC activator, TPA, was measured in the cortical slices. The chronic intermittent CCB with NIF did not affect the responsiveness of cerebral cortical α_1-AR. Neither IMI nor ECS treatment changed the responsiveness of α_1-AR in naive rats, but when given under CCB they caused an increase in the IP response to NE. Similarly, administration of NIF alone induced no change in the responsiveness of β-AR, but given during CCB imipramine and ECS, the treatments that down regulate the β-adrenergic system, produced different efects. ECS that normally did not affect the cAMP response to isoproterenol in the presence of TPA, under condition of CCB down regulated it. IMI, which normally blocks the TPA-potentiation of cAMP response, did not produce that effect when administered after NIF. Thus, the chronic changes in the AR systems induced by chronic antidepressant treatments (both in responsiveness of receptor systems and the receptor crosstalk) may be changed when antidepressants are given when Ca^{2+} channels are blocked. The importance of this finding lies in the fact that several depressed patients are also treated for circulatory disturbances, and Ca^{2+}-channel blockers are frequently used cardiac drugs.

PROLONGED CORTICOSTERONE TREATMENT ALTERS BIOCHEMICAL AND FUNCTIONAL PARAMETERS OF SEROTONERGIC NEUROTRANSMISSION ASSOCIATED WITH 5-HT_{1A} RECEPTORS

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The aim of the present study was to investigate the impact of repeated administration of corticosterone (10 mg/kg, twice daily, for 7 days) on biochemical, behavioural and electrophysiological parameters characteristic of the 5-HT neurotransmission associated with 5-HT_{1A} receptors. It was found that prolonged treatment with corticosterone increased the number of 5-HT_{1A} receptors in the raphe nuclei, prefrontal cortex and hippocampus, as measured by quantitative autoradiography and saturation binding in brain homogenates using [^{3}H]8-OH-DPAT as a ligand. The level of 5-HT in raphe nuclei was not changed by corticosterone, whereas the level of its metabolite 5-HIAA was increased. In the prefrontal cortex and hippocampus the levels of both 5-HT and 5-HIAA were increased after chronic corticosterone treatment. On the other hand, we observed that chronic corticosterone attenuated the 8-OH-DPAT evoked disruption of the prepulse-induced inhibition of acoustic startle response and decreased the 8-OH-DPAT-induced inhibition of a population spike and hyperpolarization in CA1 hippocampal neurons. Both these effects indicate that chronic corticosterone decreased the functional responsiveness to stimulation of 5-HT_{1A} receptors. It is concluded that chronic occupation of glucocorticoid receptors by corticosterone increases the density of 5-HT_{1A} receptors and 5-HT turnover. In contrast to the effects observed in binding studies, corticosterone attenuates most of the functional effects induced by 5-HT_{1A} receptor stimulation.

NEW ANTAGONISTS OF THE 5-HT_{1A} RECEPTOR

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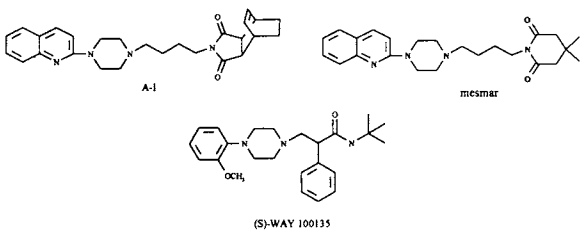
Several new analogues of buspirone, a known anxiolytic drug, were obtained. The compounds exhibited high affinity to 5-HT_{1A} receptor and were examined in the lower lip retraction and the 8-OH-DPAT induced hypothermia tests, evaluating post- and pre- synaptic, respectively, activity of the receptor ligands. It appeared that some compounds were able to reverse the 8-OH-DPAT induced lip retraction in rats in similar doses as known antagonist of the receptor (S)-WAY 100135: mesmar - 4.0, kaspar - 6.1, A-1 - 8.0, (S)-WAY 100135 - 6.0 (ED_{50} mg/kg). Mesmar and A-1 in a dose 10 mg/kg, similarly to (S)-WAY 100135 (10 mg/kg), completely reversed 8-OH-DPAT induced hypothermia in mice. Mesmar and A-1 thus behaved like functional antagonists at both pre- and postsynaptic 5-HT_{1A} sites.



Symposial 3 - Development and plasticity of sensory systems

A COMPARATIVE SURVEY OF THE ORGANIZATION OF SOMATOSENSORY NEOCORTEX IN MARSUPIALS. K. Huffman, J. Nelson, M. Sum & L. Krubitzer, Center for Neuroscience, University of California, Davis, CA, USA

This investigation is part of a broader effort to determine features of neocortical organization that are common to all mammals, and features that arose independently in different lineages. The marsupials we have studied include the Australian dunnart (*Sminthopsis crassicaudatus*), quoll (*Dasyurus hallucatus*), and striped possum (*Dactylopsila trivirgata*), and the South American short-tailed possum (*Monodelphis domestica*). In the quoll, striped possum, and short-tailed possum, the region of cortex responsive to stimulation of somatic receptors was explored using multiunit electrophysiological recording techniques. Receptive fields for neurons at multiple, densely spaced recording sites were obtained, and cortical field boundaries were determined by examining reversals in receptive field progressions, changes in neural response properties, and stimulus preferences. In all species, cortex was flattened, cut parallel to the cortical surface, stained for myelin, and reacted for cytochrome oxidase (CO). The architectonic distinctions apparent in histologically processed tissue were correlated with the cortical field boundaries established from electrophysiological recording results. In the dunnart, only myeloarchitectonic boundaries were ascertained. In all species, at least three distinct fields could be identified. The primary somatosensory area, SI, was characterized by a complete topographic representation of the body surface coextensive with a darkly myelinated, CO dense area. Neurons in SI responded to stimulation of cutaneous receptors. A moderately myelinated field immediately rostral to SI, termed the rostral deep area, R, also contained a complete representation of the body surface. However, neurons in this field responded to stimulation of deep receptors. Finally, in cortex immediately lateral to SI, a small, moderately myelinated region which was contiguous with another complete representation of the body surface was termed SII/PV. Although basic features of somatosensory cortex were similar across these species of marsupials, the internal organization differed dramatically. In SI, animals tended to have varying amounts of cortex devoted to their different morphological specializations. Also, the expansion of neocortex across these species was nonlinear with respect to body size, and was especially exaggerated in the striped possum. This expansion is possibly a result of the complex behavior patterns and social interactions that this species exhibits.

ORIGINS OF MODIFICATIONS OF CIRCUITRY WITHIN THE RAT S1 BARREL CORTEX SUBSEQUENT TO CHANGES IN TACTILE EXPERIENCE.

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When somatosensory experience is changed in the adult mammal, then modifications of both thalamic, thalamocortical and intracortical synaptic relays may contribute to receptive field plasticity of S1 cortex. We have investigated these separate aspects of reorganisation within the barrel cortex and the somatosensory (barreloid) thalamus of adult rats following 3 to 30 days of cutting all whiskers except two being cut unilaterally. Modifications to paired and unpaired (cut-whisker) inputs occur at thalamic as well as cortical levels. Both Hebbian and anti-Hebbian modifications occur. Statistical analyses of changes in spatio-temporal features of responses in homologous barrel and barreloid neuronal populations suggest that the use-dependent alterations in the thalamic relay contribute probably contribute little to alterations in processing of sensory information at the cortical level. These features will be elaborated and their relevance to models for use-dependent reorganisation of sensory processing in the somatosensory system will be discussed.

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Sensory stimulation of gene expression in neuronal plasticity

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An involvement of gene expression in neuronal plasticity has been well documented. The components of the AP-1 transcription factor (in particular, c-Fos) and Zif268 have been especially widely investigated in this regard. However, the roles of these two proteins in neurons remain speculative and include such varied functions as short-term maintenance of cellular homeostasis to long-term changes that guide neuronal plasticity. Current efforts at elucidating the physiological roles of AP-1 and Zif268 rely on assessing their expression in response to different conditions of behavioral, sensory and pharmacological stimulation. In our studies, we have examined the expression patterns of these transcription factors in the mammalian sensory cortex under different conditions, with particular emphasis on the constitutive levels and how they change after sensory deprivation, stimulation and behavioral training. A synthesis of this information implies that whereas expression of Zif268 appears to reflect ongoing synaptic activity, and thus the protein may be involved in *maintenance* of neuronal function, the expression pattern of c-Fos (or to be more precise AP-1 containing c-Fos) could be explained by either *replenishment* or *information integration* approaches to understand the role of gene expression in neuronal plasticity.

MORPHOLOGICAL AND FUNCTIONAL REORGANIZATION IN THE VISUAL SYSTEM AFTER TREATMENT OF THE DEVELOPING RETINA WITH APB

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The gradual restriction of initially multistratified ganglion cell dendrites into ON and OFF sublaminae of the IPL can be arrested by treating the developing retina with APB, the metabotropic glutamate agonist (Bodnarenko & Chalupa, Nature, 1992: Bodnarenko et al. J. Neurosci., 1995). To assess the possible functional consequences of such treatment, cats were administered a daily injection of APB from P3 until P30, with a 2 day respite on week-ends. When the animals were at least 3-months of age, extracellular recordings were made from the A and A1 laminae of the dLGN and receptive field properties were examined using computer-controlled stimulus presentations. In the dLGN layers innervated by the normal eye, all cells responded to small spots of light centered on the receptive field with either ON or OFF discharges. In marked contrast, about 40% of the cells in the layer innervated by the APB-treated eye responded to such stimuli with ON-OFF discharges. Such responses were elicited from all regions of the receptive field. At the end of the recording session, retinal ganglion cells were filled with HRP and both control and treated retinae were analyzed. Our results show, that the incidence of ON-OFF cells was nearly identical to the proportion of ganglion cells with multistratified dendrites in the APB-treated retinas.

ELIMINATION OF CALLOSAL AND CLAUSTRAL PROJECTIONS TO CORTICAL AREAS 17 AND 18 IN CATS WITH NEONATAL SEROTONERGIC LESION.

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We studied the influence of serotonin depletion on the process of developmental elimination of callosal axons. Experimental kittens were injected on the first two postnatal days with desipramine and one hour later with 5,7-dihydroxytryptamine. At the age of three months injections of Fast Blue were done into the cortical areas 17 and 18 of one hemisphere in the experimental and normal cats. In the normal animals callosaly projecting neurons were limited to the transient zone of area 17/18 and the part of area 18 representing visual field up to 10-15 deg from the vertical meridian. In the group of serotonin depleted cats callosal connections of visual areas were largely expanded. Besides of the 17/18 transient zone, they covered the representation of 5-10 deg from the vertical meridian in the area 17 and almost all of the area 18. Claustrum was another structure that changed pattern of projection after serotonin lesion. In the ipsilateral claustrum of the serotonin depleted cats total number of the labelled neurons was increased by 30-50%, while in the contralateral claustrum the labelled neurons were 2-3 times more numerous. These results show that depletion of serotonin disturbs and decreases the retrograde process of partial elimination of excessive projections that finally shapes the cortical connections.

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Symposial 4 - Molecular aspects of physiological and pathological aging in the brain

Dietary manipulation which restores the age-related decrease in membrane arachidonic acid in rat hippocampus reverses some age-related impairments in synaptic function.

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Ageing is accompanied by a number of changes in synaptic function in rat hippocampus. These changes include an impairment in ability of aged animals to sustain long-term potentiation (LTP) in the dentate gyrus, which correlates with a decrease in glutamate release. Data from several experiments have indicated that LTP is accompanied by a persistent increase in glutamate release; evidence suggests that the increase in glutamate release relies on coincident stimulation of phospholipase Cβ (PLCβ) and PLCγ by the metabotropic glutamate receptor agonist, ACPD, and arachidonic acid respectively. We have found that this interaction is impaired in aged animals. Although the underlying cause of these age-related changes is not known, it has been speculated that the decreased membrane arachidonic acid concentration, which contributes to increased membrane rigidity, may play a role. This hypothesis predicts that if membrane fluidity is restored in aged animals, then the age-related changes in synaptic function should be reversed. Evidence will be presented which indicates that dietary supplementation with arachidonic acid and gammalinolenic acid restores membrane arachidonic acid concentration in 22 month-old rats to values observed 4 month-old rats and that this change is accompanied by a reversal of the age-related (1) impairment in ability to sustain LTP, (2) decrease in glutamate release and (3) the compromised release response to ACPD and arachidonic acid. The possibility that increased expression of the proinflammatory cytokine, interleukin-1β, may contribute to some of the age-related impairments in hippocampal function will be discussed.

RECEPTOR MEDIATED SECOND MESSENGERS FORMATION AND FUNCTION IN AGED BRAIN. MODULATORY EFFECT OF AMYLOID β PEPTIDES:

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A variety of neurotransmitters, hormones, growth factors and constituents of the extracellular matrix cause a stimulation of phosphoinositides and hydrolysis of phosphatidylcholine by phospholipases C, D and A_2. GTP-binding proteins, Ca^{2+} ions and protein kinases are involved in the receptor -mediated regulation of these enzymes. The substrates and products of phospholipases play a significant role in signal trasduction, vesicle flow and trafficking. The second messengers produced by phospholipase C (PLC) and PLA_2: inositol 1,4,5-trisphosphate (IP_3), arachidonic acid (AA) and phosphatidic acid, the main mediator produced by phospholipase D (PLD) have been implicated in regulation of cytosolic Ca^{2+} concentration and mechanisms of learning and memory. We observed that brain aging significantly and selectively decreases receptor mediated, IP_3 dependent Ca^{2+} mobilisation and arachidonic acid release and enhances phosphatidic acid formation in brain cortex. Phosphatidylinositol transfer proteins, that has been implicated to play an essential role in PLC-mediated phosphatidylinositol-4,5-bisphosphate (PIP_2) hydrolysis, as well as in membrane fusion and budding processes were not affected during brain aging. In spite of alteration of lipid derived second messengers, aging diminished NMDA-receptor mediated NO dependet cGMP formation in hippocampus and cerebellum. Our *in vitro* studies on the action of amyloid β peptides (Aβ25-35, Aβ1-28 and Aβ1-40) on cholinergic and NMDA receptor mediated second messengers system showed a significant modulation of IP_3, AA and cGMP dependent processes, involved in regulation of Ca^{2+} homeostasis. These results suggest that amyloid β may be involved in alteration of neurotransmission and signal transduction processes in aged brain. The recent cloning of eucariotic phospholipases and NO synthase genes, also from human sources uncovered a novel gene family, whose members may be involved in various aspect of signal trasduction and membrane trafficking. These new findings will be helpful for the better understanding of aged related alterations occuring in brain.

POSSIBLE ROLE OF POLY(ADP-RIBOSE)SYNTHETASE IN NEURONAL DEGENERATION

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Poly(ADP-ribose) synthetase (PARS) (also termed polymerase; PARP)is a nuclear enzyme that catalyzes formation of protein-bounce $(ADP\text{-}ribose)_n$ (n=2 ~ 100<) from NAD^+. PARS is composed of three functional corniness, i.e. DNA binding(N-thermal), automodification (central), and catalytic (C-terminal). The enzyme activity is markedly stimulated by damaged DNA.

Although the precise functions of PARS remain to be elucidated, there is accumulating evidence suggesting its roles in DNA repair, genome surveillance, and cell differentiation. Furthermore, PARS has been identified as a substrate of ICE-like protease ("caspases") in apoptosis. We recently found that PARS is phosphorylated by DNA_dependent protein kinase in apoptotic lymphocytes and that the phosphorylation accelerates the cleavage of PARS at a site within the nuclear localization signal. The subsequent loose of nuclear PARS would liberate endonuclease(s) from suppression by (ADP-ribose)n and lead to nucleosomal DNA strand breaks, a hallmark of apoptosis. Activation and cleavage of PARS were also observed in neuronal cells exposed to ionizing radiation, respectively.

These results suggest that the functional PARS is essential for survival of neuronal cells under variously stressed conditions and its degradation may serve for neurodegeneration.

HB-GAM IS A NOVEL AMYLOID ASSOCIATED PROTEIN IN CEREBRAL AMYLOIDOSES.

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HB-GAM (heparin binding growth associated molecule) was recently described as a novel amyloid associated protein in amyloid β (Aβ) cerebral amyloidoses of Alzheimer's disease and Down's syndrome. Antibodies to HB-GAM immunolabelled both neuritic and diffuse (preamyloid) plaques, only when markers of neuronal injury were present, as well as a subset of neurons containing abnormally phosphorylated tau epitopes (1). It was suggested that HB-GAM is one of the co- factors associated with the cerebral plaques of Aβ and acts as a marker of neuronal injury. To explore the role of HB-GAM in other cerebral and systemic amyloidoses as well as its role as a marker of neuronal injury we performed immunohistochemical studies with anti- HB-GAM antibodies in 3 cases of Creutzfeld- Jakob disease (CJD), one with Gerstmann-Sträussler- Scheinker syndrome (GSS), one with amyloidosis of British type, 1 case of novel meningocerebrovascular amyloidosis of Hungarian type, 3 cases of hereditary cerebral haemorrhage with amyloidosis- Dutch type, 3 cases with light chain deposition and one case of gelsolin related amyloidosis. It was found immunohistochemically that HB-GAM is a co- factor associated with cerebral amyloid deposition, both in parenchymal and vessel amyloid lesions. It was absent in the systemic amyloid deposits, which we tested. The presence of HB-GAM correlated well with the presence of other amyloid associated proteins: apolipoproteins E and J, and proteoglycans in the cerebral amyloid deposits. In order to establish a role of HB-GAM as a marker of neuronal injury in certain brain regions after ischemic insult we also started preliminary studies using Western blotting with anti- HB-GAM antibodies and densitometric analysis on homogenates from different regions of the rat brain. To further explore the role of HB-GAM in amyloid deposition we performed *in vitro* studies with Aβ synthetic peptides. These *in vitro* studies showed that HB-GAM can form a high affinity complex with several Aβ peptides, with a binding constant KD= 12.1 nM for Aβ1-40, KD= 13.0 nM for Aβ1-28 and KD= 22.1 nM for Aβ1-42. The complex was concentration dependent and partially resistant to SDS, as evidenced after Laemmli electrophoresis of the preformed complex and Western blotting analysis, using anti- Aβ and anti- HB-GAM antibodies. HB-GAM was also able 3-4 fold to increase the rate of fibrillogenesis of Aβ1-40, as evidenced by using Thioflavin T method. We suggest here that HB-GAM is more specific than many other amyloid associated proteins, as we have found its distribution to be limited to cerebral deposits. HB-GAM may influence amyloidogenesis by the formation of high affinity complexes with amyloid peptides. We suggest here that in addition to its known properties as a neurite- outgrowth promoter, HB-GAM has a role a local tissue factor associated with cerebral amyloid deposits.

1. Wisniewski et al. *Neuroreport*, (1996), 7, 667-671. Supported by NIH grants NS 30455 and AG 08721, Sandoz Foundation for Gerontological Research grant and State commitee for Scientific Research grant (4.P05A.020.11) to M.L. M.L is a recipient of Foundation for Polish Science grant.

APOLIPOPROTEIN E AND DEMENTIA

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Human apolipoprotein E (apo E) is synthesized in various organs including the brain. It is a component of plasma lipoproteins serving as a ligand for cell receptors and participates in lipid transport. It plays also a role in cholesterol redistribution among cells and has an important function in the regeneration of nerves.

Apo E is polymorphic and appears in three isoforms named 2, 3 and 4 differing from each other by a single amino acid substitution and coded by three alleles at a single gene locus. The most common isoform is apo E3. Carrying a particular apoE isoform has an influence on plasma lipid level of an individual.

It was stated that apoE is a component of Alzheimer plaques and neurofibrillary tangles accompanying amyloid depositions. It was also observed that in patients with late onset Alzheimer disease apo E4 isoform is more frequent as compared with general population. On the contrary, it appears that apo E2 isoform is inhibiting the onset of Alzheimer pathology. Apo E4 frequency in other types of dementia is still controversial.

