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.

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.

Symposium 1 - Chronobiological mechanisms of retina

From photon to vision. Molecular basis of signal transduction in vertebrate photoreceptor cells.

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Vision is initiated in photoreceptor cells of retina, rods and cones, where light is captured and converted to neuronal signal. The signal is then processed in other retinal neurons and eventually sent to the brain. Responses of rods and cones to light differ in sensitivity and kinetics. Rods are sensitive at low level of illumination while cones, which are responsible for color vision, operate in bright light. A sequence of biochemical events triggered by photon appears to be the same in both types of cells and is called phototransduction. In a rod cell phototransduction begins with activation of photosensory protein rhodopsin and in a series of reactions leads to closure of cationic channels; then a recovery of the dark state of the cell takes place. Calcium ions, acting through regulatory calcium-binding proteins, play an important role at different stages of phototransduction. It is also accepted that Ca^{2+}-mediated feedback pathways underlie the light adaptation mechanisms in photoreceptor cells.

The cascade of enzymatic reactions contributing to the rod cell phototransduction as well as some new aspects of calcium ions role will be presented and discussed.

CHRONOBIOLOGICAL ASPECT OF MELATONIN IN VERTEBRATE RETINA

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Retina of different verterbare species is a major extrapineal tissue capable of synthesizing melatonin. Unlike the hormone of the pineal origin, the retinal melatonin is inactivated locally, and appears to act within the eye as a neuromodulator. Biosynthesis of the retinal melatonin takes place in photoreceptors and follows circadian rhythmicity governed by a local circadian oscillator. The activity of the rate-limiting enzyme in melatonin formation, i.e. serotonin N-acetyltransferase (NAT; which runs in parallel with melatonin level) is high at night and low during dayhours. Light is a crucial physiological signal regulating NAT activity rhythm; it entrains the daily rhythm of an oscillator, and, when applied at night, it exerts an acute suppressive effect on nocturnal NAT activity and melatonin levels. The effects of white light on melatonin biosynthesis can be reproduced by monochromatic light (green ≥ blue >> red), including ultraviolet irradiation (UV-A). Biological action of melatonin within the eye are mediated via specific melatonin receptors designated as Mel_{1b} sites. Melatonin (in concert with retinal dopamine) is considered a physiological regulator of circadian events within the eye.

CELLULAR AND MOLECULAR REGULATION OF RETINAL MELATONIN-SYNTHESIZING ENZYMES BY CIRCADIAN CLOCKS, LIGHT, AND SIGNALING CASCADES

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The synthesis of melatonin in retina has been observed in mammalian and non-mammalian species, but has been studied most extensively in chicken and *Xenopus laevis*, where it is produced in photoreceptor cells under the influence of a circadian clock. Melatonin synthesis is high at night and is regulated by circadian rhythms in the activities of tryptophan hydroxylase and serotonin *N*-acetyltransferase (NAT). The rhythm of NAT activity is controlled by an underlying circadian rhythm of NAT mRNA and independent, light-regulated post-transcriptional and/or post-translational modifications. Light has two independent influences on NAT activity: (1) it acutely suppresses NAT activity and melatonin biosynthesis, and (2) alters the phase relationship of the circadian clock. Dopamine, a retinal neurotransmitter, mimics the acute inhibitory effects of light The post-transcriptional/post-translational regulation of NAT activity involves cyclic AMP-dependent protein phosphorylation, and a model for its control by dopamine, light and darkness will be described.

CATECHOLAMINES MEDIATE THE SUPPRESSIVE EFFECT OF UV-A IRRADIATION ON MELATONIN BIOSYNTHESIS IN VERTEBRATE RETINA AND PINEAL GLAND

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Acute exposure of animals to white light at night evokes a rapid suppression of the activity of serotonin N-acetyltransferase (NAT), the rate-limiting enzyme in melatonin biosynthesis, and melatonin levels, of principal melatonin-producing tissues, i.e. pineal gland and retina. The effect of white light is mimicked by monochromatic visible light (white > green > blue > red), and - in addition - by an invisible ultraviolet (UV-A; λ_{max}=365 nm) light. The suppressive effect of UV-A irradiation was proportional to the time of light exposure (5-60 min), and seems to induce a time-related biphasic NAT response. Similar to the NAT-suppressing effects of white light, those of UV-A can be antagonized by D2-family dopamine receptor blockers (spiroperidol, sulpiride, raclopride and clozapine) in retina, and α-2 adrenoceptor blocker yohimbine in the pineal gland. These data indicate that the melatonin-generating systems of chick retina and pineal gland are sensitive to UV-A irradiation, and that dopamine and noradrenaline are respective mediators of such effects, similar to those evoked by visible light.

Diurnal variations of retinal dopamine content in blue acara reared under different light intensities or wavelengths

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Dopamine has been recognized as a major neurochemical signal for the control of light adaptive changes in the retina. It shows diurnal variations during light/dark cycles, reflecting changes in dopamine synthesis or utilization. In fish, dopamine-dependent adaptation processes are affected by rearing light conditions. It has been shown that the formation of spinules can be altered by monochromatic rearing. The aim of this study was to elucidate whether rearing in environments of different light intensities or spectral compositions would influence the dopaminergic system of the retina.

Groups of blue acara *(Aequidens pulcher)* were kept under 12:12 h light/dark cycles of white light with low (0.2 lux), intermediate (33 lux) or high (3000 lux) illuminance, and isoirradiant short wavelength (485 nm) or long wavelength (624 nm) light. At 4 h intervals endogenous dopamine and dihydroxyphenylacetic acid (DOPAC) were determined from isolated retinae, using HPLC with electrochemical detection. Autocorrelation analysis of the data series was performed to identify cyclic components of the variations in dopamine levels.

The strongest periodic component was found in retinae of the bright white light group with high dopamine levels during the light and low levels during the dark phase. Reduction of the illuminances led to a dampening and phase reversal of the amplitudes. Chromatic deprivation by rearing under long or short wavelengths abolished the diurnal cyclicity.

We conclude that the control of the dopaminergic system in fish retinae is influenced by the intensity and spectral composition of light. Monochromatic rearing abolishes the circadian cyclicity of the dopaminergic system. This may, in turn, be the reason for changes in the dopamine-dependent components of adaptation processes.

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RETINAL "CLOCK" MECHANISMS - IMPLICATIONS TO AGEING AND DISEASE

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In both mammalian and non-mammalian retinae, a number of candidate mechanisms have been identified at the cellular level which may play a role in the function of the ocular clock. Melatonin (Mel) synthesis in the retinae of numerous species, including man, is confined to photoreceptors and some bipolar cells. It appears that Mel synthesis proceeds according to a circadian rhythm in the absence of light cues and peaks during excepted night. In addition, light rapidly suppresses Mel synthesis. Mel appears to be the prime candidate as a paracrine "dark-signal". In contrast, retinal dopamine (DA) release is potently inhibited by Mel and enhanced by light, and is currently a prime candidate for a paracrine "light-signal". DA appears to regulate a multiplicity of diverse retinal functions including cell coupling, retinomotor movements, and pigment migration. Electrophysiological investigations in our laboratory have been examining these mechanisms in animal retinae, and more recently we have been assessing the role such systems might play in human vision. At the cellular level, we have been able to examine the activity of the pathway by recording from horizontal cells in the rat retina. These neurons receive DAergic input which modulates their electrotonic coupling. Our studies have revealed that the balance between Mel and DA pathways is disturbed, both in senile animals and in some forms of hereditary retinal dystrophy. In both cases, the release of DA appears to be grossly subnormal, and the implication of these findings will be discussed. We have been examining evidence for the activity of these regulatory systems in man, by investigating possible diurnal changes in the waveform of the human photopic ERG. This work suggests that the activity of cone pathways in the human retina varies according to the time od day. The principal effect appears to be a dramatic increase in the b-wave implicit time at night. We are now beginning to expand our observations to groups of clinical conditions, such as those with Parkinson's disease who express low retinal DA content. This will expand our knowledge of the mechanisms involved, and explore the possible involvement of clock abnormalities to visual disfunction.

Symposium 2 - Anxiety, depression and NMDA receptors

Introduction to the structure and function of NMDA receptors - focus on glycine site

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The NMDA receptor complex contains multiple regulatory sites sensitive to: glutamate, glycine, PCP, magnesium, polyamines, ifenprodyl, zinc, redox and pH. Interestingly, agents acting at these recognition sites produce distinct effects both in vitro and in behavioural experiments. This diversity is further increased by the existence of NMDA receptor subtypes with different sensitivity to the above mentioned agents. For example, it has recently been shown that the intrinsic activity of d-cycloserine is higher at NMDAR1/2C than at NMDAR1/2A subunit combination. In behavioural studies, NMDA receptor channel blockers impair learning, inhibit convulsions, increase locomotion, produce ataxia, myorelaxation and antiparkinsonian-like activity and also show neuroprotective effects. Competitive NMDA receptor antagonist show a similar profile except that they lack motor stimulation and are also devoid of antiparkinsonian-like activity. Antagonist acting at the glycine modulatory site (e.g. L-701,324 and new Merz agents such as MRZ 2/576) similarly produce sedation, show clear neuroprotection against mitochondrial toxin given into the NBM and in global ischaemia but fail to induce consistent anxiolytic and antiparkinsonian activity. Glycine antagonists, in contrast to NMDA channel blockers are devoid of side effects such as neuronal vacuolisation the retrosplenial/cingulate cortex, stereotyped behaviour and disruption of prepulse inhibition.

Anxiolytic activity of uncompetitive and competitive NMDA antagonists

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The ubiquitously present in the brain excitatory amino acids (EAA) control various central nervous system activities. Among others, strong environmental stimuli like stress and aversive experience enhance glutamic acid release in the limbic forebrain structures. This phenomenon is accompanied by symptoms of fear and avoidance behaviour, thus suggesting a direct involvement of EAA in the origin of emotions. In the present study the role of NMDA system in the acquisition and expression of fear controlled reactions was studied in animal models of a neophobia and conflict behaviour, with help of selective competitive and uncompetitive antagonists at NMDA receptors, and regulatory binding sites of this receptor complex.

It was shown that uncompetitive, competitive antagonists, and partial agonists at the strychnine insensitive glycine binding site exerted selective and dose-dependent anxiolytic-like effects in both tests. The most potent and selective anxiolytic activity was present after administration of CGP 37849, a competitive NMDA antagonist devoid of influence on rat motor activity, pain threshold and spontaneous water intake. Microinjection experiments showed that the dentate gyrus of the dorsal hippocampus might be involved in mediation of some central effects of peripherally administered NMDA antagonists. Subchronic experiments revealed that in contrary to diazepam or dizocylpine, repeatedly given CGP 37 849 did not cause any significant adaptive and potentially undesirable changes in the anti-fear like effects of a challenge dose of this drug. Further behavioural and biochemical studies proved that there was interaction between serotonin and EAA systems in controlling animals motor but not fear-evoked behaviour, and that dopamine might be involved in this interactive process. It is concluded that competitive NMDA antagonists may offer an interesting alternative to benzodiazepines in the treatment of some anxiety disorders.