Studies on possible mechanisms of particular apo E isoforms contribution in the development of dementia proceed into several directions. Investigations concern their binding with amyloid, their modulation of neurite extension, their interaction with microtubule associated protein.

Poster session - Development

CALCIUM-BINDING PROTEINS IN THE MATURATION OF THE RAT BASOLATERAL AMYGDALA

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In different species the basolateral amygdaloid complex consists of nuclei which have different cyto- and chemoarchitectonic characteristics. The function of this complex includes the emotional, motivational and memory processes.

The present study is a part of an ongoing study aimed at elucidating the morphology of the amygdalar nuclei during the maturation and, in particular, the distribution of different population of inhibitory neurons. Studies of other authors have shown that about 20% of neurons in the basolateral complex are GABAergic. These cells are composed of different subpopulations of neurons which contain either calbindin-D28k or parvalbumin. In the present study, we describe the distribution of cells, fibers and terminals that are immunoreactive either to parvalbumin or calbindin D28k in the basolateral complex during the maturation of the brain.

A total of 40 brains of animals at various ages starting from P0 to P60 were available in this study for immunohistochemical analysis. Care and treatment of the animals were in accordance with the guidelines for laboratory animals established by the National Institute of Health as well as by the local ethical committee. After perfusional fixation the brains were frozen and cut on the Jung cryostat 1800 in the coronal plane, and stained either with cresyl violet or standard immunohistochemical methods, using the anti-parvalbumin and anti-calbindin D-28k antibody.

Calbindin positive cells appeared just after birth and reached the maximum of density at P5 in the basolateral nucleus and at P21 in the lateral nucleus. The parvalbumin positive cells are present since P17 in the lateral nucleus and since P30 in the basolateral nucleus with the maximum peak at P21 and P30 respectively. During that time staining of both types of immunopositive cells became more intensive and dendritic arbor enlarged. The process of maturation ended at P90 when the immunopositive neurons showed long, ramified dendrites, especially for parvalbumin-positive cells; at that time the parvalbumin-immunoreactive varicose fibers and axon terminals were present around the unstained neurons and formed basket-like plexi and cartridges.

The distribution of the calcium-binding proteins in the basolateral complex during the maturation has not been described yet. Therefore, this study may provide, basic information to study the development of organization of GABAergic inhibitory circuitries in the amygdala.

SYNAPTOPHYSIN IMMUNOREACTIVITY IN THE BASOLATERAL AMYGDALA AND HIPPOCAMPUS DURING THE MATURATION

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Synaptophysin is an integral membrane protein associated with small, electron-lucent synaptic vesicles. Immunohistochemistry for synaptophysin is a sensitive method to study subtle changes in synaptic density and their distribution in various brain regions.

In the present study, the synaptogenesis was examined in the rat basolateral amygdala in comparison to the hippocampus, from the day of birth to adulthood. A total of 40 brains at various ages starting from P0 to P60 were examined. Care and treatment of the animals were in accordance with the guidelines for laboratory animals established by the National Institute of Health as well as by the local ethical committee. After perfusional fixation the brains were frozen and cut on the Jung cryostat 1800 in the coronal plane and stained either with cresyl violet or standard immunohistochemical methods using the anti-synaptophysin antibody.

Synaptophysin positive granules appeared just after birth in both structures, but their number was very low (about $0{,}18 \times 10^6$ and $0{,}19 \times 10^6$ per mm^3 in the amygdala and hippocampus respectively). In the basolateral amygdala the number of synapses increased up to $1{,}4 \times 10^6$ per mm^3 at P14 being later stabile. In the hippocampus two increases of the synaptogenesis were observed. The first at P7 (about $1{,}9 \times 10^6$ of synapses per 1 mm^3) which was followed by dramatic decrease up to $0{,}7 \times 10^6$ per mm^3 at P14. The second increase appeared later (about P90) and reached $2{,}1 \times 10^6$ per mm^3). After that time the density of synapse was stabile.

It may be supposed that the first characteristic wave of synaptogenesis observed in the hippocampus is due to the overproduction of synapses observed at that time in other cortical regions. The second wave of synaptogenesis found both in the hippocampus and amygdala is related to the great plasticity of the interneuronal connections in this period of development.

POSTNATAL DEVELOPMENT OF THE RAT CAUDATE-PUTAMEN - A STUDY USING *IN SITU* DNA END LABELING TECHNIQUES

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Caudate-putamen (dorsal striatum) is a part of the rat striatum which receives inputs mainly from the neocortex and substantia nigra. It is well known that in many regions of the brain a large number of neurons die during the development and maturation, however, we have no data about this process in the caudate-putamen. Morphological evidence of apoptotic cell death include cell shrinkage, condensation of nuclear chromatin and appearance of apoptotic bodies. At the biochemical level, apoptosis is attended by DNA fragmentation, which can be detected using *in situ* DNA end labeling technique (TUNEL method).

In the current study we calculated the number of apoptotic cells during postnatal maturation of the caudate-putamen using either cresyl violet staining or TUNEL method.

27 rat brains of various ages (postembrionic days: P0, P1, P2. P4, P5, P7, P8, P9, P10) were studied. Care and treatment of the animals were in accordance with the guidelines for laboratory animals established by the National Institute of Health as well as by the local ethical committee. All animals were decapitated under general anesthesia; after perfusion fixation, their brains were removed and placed in formalin solution. 10-μm-thick paraffin, serial sections were cut and stained with cresyl violet or TUNEL method.

We found that the postnatal development of rat caudate-putamen is related to the massive physiological death of their neurons. The greatest number of TUNEL-positive and pyknotic cells per section were observed in this structure during the first week of the postnatal life (from about 40 TUNEL-positive cells per section at P1 to 28 TUNEL-positive cells at P7); later a decrease in number of apoptotic cells was observed (about 5 TUNEL-positive cells per section at P10).

The appearance of physiological cell death during the development of the caudate putamen is probably a consequence of the elimination of neurons that have failed to receive „appropriate" neocortical and nigral afferents.

THE INSULAR AND PREPIRIFORM PARTS OF THE RAT CLAUSTRUM SHOW DIFFERENT PATHS OF DEVELOPMENT

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The claustrum is an important, telencephalic structure. It is composed of two clearly separated parts: the dorsal - insular claustrum and the ventral one - prepiriform claustrum.

As the development of both parts of the claustrum is still a matter of discussion, we tried to find similarities and differences in their development and maturation using morphometric and *in situ* DNA end labeling (TUNEL) methods.

45 rat brains of various ages (embryonic days: E15, E17, E21, postembrionic days: P0, P1, P2. P4, P5, P7, P8, P10, P21, P60, P90, P180) were studied. Care and treatment of the animals were in accordance with the guidelines for laboratory animals established by the National Institute of Health as well as by the local ethical committee All the animals were decapitated under general anesthesia; after perfusional fixation their brains were removed and placed in formalin solution. 10-μm-thick paraffin, serial sections were cut and stained with cresyl violet or were studied by using the *in situ* specific labeling of fragmented DNA (TUNEL method). Quantitative estimations of the neuronal cells population of the claustrum were performed using the morphometric methods.

On about 20th day of prenatal life the rat's claustrum is visible as a separate structure; the morphology of neurons in the insular part of this structure shows that they are less mature than neurons in the prepiriform one. Between E20 and P21, a rapid decrease of neuronal density, increase of cross-sectional area of neurons, and their nuclei and increase of volume of the insular and prepiriform claustrum were observed. According to our results, morphological features of the claustrum and its neurons do not change after 3rd week of postnatal life - neurons of both parts achieve morphological maturity. As at stage E20, the insular part of the claustrum is less mature than the prepiriform one, we may assume that dynamics of morphological changes during the development is higher in the insular claustrum.

Only in the insular claustrum we found a 30% decrease in the total number of neurons during the second week of life. The loss of these neurons, confirmed by the TUNEL method, was caused by the physiological death.

According to our results, the development of the insular and prepiriform claustrum shows striking differences which are probably based on specific functions and connections of each. These differences are expressed by higher dynamics of development and the occurrence of physiological neuronal death in the insular, phylogenetically younger part of this structure.

PARVALBUMIN IMMUNOREACTIVITY IN THE THALAMIC RETICULAR NUCLEUS

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The thalamic reticular nucleus (Rt) is a thin sheet of cells interposed between other thalamic nuclei and the internal capsule. It is composed of GABAergic cells that receive information from both thalamocortical and corticothalamic fibers. The cells in Rt are relatively homogenous. Most of them possess long dendrites which ramify up to the boundaries of Rt. GABAergic neurons of Rt hyperpolarize cells in other thalamic nuclei inhibiting their activity.

Parvalbumin belongs to calcium binding proteins. It is present in many neuronal populations of the brain, especially in GABA-ergic neurons, and plays a prominent role in calcium dependent mechanism at all stages of development. The main aim of our study was to describe the pattern of parvalbumin reactivity in the reticular nucleus during maturation.

The study was performed on 30 rats on various postnatal days. The following stages were examined: P0, P1, P2, P4, P5, P7, P10, P14, P17, P21, P30 and P90. The animals were anaesthetized and cut coronally on cryostat. Sections were incubated for three days with a mouse monoclonal antibody against parvalbumin (Sigma 1:1000). Tissue-bound antibodies were detected using the avidin-biotin peroxidase method as indicated by the manufacturer (Vector Labs, Bulingame, CA). Immunohistochemical control, in which the primary antibody was omitted did not show immunostaining.

In the central part of Rt which appeared at the earliest, the parvalbumin immunopositive cells were present for the first time at P4. Later, at P10 neurons of the central part showed quite well developed axons and at P21 numerous parvalbumin-positive axon terminals appeared. In the medial and lateral parts the cells and axonal terminals became slightly immunopositive at P10 and at P21 respectively. At P30 all parts of Rt were well developed, similarly to those in mature animals (P90).

Our observations show that maturation of parvalbumin-positive structures in Rt proceed during the first month of postnatal life.

DOES THE VOLUME OF THE CLAUSTRUM CORRELATE WITH THE DEVELOPMENT OF THE NEOCORTEX IN MAMMALS? - STEREOLOGIC STUDY

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The claustrum is a structure which posses connections with almost all cortical regions. In most of mammals two parts of the claustrum can be easily distinguished: larger - the insular claustrum and smaller - the prepiriform. The former send projection to neocortical areas, whereas the latter - to allocortical. In Primates, including Man, the subdivision of the claustrum into two parts is not so clear. The aim of our study was to find whether the size of the claustrum corresponds to the development of the cortex.

The investigations were performed on 27 brains of various mature mammals (Insectivora - *Sorex sp.*; Rodentia - Rat, Mouse, Guinea pig; Lagomorpha - Rabbit; Carnivora - cat, Primates - *Cercopithecus sp.*, *Macaca sp*, Man). The brains after removal from the skull were fixed in 8% solution of buffered formalin, and then processed according to standard histological procedure to obtain the set of paraffin sections stained with cresyl violet. The set of morphometric data concerning the volume of the brain hemisphere, cortex (iso- and allocortex) and claustrum were collected. We subdivided animals under study into lissencephalic (Insectivora, Rodentia and Lagomorpha) and gyrencephalic (Primates and Cat).

In all animals the total volume of the claustrum correlates positively with the volume of the brain and cerebral cortex. In lissencephalic mammals the increase of the volume of each part of the claustrum (insular and prepiriform) and the increase of volume of each main type of the cortex (allo- and isocortex) is similar and correlates with the increase of brain volume. In gyrencephalic mammals the increase of the total claustral volume is significantly higher than that of the allocortex and lower than that of isocortex.

We may conclude that claustrum enlarges with the development of the neocortex, but in smaller degree than the latter; that may have an impact on functions of the claustrum.

LACK OF INFLUENCE OF THE NEONATAL SEROTONERGIC LESION ON THE FORMATION OF BARREL FIELD OF SOMATOSENSORY CORTEX IN THE HAMSTER AND RAT

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In rats, after neonatal depletion of serotonin, cross-sectional area of bundles of thalamocortical axons conducting sensory input from single vibrissae to the somatosensory cortex was shrunk by about 20% (Bennet-Clarke et al, 1994). In this study we attempted to investigate maps of barrels after serotonergic lesion using the method of staining for cytochrome oxidase, compared rats with hamsters, that are born at an earlier stage of development. 5,7-dihydroxytryptamine (5,7-DHT) was injected subcutaneously in newborn animals. Desipramine was injected one hour earlier to protect noradrenergic neurons. After 14-30 days of survival, control and experimental animals were perfused with 4% paraformaldehyde. Cerebral cortex was cut tangentially to the barrel field on vibratome and the 50 μm sections were stained with cytochrome oxidase. Area of each patch, representing a barrel was measured with the Imaging Research MCID image processing system. We found that neither in rats, nor in hamsters there were any differences between the cross-sectional area of patches between the normal and serotonin depleted animals. For example, the area of the cytochrome oxidase patches corresponding with the barrels C2 was 0.103 ± 0.024 mm^2 in control rats and 0.108 ± 0.015 mm^2 in the 5,7-DHT injected animals. Similar results of measurements of the patches C2 (0.066 ± 0.0028 in control and 0.07 ± 0.0083 in the 5,7-DHT injected) were obtained in hamsters. Therefore, developmental depletion of serotonin does not seem to permanently influence the morphology of thalamocortical connections.

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PERIPHERAL NERVE GRAFTS IMPLANTATION INTO THE OPTIC NERVE AND THEIR INFLUENCE ON THE REGROWTH OF 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 predegenerated peripheral nerve grafts facilitate neurite outgrowth from the injured hippocampus and that this effect is particularly distinct when 7-, 28- and 35-days predegenerated nerve grafts were used. The purpose of the present paper was to induce and support the regrowth of injured nerve fibres as well as the survival of retinal ganglion cells. Experiments were carried out on adult male Sprague-Dowley rats. Animals were assigned into three equal groups. In the first and second group, fragment of optic nerve was excissed and subsequently a fragment of peripheral nerve was sutured into the site of excission. In the first group, the implanted nerve was freshly taken but in the second one it had been predegenerated for 7 days. In the third, control group, optic nerve was totally transected, sparing the ophtalmic artery, and both ends of cut nerve were sutured one to another. Six weeks folowing surgery fluorescent dyes were applied: FITC-DiI into the end of implants and rhodamine B to the *corpus vitreum*. After 48 hrs animals were perfused transcardially and the nerves and retinas were subjected to histological 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 predegeneration of grafts enhance their neurotrophic influence upon the injured retinal ganglion cells. In histological specimens it could be clearly observed profuse ingrowth of nerve fibres steming from the retina into the grafts. The number of surviving retinal ganglion cells was higher in the group treated with predegenerated grafts.

Małgorzata Bruska, Witold Woźniak, Piotr Kromer

Differentiation of the common afferent tract in early human embryos

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The common afferent tract is formed by afferent fibers of the ganglia of the cranial nerves that enter the brain stem and reach the developing sensory nuclei. This tract contains descending fibers of the 5, 7, 9, 10 cranial nerves, and with advancement of the development is divided into solitary tract, trigeminospinal and vestibulospinal tracts.

Present study was performed in 9 serially sectioned human embryos of developmental stages 13, 14, and 15 (5th week) belonging to the Collection of the Department of Anatomy in Poznań. In some of embryos graphic reconstructions were made.

The common afferent tract appears first in embryos at stage 13 (31 days) . All sensory ganglia of the cranial nerves are already delineated, but they are not fully differentiated. The common afferent tract forms a thick band of fibers on the ventral part of the future brain stem . It begins from the entrance of the afferent fibers of the trigeminal nerve and extends through the whole length of the cephalic neural tube, beyond the cervical flexure.

At the end of the fifth week (embryos of stages 14 and 15) the common afferent tract is joined by fibers from the vestibular nuclei. These fibers are passing on the more ventral surface of the brain, most superficially, close to the surface of the brain stem .

Witold Woźniak, Małgorzata Bruska

The development of the subthalamus in human embryos during 6th week

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Study was made on 11 embryos of stages 15, 16, and 17 (36 to 41 days). All embryos belong to the Collection of the Department of Anatomy in Poznań and they are catalougued according to developmental staging system. Embryos were serially sectioned in different planes (horizontal, frontal, and sagittal) . In 6 embryos every second or third section was impregnated with silver.

The first indication of the hypothalamus is observed in embryos of stage 15 in which the hypothalamic sulcus appears. The hypothalamus at this stage consist of subthalamus and hypothalamus proper. The subthalamus and the the hypothalamus proper are declineated by shallow sulcus which is parallel to the hypothalamuc sulcus. Between the oculomotor nucleus and the mamillary area are crossing fibers which form the supramamillary commissure. These fibers are in close relation to the group of cells in the subthalamic area. This group may be considered as primordium of the subthalamic nucleus.

In embryos at stage 16 the subthalamus is represented by large area rich in penetrating capillaries which are particularly evident in the wide intermediate layer of the subthalamus. On the periphery of of this layer pass hypothalamic fbers. The wide intermediate subthalamic zone is the source of subthalamic nuclei which may be distinguished during stage 17 in which the ventricular layer of the subthalamic region is much thinner than that of the hypothalamus.

DOES SENILE IMPAIRMENT OF CHOLINERGIC SYSTEM IN RATS CONCERNS DISTURBANCES IN ChAT EXPRESSION OR THE ACUTE DEGENERATION OF NEURONAL CELL BODIES?

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Trophic effect of nerve growth factor (NGF) on basal forebrain (BF) cholinergic neurons, a neuronal population affected during normal and pathological aging, was tested in young (4-mo-old) and aged (28-mo-old) Wistar rats. NGF was injected intraventriculary via osmotic minipump in the total dose of 80 µg. After one-month, continuous NGF delivery the morphometric parameters of BF cholinergic neurons were analyzed in medial septum, diagonal band of Broca, and magnocellular basal nucleus. Immunohistochemical procedure for ChAT and NGFp75 receptor has been applied to identify BF neurons as cholinergic. There was a reduction by as much as 60 - 90% in the number of intensely ChAT-IR profiles in all BF structures of aged non-treated rats when compared with the young ones. ChAT-IR cells appeared vacuolated and shrunken, and the neuropil staining was markedly reduced. In contrast, these same neurons stained for NGFp75r-IR were distinctly visible with perfect morphology, regardless of the age of the animals. The cell bodies were densely immunoreactive, with tapering, vericose dendrites forming an intensely stained neuropil. Treatment with NGF resulted in a restoration of cholinergic function in the BF neurons of aged rats. NGF increased a number of ChAT-IR cells and caused a significant hypertrophy of these cells in 28-mo-old rats when compared with the control, age-matched group. NGF did not influence the morphology of NGFp75r-IR neurons, which were labeling well, irrespective of treatment and age of the rats.

The ChAT staining suggests that exogenous NGF can reverse age-related impairment in cholinergic phenotype of the BF neurons. The results of NGFp75r staining provide some evidence for preservation of these BF cholinergic neurons from atrophy of cell bodies and processes during aging. These data indicate that senile impairment of cholinergic system in rats concerns disturbances in ChAT expression rather than the acute degeneration of neuronal cell bodies per se.

Movement and posture interaction: age-related decline

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Decline of integrity in many physiological systems in the elderly has a profound effect on postural stability control. Many systems function at less than optimal level; thus, the postural stability control system relies on degraded information. One of the most commonly used experimental strategy employs the measurement of an input-output characteristics of the human body during upright stance. These characteristics has been studied using sine-wave input produced by subjects themselves. The input signal consisted of the torques produced by the manipulandum movement and the output was a compound ground reactive forces as recorded by the force platform. Fifteen elderly (72.0± 8.1 years) and fifteen young (22.7±1.6 years) adults, participated in this experiment. The results confirmed decline with the age of the maximum speed. The results exhibited also a different performence of the tasks for different loads. The low resistance trials did not differe significantly in both groups. Such differences were, however, seen in the high loads in fast speed conditions. The elderly subjects reduced their postural destabilzation by either decrease of the movement range or by slowing down the movement speed.

POSTNATAL DEVELOPMENT AND MATURATION OF ADRENAL MEDULLARY CHROMAFFIN CELLS OF THE GUINEA PIG : A HISTOCHEMICAL AND BIOCHEMICAL STUDY.

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Numerous studies on differentiation of phenotypes of adrenal medulla chromaffin cells (CHc) in guinea pigs indicated that epinephrocytes (Ec) i.e. adrenaline(A)-cells were the only cells observed in those sexually mature animals, and no norepinephrocytes (NEc) i.e. noradrenaline(NA)-cells were detected (Coupland 1965, 1989; Kmieć 1976). There are nevertheless reports showing the presence of low number of NEc in adrenal medulla of those animals (Eränkö 1952, 1955; Unsicker et al. 1978).On the basis of the mutual developmental line of CHc (Anderson 1993; Unsicker 1993) originating from sympathoadrenal progenitors, and the fact that in the course of CHc differentiation NEc becomes the intermediate stage in establishing Ec phenotype maturation of those cells in the PD of the adrenal medulla in guinea pigs was investigated. There were also the attempts to determine with great precision the day of the PD in which the final and the only phenotype of adrenal medullary CHc in those animals i.e. the phenotype of Ec was established.

Adrenal glands of the guinea pigs aged 1, 3, 5, 7, 9, 11, 13, 14, 15, 18, 21, 28, 35 and 90 days (i.e. sexually mature animals) were used in the studies. Catecholamines (A and/or NA) were revealed employing histochemical methods according to Kmieć (1969), Jones (1967) and Hopsu and Mc Kinen (1966). The contents of both NA and A in adrenals were evaluated by HPLC with electrochemical detection (Nowak et al. 1992).

We showed the presence of NEc in the guinea pigs' adrenals till the age of approximately 14 day of PD. Gradual maturation of NEc to Ec happens this time. This explains the lack of NEc in the adrenals of sexually mature guinea pigs.

DIFFERENTIAL EXPRESSION OF MICROTUBULE-ASSOCIATED PROTEIN 1B PHOSPHORYLATED ISOFORMS IN THE DEVELOPING MOUSE BARREL CORTEX.

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Microtubule associated proteins (MAPs) are a family of proteins which show heterogenous spatial and temporal distribution within neurons and are thought to be involved in neuronal plasticity. Among them, MAP-1B is of special interest for it is elevated during neuronal development and down-regulated upon maturation. Two modes of phosphorylation have been identified for MAP-1B which show distinct developmental regulation.

To establish developmental changes in the distribution of the two MAP-1B phosphorylated isoforms in the mouse barrel cortex - a part of the somatosensory cortex which shows structural and functional plasticity - we performed immunohistochemical studies using monoclonal antibodies 150 and 125 against the modes I and II, respectively.

The MAP-1B phosphorylation modes I and II showed distinct developmental pattern of distribution in the mouse barrel cortex. The 125 immunoreaction first appeared in supragranular layers and in layer IV as faint stripes of puntcuate fibers on P5, then strenghtened on P12, to eventually establish a mature appearance on P21 when the immunoreaction became visible also in pyramidal cell perikarya. The 125 positive profiles were identified as pyramidal cell apical dendrites. The 150 immunopositive fibers were detectable on P5 in infragranular layers. Upon maturation the 150 immunoreaction diminished on P8 and P10 and was no more detectable on P12. On P21 the immunoreaction re-appeared in layers II/III, IV and V.

The differential and changing distribution as well as the re-appearance of immunoreactivity in the adult barrel cortex implies a possible involvemet of the investigated phosphorylated isoforms of MAP-1B in neuronal plasticity induced after the critical period for the barrels formation has ended.

Poster session - Neuropathology

PHAGOCYTOTIC CLEARANCE IN BRAIN APOPTOSIS

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Phagocytotic clearance is the process essential for restoring homeostasis at the site of the tissue injury. In the brain, phagocytosis is primarily the function of microglia, cells of which have to be transformed from resting to the active ameboid form. The transformation is stimulated by some biologically active compounds excreted by necrotic cells. In apoptosis, such compounds are not produced and microglia is not activated. The aim of the study is to answer the question which cells of the brain parenchyma participate in phagocytotic clearance of apoptotic bodies. Study was performed on rabbits. Brain apoptosis was induced by intraperitoneal injection of 0.25 mg vincristine sulfate per kg of body weight. Samples of the brains were embedded in epon and examined in electron microscope. As soon as two hours after vincristine injection, apoptotic cells and numerous apoptotic bodies were found in the tissue. Initially, apoptotic bodies were scattered throughout the brain, but four and six hours after vincristine administration, most of them were engulfed and subsequently digested by the adjacent cells. These cells included oligodendroglia, astroglia, microglia and pericytes. Seven days after treatment apoptotic bodies were sporadically found in cytoplasm of some those cells. Our results show that following brain apoptosis various type parenchymal cells are engaged in immediate removing of apoptotic bodies and suggest that these cells employ different than in necrosis, mechanism of recognition and ingestion of tissue debris.

^{1}H AND ^{31}P MAGNETIC RESONANCE SPECTROSCOPY OF THE BRAIN AND SPINAL CORD DURING ISCHEMIA AND REPERFUSION.

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Different methods are used to study pathological changes of the central nervous system under normal and pathological conditions. Nuclear magnetic resonance is a physical technique that has been used as an analytical method, to describe the structure of molecules in a specific solution. When the sample is placed in a strong static magnetic field, the atoms that have magnetic properties (e.g. 1H, 31P, 13C, 19F, etc.) interact with a pulsed radio frequency field. If the frequency of this field is in resonance with the frequency precision of the spins in the static magnetic field, it is possible to obtain information regarding the atom's fragment chemical structure, from the intensity, phase and frequency of the interaction. Magnetic resonance imaging (MRI) with high resolution images offers the possibility to study some morphological changes following ischemic brain injury, while magnetic resonance spectroscopy (MRS) allows study of certain biochemical changes, and metabolic pathways in vitro and in vivo. The great advantage of MRS is that we are allowed study of these biochemical events in real time without any disturbance of the tissue.

Investigation of brain and spinal cord changes, with ischemic - reperfusion injury using ^{1}H and ^{31}P MRS applications both *in vivo* and *in vitro* was the study's focus. The spectrometers SISCO 200/330 (4.7 T) and Varian VXR 300 MHz (7.5 T) were used for measurements. The *in vitro* study of brain tissue perchloric acid extracts, using the Varian VXR 300 MHz spectrometer, showed that with the ^{1}H spectrum it is possible to detect a few metabolites with low molecular weight. The most expressive signals were produced by creatine, inositol, aspartate, choline, N-acetylaspartate, glutamate, glutamine and lactate. During the *in vivo* study of ischemic brain tissue it was possible, using the spectrometer SISCO 200, to obtain a spectrum from the volume of interest at 150 µl. The spectral lines were broader than in the *in vitro* spectra; signals from the lactate, N-acetylaspartate, creatine, phosphocreatine and choline were detected as well. The most important findings were observed in the time - dependent increased lactate level, and in the small decreases of N-acetylaspartate. ^{31}P *in vivo* measurement shows an intensive signal from the α, β, γ ATP phosphate group, phosphocreatine (PCr), inorganic phosphate (Pi), phosphodiesters and phosphomonoesters. It was possible to detect that the signals from ATP and PCr disappeared from the spectrum, and the Pi signal became very intense after 40 minutes of ischemia It seems that MRS is a very promising method of ischemic - reperfusion injury investigation.

TRANSIENT SPINAL CORD ISCHEMIA AND NADPH-d ACTIVITY

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Silver impregnation analysis of neuronal damage and concurrent histochemical characterization of NADPH-d positive neuronal pools in the rabbit lumbosacral segments was performed during and after transient spinal cord ischemia. Strongly enhanced staining of NADPH diaphorase positive neurons and their processes appeared in the superficial dorsal horn (laminae I-III), pericentral region (lamina X) of lower lumbar segments, lateral collateral pathway, and mainly in neurons of the sacral parasympathetic nucleus in S_2 segment at the end of 40 min of abdominal aorta ligation or one day after reperfusion. Despite development of extensive neuronal degeneration in the central gray matter (laminae IV-VII) between 1-4 days after ischemia, a number of non-necrotizing neurons localized in the areas corresponding with the distribution of NADPH diaphorase positive neurons was detected suggesting a selective resistance of these pools of neurons against transient ischemic insult. It is postulated that region-specific synthesis of nitric oxide and its vasodilatatory effect during the period of incomplete spinal ischemia may account for the observed selective resistance of these spinal cord neurons to transient ischemia.

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ISCHEMIA-INDUCED ALTERATIONS OF ENDOPLASMIC RETICULUM CALCIUM STORES FROM THE GERBIL FOREBRAIN.

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It is widely accepted that disturbances of calcium homeostasis play a key role in the development of cell damage following by cerebral ischemia. Endoplasmic reticulum is believed to play an important role in the neural cell Ca^{2+} handling during normal and pathological conditions. Forebrain ischemia (5 to 15 min) was induced by ligation of both common carotids and reperfusion by subsequent release of ligature. Ca^{2+} pump mediated Ca^{2+} accumulation was shown to be depressed by15 min ischemia to 37.3% of control values. The Ca^{2+} uptake activity partially recovered after 1 hour reperfusion, however it still remained depressed to 68.1% of controls. No significant changes were detected in the kinetic parameters of Ca^{2+}-ATPase after ischemia. As detected by quantitative Western blotting, transient ischemias had no effect on the level of endoplasmic Ca^{2+}-transport proteins. 10 min. ischemia and prolonged reperfusion, however selectively down-regulated levels of $InsP_3$ receptor and plasma membrane Ca^{2+}-pump, with the most profound alterations after 72 h to 10 days. No significant changes have been detected in levels of SERCA 2b protein and of calreticulin. These findings indicate that ischemic-reperfusion insult alters membrane properties of Ca^{2+}-stores which eventually could lead to their depletion. Since the filling state of endoplasmic reticulum is in a close relation to many cellular processes, including protein synthesis and gene expression, we suggest that disturbances in reticular calcium homeostasis may contribute to ischemic cell injury.

MULTIFUNCTIONAL ROLE OF CALPAIN IN BRAIN ISCHEMIA

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Calpain (Ca^{2+}-activated cysteine protease) is one of the mediators of abnormal Ca^{2+} signal in ischemic brain. It has two well characterized isoforms, μ- and m-calpain, distinguishable on the basis of different calcium requirement: m-calpain is activated at micromolar, m-calpain at millimolar free Ca^{2+} concentration. Intracellular localization and proteolytic activity of two calpain isozymes as well as breakdown products of the calpain specific substrates were investigated in global cerebral ischemia in rats and gerbils. The SDS-PAGE and Western blot analysis of calpains together with determination of the proteolytic breakdown products were performed in soluble and membrane fractions obtained after centrifugation of brain homogenate as well as in post-synaptic densities (PSD). It seems that ischemia induces two different but interrelated effects on calcium-dependent proteolytic system:

1. The rapid activation, as was detected in the present study by: increased cleavage of calpain substrates: spectrin and protein kinase C; extensive calpain association with the particulate fraction; enhanced autoproteolysis of m-calpain proenzyme to its active, post-autolytic species.
2. Gradual, time-dependent down-regulation of the total m-calpain activity with a concomitant increase of calpastatin activity.

The ability of tissue to keep these two activatory and inhibitory processes in balance might determine the final outcome from the ischemic episode.

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EFFECT OF 7-NITROINDAZOLE ON NITRIC OXIDE MEDIATED BIOCHEMICAL AND MORPHOLOGICAL ALTERATIONS EVOKED BY BRAIN ISCHEMIA.

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In this study the action of 7-nitroindazole (7-NI) on nitric oxide synthase (NOS) activity and cGMP level during different time of reperfusion until 7 days after 5 min of ischemia in gerbil brain hemispheres and cerebellum was investigated. Moreover, we tried to identify the role of NO in membrane lipoperoxidations activated during reperfusion. Animals were treated with inhibitor which appears to have some selectivity to nNOS, 7-NI, 5 min before ischemia in a dose of 25 mg/kg b.w.. It was observed that transient forebrain ischemia enhances significantly Ca^{2+}/calmodulin dependent NOS activity and cGMP level in brain hemispheres and also in cerebellum, non ischemic part of brain. During reperfusion biphasic increase of NOS activity and cGMP level took place with two peaks 15 min and 2h after ischemia. The cGMP level that enhanced during reperfusion is NO - dependent. The amount of conjugated double bounds (CDB) in membrane lipids and the level of thiobarbituric acid reactive substances (TBARS) increase significantly during reperfusion in brain hemispheres, indicated the activation of lipid peroxidation. 7-Nitroindazole (7-NI) eliminates enhancement of NOS activity and cGMP level in brain hemispheres and cerebellum evoked by ischemia. Moreover, the NOS inhibitor decreases significantly the early phase of membrane lipid peroxidation, but it has no significant effect on the level of CDB and TBARS 2 hours after ischemic insult. These results suggest, that NO is involved in activation of membrane lipid peroxidation during the early time of reperfusion after ischemic insult. Histological examination demonstrated that 7-NI protects against death a very small population of neuronal cell in CA_1 layer of hippocampus analysed 7 days after ischemia. It is suggested that NO release during reperfusion through activation of free radicals formation and subsequently through stimulation of membrane lipid peroxidation may be involved in alteration of biochemical processes in brain leading to the degeneration of some population of neurones. Moreover, ischemia evoked NO dependent signal transmission to cerebellum may have biochemical and functional consequences. Our results indicated that 7-NI in spite of ameliorating effect on ischemia evoked biochemical alteration is not able to prevent death of neurons.

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LIPID PEROXIDATION AND MEMBRANE-BOUND ENZYMES DURING COMPLETE AND IN-COMPLETE BRAIN ISCHEMIA IN RATS

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Many functionally important neuron proteins are membrane-bound and depend on the nativity of the lipid environment; during free radical attack they suffer both from the direct chemical modification and from membrane structure disordering by the peroxidation process. In the current research we have studied the activity of Na/K-ATPase (p-nitrophenilphosphatase) and monoaminooxidase, lipid peroxidation levels and membrane fluidity, during different brain ischemia periods in rats.

Complete brain ischemia (4-vessel occlusion). P-nitrophenilphosphatase activity in the rat's brain was significantly decreased (62-85% of control level) after 15 and 45 min. of ischemia, as well as after ischemia followed by 30 min. and 24 hrs. reperfusion. The monoaminooxidase activity in brain cells mithochondria was decreased up to 85 and 60 % of the control level after 15 and 45 min. of ischemia, and reperfusion led to the enzyme activity restoration. Accumulation of lipid peroxidation products in the brain after different periods of complete ischemia was about 86-95%. The decrease of (ANS) anilinonaphtalinosulphonate fluorescence maximum after 15 min. of ischemia was not very significant (92-95% of control); but subsequent reperfusion and longer ischemia periods (45 min.) led to a further decrease of this parameter (to 68-80% of control).

Incomplete ischemia was effected, bilateral common carotid arteries occlusion for a period of 5 days. After 24 hrs. of brain ischemia, as well as during the subsequent 4 days, the neurological deficit was not significant, but all animals demonstrated decreased learning ability in the open field and T-maze management. Accumulation of lipid peroxidation products in the brain tissue homogenates in the presence of ascorbic acid and ferrous ions manifesting 1.6-fold higher in rats after 5 days of brain ischemia, when compared with the control animals. Loss of monoaminooxidase activity under these conditions was about 15%. Congruently, the activity of Na/K-ATPase in brain microsomes appeared increased 15%. We can conclude that both lipids and membrane-bound enzymes are affected during brain ischemia of different severity and longevity, however the changes appear to be different.

THE PROTECTIVE EFFECT OF CARNOSINE DURING EXPERIMENTAL BRAIN ISCHEMIA IN RATS

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Oxygen deficiency and tissue reoxygenation effects a discharge of free radicals formation, lipid peroxidation and direct oxidation damage to proteins and nucleic acids. At onset of ischemic injury, reactive oxygen species are formed in the hydrophilic cell space, and the protective role of hydrophilic antioxidants seems to be important. In the current research we have studied the effect of carnosine, a natural hydrophilic neuropeptide possessing antioxidant with membrane-protective properties, during experimental brain ischemia in rats. NMR-spectroscopy allowed us to show the significant lactate elevation, accompanied by a decreased N-acetyl-aspartate signal after 15-45 min. in brain ischemia (4-vessel occlusion). In rats treated with carnosine before ischemia, lactate accumulation was much lower and the changes in the N-acetyl-aspartate signal were not significant. We have compared cell membrane conditions and the activity of some membrane-bound enzymes after brain ischemia in rats, both treated/untreated with carnosine before the surgery. After 15 minutes of brain ischemia, the rat's brain lipid peroxidation level was about 86% of the control animals, and (ANS) anilinonaphtalinosulphonate's fluorescence maximum was about 86-95% of the control in different membrane fractions. In rats treated with carnosine (150 mg/kg body weight daily administered with the drinking water) for 10 days before surgery, the measured parameters were similar to that of the control group, to wit: lipid peroxidation level at 98% of the control, fluorescence maximum of ANS about 100%. Fifteen minutes of ischemia followed by 24 hrs. of reperfusion displayed ANS maximum fluorescence at 76%, while in rats treated with carnosine this parameter was about 98-100%. The decrease of p-nitrophenylphosphatase activity in brain synaptosomes was significant, both after ischemia (71-84% of control) and after the reperfused ischemia (65-83% of control). The monoaminooxidase activity, membrane-bound enzyme of mithochondria, was decreased to 83-90% of the control animals, also both after ischemia and after reperfused (24 hrs.) ischemia. In rats treated with carnosine before surgery, the activity of both enzymes were similar to that of the control group. We can conclude: 15 minutes of complete brain ischemia causes greater damage to membrane-bound enzymes than to membrane lipids, and it is quite possible that proteins are the primary target of the free radical attack in vivo. Natural histidine-containing dipeptide carnosine can protect both proteins and lipids from ischemic damage in the brain.

NFκB p65 SUBUNIT IN THE RAT BRAIN: UNUSUAL AXOPLASMIC PRESENCE AND LACK OF EARLY NUCLEAR TRANSLOCATION FOLLOWING NEOCORTICAL INFARCTION.

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Devascularizing infarction of the neocortex leads primarily to death of cortical and thalamic neurons, followed by a gradual atrophy and loss of cortical tissue within the infarct. The changes are accompanied by induction of nerve growth factor (NGF) and NGF TrkA receptor expression within a subpopulation of cortical and thalamic reactive astrocytes and by a decrease of NGF and TrkA expression in injured nucleus basalis magnocellularis neurons. In search for the mechanisms of postinjury neuronal degeneration and regulation of NGF expression we investigated *in vivo* the presence and activation state of the p65 subunit of the inducible transcription factor NFκB shown to mediate *in vitro* neuroprotective actions and NGF activation in astrocytes. The immunocytochemical study was carried out on adult, Wistar, male rats, 0.5h, 1.5h, 3h and 6h following unilateral cortical infarction. Naive and sham-operated animals served as controls. NFκB p65 antibody (1:1000, Santa-Cruz) and ABC Vectastain kit were used. In control rats weak constitutive perikaryonal NFκB labeling found in most of the brain neurons remained in contrast with heavy perikaryonal and axonal staining found within reticular thalamic neurons, dorsomedial, lateral, periventricular and perifornical hypothalamic area. Dense, heavily labeled fiber networks within mfb, substantia innominata, internal capsule, claustrum and septum were found. Lesion did not affect NFκB labeling pattern and intensity within subcortical areas but caused spatially restricted enhancement of perikaryonal labeling in the devascularized cortical fields. Neither p65 nuclear translocation nor nonneuronal p65 expression was found. The study documents for the first time axoplasmic localization of NFκB p65 subunit and extends data on p50 subunit presence within synaptosomes (Kaltschmidt et al., Mech. Dev. 43, 1993). Results support the view of NFκB function as a retrograde messenger mediating stimulus-response coupling following presynaptic stimulation and suggest no early (hours) NFκB involvement in postinjury neuronal and glial responses.