Anxiolytic activity of glycine partial agonists and antagonists

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The effects of compounds that act at the glycine site (glycine$_B$) of the N-methyl-D-aspartate (NMDA) receptor were examined in animal tests of anxiety and memory and compared with those of competitive NMDA receptor antagonists. The glycine$_B$ site ligands 1-aminocyclopropanecarboxylic acid (ACPC), 7-chlorokynurenic acid (7KYN) and D-cycloserine (DCS) were anxiolytic in the elevated maze. All these compounds, when administered after training, reduced step-through latencies in an avoidance task when testing was performed within 1 hour after administration. In contrast, post-training administration of glycine site ligands or DZP did not modify step-through latencies when tested 24 h later, indicating that none of these compounds produces retrograde amnesia. Similarly, pretaining administration of glycine$_B$ site ligands did not alter step-through latencies measured 24 hr later. However, under the same conditions, competitive NMDA antagonists and diazepam produced a significant reduction in latencies. These results indicate that glycine$_B$ site ligands, in contrast to benzodiazepines, do not impair learning and memory at doses that are anxiolytic. Furthermore, administration of antisense but not sense oligodeoxinucleotides directed to the NMDAR1 subunit of the NMDA receptor produced anxiolytic-like effects in the elevated plus maze. These results are consistent with the anxiolytic properties of glycine$_B$ site ligands and provide support to the hypothesis that the NMDA receptor plays a key role in the pathophysiology of anxiety related behaviors. (DGICYT PB94-0017).

ALLOSTERIC INTERACTIONS AND FUNCTIONAL BEHAVIOR OF THE NMDA RECEPTOR FOLLOWING ANTIDEPRESSANT TREATMENTS.

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Antidepressant treatments are the product of serendipitous discoveries over the past 40 years. Consequently, although the neurobiological effects of antidepressants are well known, the mechanism of action is not. Any such theory must account for the within-class specificity of antidepressant effects and the broad range of structures and actions which result in antidepressant activity. Recent studies have demonstrated that antidepressants from all major classes produce a characteristic complement of changes in the radioligand binding characteristics of the NMDA receptor in rodent cortex. Specifically, antidepressants (16/17 tested) produce a reduction in the potency of glycine to displace [^{3}H]5,7-dichlorokynurenic acid from the NMDA receptor. Similarly, antidepressants reduce the proportion of high-affinity, glycine-displaceable [^{3}H]CGP-39653 binding. These effects are dose- and time-dependent and are reversible following cessation of treatment. In addition, preclinical antidepressant screening procedures such as the forced swim test and animal analogs of depressive symptomatology produce changes in the radioligand binding characteristics of the NMDA receptor which oppose those produced by antidepressants. Moreover, the binding of [^{3}H]CGP-39653 in cortical tissue from suicide victims differs significantly from that of normal controls. Recent studies in this and other laboratories have demonstrated that these radioligand binding changes are accompanied by a reduction in the functional activity of second messenger systems related to the NMDA receptor. Specifically, antidepressants reduce the functional activity of both nitric oxide synthase and glutamate-stimulated cyclic GMP production. Moreover, antagonists of nitric oxide synthase possess significant antidepressant and anxiolytic properties, similar to those of NMDA receptor antagonists. These data provide significant support for the hypothesis that adaptation of the NMDA receptor complex is a common neural pathway of antidepressant treatments. *Supported by NIH grant, MH-53228 and the Dept. of Psychiatry, UMC.*

Antidepressant activity of NMDA antagonists

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Functional NMDA antagonists are represented by: competitive antagonists of the glutamate site such as CGP 37849, CGP 39551 and AP-7 (2-amino-7-phosphono-heptanoic acid); use dependent channel blockers such as dizocilpine (MK-801), amantadine and memantine; a partial agonist of the strychnine-insensitive glycine site ACPC (1-aminocyclopropanecarboxylic acid), an antagonist of the strychnine-insensitive glycine site 7-chlorokynurenic acid; and an antagonist of the polyamine site eliprodil. These above listed functional NMDA antagonists are active in the forced swim test in rodents, a test that predicts antidepressant activity in humans. When administered together, they enhance the antidepressant activity of "classical" antidepressants in that test. Moreover, functional NMDA antagonists administered chronically induce a β-adrenoceptor down-regulation, an effect being characteristic for majority of antidepressants. The activity of these agents in above mentioned preclinical tests sensitive to antidepressants indicate their possible antidepressive properties in humans. This notion is supported by the recent studies using chronic mild stress animal model of depression.

Effects of various NMDA receptor ligands in a realistic model of depression.

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This paper reviews results of studies designed to evaluate antidepressant activity of five ligands acting at different loci on the NMDA receptor complex. The uncompetitive NMDA receptor antagonist, MK-801, the two competitive NMDA receptor antagonists, CGP 37849 and CGP 40116, as well as the partial agonists at strychnine-insensitive glycine receptors, ACPC and d-cycloserine, were tested in a chronic mild stress (CMS) model of depression. In the CMS model, animals subjected to a variety of mild stressors for a prolonged period of time, develop a substantial decrease in their responsiveness to rewarding stimuli (anhedonia) which can be effectively reversed by chronic treatment with various classes of antidepressant drugs. Chronic (5 weeks) treatment with MK-801, CGP 37849, CGP 40116 and ACPC caused a gradual reversal of the CMS-induced anhedonia. The magnitude of the effect of MK-801 and the CGP substances and its time-course were comparable to those observed following similar administration of imipramine. However, the ACPC-treated animals recovered from the stress-induced anhedonia significantly faster then those treated with imipramine and the three NMDA antagonists. Chronic administration of d-cycloserine produced weak and inconsistent effects in the CMS model. These findings provide further support for the hypothesis that chronic inhibition of transmission at NMDA receptors may result in antidepressant effects. Moreover, these results suggest that antidepressant activity of ACPC is comparable to conventional drugs, but with a faster onset of action.

Symposium 3 - Immunology of demyelination and remyelination

ROLE OF γδ T CELLS IN INFLAMMATION IN THE CENTRAL NERVOUS SYSTEM

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γδ T cells are a minor population of lymphocytes whose exact function remains unknown. Previous studies have shown that these cells are present in multiple sclerosis (MS) lesions and that their numbers increase as the disease becomes more chronic. Studies in vitro suggest that these cells may be responding to cell surface proteins, such as heat shock proteins, displayed on the surface of activated inflammatory cells as well injured CNS glial cells. To pursue the potential contribution of these cells to the inflammatory process, we have used the mouse model of MS experimental allergic encephalomyelitis (EAE). In this model, we have shown that γδ T cells in the lesion fluctuate in association with disease activity with the highest numbers being found at the height of the disease process. Phenotypic changes are alsonoted in the percentage of cells that express the CD8 coreceptor and Fas or FasL. We have also shown that depletion of γδ T cells can protect animals against the acute inflammatory process and can alter the course of chronic disease. Pathological analysis of the tissues indicate a reduction in the inflammatory process, particularly in the macrophage component. Cytokine analysis of control and γδ T cell depleted animals shows an early burst of IL-1 and IL-6 at the onset of EAE which is absent in the depleted animals. In contrast, levels of lymphotoxin and tumor necrosis factor are not changed. Current experiments are designed to determine whether γδ T cells controll the influx or activation of inflammatory cells in the EAE lesions.

TNF RECEPTORS AND ANTAGONISTS. PERSPECTIVES OF NEW THERAPEUTIC STRATEGIES

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The many different activities of TNF are best understood as concerted activities in host defense. However, in some disease states endogenous mediators such as TNF produced in excess contribute to and maintain inflammatory conditions and thus develop highly toxic activities, suggesting the therapeutic use of TNF-neutralising agents. Two distinct cellular TNF receptors, p55TNFR and p75TNFR, have been identified. Natural soluble TNF binding proteins, fragments of the extracellular regions of both TNFR, are thought to act as TNF inhibitors, or slow TNF release reservoir. To develop TNF antagonists, recombinant TNF receptor-immunoglobulin chimaeras (TNFR-IgG) were expressed in eucaryotic cells. These dimeric molecules have a longer in vivo halflife than the monomeric natural soluble receptors and a higher binding avidity for TNF, because the two receptor moieties of one TNFR-IgG molecule bind to one TNF trimer. Binding studies revealed that p55- and p75TNFR-IgG had similar equilibrium binding affinities. However, a pronounced difference in binding kinetics was discovered, p55TNFR forming the kinetically more stable complex with TNF, p55TNFR-IgG therefore was chosen as TNF antagonist candidate. The efficacy of p55TNFR-IgG to protect from mortality in endotoxin or bacterial challenge was investigated in animal models. It was found to fully protect mice sensitised with D-galactosamine from endotoxin challenge as well as from lethal intravenous E. coli challenge. Baboons were also protected from lethal E. coli challenge, and various haemodynamic, haematologic, coagulation, metabolic and secondary cytokine disorders in these animals were significantly attenuated by the TNF antagonist treatment. A clinical phase II trial in severe sepsis and septic shock paients was completed revealing highly promising beneficial treatment effects in severe sepsis patients. Further animal studies were conducted to investigate the effect of p55TNFR-IgG treatment in chronic inflammatory diseases with autoimmune background.

ALTERATION OF THE CYTOKINE NETWORK IN MS

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Multiple sclerosis is a chronic inflammatory disease of the central nervous system. There is increasing evidence that a CD4+ T cell- mediated immune reaction against components of the central myelins (e.g. myelin basic protein; MBP) plays an important role in the pathogenesis of this demyelinating disease.

Cytokines with potent regulatory and effector functions are key mediators of this inflammatory process. Effective antigen presentation as well as clonal expansion of auto-reactive T cells is regulated by pro-inflammatory cytokines. Transmigration of activated T lymphocytes across the blood brain barrier is dependent on cytokine induced upregulation of adhesion molecules. The expression of intercellular adhesion molecule-1 (ICAM-1) and vascular adhesion molecule-1 (VCAM-1) on cereberal endothelial cells is increased upon stimulation with IL-1, TNF-α or IFN-γ. TNF-α and lymphotoxin are both expressed in chronic active lesions from MS patients. These cytokines have both direct demyelinating activity and are regarded as potent effector molecules involved in destruction of myelin and oligodendrocytes, the myelin producing cells. Alterations of the balance between Th1 und Th2 cytokines has been described and was found to be associated with disease activity. For example, detection of cell associated cytokine expression (RT PCR or in situ hybridization) revealed a correlation between clinical relapses and increased cellular mRNA levels of IFN-γ, TNF-α, lymphotoxin and IL-12 p40, whereas IL-10 and TGF-β mRNA were decreased at the same time.

As various cytokines are involved in the immunopathogenetic process of multiple sclerosis and correlations with disease activity could be detected, they are regarded as promising targets for immunotherapy (e.g. Beta-interferons, copolymer-1, roquinimex, oral tolerance and phosphodiesterase inhibitors). Initial studies indicate that disease activity, measured by gadolinium enhancement on serial magnetic resonance tomography scans, as well as cytokine expression in blood mononuclear cells may be early indicators for effective therapeutic immunomodulation.

Genetic predisposition to Multiple Sclerosis: Possible involvement of the increased frequency of the ICAM-1 gene codon 469 allele T+T and the combined -308 promoter TNF G+A/LT-alpha gene exon 3 C+C genotype

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Genetic susceptibility factors are implicated in Multiple Sclerosis (MS). The place of the genomic aberration associated with MS remains still unknown. On account of immunological system disregulations observed in MS patients we concentrated on the two proinflammatory cytokines: tumor necrosis factor (TNF), lymphotoxin-alpha (LTa) and the adhesion molecule: intracellular adhesion molecule-1 genes distribution in MS. Their involvement in the pathogenesis of the demyelinating diseases, like MS and its animal model: experimental autoimmune encephalomyelitis (EAE) have been heavily implicated. To detect TNF, LTa and ICAM-1 gene polymorphisms in Multiple Sclerosis (MS), we have developed a highly sensitive and very specific, two stage, nested polymerase chain reaction (PCR). The second stage was a specially selected condition nested PCR involving mutation specific primers. Total genomic DNA was extracted from blood cells from 57 MS patients and 81 control subjects. The results were confirmed by direct dideoxy chain termination sequencing. In the control group, the frequency of the -308 G to A mutation in the TNF promoter region was 15%. In MS patients, the frequency of this mutation was higher by 9%. The LTa gene frequency of the exon 3 polymorphic allele A was 34% in the MS patient group and 36% in controls. In MS, the combined genotype TNF G+A and LTa C+C was present six times more frequently than in controls. Also MS patients with this genotype showed higher EDSS. We found this effect to be independent from the HLA class II DR2 allele distribution in the MS. For the ICAM-1 gene the frequency of the exon 4 codon 241 G to A point mutation was 28% in control group and 18% in MS patients. However, the frequency of the exon 6 codon 469 homozygots T+T was found to be significantly higher in the MS patients (56% to 36% in controls). These results are reported for the first time and may contribute to the MS genetical susceptibility background.

MHC class I restricted killing of human oligodendrocytes by myelin basic protein peptide specific CD8 T lymphocytes

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Introduction: Multiple sclerosis (MS) is supossed to be an autoimmune disease where myelin sheats or cells of its origin oligodendrocytes(OLs) are suspected to be a target of immune injury. Human OLs express in vitro MHC class I but not MHC classII and therefore could be susceptible to MHC class I restricted killing.

Methods:Specific cytotoxic activity of MBP peptide reactive CD8 T cells isolated from MS patients and healthy volunteers, checked against Hmy2C1R cells transfected with HLA-A2 and HLA-A3 and pulsed with MBP peptide or virus peptide was determined in 5 hours 51-chromium (Cr) release assay. Cytotoxic effects of these CD8 T cells on either HLA-A2 and non-HLA-A2 human oligodendrocytes (1:10 target:effector ratio) was measured in the same assay system.

Results:Mean cytotoxic activity of MBP peptide specific CD8 T cells against HLA-A2 transfected cells pulsed with MBP peptide was 47%±2% vs pulsed with virus peptide11%±1% and vs HLA-A3 pulsed with MBP peptide 11%±1%.Mean CD8 T cells induced Cr release from HLA-A2 OLs was 15%±2% compared to 3%±0,6% in the presence of pan-MHC class I blocking antibody (W6/32) and 4%±0,5% from non HLA-A2 OLs. There was no difference between MS patients and healthy volunteers CD8 T cells.

Conclusions: Human oligodendrocytes in vitro can be susceptile to MHC class I restricted cytotoxicity mediated by MBP peptide specific CD8 T cells

Pattern of γ/δ TCR genes in multiple sclerosis versus myasthenia gravis.

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In view of the recent evidence, that in addition to α/β T lymphcytes also γ / δ T cells my have autoreactive potentials. TCR delta repertoire in peripheral blood was compared in MS and MG. Rearrangement of TCR Vδ-Jδ genes has been analysed in 20 MS and 20 MG patients using the seminested polymerase chain reaction (PCR). Oligoclonal primers specific for six Vδ regions and for Jδ1 genes were used for amplification of Vδ - Jδ junctional region, responsible for the diversity of γ / δ TCR. Majority of MS and MG patients demonstrated contrary to healthy subjects mono- or oligoclonal character of rearrangement of TCR Vδ1 - 6 to Jδ1 genes, but more frequent the rearrangement of Vδ1 - Jδ1 and Vδ2 - Jδ1 demonstrated MS cases. On the basis of the similarity of the bonding pattern in MS and MG it can be suggested that γ / δ T lymphocytes clonal expansion in vivo is despite of various acting antigens, the general reaction in autoimmune diseases, with the significant role in their pathogenesis.

Chemokine expression in experimental autoimmune encephalomyelitis

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The mechanism underlying accumulation of blood mononuclear cells within the CNS during experimental autoimmune encephalomyelitis (EAE) and multiple sclerosis (MS) remain unclear. Recent reports describe a novel family of proinflammatory chemoattractant cytokines called chemokines which mediate directional migration of different leukocyte subsets toward inflammatory sites. Using a dot-blot RT/PCR hybridization assay for mRNA and ELISA assay for protein, we monitored the expression of several chemokines within the central nervous system (CNS) during the course of acute and chronic relapsing EAE. In acute EAE mRNA expression for two chemokines MCP-1 and IP-10 occurred transiently at the earliest signs of leukocyte infiltration of CNS tissues, and was stringently restricted to astrocytes. Astrocyte chemokine expression occurred near perivascular mononuclear cell infiltrates. In chronic-relapsing EAE we monitored mRNA and protein expression for chemokines MCP-1, MIP-1 alpha, IP-10, KC and RANTES during spontaneous relapse of the disease. We found coordinate chemokine upregulation in brain and spinal cord during clinical relapse, with expression confined to CNS tissues. MCP-1, IP-10 and KC were synthesized by astrocytic cells, while MIP-1 alpha and RANTES were elaborated by infiltrating leukocytes. The results demonstrate stringent regulation of multiple chemokines in vivo during a complex organ-specific autoimmune disease.

Plenary lectures

ELECTROPHYSIOLOGY OF ANIMAL COGNITION: PLACE CELLS AND PLACE NAVIGATION

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Navigation to unmarked places not directly visible from the departure point is believed to depend on cognitive maps. Hippocampus plays a pivotal role in spatial memory and hippocampal place cells (PCs) may contribute to formation of engrams. This assumption can be verified by examining PCs in tests requiring egocentric (idiothetic) or allocentric (landmark related) orientation during dissociation or isolation of relevant inputs. Such condition can be achieved by placing the animal on a rotating arena which provides the rat and its PCs with two reference systems, a room frame, relating a place to the geometry of the room, and an arena frame, with places defined in the polar coordinates of the circular arena. Computerized tracking allows recording the rat's position simultaneously in either frame and to plot the corresponding PC firing fields (FFs). During random exploration of a rotating arena most FFs disintegrate in both frames and only few remain stable either in the room or in the arena frames. This ratio can be modified in the place avoidance task requiring the rat to avoid allocentrically or egocentrically defined regions of the rotating arena. In the place preference task visits to a definite region of the arena are rewarded by delivery of a pellet. The effects of the alternation between place navigation to the trigger zone and subsequent random search of food on PC firing will be described.

THE IMPACT OF SENSORY EXPERIENCE ON CORTICAL FUNCTION

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Properties of neurons in the sensory brain cortices can be modified by sensory experience. The somatosensory cortex of the rodent brain offers several examples of experience-induced plasticity of representational maps. The barrel cortex, which frequently serves as a model for plasticity investigations, can be modified in a number of ways. Early in development the peculiar cellular structure of the barrel field can be altered by interruption of the sensory nerve from the vibrissae. Later, the functional structure of the barrel cortex can be changed by denervation, deprivation of sensory input without injury to the nerve, or sensory learning. These processes depend upon undisturbed functioning of NMDA receptor and are associated with changes in the GABA-ergic system. Denervation of adult barrel cortex leads to reorganization of cortico-cortical connectivity and changes in dendritic spine density on pyramidal neurons. Thus sensory experience can modify gene expression, neurotransmitter levels, neurotransmitter receptor binding, axonal branching, dendritic spine density and neuronal receptive fields in the cerebral cortex.

Symposial 1 - Sleep and immune system

SLEEP AS A MODEL BEHAVIOR FOR THE STUDY OF CENTRAL NERVOUS SYSTEM - IMMUNE INTERACTIONS.

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Bi-directional communication between the CNS and the immune system occurs at multiple levels. Cytokines are key mediators of this bi-directional communication. Although cytokines were traditionally thought of as immune products, it is now known that they, and their receptors, are produced and act within the CNS. Sleep is a fundamental CNS process that is altered during microbial challenge, or in response to the administration of cytokines. Sleep alterations in response to exogenously administered cytokines share many of the features observed during pathogen-induced alterations. As such, sleep provides a unique model to study CNS - immune interactions.

The cytokines interleukin (IL)-1, tumor necrosis factor (TNF), and interferon (IFN), have been implicated in sleep regulation. Sleep of experimental animals is altered when these cytokines are administered either centrally or systemically. Blocking the actions of these cytokines alters spontaneous sleep, and pretreatment with specific receptor antagonists blocks the compensatory sleep rebound that occurs after sleep deprivation. Furthermore, cytokine synthesis inhibitors reduce spontaneous sleep in otherwise normal animals. Finally, transgenic animals, in which genes regulating cytokine protein or receptor production have been "knocked out", also exhibit altered sleep. Such observations suggest that sleep is regulated, in part, by cytokines, and point to the utility of sleep as a behavioral endpoint and model for the study of CNS - immune interactions.

CLASSICAL NEUROTRANSMITTERS AND CYTOKINES IN SLEEP REGULATION: SEROTONIN AND INTERLEUKIN-1

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The role of classical neurotransmitters (such as serotonin or ACh) in sleep regulation is amply documented. In recent years, evidence has been gathered that some cytokines play a role in sleep regulation. Although serotonin (5-HT) is one of the most extensively studied neurotransmitters with regards to sleep and interleukin-1 (IL-1) has received the most attention of any cytokine in terms of its involvement in sleep regulation, little is known of how these molecules interact and of the relevance of this interaction in sleep control.