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EFFECT OF ß-AMYLOID PEPTIDES ON CHOLINERGIC RECEPTOR-MEDIATED CALCIUM SIGNALING IN BRAIN CORTEX SYNAPTONEUROSOMES

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In this study, the effect of two fragments of ß-amyloid peptides (Aß) 1-28 and 25-35, on the resting and muscarinic cholinergic receptor (mAChR)-induced increase of cytosolic calcium concentration ($[Ca]_i$) in adult brain cortex synaptoneurosomes was investigated. Result was compared with the effect of aging of mAChR-evoked inositol trisphosphate (IP_3)-mediated increase of $[Ca]_i$. The release of IP_3 was measured after lipid prelabelling with *myo*-[^{3}H]inositol. Changes in $[Ca]_i$ were monitored by using fura-2 indicator. The effect of Aß peptides was evaluated by their preincubation with protein 1, 5, 30 and 60 min, before starting of $[Ca]_i$ measurement. It was observed that in aged brain, Ca^{2+}-dependent and mAChR-mediated IP_3 production was not changed in comparison with the adult brain over 60 min of incubation. Activation of mAChR in adult brain for 10 min increased $[Ca]_i$ by about 50-60% over its resting level, which was completely blocked by muscarinic antagonists, atropine and pirenzepine, as well as by antagonist of IP_3 receptor, TMB-8. In aged brain, there were no detectable changes in $[Ca]_i$, due to mAChR stimulation. Aß 25-35 peptide caused a time-dependent significant increase in $[Ca]_i$ which was almost five-fold after 60 min of incubation. The action of Aß 1-28 on resting $[Ca]_i$ was statistically insignificant up to 30 min, then a rapid increase of resting $[Ca]_i$ by two-fold was observed up to 60 min of incubation. Both Aß peptides decreased markedly mAChR-evoked elevation of $[Ca]_i$ in adult brain. After 60 min of preincubation with 1-28 Aß, the activation of mAChR enhanced resting $[Ca]_i$ by about 30%, whereas 25-35 Aß eliminated this receptor-dependent calcium mobilization. These results indicated that the neurotoxicity of deposited ß-amyloid may take a part in decreasing of mAChR-evoked IP3-mediated calcium mobilization and may further lead to alteration of muscarinic receptor-mediated signal transduction in brain.

NMDA AND GLUTAMIC ACID INDUCED CHANGES IN EXPRESSION OF β-APP AND TAU PROTEIN IN PAIRED HELICAL FILAMENTS (PHF) "IN VIVO"

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Accumulation of β-amyloid protein (β-A), β-amyloid precursor protein (β-APP) and appearance of bundles of paired helical filaments (PHF), the neurofibrillary tangles, has been found in Alzheimer disease. The aim of this study was to establish changes in the expression of some of domains of β-APP and tau protein in PHF in rat hippocampus after stereotactic microinjection of 1 µl of 2 or 5 mM NMDA and 1 mM or 2 mM glutamic acid to CA_1. To detect more precisely the role of NMDA receptors in modulation of β-APP processing and PHF immunoreactivity, MK-801 or CPP which are antagonists of NMDA receptors were injected intraperitoneally 30 min before experiments. Separation of proteins form hippocampal tissue by electrophoresis was followed by their Western blot analysis using antibodies against some domains of β-APP and tau in PHF. It was found in this study that application of glutamic acid or NMDA to the rat hippocampus induces significant changes in the pattern of β-APP fragments and tau in PHF. This pattern changes during time of recovery (2h, 12h, 24 h, 72 h) after injection and are more evident after NMDA then glutamic acid application. After 24 hrs and 72 hrs the enhanced immunoreactivity of all β-APP domains, particularly of βA (which was dose-dependent, sensitive to MK-801) and of the C-terminal fragment of β-APP was noted. After 72 hrs enhanced immunoreactivity of all tau epitopes in PHF was observed. Our results will be discussed in relation to PHF changes observed in Alzheimer disease.

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MODULATORY EFFECT OF BOVINE SPINAL CORD HYDROLIZATE UPON THE EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS IN THE RAT. IMMUNOCYTO-CHEMICAL STUDY

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Specific protein feeding is known method of induction of tolerance of immunologic response to this antigen. This method is reviewed recently by some authors as a possible tool in the treatment of autoagressive diseases. Experimental allergic encephalomyelitis (EAE) is the respected animal model of such autoagressive disease as multiple sclerosis. The aim of the study was evaluation of the effect of bovine spinal cord hydrolizate given orally upon the course of EAE in the rat. Experimental allergic encephalomyelitis was evoked in Wistar rats by intradermal injection of homogenate of spinal cord of guinea pig with Freund's adjuvant and Myocobacterium phlei.

Since the end of first relapse rats were fed of bovine spinal cord hydrolizate three times a week and also evaluated clinically in blind fashion. Clinical course of EAE in treated animals group was milder in comparison to the control ones. At six weeks post immunisation immunocytochemical study showed increased ratio of CD_4/CD_8, decreased immunological answer to CD_{68} and decreased reaction with αTNF antibodies in the group of treated animals with hydrolisate in comparison to the control.

In conclusion these pilot data indicate that oral treatment with spinal cord hydrolisate modulate clinical course, immunologic response and pathologic image of EAE in the rat and might also have some clinical implication.

Recovery from Acoustic Trauma: An Ultrastructural and Immunohistochemical Study on Regenration of Hair Cells in the Chick Basilar Papilla

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Acoustic overstimulation produces a loss in the auditory epithelium. In postembrionic mammals, the loss of auditory hair cells is thought to be permanent and to result in irreversible hearing deficiency. In birds, however, degenerated auditory epithelium may be replaced by new hair cells, which probably originate from the support cells of the basilar papilla.

The aim of the study was to assess by light and transmission electron microscopy the cytological and fine structural changes of the newly developing hair cells and neural elements during post-traumatic regeneration in the chick. We have also studied, by immunochistochemical method, proliferation cell nuclear antigen (PCNA) in relation to the regeneration process.

The study was performed on 1-day-old White Leghorn chicks, exposed to broad-band noise at high intensity for 5 consecutive days, 4 hours a day. The structural changes of the basilar papilla and PCNA expression were assessed on days 1, 4 and 6 after exposure.

We confirmed that the proliferation of sensory cells in the chick basilar papilla starts from the first days after noise exposure. The schedule of regeneration of hair cells and neural elements is described in detail in this study. The presence of PCNA is a good marker of cell division and correlates well with the regeneration process of hair cells in the chick basilar papilla.

Poster session - Excitatory amino acids

DEVELOPMENTAL CHANGES IN [^{3}H]KAINATE BINDING SITES IN THE BARREL CORTEX OF MICE

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L-Glutamate is the major excitatory transmitter in connections to and from the cortex and between cortical areas. Glutamate mediates excitatory activity in the neurons through its activation of three types of ionotropic receptors (NMDA, AMPA, kainate) and metabotropic receptors. The present work is focused on developmental changes of distribution of kainate receptors present in the specific area of somatosensory cortex - the barrel cortex. The barrel cortex is a locus of representation of sensory whiskers of the snout of mice. To examine the pattern of distribution of [^{3}H]kainate binding sites the quantitative receptor binding autoradiography was used. We found that kainate binding sites were present in the cortex of newborn mice (P0). During the initial three days of life [^{3}H]kainate binding rapidly increased. Then, from postnatal day 8 (P8), binding rose slowly, and the adult level (500 fmol/mg protein) was reached at P12. Later the labeling remained quite stable until P28. No barrel-like pattern of [^{3}H]kainate binding sites could be discerned in tangential sections obtained from cortex of adult mice. Clear developmental interlaminar changes of [^{3}H] kainate binding in the barrel cortex were registered. At P3 in immature layer IV the highest binding of [^{3}H]kainate was observed. By P5, a high labeling is also present in layer V. At P12, when the adult pattern of receptor binding distribution is established, the densest labeling is concentrated in layers V/VI, moderate in layer IV and the lowest in supragranular layers. Thus, in contrast to AMPA receptors, kainate binding is concentrated in infraglanular layer from the second postnatal week.

THE NEONATAL BLOCKADE OF NMDA RECEPTORS ALTERS THE DOPAMINERGIC NEUROTRANSMISSION AND SENSORIMOTOR GATING IN RATS.

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Recently it has been suggested that glutamate driven impairments of neurodevelopment may lead to hyperactivity of dopaminergic neurotransmission and in consequence to the psychoses in adult life. Therefore, the present study investigated the effects of neonatal administration of a competitive NMDA receptor antagonist CGP 40116 on functional parameters characteristic of the dopaminergic neurotransmission, i.e. sensitivity of rats to amphetamine and quinpirole, and on the density of dopamine D-1 and D-2 receptors, as measured by an autoradiography (using [^{3}H]SCH 23390 and [^{3}H]spiperone as ligands, respectively). We found that chronic neonatal administration of CGP 40116 enhanced exploratory activity of rats and augmented the locomotor stimulant effects of amphetamine and quinpirole. Such a functional supersensitivity was accompanied, at receptor level, by an increase in the number of D-1 receptors in substantia nigra. It was also found that neonatal administration of NMDA receptor antagonist abolished the prepulse-induced inhibition of acoustic startle response, this effect being antagonized by clozapine. It is concluded, for the first, that blockade of NMDA receptors during development may lead to the overactivity of dopaminergic systems, and secondly, that it induces impairments in experimental animals which resemble some deficits seen in patients suffering from schizophrenia.

AGED RELATED CHANGES OF NMDA RECEPTOR MEDIATED NITRIC OXIDE DEPENTED SIGNALING PATHWAY IN BRAIN. EFFECT OF AMYLOID β PEPTIDES.

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In central nervous system, activation of glutaminergic receptor NMDA type was shown to induce Ca^{2+}- dependent NO synthesis, which activates soluble guanylate cyclase and leads to the formation of cGMP. Both compounds appear to be important mediators in long term potentiation (LTP), mechanism responsible for processes of learning and memory. Aging is an important risk factor for impairment of memory and dementia. In these studies the mechanism of basal and NMDA receptor mediated, cGMP formation in different part of adult and aged brain was analyzed. The studies were carried out using hippocampal, brain cortex and cerebellum slices from 4 months and 27 months old animals. The relative function of the NO cascade was determined by measurement of NO synthase and guanylate cyclase using radioimmunochemical methods. In these assays specific agonist and antagonist of NMDA receptor and selective enzyme inhibitors were used. In addition, the effect of different synthetic Aβ peptides: Aβ25-35 and Aβ1-40 on the NO/cGMP messenger system was evaluated. It was found that brain aging is coincident with a decrease of a basal level of cGMP as a consequence of more active degradation of cGMP by phosphodiesterase as compared to adult brain. Moreover a loss of NMDA receptor response evoking an enhancement of cGMP level determined in the presence of cGMP-phosphodiesterase inhibitor (IBMX) was found in hippocampus and cerebellum, but not in brain cortex of aged matched controls. A significant enhancement of NO synthase activity, by about 175% and 160% in hippocampus and cerebellum of aged brain, observed in our studies, may be responsible for NO-dependent alteration of receptor or enzyme protein. It was found that Aβ 25-35 affected significantly NMDA receptor mediated NO dependent signaling pathway. These alterations may have functional consequences in disturbances of learning and memory processes in aged brain.

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EFFECTS OF THE PENTYLENETETRAZOLE-INDUCED KINDLING ON THE NMDA RECEPTOR GENE EXPRESSION IN THE RAT HIPPOCAMPUS

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A number of data indicate an important role of NMDA receptors in seizure phenomena. Agonists of NMDA receptors evoke hypersynchronous discharges and induce behavioral seizures, whereas their antagonists suppress the seizure activity and prevent development of experimental epileptogenesis. It has been hypothesised that changes in the NMDA receptor biosynthesis may be involved in the mechanism of kindling - an animal model of epilepsy whereby periodic subthreshold electrical or chemical stimulation leads to long-lasting neuronal hyperexcitability. In order to test this hypothesis, in a time course study we investigated the effects of pentylenetetrazole kindling on the expression of a gene coding for NMDAR1 and the density of NMDA receptors in the rat hippocampal formation. As shown by an in situ hybridization study, the pentylenetetrazole kindling decreased the NMDAR1 mRNA level in the CA1 field and dentate gyrus at 3 and 24 h after the last injection of the convulsant. A receptor autoradiography showed an increase in the [^{3}H]MK-801 binding density in the stratum oriens, stratum radiatum and stratum moleculare of the hippocampus of kindled rats. However, the alterations in both the NMDAR1 mRNA level and the density of [^{3}H]MK-801 were back to control values on day 15 after the last injection of pentylenetetrazole. The transience of the above changes in the biosynthesis of hippocampal NMDA receptors suggests that they are rather an adaptive response to repeated seizures rather than a cause of permanent neuronal hyperexcitability.

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MODULATION OF NMDA-EVOKED PROSTAGLANDIN D_2 RELEASE IN RABBIT HIPPOCAMPUS IN VIVO

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Prostaglandin D_2 (PGD_2), a biologically active product of cyclooxygenase, is involved in various brain functions. Our previous studies with in vivo microdialysis demonstrated NMDA-induced, Ca^{2+} dependent eicosanoid release in the rabbit hippocampus. Here we characterise NMDA receptor dependence of PGD_2 production in this experimental model, its relation to extra- and intracellular Ca^{2+} pools, and to nitric oxide (NO). All drugs were applied with microdialysis medium, dialysates were analysed for concentrations of PGD_2 (with RIA), of amino acids (with HPLC), of NO (with haemoglobin trap), and for changes in $^{45}Ca^{2+}$ efflux. The results: a dose-response relation between NMDA concentration and PGD_2 release and its inhibition by competitive NMDA receptor antagonists, demonstrate the role of NMDA receptors. NMDA-evoked PGD_2 release was accompanied by a drop of $^{45}Ca^{2+}$ efflux, indicating a decrease in extracellular Ca^{2+} concentration due to its influx to neurones, and by release of taurine and phosphoethanolamine, known to be partially Ca^{2+} dependent. These effects were resistant to dantrolene and ryanodine modulation suggesting a marginal role of the ryanodine sensitive pool of intracellular Ca^{2+}. NMDA application resulted also in NO release to dialysate, which was sensitive to L-NAME. This NO synthase antagonist had no effect on NMDA-evoked decrease in extracellular Ca^{2+} concentration, but inhibited PGD_2 release, which is consistent with a direct cyclooxygenase activation by NO.

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CHARACTERIZATION OF METABOTROPIC RECEPTORS FOR EXCITATORY AMINO ACIDS WHICH STIMULATE CYCLIC AMP ACCUMULATION IN RAT BRAIN

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Glutaminic acid (Glu) is a major excitatory neurotransmitter in the central nervous system. It activates several subtypes of ionotropic glutamate receptors, as well as metabotropic glutamate receptors (mGluR). Stimulation of mGluR leads to formation of different second messengers in the cell. The aim of our experiments was to investigate the effect of different agonists of mGluR on cyclic AMP formation in slices from rat brain cerebral cortex. The formation of cyclic AMP was measured using a prelabelling technique with preincubation of slices with ^{3}H adenine. We found that the endogenous transmitter Glu in doses up to 2500 uM induces a dose dependent statistically significant increase in cyclic AMP formation with the EC_{50} of 440 μM, after higher doses a gradual decrease in cyclic AMP formation was observed. Substances which activates several subtypes of mGluR such as ibotenic acid or 1S,3R-1-aminocyclopentane-1,3-dicarboxylic acid (1S,3R-ACPD) also induced a dose dependent increase in cyclic AMP accumulation with EC_{50} of 142 and 70 μM, respectively. Quisqualic acid which stimulates group I of mGluR did not affect cyclic AMP formation as well as and agonist of III group of mGluR - L-SOP. However compounds which are rather selective towards group II of mGluR such as (L-CCG-I), 3-carboxy-4-hydroxyphenylglycine (3C4HPG) and 4-Carboxy-3-hydroxyphenylglycine (4C3HPG), also induced a dose depenendt increase in cyclic AMP formation with the EC_{50} of 142, 227 and 209 μM, respectively. This indicates that the stimulation of II subtype of mGluR is responsible for an increased formation of cyclic AMP. This hypothesis is further supported by the fact that the action of mGluR II agonists was inhibited by (RS-alpha-methylserine-O-phosphate-monophenyl ester (MSOPPE), which is an antagonist of the II group of mGluR.

LACK OF THE RELATIONSHIP BETWEEN THE CONCENTRATION OF MAGNESIUM IN BLOOD PLASMA AND THE DEATH RATE IN RATS AFTER ADMINISTRATION OF N-METHYLO-D-ASPARTATE (NMDA) INTO THE 3rd CEREBRAL VENTRICLE

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Numerous experimental studies performed on animals and also clinical reports indicate the suppressing action of magnesium salts on the cytotoxic effect of excitatory amino acids.

The aim of the studies was to find the relationship between the concentration of magnesium in blood plasma and the death rate of animals after the infusion of NMDA into the 3rd cerebral ventricle.

The experiments were carried out on 30 adult male rats. In a stereotaxic apparatus, under i.p. barbiturate anaestesia all animals were infused intracerebroventricularly with 10 μL of a solution containing 400 nmol of NMDA. Prior to the NMDA infusion blood from the tail vein was collected in order to assess the concentration of magnesium ions.

After the NMDA infusion 8 animals died within 25 - 60 minutes and 7 rats died after several hours up to 34 days. In the group of animals which survived 80 days after the NMDA infusion the average plasma concentration of magnesium ions was 1.90 ± 0.05 mEq / L plasma (within the range of 1.64 - 2.22 mEq / L). In the group of animals which died after the NMDA infusion the average plasma concentration of magnesium ions was 1.87 ± 0.04 mEq / L plasma (within the range of 1.62 - 2.40 mEq / L).

The significant difference in the concentration of magnesium ions in blood plasma in surviving animals and in those that died after the NMDA infusion was not ascertained.

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LACK OF PROTECTIVE EFFECTS OF MAGNESIUM SULPHATE INTRA VENOUS INFUSION ON THE DEATH RATE AFTER NMDA INJECTION INTO THE 3rd CEREBRAL VENTRICLE IN RATS.

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Recent evidence suggests that the neurotoxicity of endogenous exitatory amino-acids plays an important role in the pathogenesis of the brain damage. The magnesium exerts protective effect by blocking the calcium influx through ion channels which are coupled to the N-methyl-D-aspartate (NMDA) receptors.

The present study was undertaken to determine whether a magnesium sulphate infusion into the veins of rats before or after the NMDA injection into the 3rd ventricle may have an influence on the survival of the rats.

80 adult male rats were divided into five experimental groups. Anaesthetized rats received a single injection of 10 μl of 0.9% NaCl solution (control group) or 200 nmol (low dose) and 400 nmol (high dose) of NMDA to the 3rd ventricle. The NMDA was injected 30 minutes before or 30 minutes after the 12.5 % $MgSO_4$ infusion into the vein.

In the control group all rats survived the experiment, which was carried out over 80 days. The low dose of NMDA injected into the 3rd ventricle caused the death of 27 % of rats, while the high dose of NMDA caused the death of 80 % of rats. The animals died after several minutes or a few days. This effect was dependent on the dosage of NMDA but not on the moment of $MgSO_4$ infusion into the vein. These data demonstrate that magnesium sulphate infusion into the vein cannot prevent the neurotoxic effect of NMDA injections into the 3rd ventricle in rats.

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CHANGES IN GONADOTROPIN RELEASING HORMONE (GnRH) CONCENTRATION IN THE DIENCEPHALON AND PITUITARY AFTER N-METHYL-D-ASPARTATE INFUSION INTO THE 3-RD CEREBRAL VENTRICLE IN MALE RATS.

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There is evidence that N-methyl-D-aspartate (NMDA) by stimulation of the hypothalamic gonadotropin releasing hormone (GnRH) is involved in the control of LH secretion.

We investigeted the effect of intraventricular administration of NMDA on GnRH concentration in the diencephalon and pituitary in adult male rats. The hexobarbital anasthetized animals were placed in a stereotaxic apparatus and through a cannula inserted into the 3rd cerebral ventricle infused with NMDA in three doses: 100, 200 and 400 nmol. The dose of 400 nmol proved to be lethal in 50 % of animals. After 80 days all animals, which survived were decapitated. The brains were removed and diencephalon and pituitary were isolated. In tissue extracts the GnRH concentration was determined by radioimmunoassay.