Interleukin-1 (2.5 ng) induces a biphasic increase in slow wave sleep (SWS) when injected intracerebroventricularly in freely behaving rats, at dark onset. The first phase of IL-1-induced SWS enhancement is abolished following brain serotonin depletion (by pretreatment with *para*-chlorophenylalanine). It has also been shown that the first phase of IL-1-induced SWS enhancement is specifically associated to an early increase in serotonergic activity (measured by means of *in vivo* voltammetry) in the Medial Preoptic Area. Phasic, state specific changes (which have been described in physiological sleep) are superimposed on this tonic, overall increase in serotonergic activity.
These results suggest that i) the first phase of IL-1-induced SWS enhancement may be mediated by the serotonergic system, and ii) IL-1 does not alter the normal physiology of the serotonergic system, as far as the sleep-wake cycle is concerned.

CIRCADIAN AND HYPNOTIC ACTIVITY OF MELATONIN

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Melatonin is a principal hormone of the pineal gland. Its synthesis by the gland, and secretion into the blood stream, follows circadian rhythmicity, with high values at night and low values during dayhours. Such a rhythm is driven by the master circadian oscillator localized to the hypothalamal suprachiasmatic nuclei (SCN). Photic signal, which under natural or imposed daily light-dark conditions provides cues entraining the SCN clock (and thus melatonin formation) to approximately 24 hour rhythm, exerts in addition (i.e. when exposure to light occurs at night) an acute suppressive effect on nocturnal activity of serotonin N-acetyltransferase (NAT), the rate-limiting enzyme in melatonin biosynthesis. Physiological effects of melatonin are mediated by specific receptors, designated as Mel_{1A}, Mel_{1B} and Mel_{1C} sites, which in mammalian central nervous system show highly uneven distribution, with highest densities occurring in the SCN and the pituitary *pars tuberalis*. Among diverse biological actions of the hormone are those related to a daily sleep-wake cycle. In fact, the role of melatonin as a sleep-promoting agent finds support in its well described hypnotic (or soporific) activity. For this reason melatonin appears to be an effective drug in chronobiological sleep disfunctions, especially in elderly and/or blind individuals.

STATES OF THE BRAIN DURING SLEEPING AND WAKING

Anton M.L. Coenen

Wakefulness is accompanied by a low amplitude high frequency EEG, due to the fact that thalamocortical neurons independently fire in a state of tonic depolarization. Information can easily pass the low-level threshold of the neurons, leading to a high transfer ratio. The complexity of the EEG during waking is high, expressed in a high correlation dimension. Accordingly, the level of information processing is high. Oscillating phenomena are related to drowsiness and mark the transition from wakefulness to sleep. This occurs when cells undergo a moderate hyperpolarization. Increased inhibitions result in a reduction of afferent information with a lowered transfer ratio. Information processing subsides, which is expressed in a diminished correlation dimension. Vigilance is further decreased at the onset of slow wave sleep. Neurons undergo a further hyperpolarization leading to a synchronized burst-pause firing pattern, expressed in a high voltage, low frequency EEG. Inhibitory activities are so strong that the transfer ratio further drops, as well as the correlation dimension. Thus, sensory information is largely blocked and information processing is on a low level. Finally, REM sleep is associated with a 'wake-like' EEG. Just as during wakefulness, this is the manifestation of a tonic depolarization of thalamocortical neurons. The transfer ratio of REM sleep is not yet been determined, but seems to vary. Evidence exists that this type of sleep, associated with dreaming, is involved in processing of 'internal' information. In line with this, REM sleep has higher correlation dimensions than slow wave sleep. It is assumed that the 'near-the-threshold' depolarized state of thalamocortical neurons is a necessary condition for perceptual processes and high levels of vigilance, associated with waking and in an altered form with REM sleep.

THE ROLE OF OPIOIDS AND CYTOKINES IN SLEEP-WAKE MECHANISMS

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The behavioral effects provoked by opioids depend on dose, types of receptors and the CNS loci. Systemic administration of morphine suppresses slow wave sleep (SWS) and REM. Animal studies during insomnia have revealed that opioids cause a typical EEG behavioral dissociation, i.e. high voltage slow-frequency waves are present during wake state. In cats μ and δ agonists microinjections into nucleus of the solitary tract enhance SWS, medinal pontine reticular formation α receptors mediate REM inhibition.

Endogenous opioids affect the immune system either through direct interaction with receptors with receptors on the effector cells or indirectly through the ligation of receptors found within the CNS involving secondary pathways like Hypothalamic-Pituitary-Adrenal (HPA) axis ultimately resulting in immunomodulation. The interaction is reciprocal.

The cytokines network has been found to interact with endogenous opioids in CNS. Concerning sleep mechanisms interleukin-1 β (IL-1 β) is the most interesting being proposed as man regulator in sleep-wake cycle.

There are several points where IL-1 β and opioids interact in CNS. One cellular target of immune system activation is the astrocytes which respond to IL-1 β with an increased μ receptor mRNA expression and proenkephalin synthesis. It has also been recently described that central opioids are effective modulators of the inflammatory cytokine IL-6 response induced by IL-1 β. A few reports suggest that cytokines may increase the production of endogenous opiates in the neuroendocrine system. IL-1 enhances proopiomelanocortin gene expression in pituitary cells. It has been reported that the HPA axis which is the pathway of sleep-wake modulation by IL-1 β is stimulated in rats by κ opioid receptor agonist at the hypothalamic and the pituitary level what supports the hypothesis that endogenous κ opioids not only act the pituitary level to increase ACTH output, but may also act at the hypothalamic level to increase CRH release.

INFLUENCE OF MELATONIN ON DISTURBED SLEEP IN HUMANS

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The influence of melatonin on the sleep was observed in a group of 45 patients, in the age ranged from 25 to 87 (mean age 52 years), consisted of 22 females and 23 males. In 32 chronic insomnia was diagnosed, 23 in course of depression. In 3 subjects the main problem was dependence of hypnotic (benzodiazepines), in 4 subjects post traumatic stress disorder was diagnosed, 1 patient was with idiopathic insomnia, and 1 with schizophrenia. 10 patients had circadian rhythm sleep disorders. The majority of patients came to the ambulatory after beginning of melatonin administration by themselves. The period of observation was from 1 month to 6 months. In all patients melatonin was an additional drug to basic treatment.

In none of the patients but one adverse events were observed (in case of one patient the insomnia was exaggerated). In 17 patients considerable improvement were observed, 10 of them suffered from sleep-wake rhythm disorder. In 13 patients no effect of melatonin was observed.

Oral communications I - Learning and memory

c-FOS EXPRESSION IN MOUSE BRAIN AFTER FEAR CONDITIONING; EFFECT OF FYN MUTATION

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c-Fos expression is recognized as a marker of "important" neuronal activity and has been used to map brain structures involved in specific behaviors. We employed c-Fos immunocytochemistry to study changes in mouse brains after fear conditioning. Both wild-type and *fyn* heterozygous mice were used and divided into four groups each: i. naïve, ii. control animals (habituated to a shocking chamber for four consecutive days), iii. non-shocked (introduced once to the shocking chamber), iv. shocked (not habituated and shocked once). Two hours after the exposure to the chamber (and) the electric shock the animals were anaesthetized, perfused and the expression of c-Fos was assessed in coronal sections with *in situ* immunocytochemistry. We observed no differences between wild-type and *fyn* heterozygous mice. Naïve and control animals had very little level of c-Fos expression. It was elevated in both shocked and non-shocked animals. This increase was observed especially in the cortex and amygdala but not in the hippocampus. In the cortex, the increased c-Fos immunoreactivity was noted in both piriform and somatosensory corticies, in barrel cortex especially. Here the stronger reaction was observed in the brains from non-shocked animals. In amygdalar nuclei, the medial one especially, the stronger c-Fos immunostaining was noted in shocked animals. These results suggest a cosiderable involvement of cortex and amygdala but not of hippocampus in fear conditioning.

THE INVOLVEMENT OF MESOLIMBIC DOPAMINERGIC SYSTEM IN FACILITATORY EFFECT OF ANGIOTENSINS ON RECOGNITION MEMORY IN RATS.

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We have previously reported that the dopaminergic projection from A10 ventral tegmental neurons and A9 neurons of substantia nigra to the central amygdala (CA) and to the CA4 field of the hippocampus (HI) is, in part, responsible for the facilitatory effect of angiotensin II (AII) and its 3-7 fragment [AII(3-7)] on the retrieval of information in memory motivated affectively. In this study the influence of both angiotensins, given intracerebroventricularly at the dose of 1 nmol each, in rats lesioned bilaterally with 6-OHDA to CA, CA4 field of HI, the nucleus accumbens septi (NAS) and to the nucleus septi lateralis (NSL) on the recognition memory was evaluated. In addition, evaluation of the locomotor and exploratory activity in open field was conducted in all, lesioned and sham-operated groups of animals. AII and its 3-7 fragment significantly improved object recognition in the all sham-operated groups of rats. Bilateral 6-OHDA lesions to CA and NAS totally abolished, while to NSL significantly attenuated facilitatory effect of both angiotensins on object recognition. Bilateral destruction of dopaminergic endings in the CA4 field of HI had not influence on angiotensins facilitation recognition of objects. Some increase of the locomotor and exploratory activity in CA lesioned animals and diminution in rats lesioned to NAS, NSL and CA4 field of HI was unlikely to interfere with the cognitive effect of both angiotensins. These results suggest that the anatomical substrate of facilitating recognition memory by AII and AII(3-7) is closely related to the dopaminergic projection from ventral tegmental area and substantia nigra to the septum and the amygdala.

Acamprosate involvement in working memory by using the three-panel runway apparatus in rats.

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Acamprosate [ACAM] is one of the recently developed drugs used in the treatment of alcoholism (Chick J., 1995, Whitworth A.B., et al., 1996), but many aspects of its pharmacological profile seem to be opened (Littleton J., 1995). In our previous studies we found that ACAM amplified the long-term memory especially in older rats (Okulicz-Kozaryn I., et al., 1996) and in chronically ethanol treated rats (Mikołajczak P., et al., 1994) using passive avoidance test. The aim of this study was to evaluate the effects of multiple ACAM administration on working memory by using three-panel runway modified Hill's apparatus (Furuya Y., et al., 1988). For the comparison, a scopolamine was chosen as a model compound due to its well known property to disrupt learning and memory in animals. *Materials &Methods*: Animals were premedicated with ACAM (500 mg/kg/day, p.o.), Scopolamine hydrobromide - [SCOP] - (0.5 mg/kg/day, i.p.) or ACAM and SCOP together for 10 consecutive days (10x) during the test procedure and working memory tasks were measured (Furuya Y., et al., 1988). Control animals [CR] received the vehicle according to the same schedule. *Results:* 10xACAM administration reduced significantly errors and latency in CR group in comparison to no-drug treated animals. Multiple SCOP treatment had a significantly negative effect on the rats' ability to complete the tasks. The rats which were given both ACAM and SCOP concomitantly, performed better; they made fewer mistakes and displayed shorter latency when compared to the rats which were given SCOP only. Moreover, these last-mentioned rats completed the tasks in a similar manner to CR group, although the number of mistakes made by these ACAM and SCOP rats was greater. *Conclusion:* The observed positive action of ACAM may be considered for the use of this drug not only in maintaining abstinence but also in improving memory disturbances caused by alcoholism. Confirmation of this ACAM activity requires further studies on the experimentally induced alcoholism.

LEARNING AND PERFORMANCE OF AN AUDITORY RECOGNITION MEMORY TASK IN DOGS – EFFECT OF EXPERIMENTAL DESIGN

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Eleven male mongrel dogs were tested in an auditory Delayed Matching to Sample task (DMS) with trial-unique stimuli *(Kowalska D.M., 1996, ENA Abstracts 2: 126)* in two different experimental designs. Seven dogs were tested with approach instrumental response (design A) in which feeders were located in 2.5 m. distance from the starting platform. Four dogs were trained with bar-press instrumental response (design B) with manipulanda located on the platform close to the dog. In both situations the training consisted of: preliminary stages I & II (instrumental conditioning), stage III (go-no go auditory recognition task) and stage IV (auditory DMS training with 1.5 s delay to a criterion of 90% correct responses in 100 (design A) or 90 (design B) consecutive trials). After 14-day control pause the dogs were retrained in the auditory DMS with 1.5 s delay to the same criterion (stage V). On stage VI dogs were tested in the auditory DMS with delays 10, 30, 60 and 90 s in blocks of 100 or 90 trials (for design A and B, respectively).

The dogs needed significantly less trials to reach the criterion in the design A than in the design B on stages I (medians: 20 and 255) and IV (medians: 80 and 427.5). No difference was encountered during other stages, including retraining (stage V).

On Stage VI, in both designs, a significant effect of delay was observed, showing decay of performance with extended delays (10 s - 86%, 30 s - 76%, 60 s - 69%, 90 s - 64%). However, the effect of the experimental design on performance level and of the interaction between delays and design were not significant.

During DMS training latencies of responses for dogs trained in design B were significantly shorter than those trained in the design A.

Approach and bar-press experimental designs influenced level of performance only in two stages of learning. The effect of response latencies on the performance level at these stages was analyzed.

Neuropsychological sequelaes from heart revascularization in ischemic heart disease: an analysis of selected cognitive and motor functions

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Course of changes in cognitive functions associated with cardiac surgery was observed in thirty patients with ischemic heart disease (IHD) underwent coronary artery bypass grafting (CABG). They were examined using a BVRT, story recall task, letter cancelation task, Finger-Tapping Test and Raven´s Progressive Matrices (RPM). The subjects were tested three times: before surgery, after the operation (approximately 10-12 days) and six months later. There were two control groups: one consisted of patients with IHD pharmacologically treated only (n=30) and healthy persons (n=30) composed another.Control subjects were examined once. After CABG deficits of the cognitive and motor functions on measures of immediate memory of the visual-spatial material, concentration,speed of the finger´s oscilation and problem-solving ability were observed. Immediate memory of the auditory-verbal material was preserved. The long-term assessment revealed that deficits following CABG remitted six months later. Moreover, six months after surgery the patients´ performances were better than before in memory, concentration and intelligence tasks. Comparison of scores of two control groups revealed that ischemic heart disease was characterized by decrement of visual memory (BVRT) and reasoning ability (RPM). These results confirm hypothesis that postoperative cognitive impairment associated with CABG is transient and they also suggest that open-heart surgery is more effective than pharmacological treatment for cognitive functioning.

Visual Evoked Potentials and Risk Taking Behavior in Humans, Cats and Rats

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Risk taking, also described as sensation seeking, is a character trait expressed in many behaviors (e.g., gambling, dangerous sports, criminal acts and drug use). Individuals vary on this trait from being extreme risk takers to being excessively cautious and anxious under conditions of even minimal risk and uncertainty. The project described here investigates the neurophysiological basis of these behavioral types. High risk takers exhibit increasing amplitudes of cortical VEPs to increasing intensities of light flash (VEP augmenting); low risk takers show **decreased** VEP amplitudes as a function of flash intensity (VEP reducing). We describe an animal model using cats and rats that shows comparable risk taking behaviors associated with VEP augmenting and reducing. Our rat work, using two selectively bred lines (Roman high- and low-avoidance rats), provides evidence for a heritable basis for the described neural and behavioral differences. We also describe our research program and initial findings on the neurotransmitters and receptors that modulate neural activity of the visual cortex to result in VEP augmenting or reducing.

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Symposial 2 - The role of calcium in neurotropic drug's action

Dihydropyridine sensitivity of the addictive drug action

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Reinforcing effects of morphine, cocaine, amphetamine, caffeine, nicotine and ethanol were shown to be affected by calcium channel modulators of dihydropyridine (DHP) group. DHP calcium channel blockers inhibited drug reward as measured by i.v. self-administration and conditioned place preference paradigms, whereas calcium channel activator BayK 8644 exhibited the opposite action when tested against all tested typical addictive substances except ethanol. In the ethanol studies, BayK 8644 inhibited ethanol reinforcement at the maximal effective concentration but failed to shift concentration-response curve to the left. In the separate experiments the stereospecificity of isradipine effects was found. We failed to demonstrate the inhibitory effects on drug reinforcement with verapamil, flunarizine and diltiazem, although reportedly verapamil also inhibits cocaine and ethanol reinforcement.

Behavioral mechanism underlying DHP influence on substance induced reward remains to be discovered. One possible behavioral mechanisms could be that DHPs: 1)Share common stimulus properties with addictive drugs which are sufficiently similar to allow for stimulus substitution; 2) "Antagonistic" effect of DHPs in relation to drugs' reinforcing properties; 3) Finally, it might be proposed that typical addictve substances acquires aversive properties after pretreatment with the DHPs.

The possible neurophisiological basis for the effect of DHPs also remains to be found. DHPs might affect rewarding effects of drugs of abuse by the means of: 1) Direct interaction with the excitability of the postsynaptic membranes due to the modulation of calcium fluxes through L-type calcium channels; 2) Indirect influence on the drug-induced release of a)dopamine; b) opioid peptides; 3) Interaction with adenosinergic mechanisms, which regulate the dopaminergic pathways; 4) Influence on the intracellular second messengers related to the system of cAMP.

It is concluded, that DHP sensitive mechanism underlies the reinforcing and craving effects of all typical drugs of abuse. Thus, the possible pharmacotherapy for addictive disorders might be found among centrally active DHP calcium channel modulators.

ROLE OF METABOTROPIC GLUTAMATE RECEPTORS IN INTRACELLULAR CALCIUM HOMEOSTASIS

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Metabotropic glutamate receptors mGluR1 and mGluR5 stimulate phospholipase C, leading to an increase of IP_3 level and to Ca^{2+} release from intracellular stores. The specific roles of mGluR1 and mGluR5 in intracellular Ca^{2+} homeostasis are poorly understood. Using Fura-2 fluorescence imaging, we examined the increases of intracellular free Ca^{2+} concentrations ($[Ca^{2+}]_i$) induced by mGluR agonists in HEK-293 cells transiently transfected with rat mGluR1a, and mGluR5a receptors. The time patterns of Ca^{2+} mobilization induced by mGluR agonists glutamate, quisqualate and *trans*ACPD were different in cells transfected with mGluR1a and mGluR5a. Activation of mGluR1a receptors evoked transient $[Ca^{2+}]_i$ increases, suggesting a rapidly-desensitizing response. Analysis of dose response data indicated that individual transfected cells responded to a treshold stimulation and that the mean dose-dependent response reflected both the amplitude of Ca^{2+} signals and the number of individual responding cells. In contrast to mGluR1a, the activation of mGluR5a evoked $[Ca^{2+}]_i$ oscillations. Both the amplitude of Ca^{2+} signals and the period of oscillations depended on agonist concentration. Ca^{2+} signals induced by mGluR1a, but not those elicited by mGluR5a were inhibited in a non-competitive manner by cyclothiazide. The analysis of Ca^{2+} signals induced by two chimeric receptors in which the N-terminal extracellular domain of mGluR1a was substituted with the corresponding homologous domains of either mGluR2 or mGluR3 suggested that the modulatory effect of cyclothiazide is not determined by interactions with the agonist-binding N-terminal domain of mGluR1a but possibly with one of the extracellular loops which are preserved in the chimeric receptors. Our results indicate that two phospholipase C-coupled mGluRs elicit different $[Ca^{2+}]_i$ responses and contribute in distinct ways to the regulation of intracellular calcium homeostasis.

THE ROLE OF VOLTAGE-DEPENDENT Ca^{2+} CHANNEL IN ADAPTIVE CHANGES INDUCED BY NEUROLEPTICS

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The aim of the study was to investigate the role of Ca^{2+} channel in biochemical and behavioral events appearing as adaptive changes after chronic treatment with neuroleptics. Chronic treatment of rodents with neuroleptics leads to development of tolerance and on withdrawal supersensitivity of the dopamine system. As we intended to investigate the effect of Ca^{2+} channel blockade on the haloperidol-induce withdrawal syndrome, it seemed of interest to investigate also the syndrome observed after withdrawal of a neuroleptic which itself has Ca^{2+} channel antagonistic properties: pimozide. The experiments were carried out on male Wistar rats, weighing 230-260 g. The neuroleptics (haloperidol 1, pimozide 4 mg/kg) were given for 14 days alone or shortly after injection of CA^{2+} channel antagonists (CCA, nifedipine 5 mg/kg, verapamil 10 mg/kg). We have found that withdrawal effects after haloperidol and pimozide were different. After haloperidol treatment we observed an increase in cortical Ca^{2+} channel ([^{3}H]nitrendipine binding sites) and limbic dopamine D_1 receptor ([^{3}H]SCH-23390 binding sites) and in behavioral studies we observed an increase in spontaneous motor activity and response to apomorphine-induced hyperactivity and stereotypy. In contrast no biochemical changes were observed during pimozide withdrawal, and locomotor activity and responses to apomorphine were depressed. Co-administration of CCA with haloperidol prevented the observed biochemical and behavioral symptoms of withdrawal. Nifedipine administration did not change the depressant effects of pimozide. Our results suggest that the voltage-dependent Ca^{2+} channel is involved in the observed withdrawal syndrome of neuroleptics and suggest that Ca^{2+} channel blockers may be useful in combination with neuroleptics lacking potent Ca^{2+} channel-blocking properties, as they may counteract the development of dopaminergic supersensitivity.