Intraventricular administration of NMDA in the dose of 200 and 400 nmol significantly ($p = 0.001$) increased GnRH concentaration in the pituitary in comparison with intact animals. The increase after 100 nmol of NMDA was not statistically significant. Infusion of three doses of NMDA resulted in an insignificant increase in GnRH concentration in the diencephalon as compared with the control, intact animals.

The present study indicates that intraventricular administration of NMDA increased GnRH concentration in the pituitary and diencephalon probably by affecting the process of GnRH synthesis and release.

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INFLUENCE OF INTRASTRIATAL INJECTIONS OF 5,7-DICHLOROKYNURENIC ACID, AN ANTAGONIST OF GLYCINE SITE OF THE NMDA RECEPTOR, ON THE HALOPERIDOL-INDUCED MUSCLE TONE.

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The consequence of a dopamine deficit in the striatum of parkinsonian patients seems to be a shift of the equilibrium between dopamine and glutamate towards the glutamatergic system. Animal studies indicate that competitive and non-competitive antagonists of NMDA receptors are able to restore the dopaminergic-glutamatergic balance and thus induce an antyparkinsonian effect. However, these drugs produce a lot of side-effects. Antagonists of the modulatory glycine site of the NMDA receptor seem to be devoid of such effects.

The aim of the present study was to find out whether 5,7-DCKA, an antagonist of the glycine site of the NMDA receptor, counteracted the haloperidol-enhanced muscle tone in the rat, a model of parkinsonian rigidity. The experiments were carried out on male Wistar rats which were injected bilaterally with 5,7-DCKA in doses of 1, 2.5, 4.5 μg in a volume of 0.5 μl in the rostral part of the striatum. The muscle tone was measured as mechanical resistance of the hind foot, developed in response to passive movements in the ankle joint. The EMG activity of the gastrocnemius and tibialis anterior muscles was simultaneously recorded. 5,7-DCKA, injected intrastriatally, caused a significant and dose-dependent decrease in the haloperidol-induced muscle tone. Similarly, a tendency to decrease the EMG activity in the musculus tibialis anterior during extension and in the musculus gastrocnemius during flexion was observed .

The present results indicate that blokade of the glycine site of the NMDA receptor complex in the rostral part of the striatum may be important to the antyparkinsonian effect of drugs.

ANTIPARKINSONIAN ACTION OF L-701,324, AN ANTAGONIST OF THE GLYCINE SITE OF THE NMDA RECEPTOR COMPLEX, ON THE HALOPERIDOL-INDUCED MUSCLE RIGIDITY IN RATS.

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L-701,324, an antagonist of the glycine site of the NMDA receptor complex has been shown in a number of tests to have a pharmacological profile of an atypical neuroleptic, devoid of extrapyramidal effects in rats. The aim of the present study was to find out whether this compound affected the haloperidol-induced muscle rigidity of the parkinsonian type, which was recorded mechano- and electromyographically in rats (MMG/EMG).

The MMG/EMG method measures the muscle resistance of the rat's hind leg, evoked by passive flexions and extensions in the ankle joint, as well as the simultaneous electromyographic activity (EMG) observed in flexor (tibialis anterior) and extensor (gastrocnemius) muscles of the hind leg during movements. Our previous experiment shoved that haloperidol (0.5-10 mg/kg) induced dose-dependent muscle rigidity of the parkinsonian type. This drug simultaneously - especially at higher doses - increased muscle resistance and late components of the reflex EMG activity to movements.

L-701,324 in doses of 2.5-10 mg/kg decreased the muscle rigidity induced by 1 mg/kg of haloperidol; used in doses of 10-40 mg/kg, it diminished the muscle rigidity evoked by 5 mg/kg of haloperidol. However, L-701,324 given alone in doses equal to or higher than 5 mg/kg induced ataxia related to muscle rigidity.

The present results indicate that glycine antagonists used in doses devoid of motor side-effects may be useful in the treatment of parkinsonian rigidity.

CGP 40116, A COMPETITIVE ANTAGONIST OF NMDA RECEPTORS, AND THE PROTECTIVE POTENCY OF COMMON ANTIEPILEPTIC DRUGS AGAINST MAXIMAL ELECTROSHOCK

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Antagonists of NMDA-induced excitation, apart from their anticonvulsive effects per se (Czuczwar and Meldrum, Eur. J. Pharmacol., 83,335,1982), may augment the anticonvulsive activity of conventional antiepileptic drugs against maximal electroshock (Czuczwar et al., Eur. J. Pharmacol., 100,357,1984; Czechowska et al., Eur. J. Pharmacol., 232,59,1993). However, some combinations exerted serious adverse effects (Żarnowski et al., J. Neural. Transm., 97,1,1994; Neuropharmacology 33,619,1994). CGP 40116 (an active isomer of CGP 37849) was consequently combined with antiepileptic drugs. An influence of CGP 40116 upon the free plasma levels of antiepileptic drugs as well as their adverse effects was also evaluated. At a subprotective dose of 0.5 mg/kg (90 min before the test) against electroconvulsions, CGP 40116 reduced the ED_{50} value of carbamazepine against maximal electroshock from 12 to 5.3 mg/kg, that of diphenylhydantoin - from 12.3 to 4.7 mg/kg, that of phenobarbital - from 16.5 to 8 mg/kg, and that of valproate - from 254 to 145 mg/kg. At 0.125 mg/kg, CGP 40116 was still able to potentiate the protective potency of these antiepileptics. In no case the NMDA receptor antagonist affected the free plasma levels of antiepileptic drugs as measured by immunofluorescence. Only its combinations with valproate were associated with impaired motor coordination and long-term memory. Considering these results, one may postulate a clinical significance for these combinations, especially in epileptic patients with increased plasma levels of excitatory amino acids.

INFLUENCE OF LY 300164 (A NOVEL NON-NMDA ANTAGONIST) ON THE ANTICONVULSIVE ACTIVITY OF ANTIEPILEPTICS

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Excitatory amino acid antagonists affecting NMDA or non-NMDA receptors have been shown to enhance the protective effects of conventional antiepileptic drugs. Some combinations resulted, however, in profound adverse effects (for review see Czuczwar et al., Metab. Brain Dis., 11,143,1996). It was therefore of interest to examine the influence of a novel non-NMDA antagonist, LY 3001647 [7-acetyl-5-(4-aminophenyl)-8,9-dihydro-8-methyl-7H-1,3-dioxolo(4,5-H)-2,3-benzodiazepine], on the protective effects of antiepileptic drugs against electroconvulsions. LY 300164, up to 2 mg/kg, did not influence the threshold for electroconvulsive seizures. In doses of 2.5 - 4 mg/kg, LY 300164 significantly raised the threshold. In subprotective doses against electroconvulsions, this excitatory amino acid antagonist enhanced the protective activity of valproate, carbamazepine and diphenylhydantoin against maximal electroshock-induced convulsions in mice. The anticonvulsive action of phenobarbital was potentiated by LY 300164 only at 2 mg/kg. The non-NMDA receptor antagonist did not affect the plasma levels the antiepileptic drugs, so a pharmacokinetic interaction is not probable. The combined treatment of LY 300164 (2 mg/kg) with the antiepileptics studied (providing a 50% protection against maximal electroshock) did not impair the motor performance of mice, evaluated in the chimney test. Valproate, at its ED_{50} of 280 mg/kg against maximal electroshock, produced motor impairment. As shown in the passive avoidance task, combination of LY 300164 (2 mg/kg) with valproate or diphenylhydantoin resulted in impairment of long-term memory. Among antiepileptic drugs alone, valproate (280 mg/kg) and phenobarbital (28.5 mg/kg) disturbed long-term memory. The results suggest that the blockade of glutamate-mediated events via non-NMDA receptors leads to the enhancement of the anticonvulsive activity of conventional antiepileptic drugs. Some combinations of LY 300164 with antiepileptic drugs were superior than these antiepileptics alone in terms of adverse effects.

RILUZOLE, AN INHIBITOR OF GLUTAMATE RELEASE, POTENTIATES THE PROTECTIVE ACTIVITY OF CONVENTIONAL ANTIEPILEPTIC DRUGS AGAINST ELECTROCONVULSIONS IN MICE

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Agents blocking ionotropic receptors for excitatory amino acids have been documented to augment the anticonvulsive action of antiepileptic drugs against maximal electroshock-induced seizures in mice (Borowicz et al., Eur. J. Pharmacol., 281,319,1995; Epilepsia 37,618,1996). A question arises whether such an activity may be shared by the glutamate release inhibitor, riluzole. Riluzole alone (1.25 and 5 mg/kg, 30 min prior to the test) significantly raised the threshold for electroconvulsions (tonic extension of the hind limbs taken as the endpoint) from 6.2 to 7.8 and 9.2 mA. In lower doses, the inhibitor of glutamate release did not affect the threshold. When combined with antiepileptic drugs, riluzole at 0.625 mg/kg potentiated the protective potency of carbamazepine, diphenylhydantoin, and phenobarbital. The potentiating action of riluzole was the most evident in the case of carbamazepine and diphenylhydantoin whose ED_{50}s against maximal electroshock-induced seizures were reduced from 14.4 to 8.7 mg/kg and from 10 to 5.7 mg/kg, respectively. The combined treatment of riluzole with antiepileptic drugs did not affect the motor coordination of mice evaluated in the chimney test. As regards long-term memory (evaluated in the passive avoidance task), only a combination of riluzole with diphenylhydantoin impaired this parameter. It may be suggested that similarly to the blockade of glutamate-mediated events, an inhibition of its release in the central nervous system may lead to the enhancement of the antiepileptic drug-induced protection against maximal electroshock, a widely recognized model of generalized tonic-clonic seizures in humans.

7-NITROINDAZOLE (A SELECTIVE NEURONAL NITRIC OXIDE SYNTHASE INHIBITOR) DIFFERENTIALLY AFFECTS THE ANTICONVULSIVE ACTIVITY OF CONVENTIONAL ANTIEPILEPTICS IN MICE

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Nitric oxide (NO; a small membrane-diffusible molecule) is probably involved in the modulation of seizure activity. Both, pro- and anticonvulsive actions of NO have been documented. Consequently, it was of interest to study the effect of 7-nitroindazole on the protective activity of conventional antiepileptic drugs against maximal electroshock-induced seizures. At 25 and 50 mg/kg 7-nitroindazole, 30 min before the test, did not influence the electroconvulsive threshold. However, at 50 mg/kg, it enhanced the anticonvulsive activity of phenobarbital against maximal electroshock (the ED_{50} value of phenobarbital was decreased from 17.7 to 7.3 mg/kg) and did not affect that of carbamazepine, diphenylhydantoin, and valproate. L-Arginine (500 mg/kg) did not modify the protective activity of phenobarbital alone or the 7-nitroindazole-induced enhancement of its anticonvulsive potency against maximal electroshock. 7-Nitroindazole did not alter the plasma levels of antiepileptic drugs, so a pharmacokinetic interaction, in terms of total and free plasma levels, is not probable. 7-Nitroindazole combined with the antiepileptics resulted in motor disturbances, except of the combination with phenobarbital. On the other hand, the combined treatment of 7-nitroindazole with carbamazepine or phenobarbital produced effects superior to those produced by single drugs, as regards long-term memory. Our results indicate that the protective activity of carbamazepine, diphenylhydantoin, or valproate against maximal electroshock may be not dependent upon the central NO level. The enhancement of the anticonvulsive action of phenobarbital by 7-nitroindazole is probably not related to the decrease of NO in the central nervous system.

NMDA ANTAGONISTS COUNTERACT CHRONIC STRESS-INDUCED DEFICIT OF AGGRESSION

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It has recently been suggested that the NMDA subtype of glutamate receptors may be involved in the mechanism of action of antidepressant drugs. Our previous studies have shown, that the rats, subjected to chronic stress procedure, exhibited a decreased footshock-induced fighting behavior and that prolonged treatment with antidepressants counteracted this effect of chronic stress.

In the present study, we investigated the effect of two non-competitive NMDA antagonists: dizocilpine (MK-801) or memantine, given in the single dose or for 14 days, on footshock-induced fighting behavior and exploratory activity in normal or chronically stressed rats.

It was found that repeated application of various kinds of stressors decreased the footshock-induced fighting behavior and that prolonged treatment with memantine (2.5 mg/kg/day) or MK-801 (0.1 mg/kg/day) restored the intensity of fighting behavior to control level. A similar effect was observed in stressed rats receiving the single dose of MK-801 (but not of memantine), but this effect was accompanied by locomotor stimulation.

The results of the present study indicate that non-competitive NMDA antagonists can reduce the behavioral deficit produced by chronic stress. This effect is similar to that of antidepressant drugs.

Role of NMDA receptors in ultrasonic vocalizations in 50 kHz band during acquisition of sexual behavior in male rats

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Acqusition of copulatory reaction by male rats exposed to receptive females provides an interesting model of learning processes. We have previously shown that shortening of mount and intromission latencies occurs rapidly, within 1-2 traning sessions (each session up one ejaculation only) during such training. To the contrary, decrease in ejaculation latency as well as increase in ultrasonic vocalizations in 50 kHz band (U-bc) emitted by males during 5 min of habituation to copulatory cage, before introduction of female are requiring at least three training session to be significant. To test a role of NMDA receptors in these phenomena, we trained the naive males up to 10 copulatory sessions (single ejaculation in each session). One group of animals 25 minutes before each session received injection of Ringer's solution (RS). The second received injection of 0.1 mg/kg MK-801 at the same regime. The third was treated to injection of RS before the first five training sessions, and afterwards - the males received MK-801. In the fourth group, the order of treatment was reverse. We suggest that the increase in number of U-bc corresponds with learning process and NMDA receptors play an important role in this phenomena.

The increasing of susceptibility for kyotorphin in picrotoxin-induced kindling

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Kyotorphin was shown to suppress seizures in cases of acute epileptic activity in rats and mice. We performed the investigations aimed to study the kyotorphin effectivity in picrotoxin (PTX) kindled rats.

Kindling procedure was performed by daily i.p. PTX injections in subthreshold (1.3 mg\kg) dose. Peptide was injected i.c.v. in dose of 10 nmoles ½ hr before the testing PCT injection after the kindling termination (24-th PTX injection).

Kyotorphin showed significant antiepileptic efficacy in kindled animals which was expressed both in seizure latency prolongation (on 44%, $P<0.01$) and seizure severity decreasing ($P<0.05$). This antiepileptic effect was blocked by preliminary naloxone (0.1 mg\kg).

Hense, the data obtained revealed the expressed antiepileptic profile of kyotorphin effects. It should be stressed the singificance of kindled seizures inhibition - the very complicated form of seizures and considered to be a drug-resistant form of seizures. Named effect is realized via μ-opioid neurotransmitter system.

The effect of ACPC on rewarding properties of some drugs of abuse.

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The majority of psychoactive substances that are abused by people have rewarding properties, and therefore, the understanding of neurochemical mechanisms underlying these effects is one of the major purpose in modern studies on psychobiological processes involved in the development of drug dependency and its therapy. A growing number of preclinical data suggest that some aspects of drug abuse and dependence may be attenuated by the NMDA receptor antagonists. However, there are also reports suggesting that these agents have strong reinforcing and psychotomimetic properties. Therefore, in recent years intensive studies have been carried out to identify compounds which would inhibit function of the NMDA receptors indirectly, e.g. acting at strychnine-insensitive glycine sites coupled to the NMDA receptor complex. There reasons to believe that these compounds share many of the pharmacological actions of both uncompetitive and competitive NMDA antagonists without their unwanted side-effects. The present paper reviews results of studies designed to evaluate the effect of ACPC, a high affinity, partial agonist at strychnine-insensitive glycine sites, on the acquisition and expression of a place preference conditioning induced by morphine, cocaine, amphetamine and diazepam. These results will be discussed in terms of the involvement of the NMDA receptors in brain mechanisms of rewarding activity of those drugs of abuse and the possible use of various ligands of the NMDA receptor complex in the therapy of drug dependence in humans.

Poster session - Peptides

OPPOSITE DOPAMINERGIC REGULATION OF NEUROPEPTIDE Y AND CORTICOTROPIN RELEASING FACTOR IN THE RAT AMYGDALA

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Previous studies indicated that dopaminergic (DA) innervation may affect the expression of certain neuropeptides in some brain structures. In the present study we performed a selective unilateral 6-OHDA lesion of mesencephalic DA neurons projecting to the amygdala. The effect of denervation was observed using immunohistochemical methods in two different neuronal populations in the amygdala: neuropeptide Y (NPY) and corticotropin releasing factor (CRF) neurons. The NPY or CRF immunoreactive (-ir) neurons in the amygdala were counted in microscopic sections, and comparisons between lesioned and contralateral sides, and versus sham-operated controls were made.

A significant increase in the number of NPY-ir neurons was found in the amygdala on the lesioned side in comparison with the contralateral one (170%) and sham-operated rats. The number and staining intensity of CRF-ir neurons decreased in the ipsilateral amygdala (40% of contra). The obtained results indicate an opposite DA regulation of the NPY and CRF expression in neurons of the rat amygdala.

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EFFECTS OF NEUROPEPTIDE Y ON EPILEPTIFORM ACTIVITY IN THE CORTEX IN VITRO.

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The 36 amino acid peptide, neuropeptide Y (NPY) is the most abundant peptide in the mammalian brain. High concentrations of NPY are found in the cortex. Several studies have shown marked increases in cortical content of NPY after experimentally induced seizures, which leads to a hypothesis that NPY may serve as an endogenous anticonvulsive agent. In fact it has been reported that NPY inhibits some forms of epileptiform activity in the hippocampus and cortex in vitro. The aim of this study was to determine the effect of NPY on epileptiform activity in the rat frontal cortex in vitro.

Perfusion of a cortical slice with saline containing nominally zero Mg^{2+}, results in the appearance of synchronous discharges. NPY potently and reversibly inhibited the frequency of the discharges. This effect was mimicked by the Y1 receptor agonist (Leu^{31}, Pro^{34}) NPY but not the Y2 receptor agonist NPY_{13-36}. One possible explanation for the inhibition of epileptiform activity is that NPY has effects on electrical properties of cortical neurons or on glutamate release. Neither the resting membrane potential, input resistance, discharge properties nor the excitatory postsynaptic potentials evoked by electrical stimulation were affected by NPY application. The possible target of NPY action still remains to be elucidated. The presented data support the hypothesis that NPY may play the role of an endogenous antiepileptic agent in the cortex.

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THYROLIBERIN (TRH) AFFECTS THE OXYTOCIN AND PROLACTIN RELEASE IN FEMALE RATS DURING MIDLACTATION

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TRH is possibly engaged in functional regulation of the hypothalamo-neurohypophysial system. This experiment was performed to study the effects of TRH on oxytocin (OT) and prolactin (PRL) release in lactating female rats not suckled or suckled. Primiparous female Wistar rats in midlactation, isolated from their litters 12-14 hrs before the experiment, were used. The animals were divided into two groups: A - rats injected intracerebroventricularly (i.c.v.) with 10 µl of 0.15 M NaCl; B - rats treated with TRH administered i.c.v. at a dose of 200 ng dissolved in 10 µl of 0.15 M NaCl solution. In each group two subgroups were set up: I - females not suckled; II - females suckled during 30 minutes. On the day of experiment, a permanent cannula was implanted into the left cerebral ventricle of urethane-anaesthetized animals. At the end of surgery the rats were given intravenously (i.v.) propranolol (250 µg/kg b.w.) to facilitate the milk ejection reflex. One hour later a blood sample (time „0") was collected. Immediately thereafter the animals were injected i.c.v. with respective solution and the pups were allowed to suck the appropriate females for 30 minutes (subgroups A-II and B-II). In all animals, the blood samples were also collected 5, 10, 15 and 30 minutes (time „5", „10", „15" and „30") after i.c.v. infusions.

Plasma OT concentration in TRH-treated lactating but not suckled females (subgroup B-I) increased distinctly 5, 10 and 15 minutes after injection in comparison with not suckled females treated with vehicle; at time „30" it returned to the control level. In females suckled and treated with normal saline (subgroup A-II) OT plasma level increased at 5th and 10th minute of suckling. A single i.c.v. dose of TRH inhibited the OT release in response to suckling. Plasma PRL concentration in not suckled females remained unchanged from time „0" up to the end of experiment. I.c.v. injection of TRH distinctly raised PRL plasma level in not suckled females. The PRL plasma level of suckled females raised at 15-th minute of suckling. Contrary to TRH effect in not lactating animals, TRH caused significant decrease of PRL level in blood plasma of lactating females suckled by their litters over 30 minutes.

It is concluded that the effects of TRH on OT and PRL release in lactating females are different in not suckled and suckled animals.

THE EFFECT OF MELATONIN ON OXYTOCIN RELEASE UNDER STRESS CONDITIONS

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Melatonin (MEL) modifies the release of oxytocin (OT) as brought about by some pathological conditions such as dehydration or hemorrhage. The aim of the present investigations was therefore to study the effect of MEL on OT release under immobilization stress.

Male Wistar rats were divided into two groups: A) animals injected once daily for two weeks with melatonin vehicle (1% ethanol in 0.9% NaCl; 0.1 ml per 100 g. b.w.), B) animals similarly injected with melatonin solution (50 µg/100 g. b.w.). In each group three further subgroups were chosen: 1 - rats euhydrated, i.e., they had free access to food and tap water during whole experiment; 2 - animals dehydrated (i.e., they did not have access to food and tap water) for 24 hours before decapitation; 3 - animals dehydrated as animals of group 2 and additionally immobilized (i.e., singly transferred into small cages where they could not move freely) for 24 hours. The animals were decapitated between 9.00 - 9.30 a.m. and the OT content in the neurohypophysis (NH) as well as plasma levels of OT and ACTH were radioimmunoassayed.

Dehydration for 24 h did not modify the OT content in the NH. However, immobilization decreased the content of OT in NH both in vehicle- and MT-treated rats. Neither dehydration nor immobilization modified the plasma OT levels in vehicle-treated rats. However, after MT treatment plasma OT level was diminished in euhydrated animals but slightly increased in immobilized rats. Melatonin injections significantly inhibited the rise in plasma ACTH levels as brought about by immobilization.

On the base of the present results we conclude that MEL seems to increase OT release but inhibit ACTH release under conditions of immobilization stress.

LHRH - A NEUROMODULATOR OF THE HYPOTHALAMO-NEUROHYPOPHYSIAL SYSTEM ACTIVITY

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The secretory activity of the hypothalamo-neurohypophysial system (HNS) is affected by a number of factors of neural as well as hormonal origin. We have found recently that intracerebroventricularly injected luteinizing hormone releasing hormone (LHRH) alters the vasopressin release in the rat. The present study was designed to investigate in vitro some mechanisms possibly involved in the LHRH - HNS interactions. The hypothalamo-neurohypophysial complex (the intact pituitary stalk was preserved) obtained from male Wistar rats was incubated in 1 ml of the Krebs-Ringer buffer (KRB) gassed with carbogen at 37°C. The medium was changed every 20 min. After a 60-min. equilibration period, the HNS was incubated successively in: (1) the normal Krebs-Ringer fluid (B1), (2) the hypertonic KRB with excess of sodium (osmolality = 320 mOsm/kg H_2O) or in the KRB containing 40 mM potassium (S1), (3) normal medium alone or with 40 nM LHRH (B2), (4) the incubation fluid as (2) in the presence or absence of LHRH (S2). Vasopressin concentrations in the samples were radioimmunoassayed and the ratios B2/B1 and S2/S1 calculated and compared using Wilcoxon test. LHRH significantly ($p < 0.01$) inhibited basal vasopressin secretion, the ratio B2/B1 decreasing from 1.7 ± 0.3 to 0.9 ± 0.2. Similarly, LHRH reduced both hyperosmotically challenged vasopressin secretion (the S2/S1 ratio from 0.85 ± 0.12 to 0.47 ± 0.07; $p < 0.05$) and K^+-stimulated hormone release (the S2/S1 ratio from 1.4 ± 0.4 to 0.7 ± 0.1; $p < 0.01$).
We conclude that LHRH is involved in the regulation of the HNS function by the inhibitory impact on the vasopressinergic neurons' activity.

INFLUENCE OF OSMOTIC STIMULATION ON VASOPRESSIN (AVP) AND CARDIODEPRESSANT FACTOR (CDF) RELEASE INTO THE BLOOD

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The aim of this study was an attempt to reveal if CDF and AVP were simultaneously released from the pituitary into blood after osmotic stimulation, and if excitatory amino acids blockers influence AVP and CDF release to osmotic stimulation. The samples of dialysates of the blood outflowing from the sella turcica region and from the femoral vein were collected in anaesthetized rats. At the beginning of the collection of the 2nd 30 min dialysate samples hypertonic saline and/or 200 μg of selective receptor antagonist D, L-2-amino-5-phosphonopentanoic acid (AP-5) or non-selective receptor antagonist 6,7-dinitroquinoxaline-2,3 (1H,4H)-dione (DNQX) was infused intraarterially. In dialysate samples AVP concentration was measured by RIA and CDF on spontaneously discharged pacemaker tissue of the right auricle of the right heart atrium of a two-day-old rat. Osmotic stimulation simultaneously caused increase in AVP and CDF concentration in the blood dialysate from the sella turcica and from the femoral vein. Blockade of excitatory amino acids receptors by AP-5 and by DNQX significantly decreased in the blood dialysate AVP and CDF concentration elicited by intraarterial injection of hypertonic saline. Excitatory amino acids are involved in the mechanism of the release of blood AVP and CDF to osmotic stimulation.

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INFLUENCE OF THE SYMPATHETIC EFFERENTS ON NEUROHYPOPHYSIAL HORMONS RELEASE IN RAT

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The aim of the present study was to investigate whether the stimulation of the superior cervical ganglion (SCG) or superior cervical ganglionectomy (SCGx) have the influence on the vasopressin and oxytocin release from the posterior pituitary lobe. The experiments were performed out on male rats under general anaesthesia. The animals were divided into five groups: 1) control, 2) 20 days after SCGx, 3) immediate after SCGx, 4) after the preganglionic fibers of the SCG stimulation, 5) after blooding (1 % b.w.).The venous blood from the sella turcica region dialysis was carried on. Vasopressin and oxytocin content was determined in the dialysates by radioimmunoassay. In chronic animals 20 days after bilateral SCGx the oxytocin and vasopressin content in dialysates were 4 times higher per time unit then in control animals. Superior cervical ganglionectomy immediate before dialysis evoked several times increase in vasopressin and oxytocin release like after blooding. SCG stimulation increase four times vasopressin and oxytocin release.
On basis of results obtained, it my be presumed that noradrenergic efferents probably decrease in neurohypophysial hormones release, but stimulation of the superior cervical ganglia increase its release.

The study was supported by a grant for Medical University of Lodz.

Involvement of histamine in the CRH and vasopressin-induced pituitary-adrenocortical response.

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Vasopressin (AVP) derived from the hypothalamic paraventricular nucleus is a well documented coregulator of ACTH release from the anterior pituitary. The release of both corticotropin-releasing hormone (CRH) and vasopressin from the hypothalamic neurons and ACTH from the pituitary corticotrops is coregulated by neurotransmitters. We found that stimulatory effect of CRH on the pituitary-adrenocortical axis does not markedly depend on central histaminergic mechanisms. The secretion of corticosterone induced by systemic or intracerebroventricular administration of CRH in rats was not substantially affected by ip or icv pretreatment with the histamine H_1- and H_2-receptor antagonists, mepyramine and cimetidine. Also hypothalamic and hippocampal histamine levels were not markedly altered by treatment with CRH.
A possible involvement of brain histamine and its receptors in the AVP-induced ACTH and corticosterone response is not clear. Vasopressin given ip significantly increased the hippocampal histamine level as well as serum corticosterone concentration. Histamine synthesis inhibitor α-fluoromethylhistidine considerably decreased the AVP-elicited hippocampal histamine and serum corticosterone levels. Histamine H_1- and H_2-receptor antagonists, mepyramine and cimetidine, also markedly impaired the vasopressin-induced increase in the hippocampal histamine and serum corticosterone levels. Pretreatment with the histamine H_3-receptor antagonist thioperamide significantly diminished the AVP-elicited corticosterone response, but did not alter the histamine content in either brain structures examined. These results indicate that histamine H_1-, H_2- and H_3-receptors and hippocampal histamine are involved in mediation of the AVP-induced pituitary-adrenocortical response, whereas histaminergic mechanisms are not involved in the response elicited by CRH.

Prostaglandins and nitric oxide regulate adrenocortical response to vasopressin in stressed rats

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The significance of prostaglandins (PG) and nitric oxide (NO) in the hypothalamic-pituitary-adrenal (HPA) response to vasopressin (VP) was investigated in rats crowded for 3 days. We have found that vasopressin is almost as potent as corticotropin-releasing hormone (CRH) in stimulating the HPA axis. Social crowding stress considerably diminished the ACTH and corticosterone response to VP but not to CRH or interleukin-1β. Indomethacin, an inhibitor of constitutive cyclooxygenase (COX-1) and PG synthesis significantly reduced the ACTH and corticosterone response to VP in control rats and further reduced this response to VP already diminished by crowding stress. Dexamethasone (DEX) abolished the HPA response to a short restraint stress and the response to VP in both control and crowded rats. Although DEX inhibits the HPA axis by negative feedback mechanisms it also inhibits the induction of COX-2 and nitric oxide synthase (NOS). Nitric oxide synthase is present in perikarya of the hypothalamic nuclei closely associated with the regulation of pituitary activity, in the median eminence and the pituitary itself. A nitric oxide synthase antagonist Nω-nitro-L-arginine-methylester (L-NAME) significantly enhanced, and L-arginine, a NO donor, considerably diminished the HPA response to VP or stress, indicating that NO inhibits centrally the HPA stimulation.

The results indicated a significant interaction of PG and NO systems in activation of the HPA axis by VP during stressful circumstances.

Modulatory effects of galaninergic system in the rat striatum

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Galanin is a peptide identificated in the CNS of humans and different species of animals. Galanin exerts modulatory effects on different central neurotransmitter systems. We tried to reveal the possible interaction of galanin with the different neurotransmittive systems in the CNS.

Experiments were performed on male CFY rats. Rat galanin (2,10,50 ng) was administered intrastriatally (i.s.) either alone or 10 min after kainic acid (KA) administration (20-100 ng). Naloxone (1.0 mg\kg), ketamine (5.0 mg\kg) and atropine (1.0 mg/kg) were used i.p. 10 min prior to KA or galanin. We investigated the locomotor activity in the "open field" test and muscle tonus.

We revealed the development of KA-induced bradykinesia, ptosis and muscle tonus increasing. Galanin coadministration with KA (20 ng) into the striatum dose-dependently resulted in the development of behavioral disturbances reminiscent of those obtained after KA i.s. administration. Named behavioral disturbances were partially blocked by naloxone, ketamine or atropine. It is concluded that galanin potentiates the action of i.s. KA in inducing of specific behavioral impairments. This effect can be realized via galanin modulatory effects on the opiate, excitatory amino acids or cholinergic transmission.

EFFECTS OF ANGIOTENSIN II AND ITS PEPTIDE AND NONPEPTIDE RECEPTOR ANTAGONISTS ON A WRITHING TEST

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The writhing test (acetic acid 1 % ip) was used. Writhings were counted at 5 min intervals for 30 minutes. Angiotensin II (ATII) at doses of 0.1 and 1µg icv significantly decreased writhings within all 30 min period. ATII analogue saralasin (0.5 µg icv) decreased writhings only within first 5 min, and increased them within 10 min. Pretreatment of saralasin blocked the effect of ATII (0.1 µg) at 15 min. ATII analogue sarmesin - [Sar^{1}Tyr^4(Me)]ATII (0.5, 1, 5µg icv), decreased writhings during the whole 30 min observed period with a dose of 5µg. Sarmesin (0.5 µg) blocked the effect of ATII (0.1 µg) from 10th to 30th minutes. The non-peptide AT_1 receptor antagonist DuP753 (25, 50µg) showed well expressed analgetic effect. DuP753 (25 µg) did not block the analgetic effect of AT II (0.1 µg). The non-peptide AT_2 receptor antagonist PD 123319 (5, 10 µg) showed algetic effect (increased number of writhings) at 10th and 15th min. PD 123319 blocked the analgetic effect of ATII (0.1 µg). Taken together it might be accepted that ATII realizes its analgetic effect on writhing test through AT_2 receptor subtype.

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Effects of peptide and nonpeptide angiotensin II receptor antagonists on acute hypoxia in mice.

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The effects of i.c.v. administered angiotensin II (AT II) peptide receptor antagonists saralasin (Sar) and sarmesin (Sarm), as well as of nonpeptide receptor antagonists DuP 753 and PD 123319 on acute hypoxia (asphyctic and haemic - sodium nitrite 300 mg/kg, s.c.) were studied in male mice. Latencies (in min) until the first clonic seizures and animal death (survival time) were measured as end points of hypoxia. It was found that both Sar (1, 5, 10 µg) and Sarm (1, 5, 10 µg) increased latencies until seizures the survival time (asphyctic hypoxia). In haemic hypoxia only Sar in the dose of 10 µg increased the two studied parameteres and Sarm did not influence them. DuP 753 (50, 100, 200 µg) increased latencies until seizures and the survival time (both types of hypoxia). PD 123319 (5, 10, 20 µg), also influenced both parameters in asphyctic hypoxia and decreasing them in (haemic hypoxia). Taken together, the results show that the balanced participation of both AT_1 and AT_2 receptor subtypes is necessary for regulation of acute hypoxia.

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Poster session - Motor control

CHANGES IN LOCOMOTION AFTER PARTIAL SPINAL LESIONS IN RATS

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In 7 intact rats and 5 rats with partial spinal lesions, performed at the low thoracic level, the main indices of gait during unrestrained locomotion were compared. The animals were taught to move on 2 m long and 12 cm wide platform placed 1.5 m above the ground. The platform was covered with conductive material connected to low voltage DC source. The animals wore on palmar surface of each limb small cooper wire contact electrodes, which allowed to record the stance and swing phases of each limb. The locomotion velocity was measured using photocells placed every 25 cm. In the operated group, in 3 rats the lesion was essentially confined to the dorsal columns, while in the remaining 2 animals it was much larger, involving dorsal quadrants of the spinal cord. The operated animals with smaller lesions were tested both before and after surgery, while the animals with larger lesion were tested only postoperatively. No essential changes were found between pre- and postoperative data in rats with smaller lesions, nor between the animals with lager lesion and the group of intact animals with regard to locomotor velocity, swing, stance and step cycle durations in the fore- and hindlimbs, the lateral and diagonal phase shifts and their relationships with the step cycle duration. The coupling of the fore- and hindlimb movements, measured by the correlation of time intervals between the onsets of each limb stance phase and the offsets of these phases in the homologous and the diagonal limbs, were not changed in animals with smaller lesions, while in the 2 animals with larger lesions, it was less accurate as shown by weaker correlation between the measured time intervals. This means that in latter animals the movements of the hindlimbs were less dependent on the movements of the forelimbs than in the remaining operated animals. Our results show that neither dorsal columns nor the dorsal quadrants are not essential for locomotor movements, although the latter lesion impair some indices of fore- hindlimb coordination.

PARTIAL RESTORATION OF HINDLIMB FUNCTION IN ADULT SPINAL RATS AFTER TRANSPLANTATION OF EMBRYONIC RAPHE NUCLEUS INTO THE SPINAL CORD BELOW THE TRANSECTION.

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Monoaminergic pathways are known to influence the circuitry of the spinal cord involved in producing locomotor movements (central pattern generators - CPGs). Following spinal cord transection, the CPGs, although still present in the disconnected part of the spinal cord, are unable to generate coordinated movements. Administration of serotonergic agonists to spinal cats improves their locomotor movement. Another method of administering serotonin to the spinal cord is by grafting of embryonic cells from raphe nucleus. The aim of our study was to activate the CPGs by using the grafted cells which were supposed to release serotonin.

The graft of embryonic, serotoninergic cells survives transplantation and integrates with the host neuropil. Moreover, the grafts seem to encourage functional improvement. Our study showed that the embryonic tissue transplanted into the separated part of the spinal cord one month after its transection at the Th9-10 level resulted in more coordinated hindlimb movement of Wistar rats. Three months after transplantation the functional improvement of motor function was assessed by behavioral and electromyographic analysis. Locomotor activity was tested in animals held with their forelimbs on the moving trolley and their hindlimbs walking on the pathway with simultaneous stimulation of the tail. A rhythmical locomotor activity with a pattern similar to that observed in intact animals (alternating movements of right-left hindlimb and complete flexion-extension movements) was regularly obtained in grafted rats. Although, some rhythmic alternating movements were also observed in spinal animals (without graft), the coordination between the hindlimbs was improved in the grafted spinal animals. The electromyographic activity recorded from soleus and tibialis anterior muscles during regular locomotion showed a typical alternating pattern of extensor and flexor activity. However, unlike in intact animals, in the grafted rats the burst duration of the flexor muscle was related to the step cycle duration.

The results seem to indicate that transplantation of defined groups of neurones to the transected spinal cord made use of the residual circuitry in the lesioned spinal cord to improve the recovery of motor functions after lesions.

SURVIVAL AND GROWTH OF EMBRYONIC CELLS FROM RAPHE NUCLEUS TRANSPLANTED INTO TRANSECTED SPINAL CORD IN RATS.

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Recently, fetal neuronal transplants have been used for supplying new cells for anatomic and neurophysiologic integration into the host CNS to restore lost function. In our experiments the circuitry of the spinal cord (central pattern generators - CPGs) involved in producing locomotor movements of hindlimbs was separated from the supraspinal inflow by transection of the spinal cord at the lower thoracic level (Th9-Th10). Following spinal cord transection, the CPGs, although still present in the disconnected part of the spinal cord, were unable to generate coordinated movements. One of the main reasons of hindlimb movement deficits is a destruction of supraspinal monoaminergic pathways which are known to influence the CPGs activity responsible for producing locomotor movements. Recent results show that administration of monoaminergic (serotonergic and noradrenergic) agonists to spinal cats improves their locomotor performance. In order to administrate serotonin to the disconnected spinal cord in rats, the embryonic tissue of raphe nucleus region (containing serotoninergic cells) was transplanted one months after spinal cord transection.

Three months after transplantation, the rats were deeply anesthetized with injection of sodium pentobarbital (60mg/kg) and perfused transcardially with 0.9% saline followed by 4% paraformaldehyde. After dissection, the spinal cord was prepared for further immunocytochemical treatment in order to establish the grafts survival. The 40 μm cryostat sections of the spinal cord were processed for immunocytochemical detection of serotonin (5-HT). The transplanted rats showed many immunoreactive 5-HT perikarya in the graft area, some at a distance of up to 15mm, and a progressive innervation of the whole grey matter extending over at least 20mm from the graft site.

Our behavioral experiments showed the improvement of motor function which may confirm that the grafted cells of embryonic raphe nucleus, after integration with the host neuropil, are able to release serotonin into the separated part of the spinal cord and to encourage recovery of hindlimb locomotor functions.

THE INVESTIGATION OF MOTONEURONAL CHARACTERISTICS IN HUMAN

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The relationship between two characteristics of human motoneurons, the afterhyperpolarization (AHP) duration and the relationship between the standard deviation of the interspike intervals (ISIs) and their mean value, $s(T_m)$, was studied in two series of experiments. During the first one, the rhythmic firing of voluntarily activated single MUs of two muscle types: fast - biceps brachii (BB) and slow - soleus (SOL), was recorded and stationary fragments were analyzed off-line to get their statistical characteristics. In the second series of experiments, the tibial nerve was stimulated with paired stimuli of varied interpulse interval. Responses of single MUs from SOL were recorded and the AHP duration was estimated from the recovery period of the motoneuronal excitability after the first stimulus.