NMDA-EVOKED Ca^{2+}-INDUCED Ca^{2+} RELEASE IN RAT HIPPOCAMPUS IN VIVO: MODULATION BY SOME NEUROTROPIC DRUGS

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Numerous in vitro studies demonstrated that Ca^{2+}-induced Ca^{2+} release (CICR) significantly contributes to NMDA-evoked rise in intracellular Ca^{2+} concentration in cultured neurones. Thus, in vivo in the specific brain regions CICR may play a key role in cellular signalling and serve as a target for neurotropic drug's action. Utilising an in vivo microdialysis technique combined with measurements of $^{45}Ca^{2+}$ efflux from the prelabelled rat hippocampal formation we have demonstrated a phenomenon of NMDA-evoked $^{45}Ca^{2+}$ release in the dentate gyrus (DG). Specific pattern of its modulation by ryanodine and dantrolene indicates that it reflects the activity of CICR in the rat neurones. This model was used for experimental verification of the hypothesis that independently from other mechanisms, caffeine applied in vivo in pharmacological concentrations may stimulate ryanodine receptors and inhibit IP3-sensitive receptors. Caffeine, 40 mg/kg followed by 80 µg/kg/min infusion, was applied intraperitoneously. NMDA-evoked $^{45}Ca^{2+}$ release in DG and dantrolene-insensitive spontaneous Ca^{2+} efflux in CA1 served as approximate measures of CICR and IP3 receptor activity, respectively. Caffeine had no significant effect on NMDA-evoked $^{45}Ca^{2+}$ release in the rat DG, however preliminary experiments demonstrated its inhibition of a dantrolene-insensitive portion of a spontaneous $^{45}Ca^{2+}$ efflux from the CA1. This may suggest that caffeine in vivo inhibits IP3-induced Ca^{2+} release. Based on this and other data we will discuss utility of our model for studies on the mechanism of some malignant side-effects of antidepressants and halothane in the brain.

THE BEHAVIORAL AND NEUROCHEMICAL CONSEQUENCES OF CHRONIC ADMINISTRATION OF LSD: PREVENTION OF DOPAMINE METABOLISM DISTURBANCES AND COGNITIVE IMPAIRMENT BY Ca^{2+} CHANNEL BLOCKADE

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Lysergic acid diethylamide (LSD) is a hallucinogenic drug of abuse that after prolonged administration causes psychoses. In most animal studies the effects of single LSD doses were usually investigated. To obtain data more related to the situation of abusers, we studied now the effect of multiple doses of LSD on dopamine and serotonin metabolism in the rat limbic and extrapyramidal brain structures and on cognitive functions in mice, and to find out if the induction of such changes would be prevented by a Ca^{2+} channel blockade during LSD administration. We found that the neurochemical effects of a single dose were negligible, while chronic administration resulted in profound activation of dopaminergic system in the striatum and nucleus accumbens, and less evident in the cortex. The effect of a low dose (0.1 mg/kg) differed from that of the high one (2 mg/kg), as the former caused apparently an intraneuronal release of dopamine. The changes were much more marked 24h than 2 h after the last dose, suggesting that they represent adaptive changes. Chronic administration of 0.2 mg/kg LSD to CD-1 mice before and during the shuttle-box training resulted in impairment of acquisition of the conditioned avoidance responses. LSD produces no such changes when given under conditions of Ca^{2+} channel blockade (nifedipine, 5 mg/kg before each dose of LSD). The results suggest that the LSD-induced psychosis is an adaptive phenomenon following acute hallucinogenesis, thatCa^{2+} channels play a role in its development, and that Ca^{2+} channel blocking agents may be useful in combating the effects of LSD abuse.

Poster session - Neuroanatomy

Afferents to the anterior suprasylvian cortex in the cat

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Because electrophysiological maps of auditory cortex in cats have implicated the anterior suprasylvian cortex plays a role in audition, the present study was undertaken to examine the anatomical basis of these observations. The afferents to the suprasylvian cortex were studied using retrograde transport of horseradish peroxidase. The widely inclusive bulk-filling method was used to maximize the likelihood that virtually all afferents to the area would be labelled.

The present results cast doubt on the direct involvement of the suprasylvian cortex in the auditory system. No labelled cells were found in medial geniculate nucleus or in the dorsolateral part ot the posterior nucleus. Moreover, only a few cells were labelled in the primary auditory cortex of the nearby ectosylvian gyrus. Instead, the results show that the strongest thalamic input to the cortex of the suprasylvian gyrus originates in the pulvinar-lateral posterior complex. Lesser input may originate in ventral lateral and ventral anterior nuclei and still lesser from a variety of non-specific thalamic nuclei.

Therefore, there seems to be no hodological substrate for auditory involvement in the suprasylvian cortex. Nevertheless, there are too many reports of electrophysiological experiments suggesting the suprasylvian cortex involvement to be ignored. Auditory activity may reach the suprasylvian cortex by a more circuitous route than one denied by the present results.

LATERALITY OF PARABIGEMINO-GENICULATE PROJECTION IN THE HAMSTER

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In several species of mammals like squirrel, gopher, rat or opposum projection of the parabigeminal nucleus (PBG) to the dorsal lateral geniculate nucleus is mostly crossed, whereas in the cat it is bilateral and in the primates it is primarily ipsilateral. There is no data about the parabigemino-geniculate projection in the hamster.

In the present study we injected the dorsal lateral geniculate nucleus in the adult, pigmented hamsters on one side with one retrogradely transported fluorescent dye (Fast Blue) and on the other side with another such dye (Diamidino Yellow). After three to four days of survival the animals were perfused transcardially with saline followed by 4% paraformaldehyde in the phosphate buffer (pH 7.4).

We have found up to 1700 labeled neurons in each PBG, of which only 4 - 5% were labeled by the ipsilateral injection and the remaining majority by the contralateral injection. The highest density of the labeled neurons was observed in the rostral half of the nucleus, whereas the majority of ipsilaterally projecting neurons were placed in its caudal part. Therefore, this projection is similar to the projection in the rat.

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DESCENDING CONNECTIONS OF THE CLAUSTROCORTICAL NEURONAL LOOP IN THE RAT AND RABBIT

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Claustrum is the subcortical nucleus closely associated with large areas of the neocortex. Its connections with the cerebral cortex seem to have a bi-directional character, forming the cortico-claustral loop composed of ascending and descending projections. The ascending claustrocortical projection has been extensively studied. In many species including the rat and rabbit the descending projection has not been investigated by means of the method of axonal retrograde transport of fluorescent tracers.

The studies were performed on 20 Wistar rats and 15 New Zealand rabbits. Two different fluorescent tracers (Fast Blue, Fluoro-Gold) were administered streretactically into the anterior, central and posterior part of the insular claustrum by means of the iontophoretic apparatus (5 μA continuous current; 10 min). 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. The survival time ranged from three to eight days. The brains were fixed and cut coronally with the frozen microtome into 40-μm-thick sections which were studied using fluorescent microscope with UV-filter system.

After administration of the fluorescent tracers into the claustrum, the labeled neurons were detected in the cortex in both species. Injection of tracers into the anterior portion of the claustrum resulted in labeling of neurons in the motor cortex. After administration of tracers into the central portion labeled neurons were present both in the motor and somatosensory cortex. Injection of tracers into the posterior portion resulted in labeling of cortical neurons in the visual cortex. The cortical neurons projecting to the claustrum were localized mainly in layer VI of the neocortex in both species. In the motor cortex of rabbit the labeled neurons were also present in layer V.

Administration of fluorescent tracers into the insular claustrum of the rat resulted in labeling of cortical neurons in corresponding areas of both hemispheres. In the rabbit cortical neurons projecting to the claustrum were detected only in the ipsilateral hemisphere.

Our studies suggest that both in the rat and rabbit the cortico-claustral projection, like the claustrocortical one, is organized topographically in the anteroposterior direction. The bi-directional connections of the claustrum with the neocortex in both species strongly confirm the existence of the claustrocortical loop revealed in other species.

THE STRUCTURAL BASIS OF INTERACTION OF LIMBIC AND MOTOR SYSTEMS IN STRIATAL NUCLEI OF THE DOG AND PRINCIPLES OF ITS FUNCTIONAL SPECIALIZATION.

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The study is aimed at investigation of the function of dog's striatum based on knowledge of the detailed spatial organization of striatum afferent projections from structures connected with regulation of motor behavior (motor cortical areas, substantia nigra) or emotional and incentive (limbic cortical areas, ventral tegmental area, amygdaloid body) aspects of behavior. Experimental research has been carried out by the method of axonal transport of retrograde markers. Proceeding from data obtained, the "limbic" and "motor" segments as well as zones with overlapped terminal areas of the projection fibers from the different functional structures can be distinguished in the nucleus caudatus, the putamen and the nucleus accumbens. Thereby, organizational peculiarities of the afferent projection system of the nuclei aforesaid can be considered as morphological substrate of its heterogeneity. Moreover, the tentative scheme suggests an integration and a segregation of the neuronal signals that is conveyed through the ventral striatum.

The work was supported by RFFR (N95-04-11518a).

LOCALIZATION OF ACETYLCHOLINESTERASE-POSITIVE NERVE STRUCTURES IN THE PINEAL GLAND OF MALE ADULT ALBINO RATS BY MEANS OF THE HISTOCHEMICAL METHOD OF TAGO AND COWORKERS.

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The presence of the cholinergic innervation in the pineal gland of the different mammalian species including rat is still the matter of numerous controversies, deriving from the contradictory reports on this subject. Application of the histochemical method for testing acetylcholinesterase, showed lack of AChE-positive nerve structures in the superficial part of the pineal gland of male adult albino rats. AChE-positive nerve fibers-like structures were found in the pineal stalk. This result may good corroborate with the fact of different innervation of the particular parts of the rodent pineal gland. It is well known that the deep pineal gland and the pineal stalk of rodents are the primary target of the central innervation derived from the forebrain and the midbrain via the stria medullaris thalami and the habenular complex. It appears possible that ACh-containing neurons in the medial habenular nuclei may project to the pineal gland via the pineal stalk. The presence of AChE-positive nerve structures only in the pineal stalk of male albino rats, may indirectly support existence of cholinergic innervation of the subpopulation of the rat pinealocytes belonging to the pineal stalk and/or superficial pineal gland.

Poster session - Neurochemistry

CHRONIC ELECTROCONVULSIVE TREATMENT INCREASES THE ACTIVITY OF NITRIC OXIDE SYNTHASE IN THE RAT BRAIN

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Antidepressant drugs and electroconvulsive shock induce adaptive changes in the NMDA receptor complex. This adaptation consists of a reduction in the potency of glycine to displace [^{3}H]5,7-dichlorokynurenic acid from strychnine-insensitive glycine sites and a reduction in glycine-sensitive [^{3}H]CGP-39653 binding to glutamate sites of the NMDA receptor complex. Activation of this receptor complex increases the intracellular free Ca^{+2} concentration, which in turn influence a nitric oxide synthase (NOS) activity. In this study we investigated the NOS activity after acute and chronic electroconvulsive treatment in different rat brain areas. Chronic (10 daily treatments) and acute (single treatment) electroconvulsive shocks were administered to male Wistar rats. Twenty-four hours after the last treatment animals were decapitated, their brain dissected and stored until they were assayed (24 hs). NOS enzyme activity was assesed by measuring the formation of [^{3}H]citruline from [^{3}H]arginine.

Chronic, but not acute electroconvulsive treatment significantly increased the NOS activity by 60% in the cerebral cortex and by 20-30% in hippocampus and cerebellum. The increased NOS activity might be a compensatory mechanism which balanced the reduced NMDA receptor complex reactivity. In fact, the adaptation of the NMDA receptor complex induced by chronic antidepressants and electroconvulsive treatment measured at the receptor level suggests the subsensitivity of that complex.

Present results support the hypothesis of the critical role of the NMDA receptor complex in the mechanism of antidepressant action.

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KAINIC ACID-STIMULATED RELEASE OF TAURINE FROM THE RAT SUBSTANTIA NIGRA (SN): *IN VIVO* MICRODYALISIS STUDY

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Previous microdialysis studies in the freely moving rat have demonstrated that direct application of the excitatory amino acid receptor agonist, kainic acid (KA), to the SN induces a DNQX-sensitive local release of GABA (Bianchi et al.,1994). The object of the present experiment was to examine the release of endogenous taurine, as well as GABA, under similar conditions. Rats were implanted with a microdialysis probe in the SN pars reticulata. After 24h the probe was perfused with artificial CSF (1.2 ml/min) The SN was then perfused with 100 μM KA (20 min) alone or in the presence of 10 μM or 100 μM DNQX or 10 μM tetrodotoxin (TTX). Fractions (20 min) were collected for 2 h before and up to 3 h after the KA perfusion. Following derivatisation and separation (HPLC) the amino acids were detected fluorometrically. The perfusion of SN with KA stimulated the release of both taurine and GABA. The enhanced release of both amino acids was partially attenuated by the inclusion of 10 μM DNQX in the perfusion medium and completely blocked by inclusion of 100 μM DNQX. The enhanced release was also blocked by 10 μM TTX.

These results suggest that the release of taurine and GABA in SN in response to KA is, at least part, receptor-mediated and dependent of action potentials. The data are consistent with the presence of excitatory amino acids in some of the SN afferents and the presence of non-MNDA excitatory amino acids receptors. Furthermore, they provide additional support for the possible association of taurine with the striatonigral pathway, but do not preclude the notion that at least part of the released taurine and GABA is derived from non-neuronal sources.

Pentylenetetrazole-induced seizures upregulate expression of mRNAs for TBP1 and Mss4 in rat hippocampus.

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Differential RNA display was used to compare RNA species expressed in hippocampi of untreated rats and rats 6 h after seizures induced pentylenetetrazole (PTZ). This treatment was used as a model of some aspects of neuronal plasticity and/or neurodegeneration. We identified several bands that had been regulated in animals after seizures. These bands, which may represent differentially expressed RNAs, were cloned, sequenced, and compared to sequences in data banks. The results of differential RNA display were verified by northern blots either with the cloned sequences or a synthetic oligonucleotide.

We found that PTZ induced seizures caused upregulation of mRNAs for tat-binding protein 1 (TBP1) and mammalian supressor of Sec4 (Mss4) in the rat hippocampus. TBP1 protein forms (or is closely homologous to) the 19S - regulatory - subunit of the proteasome. It has also been shown to regulate transcription. Mss4 is a nucleotide exchange factor for monomeric G proteins of the Rab family, known to regulate synaptic transmission.

The levels of induction were moderate: a 1.5-2 fold increase, with maximum after 2-6h after seizures. Expression of Mss4 analysed by *in situ* was predominantly dendritic.

The effects of the peripheral denervation on levels of mRNA coding for glutamate decarboxylase (GAD) and GluRB subunit of glutamate AMPA receptor in the adult somatosensory cortex of adult mice.

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The interactions between excitatory and inhibitory systems seem to be essential for brain cortex plasticity. Alterations in both systems temporally correlate with plastic changes observed in the nervous system. It was demonstrated previously that peripheral denervation resulted in marked decrease in GAD immunoreactivity in the primary somatosensory cortex receiving input from the injured nerve and that eye lid suture led to changes in levels of mRNAs coding for AMPA receptor subunits in the visual cortex.

We investigated the short-term effects of partial denervation of the barrel field in mice (which produces changes in sensory cortical map), upon the levels of expression of gene coding for GAD67 and GluRB as measured by an in situ hybridization method. 35S-labelled oligodeoxynucleotides were used as sense probes. Unilateral lesion of all whiskers except row C was performed in 6 week old mice. Hybridized, washed and dried slides with 15μm brain sections mounted on were exposed to X-ray sensitive film along with 14C-standards. The autoradiograms were analyzed with computerized image processing system (MCID).

Level of GAD67 mRNA was significantly diminished in cortical representation of the damaged vibrissae 1 week after injury as compared to level in the intact C row. In the contralateral i.e. control hemisphere levels of GAD67 mRNA in rows A,B,D,E of barrels remained unchanged as compared to row C. Also in control animals the labelling was similar in all rows of both hemispheres. Levels of GluRB mRNA were not affected by the treatment. These results indicate that downregulation of inhibitory system depends on decrease of transcriptional activity of the gene coding for GAD67, one of the two isoforms of the enzyme synthesizing γ-aminobutyric acid (GABA).

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p30, A NOVEL PROTEIN TARGET OF BRAIN CALCYCLIN

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Calcyclin is a calcium binding protein belonging to the S100 family present in fibroblasts, epithelial cells and neurons. The role of calcyclin is not yet understood, although there are data suggesting several functions and interactions with possible targets. In the current work, using a gel overlay method, we have found that mouse calcyclin interacts with a novel target, a 30 kDa protein present in Ehrlich ascites tumour (EAT) cells. Interaction of calcyclin with this 30 kDa protein (p30) depends on the presence of calcium ions. The binding of p30, evidenced by the reaction with ^{125}I-labelled calcyclin, was found to be of higher affinity than the binding between mouse calcyclin and annexin II or glyceraldehyde-3-phosphate dehydrogenase. Examination of tissue extracts by the gel overlay method has shown that p30 is present not only in the EAT cells but also in the mouse brain and spleen. Purified p30 was digested with α-chymotrypsin and a partial aminoacid sequence of one of the resulting peptides was established. A data base search analysis revealed that the sequence is unique suggesting that p30 represents a novel protein. From the established amino acid sequence the oligonucleotides were designed and comercially synthesized. The cDNA mouse brain library was screened for clones representing the p30 coding region using oligonucleotides described above. Several clones were identified and they are currently undergoing sequencing. The results of this work will allow us to identify the clone of the novel target of calcyclin and will help to understand the function of this Ca^{2+}-binding protein in the brain.

Fe^{2+}-INDUCED INHIBITION OF GERBIL FOREBRAIN ENDOPLASMIC RETICULUM Ca^{2+}-ATPase

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In the central nervous system, free radicals and oxidative stress are thought to be involved in the pathophysiology of some neurodegenerative diseases, brain trauma, and ischemia-reperfusion injury. The aim of this work was to study the Fe^{2+}-induced inhibition of gerbil forebrain microsomal Ca^{2+}-ATPase. The incubation of the gerbil forebrain microsomes in the presence of ferrous sulfate and EDTA led to the inhibition of Ca^{2+}-ATPase in both a concentration- and time-dependent manner. The preincubation of microsomes either with stobadine or stobadine-glutathione mixture prevented the inhibition of Ca^{2+}-ATPase, however, the effectivity of prevention was dependent on the Fe^{2+} concentration. The results indicated that the best stobadine-glutathione ratio was close to 1:1. Inclusion of glutathione had no significant protective effect on the inhibition of Ca^{2+}-ATPase, indicating that oxidation of SH-groups is not probably involved in this inhibition. In addition, the inclusion of aminoguanidine also prevented the inhibition of Ca^{2+}-ATPase in a Fe^{2+} concentration-dependent manner. Since the Ca^{2+}-ATPase is also inhibited by carbonyl compounds we suppose that both changes in lipid-protein interactions and modification of polypeptide chain, by lipid peroxidation products, play an important role in the Fe^{2+}-induced inhibition of Ca^{2+}-ATPase.

STUDIES OF REGULATION OF PLASMA MEMBRANE Ca^{2+}-ATPase PURIFIED FROM RAT BRAIN.

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The plasma membrane Ca^{2+}-pump is a calmodulin regulated P-type ATPase that is a crucial element in controlling intracellular Ca^{2+} concentration. Using different approaches we have examined the regulation of the activity of this enzyme, purified from three regions of rat brain : cortex, cerebellum and hippocampus, by neuroactive steroids and by phosphorylation processes. Our previous studies revealed that neuroactive steroids primarily acts on membrane Ca^{2+}-ATPase, and suggested that some hormones could modulate neuronal Ca homeostasis in rat brain. Experiments are in progress to elucidate the mechanism of hormones interaction with the enzyme.
The purified Ca^{2+}-ATPase from selected regions of rat brain was phosphorylated *in vitro* by PKA and PKC. The incorporation of ^{32}P was different in cortical, cerebellar and hippocampal enzyme, and was more pronounced with PKC. These differences are probably the physiologically important consequence of the different isoforms combinations in particular regions of rat brain. Immunostaining experiments with anti P-Ser and anti P-Thr showed that phosphorylated amino acids were also detected in the Ca^{2+}-ATPase, before the phosphorylation catalysed by protein kinases. It could suggest that phosphorylation of plasma membrane Ca^{2+}-ATPase is a naturally existing process. The presented results give new information about the potentially important mechanisms of Ca^{2+}-ATPase regulation in rat brain.

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Calcium phosphate transfection of the dentate gyrus cells in the primary culture.

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Dentate gyrus of the hippocampus is known to be an important structure for studying neuronal plasticity. We have developed an *in vitro* culture of dentate gyrus neurons from 5-day old rats. To study a role of transcription factors: AP-1 and CREB in neuronal responses to excitatory amino acids, we have developed a method of DNA transfer into dentate gyrus cells in primary culture using calcium-phosphate procedure. For transfection we have employed a vector containing a gene encoding the green fluorescent protein (GFP) under control of CMV promotor. The transfected cells were visualized under fluorescent microscope. One to two percent of neurons were found to be transfected. We have cloned genes encoding: TAM67 (dominant negative mutant of *c-jun*), CREBA119 (dominant negative mutant of CREB) under control of two different promoters: constitutive (CMV) and inducible (tetracycline-dependent). At present we are studying regulation of TIMP-1 (tissue inhibitor of metaloproteainases 1) by glutamate using this system. The results of these experiments will be presented.