The relationships $s(T_m)$ of single MUs were slightly different from those reported in the literature for the pooled data. The plateau for shorter intervals was scarcely observed. Instead, the typical relationship for a single MU consisted of two segments of a straight line with different slopes. Some of the relationships for MUs from BB could not be approximated by two straight lines but were curvilinear. There was a significant scatter of the break-points for MUs from the same muscle type of all subjects and even for MUs from one subject only. As expected, the range of the break-points for BB was shifted towards the shorter ISIs as compared to SOL. For SOL MUs, the break-point range corresponded to the intervals shorter than the range of the AHP duration.

The results presented above provide evidence that the statistical parameters of a single MU activity are dependent on the motoneuron properties. These properties for a motoneuron pool of a given muscle are not uniform.

The correlation between the statistical parameters of the MU activity and the AHP duration established in this study may be significant for clinical studies of neuromuscular diseases. The differences in this respect were recently revealed to exist between dystrophic and normal muscles.

SOLEUS DISTINCTIONS IN CLINICAL ELECTROPHYSIOLOGICAL EXAMINATIONS

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Motor evoked potentials (MEPs) were recorded by transcranial magnetic stimulation (TMS) combined with electrical stimulation of pertinent pheripheral nerves. This procedure allows to estimate central conduction in segment: cortex-spinal motoneurones (CCT-F). Four muscles of low limbs: VL, TA, EDB and SOL were examined bilaterally at rest. (240 muscles in 30 healthy subjects). From theoretical point of view CCT-Fs should be successively longer depend on descending metameric localisation of motoneurons innervating examined muscle.However CCT-F for SOL revealed to be relative short. Moreover the mean amplitude of „cortical" C-MEP from SOL was relatively the smallest one in comparison with the C-MEPs from other muscles. On the contrary the amplitudes of peripheral CMAP and F-wave were the highest ones. Standarized distal motor latency (DML/cm) was in SOL the longest. Differences were statistically significant. The cause of SOL distinctions may be explained by different structure and innervation of this muscle (slow - red, tonic muscle).

DIRECTIVITY OF INFORMATION FLOW BETWEEN LIMBIC AND MOTOR STRUCTURES FOR VARIOUS FREQUENCY BANDS IN MOVING RAT

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Investigation of functional role of limbic-motor connections involved in emotional behavior and initiating the performance of motor reaction was the aim of our experiment. Information flow among a nodal point between the motivational and executory systems in the brain – the n. accumbens (ACC), its main input structures the basolateral amygdala (BLA) and ventral subiculum (VSB), and its output to the motor system, the subpallidal area (SPL), was analyzed. The EEG signals, recorded with chronic electrodes in various experimental situations (emotional states) in freely moving rats, were analyzed with the use of directed transfer function (DTF). The method enabled analysis of the direction and intensity of information flow among structures, taking into account all signals simultaneously, and providing spectral characteristics of information flow among structures. The DTFs were normalized in a way enabling comparison of information flow for various behavioral situations in 6 selected frequency bands from the range of 1–90 Hz. Thus, we estimated the differences of the strength of information flow within the BLA-VSB-ACC-SPL circuitry, and as a result we obtained a pattern of connections for which flows in various situations were different. Comparison of DTFs for the rest state and well trained locomotion (i.e., a low emotional state) showed that the flows for frequencies above 30 Hz did not differ for both situations. Most of the differences were present in the theta band (7.9–9.3 Hz), the interactions among all structures were significantly higher during locomotion, when compared with the rest state. During the preparatory phase to locomotion the flow from all structures to ACC was higher than in the rest state, while after the locomotory phase the flow between ACC and SPL was the same as in the rest state. During maze exploration some of the flows in the theta band were also higher, when compared to the rest state. In the band of 67.7–72.2 Hz the comparison of the DTF values calculated for various experimental situations showed that they were different for exploration of maze in comparison with the locomotion along a wide runway. Inducing the bell stimulus during locomotion on a wide runway resulted in changes of the DTF values for the information flow from ACC to SPL only. Summarizing the results one can say that patterns of information flow among structures are different for various frequency bands, depending on the experimental situation (i.e., emotional-motivational and motor components of the task).

Lesions of the anterior intraparietal area induce prehension deficits

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Recently, it has been shown in non-human primates that the posterior parietal cortex is involved in coordination of arm and eye movements in space, while the anterior lateral bank of the intraparietal sulcus (anterior intraparietal area, AIP) plays a crucial role in grasping. In this study we show by kinematic recordings that patients with cortical lesions involving the anterior part of the intraparietal sulcus and the human homologue of AIP have selective deficits in the coordination of finger movements required for object grasping whereas the reaching is much less disturbed. fMRI data obtained during reaching and grasping movements provide complementary evidence that the same area is specifically activated during grasping. Taken together, the combined lesion and activation study is suggestive that AIP mediates the processing of sensorimotor integration of precisely tuned finger movements in humans.

Poster session - Sensory processing

CHANGES OF EVOKED POTENTIALS INDUCED IN THE SOMATOSENSORY BARREL CORTEX BY COOLING

E. Kublik, P. Musiał and A. Wróbel

It is known that low temperature inhibits electrical activity of the cortical tissue. We utilized this mechanism to evaluate contribution of various populations of cells in barrel cortex into the potential evoked (EP) by vibrissae stimulation.

Three hooded rats were used in the experiments. Under urethan anaesthesia the scull was opened to expose the barrel field. The multichannel electrode was then placed into the cortex at the depth of 0, 300, 600 1200 μm from the surface. The silver plate attached to narrow plastic tube was placed on the surrounding surface of the barrel field around the electrode. By stroking each vibrissa with the piezoelectric device we recorded evoked potentials (EPs) to obtain four maps of EP amplitudes associated with each recording site of the electrode. The principal whisker (PW) - corresponding to the biggest EP - was then continuously stimulated with a 5s interstimulus interval and the EPs were on-line digitized and stored on PC computer by the Spike2 software. The "Freeze 75" was sprayed into the plastic tube in order to cool the silver plate and the underlying cortex. This method allowed to lower the temperature rapidly and deactivate the cortex throughout the whole its depth. This procedure resulted in disappearance of EPs followed by their slow restitution with the increasing temperature. After a few seconds EPs reappeared with amplitudes comparable to control but with twice as long latencies. The control shapes of EPs were observed only after a few minutes.

VISUAL TASK STIRS CORRELATED ACTIVITY IN CAT'S THALAMO-CORTICAL SYSTEM

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We have previously shown (Bekisz and Wróbel, Acta Neurobiol. Exp. (1993), 53: 175-182) that local field activity (LFP) recorded from many sites in lateral geniculate nucleus (LGN) and primary visual cortex (VCx) of cats attending to visual stimuli during conditioning task contained enhanced amount of power within 20 Hz band as compared to auditory or erroneously ended visual trials.

We now analyzed the same data by calculating the normalized cross-correlation coefficient with zero lag (CC) for (1) band-pass filtered (16-24 Hz) LFPs and (2) amplitude envelopes of these filtered signals, for all possible pairs between recording sites.

The analysis revealed positive synchronization between filtered signals from majority of electrodes during nonvisual situations which indicated common oscillatory rhythm within the investigated part of the visual system. The level of synchronization decreased during periods involving visual attention. In few pairs of recording sites of CC>0.8, the synchronization of such an activity increased. Similar direction of CC changes were obtained when calculating the amplitude envelopes for the given pair of electrodes. We therefore conclude that main contribution to CC values is from simultaneous, large amplitude bursts with in-phase oscillations and not from continuous synchronization of phase between both signals.

We hypothesize that visual attention activates the specific mosaic of functional connections with the use of the 20 Hz oscillatory carrier. This activation changes the basic, global synchronization into organized visual network for the process off attentive seeing.

IMMEDIATE CHANGES OF INTRACORTICAL INFORMATION PROCESSING RELATED TO THE CONDITIONING PROCEDURE

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We used for this study five unanesthetized rats with chronic electrodes implanted in the barrel cortex. Before recording animals were trained to rest in a plexiglass tube with head restrained in a holder. After implantation of electrodes the chosen vibrissa was stimulated with piezoelectric device and evoked potentials (EP) were recorded from the barrel cortex. Five habituation sessions were followed by a conditioning session and in all of them the animal received 100 vibrissa stimulations with intervals randomly scattered from 30 to 45 s. In the conditioning session the first 30 vibrissal stimulations allowed for stabilization of the EPs. All remaining stimulations were followed by a mild electric shock (unconditioning stimulus) applied with a 250 ms delay to the ear on the same side. The whole conditioning session lasted for about an hour.

The first negative component (N1) of EP consisted of two peaks with latencies differing by 1.5-2 ms. The contribution of these subcomponents of N1 to the integral value of EP was calculated within the 5 ms period containing both peaks. This procedure allowed to classify EPs with respect to relative amplitude of the two subcomponents. The second class differed from the first one by increased amplitude of the second subcomponent. Introduction of the conditioning procedure changed the control ratio of the two classes in such a way that number of EPs with the enhanced amplitude of later subcomponent rapidly increased.

We hypothesize that the two N1 subcomponents might reflect the successive stages of sensory information flow within the barrel cortex. The conditioning procedure would recruit larger population of cells at the higher processing level (delayed by one synapse) and thus enhance the amplitude of the second subcomponent.

INTERACTION BETWEEN NITRIC OXIDE SYNTHASE AND OXOTREMORINE IN ACUTE AND CHRONIC PAIN.

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The present research was aimed to find out, using behavioral and immunohistochemical methods, whether the cholinergic analgesia induced by the muscarinic agonist oxotremorine was modulated by nitric oxide in different tests of nociception. The preliminary experiment showed that intraperitoneal administration of the nitric oxide synthase inhibitors NG-nitro-L-arginine-methyl ester (L-NAME) and NG-nitro-L-arginine (N-ARG) in doses ineffective per se significantly enhanced the analgesic effect of oxotremorine in CD1 mice in a tail-flick test. Moreover, an interaction between oxotremorine and NO was also reported when the effects of those drugs were studied at a spinal level. Intrathecal administratin of L-NAME (400 μg) increased the antinociceptive action of oxotremorine (1ng) in both the paw pressure and tail flick tests in Wistar rats.

We also investigated the interaction between L-NAME and oxotremorine in modulation of the response to a prolonged nociceptive stimulus induced by intraplantar injection of formalin to rats. Behavioral and immunohistochemical methods were used.

Intraplantar formalin injection significantly enhanced the characteristic behaviour (paw jerks) and increased the number of NO synthase labelled neurons in laminae I-III, IV and X, but not in laminae V-VI. Oxotremorine and L-NAME inhibited the paw-jerk frequency, however only in the second phase of the formalin-induced behavior. Both oxotremorine and L-NAME suppressed the formalin-induced increase in the number of NO synthase neurons, mainly on the ipsilateral side of the lumbar spinal cord.

In summary, our study supports the hypothesis that inhibition of NO synthase in the spinal cord may facilitate antinociception. At the same time, we have also obtained some evidence that oxotremorine may either increase or reduce the the number of NO synthase-labelled neurons in the formalin model.

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ANTINOCICEPTIVE EFFECTS OF HYPOTHALAMIC REWARDING STIMULATION IN THE FORMALIN TEST IN YOUNG AND ADULT RABBITS

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In 20-25-d-old and adult rabbits anaesthetized with urethane and chloralose the inhibitory effects of electrical stimulation of hypothalamic reward sites on the evoked potentials (EPs) recorded in the thalamic intralaminar nuclei in response to the electrodermal stimuli applied to the hindpaw were investigated before and after injection of dilute formalin (F) in the contralateral hindpaw. Before F we determined the intensity of rewarding stimulation that induced the decrease of amplitude of EPs but not their complete inhibition. F produced the biphasic (in 5-10 min and 20-60 min after injection of F) enhancement of the inhibitory effect of rewarding stimulation manifesting as the complete inhibition of EPs recorded in response to electrodermal stimuli applied to the contralateral hindpaw. In 20-25-d-old rabbits the enhancement of the inhibitory effect of rewarding stimulation was more pronounced as compared to the adults. The results of the present study are in agreement with our data about the enhancement of the antinociceptive effects of stimulation of the reward sites in 20-40-d-old rabbits (Butkevich and Kassil,1989), as well as with the behavioral data according to which the decrease of the threshold of the reflex withdrawal response of the contralateral paw to noxious stimuli after F includes two phases, an early phase and a late one (Dubuisson and Dennis, 1977).

THE SPATIAL STRUCTURE OF THE VISUAL RECEPTIVE FIELDS OF CAT'S PRETECTAL NEURONES

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The aim of this study was to examine spatial organization of the receptive fields of visually sensitive neurones in the pretectal region. The 21 neurones was examined using extracellular recording of single units in the pretrigeminally transected cats. After establishing the preference for the stimulus size and velocity of movement, cells were tested with a decreasing range of stimulus movement around the centre of the receptive field and with a small range of movement around the locations placed in equal intervals along the horizontal axis of the receptive field.

The intensity of cellular responses varied depending on the range of the stimulus movement and the location within the receptive field. The intensity of responses gradually decreased with the decrease of the range of movement. The majority of units required a quite large range (2.5° - 20°) of the stimulus movement to evoke their response. Only one neurone responded to the range as small as 1°. The smallest range of the stimulus movement was achieved for a preferred stimulus size and velocity of movement and could differ between ipsi- and contralateral eye inputs. The majority of cells did not change their directional selectivity with a decreasing range of the stimulus movement. Only one out of all tested cells showed changes in the directional selectivity index depending on the location of the stimulus within the receptive field.

The pretectal cells require large region of summation to evoke the response and show high level of homogeneity of their receptive fields. These properties of receptive fields of neurones in the pretectal region distinguish them from highly heterogenous receptive fields of collicular cells which are unusually sensitive to small range of the stimulus movement.

Effect of acoustic stimulus characteristic on startle response in rats

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The startle reflex is an animal response to a sudden intense stimulus e.g. sound pulse. The acoustic startle response (ASR) depends not only on the pulse amplitude but is also modulated by a stimulus frequency. In the experiment amplitude and latency of the acoustic startle reflex were assessed for a variety of stimulus frequencies ranging between 3 and 23 kHz. The responses were studied in 11 adult hooded rats by means 2-ms tone pulse of different frequency presented without or with 70 dB white noise background. A main effect for frequency was found with responses to low frequency stimuli. Analysis of the ASR amplitude for each testing frequencies showed significant differences ($F(5,50)= 48.375$, $P<0.001$). The low-frequency stimulation clearly differed in their effects on startle behavior. Significant differences in the ASR amplitude were clearly pronounced for stimulus frequencies of 3, 7, 10 kHz. The rats responded more readily for lower frequencies. For the frequencies of 15, 20 and 23 kHz, however, statistical analysis did not revealed any differences in the amplitude of startle reaction.

Poster session - Learning, memory and cognitive functions

CHOLINERGIC MODULATION OF LONG-TERM DEPRESSION IN HORIZONTAL CONNECTIONS OF RAT MOTOR CORTEX

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Recent studies indicated that the activation of cholinergic receptors may play a role in the induction of long-term increases of synaptic efficacy in certain cortical areas. Here we have investigated the influence of cholinergic activation on the threshold of long-term depression (LTD) in local horizontal connections within layers II/III of rat motor cortex, using field potential recording in brain slices *in vitro*. In standard incubation conditions 1500 pulses applied at 3 Hz routinely induced LTD (-25±15% decrease, n=6), measured 20 min after cessation of the stimulation. The application of 1000 pulses induced only small changes (-10±4%, n=6), and 500 stimuli delivered at 3 Hz did not produce any marked changes in the response amplitude (n=3). In contrast, 1000 pulses applied in the presence of a cholinergic agonist, carbachol (0.5 μM) in the bathing fluid, induced marked LTD of responses (-20±3%, n=5). The effect of 1500 pulses applied in the presence of carbachol (-25±4%, n=5) was not different from control.

These results, together with an earlier communication (Hess and Krawczyk, Eur. J. Neurosci. Suppl. 9: 201, 1996), suggest that cholinergic modulatory effects may facilitate plastic synaptic rearrangements within horizontally directed intrinsic pathways of rat motor cortex.

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THE ROLE OF NMDA RECEPTORS IN ACTION OF ARGININE-VASOPRESSIN AND ANGIOTENSIN II IN LEARNING AND MEMORY

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Date from literature indicate that ionotropic receptors of glutaminergic system - NMDA receptors play a role in central action of neuropeptides, especially in learning and memory processes. We studied the influence of various antagonists of NMDA receptors on effects of arginine-vasopressin (AVP) and angiotensin II (AII) improving learning and memory in rats. We used the following compounds: competitive antagonist - AP-7, noncompetitive - MK-801, antagonist of polyamines site - arcaine and antagonist of glycine site - HA-966 in these processes. The experiments were carried on the male Wistar rats. All compounds were injected intracerebroventriculary (the antagonists 15min before neuropeptides).

We have shown that:

1. Mk-801 significantly reduces the acquisition in conditioning avoidance responses (CARs), which was increased by AII and decreases the beneficial effect of AVP on the consolidation in CARs. MK-801 diminishes the adventageous effect of AII on acquisition and remembering in passive avoidance situation.
2. AP-7 impairs the action of AII on the acquisition of CARs, acquisition and remembering in passive avoidance responses. AP-7 does not change of action of AVP.
3. Arcaine significantly diminishes the effects of AVP on the remembering in passive avoidance behavior and decreases the effect of AII on the acquisition.
4. HA-966 diminishes, but not significantly, effects of vasopressin on the remembering in passive avoidance responses and does not influence on the effects of AVP on the consolidation in CARs. In used doses no compound change the motor activity of animals in open field test.

THE CONTRIBUTION OF AT2 ANGIOTENSIN RECEPTORS TO THE COGNITIVE EFFECTS OF ANGIOTENSIN II AND ITS 3-7 FRAGMENT.

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We have previously shown that angiotensin II (Ang II) and its 3-7 fragment [Ang II(3-7)] facilitate acquisition and recall of certain behaviours in rats. In this study we assessed the role of AT2 angiotensin receptors in the cognition enhancing activity of both peptides using selective AT2 receptor inhibitor PD 123319 (1-[[4-(dimethylamino)-3-methylphenyl] methyl]-5-(diphenylacetyl)-4,5,6,7-tetrahydro-1H-imidazo[4,5-c]pyridine-6-carboxylic acid; PD). Male Wistar rats (160-180 g), after surgical preparation, were given into the left lateral cerebral ventricle, 2μl of 0.9% NaCl with or without 1.5μg of PD. Five min later the animals were given, into the right cerebral ventricle, 2μl of 0.9% NaCl with or without 1 nmole of Ang II or Ang II(3-7). Following the next 15 min recall of a passive avoidance, acquisition of an conditioned avoidance responses (CARs), apomorphine (1 mg/kg i.p.) stereotypy, anxiety in the elevated 'plus' maze, and motor activity in an 'open field' were evaluated. Also,the discrimination rate between some familiar and unfamiliar objects was assessed 60 min after the second intracerebroventricular injection (recognition memory). Pretreatment of rats with PD, inactive on its own in all tests, diminished the improvement of recall and recognition memory caused by Ang II and Ang II (3-7). It also abolished enhancement of stereotypy and increased rate of CARs acquisition by the peptides. In the elevated 'plus' maze PD only partially diminished anxiogenic action of Ang II. No statistically significant effects were obtained in 'open field' except for some decrease by PD of motor activity in Ang II pretreated animals.

It appears that memory improving activity of Ang II and Ang II(3-7) is mediated by similar mechanisms and AT2 receptors are substantially engaged in these processes.

EFFECTS OF AT II AND ITS RECEPTOR ANTAGONISTS (PEPTIDE AND NON-PEPTIDE) ON MEMORY PROCESSES OF RATS.

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The effects of AT II (0.1, 0.5 and 1 μg) and of its peptide receptor antagonists saralasin (1 and 5 μg) as well as nonpeptide receptor antagonists for AT_1 subtype DuP 753 (50 and 100 μg) and for AT_2 subtype, PD 123319 (5, 10 and 20 μg) were studied through passive avoidance (step through) and active avoidance (shuttle box) paradigms on male albino rats by i.c.v. administration made immediately after the last training session. AT II (0.1 μg) lead to improvement of retention at both paradigms tested 24 h and 7 day after training. Peptide and non-peptide AT II receptor antagonists did not induce any changes on retention both at 24h and 7 day after training procedure, in both paradigms; however they antagonized the effects of AT II. Taken together, the results show the balanced participation of AT II receptors (both AT_1 and AT_2 subtypes) in regulation of memory processes.

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THE EFFECT OF 1S,3R-ACPD ON COGNITIVE PROCESSES AFTER BLOCKADE OF NMDA RECEPTOR IN RATS.

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We have previously shown that 1-amino-cyclopentano-1,3-dicarboxylic acid (1S,3R-ACPD), an agonist of metabotropic glutamate receptors improves learning and memory in a passive avoidance situation. In this study we attempt to asses the role of NMDA receptor in cognitive effects of 1S,3R-ACPD. The compound was given 30 min before learning trial when its influence on acquisition was tested, and immediately after it for the evaluation of its influence on consolidation processes. 30 min before intracerebroventricular (icv) injection of 100 nmole of 1S,3R-ACPD, half of the animals received icv AP7 (10 nmole), or MK-801 (5 nmole), competitive and non-competitive antagonist of NMDA receptor, respectively. Control animals were icv injected with 0.9% NaCl. Retention of a passive avoidance behaviour was tested 24 h after the learning trial. In addition, evaluation of the locomotor and exploratory activity in open field was conducted in all groups of rats 2 h prior to the retention testing. 1S,3R-ACPD significantly facilitated both, acquisition and consolidation of information in a passive avoidance situation. AP7, the competitive NMDA receptor antagonist, and MK-801 the non-competitive NMDA receptor antagonist totally abolished the positive effect of 1S,3R-ACPD on acquisition of information, while only AP7 abolished the facilitatory effect of 1S,3R-ACPD on consolidation processes. The locomotor and exploratory activity of experimental groups injected with 1S,3R-ACPD, AP7, MK-801 and AP7 or MK-801 before 1S,3R-ACPD did not differ from the activity of control rats. The results of the present study indicate that NMDA receptor plays an important role during the activation of the metabotropic glutamate receptors by 1S,3R-ACPD.

CATECHOLAMINERGIC BRAIN SYSTEM AND BEHAVIOR OF RATS WITH DIFFERENT EMOTIONAL REACTIVITY TO STRESS

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On male Wistar rats with different emotional reactivity to acoustic stressful stimulus the effect of damage of catecholaminergic (CA) brain structures (6-oxydophamine(6-ODA),intraventricularly,150γ) on realization of a number of inborn and acquired behavior forms and brain biogenic amines (BA)turnover are studied. In both groups of rats :emotional-nonreactive (ENR) and emotional-reactive(ER) the influences of toxin decrease of orienting-exploratory activity in "open field"test, "holeboard" test, reactivity to sensory stimuli(tactile, visual,olfactory),changes of learning processes of conditional-food-directed reaction are observed. The degree of changes of this index is more prominent in ENR rats in comparison to ER. The more pronouced decrease of exploratory activity in ENR rats is correlated with the delay of CFR acquisition.Injection of 6-ODA in ER rats is accompanied by acquisition enhancement of CFR. Thus,CA-ergic system of brain involves different ways in regulation of cognitive processes in rats,oridinally differing by the level of these systems activity (the increased noradrenaline level in ENR rats and increases dopamine and serotonin levels in ER-ones) and by emotional reactivity to extremal stimuli.

MOTION DETECTION LEARNING OF RANDOM DOT PATTERN IN VISUALLY DEPRIVED CATS

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It was found previously that detection learning of a moving simple light spot was surprisingly difficult not only for binocularly deprived cats (BD cats) but also for control cats reared with open eyes (C cats). In this study presumably stronger, random dot stimulus was used. Two BD cats and 2 C cats were used. The cats were trained in a two-choice discrimination apparatus for food reward. The stimuli were a moving vs. a stationary random dot pattern. In stage 1 of training, the size of a stationary stimulus was 30% of a moving stimulus and then was enlarged in steps till 100%, the velocity of the moving stimulus was 7°/s. The BD cats reached easily criterion for discrimination performance with a 20% or 30%. size difference between moving and stationary stimuli, the C cats committed 20 times more errors with a 40% or 30% size difference. In stage 2, both stimuli were of equal size and the velocity was 20°/s. The BD cats did not reach criterion within 50 sessions, whereas the C cats reached criterion easily. Thus, increase of the stimulus velocity did not chang performance of the BD cats, whereas for the C cats it was beneficial. For BD cats the size of the stimulus dominated over the motion parameter.

SACCADIC EYE MOVEMENTS ENHANCE THE STIMULUS SPECIFIC ADAPTATION IN INFEROTEMPORAL CORTEX OF THE MACAQUE

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We examined whether saccadic eye movements (SEMs) influence a memory effect (repetition decrement or stimulus specific adaptation - SSA), seen in many cells in inferotemporal cortex (IT). The effect consists in a more vigorous response to a novel than to a subsequent re-presentation of an image. At the beginning of a trial a fixation mark was foveated by the monkey for 400 ms, the mark was extinguished, and 200-600 ms later a dot (visual cue) appeared for 14 ms. The visual cue was presented either at a position 10 deg displaced from the fixation mark, which required that the monkey made a SEM to the cued position, or at the original fixation mark, in which case no saccade was required. The image was then displayed at the cued position for 400 ms. Each image was presented twice with the variable number of intervening images. The main result came from the stimulus-selective units recorded from IT. These cells showed a significantly greater SSA with images presented to the fovea via a saccade than with images presented, foveally, to the fixating monkey. This was true for conditions in which the re-presentation was made without intervening images (paired t-test, $t=3.5$, $P<0.001$), and conditions in which the re-presentation was made with one intervening image (paired t-test, $t=3.3$, $P<0.01$).

Environmental enrichment increases NGF levels in hippocampus and visual cortex and improves spatial learning in elderly rats.

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Available evidence indicates the aging brain shows plastic changes in response to environmental influence. In previous work we found that adult rats housed for 30 days in enriched environmental condition (EC) compared to animals housed in impoverished condition (IC) had higher nerve growth factor (NGF) levels in hippocampus and were better in spatial learning. In the present study rats were housed in EC for a much longer period - during adulthood and as they approach old age. Male Sprague-Dawley rats were housed in EC or IC for 14 months, tested for spatial learning and sacrificed for analysis of NGF levels in olfactory bulbs, frontal cortex, occipital cortex, hippocampus, striatum, hypothalamus and cerebellum. EC rats were better in spatial learning than IC rats, and had higher NGF levels in the hippocampus and visual cortex. These results provide further evidence for the critical involvement of NGF in EC-induced neural and behavioural plasticity, and are compatible with the proposition that the brain maintains its capacity for structural reorganization with increasing age.

THE EFFECT OF INFANTILE NONAVERSIVE AND AVERSIVE STIMULATION ON ADULT EMOTIONAL REACTIVITY IN RATS

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Two groups (4 ss each) of 3 week old rat pups were exposed to different behavioral treatment in attempt to determine the experimental manipulation influence on adult behavior. Every day for two weeks Group NA obtained 15 minute handling whereas Group A in the same time was treated with various aversive stimulators. The applied aversive stimuli were changed every day according to following list: immobilization in a small box or by fixing rat's tail to the ground, shaking in an enclosure, swimming in 20° C cold water, exposure to: 70 dB noise, to 2 kHz tone, to a sharp light, to a dog, and finally tilting in a cage. The acoustic startle response (ASR) was measured after 5 day break and then repeated after next four weeks. The latency of the ASR was significantly shorter in Group NA in comparison to Group A, indicating less tolerance to novelty in rats with the infantile nonaversive stimulation. Twenty days after finishing ASR tests classical defensive response was trained using the CER method. In contrast to Group A, low base-line level of the alimentary instrumental responding, and markedly generalized fear on contextual and conditional cues were observed in Group NA. These results suggested that early nonaversive stimulation clearly enhanced adult emotional responding whereas infantile aversive stimulation decreased rats emotionality.

The role of primary experimental experience on further discrimination learning in rats.

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When either forward or backward procedures for classical defensive conditioning have been superimposed on bar pressing for food they evoked opposite effects on instrumental responding. The forward conditioned stimulus elicited suppression of bar presses indicating acquisition of fear. The backward stimulus caused enhancement of bar presses indicating acquisition of opposite motivation: the safety state. Previously it has been shown that rats did not discriminate darkness and noise conditioned stimuli both paired with shock according to the same contingency. However, the enhancement elicited by backward stimuli was more prononunced during noise than during darkness presentation (Walasek et al. 1995*).

In the present experiment of either forward or backward CER training on discrimination of darkness and noise stimuli introduced subsequently and presented according to the opposite contingency was studied. The signalling value of discriminated stimuli was tested in active avoidance situation. Additionally, retention of signalling properties of stimuli was checked in original CER situation. During transfer test in two-way avoidance situation the tendency for better discrimination between darkness and noise stimuli was observed in rats starting with the forward procedure. Performance during retraining session showed full retention of signalling values of conditioned stimuli acquired during discrimination stage.

*) Walasek G., Węsierska M., Zieliński K. (1995) Conditioning of fear and conditioning of safety in rats. Acta Neurobiol.Exp. 55: 121-132.

Effect of social housing conditions on reactivity of rats

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The effect of social conditions on learning abilities was studied using conditioned emotional response (CER) paradigm. In two groups of rats the forward procedure for classical defensive conditioning was superimposed on ongoing alimentary instrumental bar presses. Typically, the forward conditioned stimulus elicited a suppression of bar presses, indicating acquisition of fear.

Twenty two rats were used. During the first two months of life, rats were reared in a standard colony. Then animals were housed individually (Group IN), i.e. one rat in one cage (16cm x 40cm x 25cm). Remained twelve animals (Group SO) were housed commonly, i.e. six rats in one cage (48cm x 40cm x 25cm). Additionally, only rats from Group SO were subjected to special handling procedure in an enriched environment.

Generally, in Group SO significantly lower level of bar presses rate was seen than in Group IN. Moreover, generalized freezing to contextual and conditioned cues have been observed, resulting in a weak acquisition of classical defensive conditioning. Fast weakening of the suppressive properties of the second part of defensive stimulus has been noticed already from the second day of CER training. On the contrary, in rats from Group IN a clear discrimination between contextual and signalling cues has been observed already on the second day of conditioned training.

These results suggest that different housing conditions may caused changes in animals emotionality resulting in a strong deficit of learning abilities revealed by a weak acquisition of defensive conditioned response.

THE TWO HEMISPHERES OF THE HUMAN BRAIN DIFFER IN VISUAL INFORMATION PROCESSING CARRIED BY MAGNO- AND PARVOCELLULAR CHANNELS: A PERCEPTUAL TEST

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The processing of visual information in primates is accomplished by two parallel visual pathways: magno- and parvocellular channel. The magnocellular channel is more sensitive to low spatial frequencies. This system is thought to be involved in global analysis of visual scenes. The parvocellular channel is more sensitive to high spatial frequencies and is involved in identification of visual patterns, especially small, local details. It has been hypothesized that the two hemispheres differ in their ability to process visual information carried by these two visual channels (Sergent 1983, 1987). The present experiment aimed at testing this hypothesis by using a task in which figures of various sizes and complexity were compared. The stimuli were presented in pairs, one after another, each for 100 ms, at an interstimulus interval (ISI) of 50 - 500 ms. The subject's task was to indicate (by pressing one of three buttons) whether the second stimulus was the same, smaller or bigger than the first one. The first stimulus in each pair was exposed unilaterally, randomly in the left (LVF) or right (RVF) visual field, and the second one was presented at the centre of the visual field. The reaction times analysis shoved significant interaction between stimulated hemifield and stimulus size, and between stimulated hemifield and stimulus complexity. Small and more complex stimuli were processed faster in RVF presentation conditions than in the LVF presentation conditions. Large and less complex stimuli were processed faster in LVF presentation conditions than in the RVF presentation conditions. Our data support the view that the two hemispheres may differ in their ability to process visual information carried by magno- and parvocellular channel.

SUBJECTIVE CONTOUR ILLUSION: SEX RELATED LATERALIZATION EFFECT

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It is widely expected that right hemisphere processes the incoming information in a global, holistic way whereas the left hemisphere does it in an analytical or sequential manner. One could expect, therefore, the visual illusions to operate more strongly within the right than within the left hemisphere, as they depend on global, configural properties of perceived patterns. The aim of our study was to investigate the effect of left and right brain damage on subjective contours illusion. Twenty three patients with right hemisphere damage, 27 patients with left hemisphere damage and 18 control subjects participated in the experiment. They were presented with a series of six subjective contour triangle configurations.The series was constructed in such a way that the length of inducing elements (notches) gradually increased. In the first configuration notches were very short, thus producing no, or just a weak subjective contour effect; in the last configuration they were pretty long, thus evoking strong illusion. The subjects were presented once with each version and were asked to describe all figures they could detect. The strength of illusion was determined as a number of configurations in the series which produced the illusory percept.The results indicate that subjective contour illusion diminishes due to brain lesion, but this effect does not depend on the localization (anterior/posterior, frontal/temporal/parietal) of lesion. A lateral effect associated with the subjects' gender was observed: in female subjects both left and right hemisphere damage had the same disturbing effect, whereas in male subjects only the damage to the right hemisphere diminished the strength of the perceived illusion.The data support the view that the two hemispheres might play a differential role in subjective contour illusion. This hemispheric asymmetry effect however, is limited to male subjects. The finding confirms the notion that males' brain is more lateralized than females' brain.

Masculinity, femininity and transsexuality.

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Gender-related traits and their relation to brain function have recently gained wide interest. In contrast to earlier studies, presently most researchers accept the view that individuals need not to be either masculine or feminine but can be and often are both (androgynous). The present study tested the distribution of masculinity or femininity traits in transsexuals i.e. individuals who have a desire to live and be accepted as members of the opposite sex. This was done to examine whether transsexuals exhibit gender traits typical for their anatomical or 'mental' sex. One thousand and thirty seven students (598 females and 439 males) and 136 transsexuals (111 transsexual females and 25 transsexual males) participated in this study. Femininity and masculinity traits were measured by administrating the Polish version of Bem Sex-Role Inventory. The individuals were classified into four groups: androgenous (high femininity and high masculinity traits), masculine (high masculinity and low femininity traits), feminine (high femininity and low masculinity traits) and undifferentiated (low femininity and low masculinity traits) according to the median split method.
The results showed that transsexuals differ reliably from both controls of the same anatomical sex and controls of the same 'mental' sex. Male-to-female transsexuals possess extremely high femininity traits (in comparison to both control females and males), whereas female-to-male transsexuals are more androgenous than both control males and females. This data are related to specific sex ratio (prevalence of female-to-male over male-to-female transsexuals) observed in Poland.

Heart rate patterns in rats during Pavlovian aversive conditioning

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The changes in heart rate (HR) are often used as one of experimental measures of fear in Pavlovian conditioning paradigms. The exact meaning of this index is not, however, quite clear. The type of reaction (acceleration, deceleration or polyphasic pattern) to the conditioned stimulus (CS) in classical conditioning depends on the species used, the age of subject as well as on experimental arrangements (restrained vs. free-moving animals). Moreover HR patterns differ sometimes from one subject to another in the same experimental procedure what reflects presumably the individual emotionality of the animals.

In order to make the meaning of HR changes more clear we performed Pavlovian aversive conditioning experiments on rats restrained in special apparatus enabling the animals to run on the treadmill. Tailshock (3 mA, 100 ms) was used as a unconditioned stimulus (US). The effects of the following factors were tested: modality of the CS (5s light or tone), the presence and modality of conditioned inhibitor (CI tone or light respectively) that overlapped the last 3s of CS, administration (i.p.) of anxiolytic and anxiogenic drugs (diazepam - DZ and pentylenetetrazol - PTZ).

It has been found that the mean pattern of HR reaction to the CS depended on the CS modality. The light-CS produced HR deceleration while tone-CS resulted in acceleration followed by the deceleration. The difference might be associated with the fact that light is a natural aversive stimulus for rats. CI, irrespective of the modality, induced HR acceleration. The mean HR reaction to CS as well as to CI was not influenced by DZ (10 mg/kg) and by PTZ (10 mg/kg). The consistent effect of CI could be interpreted as a reduction of fear. On the other hand the lack of drugs influence leads to some doubts. One explanation is that the effectiveness of drugs in the doses used is too weak to affect such autonomic response like HR. It might be also supposed that in our model the HR changes do not reflect the anticipatory fear reaction *per se*. The HR may be associated with attention (the excitatory and inhibitory trials were randomly scheduled in each session) and/or with the coping strategy i.e. response intention (immobility vs. running).

Cognitive functions of children after suffering from purulent meningitis in the past

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Purulent meningitis as infection of nervous tissue may reduce working of central nervous system. Therefore in many cases depreciating of intellectual functions might be its result.

Sixteen children from 3 to 18 years old, who had been hospitalised because of purulent meningitis, were put through an examination at Medical Academy of Gdańsk. To confirm possible long-term effects of this illness there was done medical and psychological diagnosis. During medical researches no negative results of suffering from purulent meningitis in the past were found. But psychological researches showed some. In seven cases there were found symptoms of organicity, intellectual status of six children was lower then expected for their age. To realise these examinations were used: Short Intelligence Scale (adapted by M. Choynowski), The Bender - Gestalt Test, Raven Matrices Test, Benton - Visual Retention Test.

Statistic analysis of obtained results took into consideration: term of remaining of the infection, its term of occurrence symptoms of illness, eventual complications and their influence at cognitive functions of patients.

CONTRAST SENSITIVITY IN DYSLEXIC CHILDREN

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Developmental dyslexia or Specific Reading Disorder is defined as failure to learn to read not attributable to factors such as subnormal intelligence, widespread brain damage, impairment of oral language skills and severe sociocultural handicap. Despite a long lasting research in this field the phenomenon of dyslexia remains unexplained. Recent hypothesis (Lovegrove,W. et al. 1986) proposes that dyslexia may result from impairment of the magnocellular channel in the visual system. As there is substantial evidence that magnocellular pathway responds optimally to low spatial frequency patterns and is relatively highly sensitive to contrast, one might expect contrast sensitivity function in dyslexics to differ from that of good readers (especially within the low frequency range). Contrast sensitivity was measured in 12 dyslexic and 11 control children. Black and white sine-wave horizontal gratings of various (0.5; 1.5; 3.8; 8.3 c/deg) frequencies were presented on a computer screen, and the method of limits was used to establish the threshold values. The two subjects' groups were matched in age, intelligence and education. Contrast sensitivity function in dyslexics showed much higher variability than that in controls. No frequency related decrement was observed in dyslexics.

Lovegrove, W., et al. 1986. Perception and Psychophysics, 40, 440-444.

A neuropsychological test battery for the assessment of language disorders in right brain damaged patients.

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There is an increasing awareness of the language and communication disorders that can arise after right hemisphere (RH) damage. The preparations of a neuropsychological test battery for the assessment of these impairments will be presented. The battery was developed following the example of the Right Hemisphere Language Battery by Bryan (1989). The present experimental battery involves the tests of metaphor, inference, humour, and lexical-semantics. The qualitative and quantitative results of a pilot study in RH damaged and aphasics patients will also be analysed.

Test of acalculia: A pilot study.

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An experimental version of the test assessing basic facility with calculation in brain damaged patients will be presented. The test is designed to measure the ability to perform routine arithmetic calculations, the knowledge of numbers and arithmetic signs, abilities to solve arithmetic problems in text tasks. The results of pilot study involving brain damaged and healthy persons will be presented. The data will be analysed in terms of theoretical models of acalculia shown in the approach of cognitive neuropsychology.