IN VIVO MODULATION OF RAT CORTICAL ACh RELEASE

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Interest in the cholinergic system has increased since studies in humans provided evidence that this system is severely affected in Alzheimer-type and other dementias. The modulation of the basal forebrain cholinergic neurons by other neurotransmitters and, in particular, glutamate needs to be clarified. The transversal microdialysis technique was used to investigate the modulation of cortically-projecting cholinergic neurons by glutamatergic inputs in freely moving male Wistar rats (250-300g). Under chloral hydrate anesthesia a transversal micricialysis membrane was positioned in the frontoparietal cortex, and ACh release was measured by HPLC with electrochemical detection. Drugs were administered either through the dialysis membrane or i.c.v. The competitive NMDA antagonist CPP (1, 2.5 and 5 nmol/5 μl saline) administered i.c.v. to rats elicited a long-lasting, dose-dependent increase in cortical ACh output by 42%, 84% and 95%, respectively. Muscimol (5 nmol/5 μl saline, i.c.v.) temporarily inhibited the effect of CPP (5 nmol/5 μl saline). The effect of CPP was not abolished by administration of NMDA (200 μM) to the cortex through the dialysis probe, and was completely prevented by application of TTX. In some experiments, a second membrane was inserted either in the medial septum, or in the nucleus basalis magnocellularis (NBM). CPP (100 μM), locally administered to NBM, decreased cortical ACh release. CPP (100 μM), administered to the septum, increased cortical ACh release by 82%, this effect being abolished by concomitant administration of 50 μM muscimol, while bicuculine (50 μM) increased ACh release by 190%.

Our data indicate that a double regulation of the cholinergic pathways ascending to the cortex exists: i) a direct excitatory regulation mediated through NMDA receptors at the level of nucleus basalis, and ii) an indirect inhibitory regulation mediated through NMDA receptors located on GABAergic interneurons at cortical and septal levels.

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L-ARGININE IS AN ENDOGENOUS MODULATOR OF CEREBRAL MITOCHONDRIAL GLUTAMATE TRANSPORT.

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Uptake of 0.2 mM L-[14C]Glutamate (GLU) into nonsynaptic mitochondria isolated from rat cerebral hemispheres was measured in the presence of potential modulators of amino acid transport. L-aspartate (ASP) at 0.2 mM concentration virtually abolished GLU uptake pointing to the involvement of GLU/ASP antiport. L-arginine (L-ARG) inhibited GLU uptake in a dose dependent manner at concentrations ranging from 0.1 mM to 5.0 mM to maximum inhibition of 85%, and the inhibition was pH-independent in the pH range of 6.5-8.0. Of the major L-ARG metabolites added at 5 mM concentration, ornithine, putrescine or ammonia had no effect on the uptake, while creatine and the NO generator, sodium nitroprusside, increased the uptake by 73% and 57%, respectively. D-ARG was three times less effective in inhibiting GLU uptake than L-ARG at 5 mM concentration. The L-amino acids lysine, histidine, tyrosine, phenylalanine, proline, leucine, isoleucine, tryptophan, glycine, methionine, valine, serine, tyrosine, taurine, alanine or cysteine did not affect the uptake at 2-5 mM concentration. L-glutamine (2 mM), and the dicarboxylate carrier ligands α-ketoglutarate (2 mM) and phenylsuccinate (5 mM) inhibited the uptake by <20%. The results indicate that L-ARG functions as a specific endogenous modulator of GLU transport in rat cerebral mitochondria.

EFFECT OF THIOACETAMIDE-INDUCED HEPATIC ENCEPHALOPATHY IN RATS ON THE SYNAPTOSOMAL UPTAKE OF GLUTAMATE PRECURSORS

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In this study we tested one of the hypotheses linking hepatic encephalopathy (HE) to disturbed metabolic trafficking between astrocytes and neurones. The kinetics of uptake of two astroglia-derived glutamate (GLU) precursors-α-ketoglutarate (α-KG) and glutamine (GLN) were determined in synaptosomes derived from rats with acute hepatic encephalopathy (HE) induced with a hepatotoxin, thioacetamide (TAA). TAA treatment increased by 33% Vmax for the high affinity, low capacity α-KG uptake, without influencing its Km. The increase of the uptake capacity for α-KG may represent a compensatory response of the GLUergic nerve terminals to the decreased cerebral α-KG content, which during HE is associated with depressed activity of pyruvate carboxylase, an enzyme that replenishes α-KG in astrocytes. The results is thus consistent with the notion that HE affects the astroglial control of GLUergic neurotransmission. The Km and Vmax for the low affinity, high capacity GLN uptake was not affected by TAA treatment.

REGIONAL AND CELLULAR DISTRIBUTION OF MONOAMINO OXIDASE A AND B IN RAT BRAIN

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Monoamino oxidase (MAO, E.C. 1.4.3.4.) is the main enzyme of biogenic amines metabolism in CNS. We improved the coupled peroxidatic oxidation method of MAO histochemistry. Serial cryostat section of fresh-frozen brain samples and selective inhibitors of MAO A and B forms (deprenyl and clorgyline respectively) were used to study the regional and cellular distribution of MAO A and B in rat brain. The intensity of the histochemical staining reflecting MAO activity in the brain structures can be measured cytophotometrically. Maximum MAO A activity was found in adren- and noradrenergic neurons in which MAO B activity was not detected. The highest MAO B activity was seen in serotoninergic and histaminergic neurons as well as in ependimocytes covering brain ventricles, in which MAO A activity was very low. Some structures possess high (n. interpeduncularis) or intermediate (blood capillary endothelium) activity of the both enzyme forms. It was found that alcohol and morphine administrations significantly affect the MAO A and B activities in the specific brain structures. In details, the chronic (6 months) alcohol consumption increased the MAO A activity and the contribution of it to the total MAO activity in rat brain structures; both the MAO forms in all structures were more active in the ethanol preferring rats as compared to water preferring animals. This histochemical approach can be suggested for a comparative mapping of MAO A and B in CNS and measurement of the local enzyme activities under experimental and pathological conditions.

AN INTERACTION BETWEEN HISTAMINE AND VASOACTIVE INTESTINAL POLYPEPTYDE (VIP) ON cAMP PRODUCTION IN DIRECTLY PHOTOSENSITIVE PINEAL ORGAN

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Directly photosensitive pineal organ of avians and lower vertebrates displays many morphological and biochemical features in common with vertebrate retina. One of such features is the ability of both tissues to synthesize melatonin (MEL) in circadian rhythm, with high values at night and low values occurring during dayhours. The daily variation in the hormone production depends on rhythmic nocturnal induction of a penultimate and rate-limiting MEL-synthesizing enzyme, ie serotonin *N*-acetyltransferase (NAT). The process of NAT induction in pinealocytes (or retinal photoreceptors) is under control of Ca^{2+}- and cAMP-related mechanisms. In vertebrate pineal gland, VIP is capable of activating the synthesis of both cAMP and MEL. Recently we have shown in chick pineal that histamine (HA), which is locally synthesized and inactivated by the gland, is a potent stimulator of cAMP formation; yet, in organ-cultured chick pineals HA only weakly affected MEL release, suggesting that the HA-evoked cAMP effect in the gland may be unrelated to MEL biosynthesis. Here we describe an interaction between HA and VIP on cAMP production in chick pineal gland, but not in chick cerebral cortex. This observation can reinforce the idea that HA, in addition to VIP, may be considered a physiological regulator of the pineal organ activity in vertebrates.

THE ROLE OF CALCIUM IONS IN THE HISTAMINE-EVOKED STIMULATION OF cAMP SYNTHESIS IN AVIAN CNS

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Histamine (HA) is a powerful stimulator of cAMP synthesis in avian pineal gland and cerebral cortex. The molecular mechanism underlying this HA action remains unknown in nature, since the amine is a weak stimulator of adenylate cyclase activity. As HA has been shown to increase Ca^{2+} influx into isolated chick pinealocytes, in the present study we examined whether Ca^{2+} ions are involved in the stimulatory effect of the amine on the cAMP generating system in chick pineal gland and cerebral cortex.

HA (0.1-1000 μM) potently stimulated [^{3}]cAMP formation in pineal gland and slices of cerebral cortex of chick prelabeled with [^{3}H]adenine in a concentration-dependent manner. This action of HA was not modified by compounds increasing Ca^{2+} transport across the cell membrane, blockers of voltage-sensitive calcium channels, and by inhibitors of calmodulin. On the other, the HA-evoked increase of cAMP formation was inhibited by divalent cations, i.e., Cd^{2+}, Co^{2+}, Ni^{2+}, Mn^{2+}, and Ba^{2+}.

It is suggested that calcium-related mechanisms are not of a major importance in the action of HA on the cAMP generating system of chick pineal gland and cerebral cortex. The observed inhibitory effects of divalent cations likely result from their direct interaction with the catalytic domain of adenylate cyclase.

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EFFECTS OF NEAR-ULTRAVIOLET (UV-A) LIGHT ON THE NOCTURNAL MELATONIN BIOSYNTHESIS IN RAT PINEAL GLAND

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In pineal glands of various vertebrate species melatonin (MEL) level and activity of serotonin N-acetyltransferase (NAT, a key regulatory enzyme in MEL biosynthesis) fluctuate in a circadian rhythm, reaching high values during the dark phase of a light:dark illumination cycle. Visible light is a predominant environmental factor controlling MEL production. The aim of this study was to analyze effects of near-ultraviolet light (UV-A; λ_{max} = 365 nm) on the nocturnal NAT activity of rat pineal gland.

Exposure of rats to UV-A light in the middle of the night produced a potent decline of the nocturnal NAT activity of pineal gland. The magnitude of the UV-A light-induced suppression of the enzyme activity was dependent on the duration of the light pulse. 1 min and 5 min exposure to UV-A decreased the nocturnal NAT activity of the rat pineal gland by 32% and 70%, respectively. In rats that were exposed to a 1-min pulse of UV-A light and then returned to darkness for a period of 5-180 min, NAT activity of the pineal gland continued to decline during 40 min, then started to increase, and reached control values after 3 hours in the dark.

Our data demonstrate that the MEL-generating system of the rat pineal gland is sensitive to nonvisible light of the near-ultraviolet range.

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Poster session - Dopamine

THE ROLE OF DOPAMINE D_2 RECEPTOR IN THE BEHAVIORAL EFFECTS OF IMIPRAMINE - STUDY WITH THE USE OF ANTISENSE OLIGONUCLEOTIDES

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We have used the intracerebroventricular (*icv*) injection of antisense oligonucleotides (ODNs) to examine the contribution of D_2 dopamine receptor to the behavioral effects of imipramine. Antidepressant drugs, including imipramine, have been shown to shorten the immobility time in the forced swimming test (Porsolt test). It has been postulated that this effect results from the enhanced dopaminergic transmission. In the present study the antisense ODNs were injected *icv* twice a day for 5 days, in the concentration of 1 nmol/µl. Using radioreceptor binding assay it has been found that specific binding of [^{3}H]spiperone was decreased both in the caudate putamen as well as in the limbic forebrain of the rat, what may indicate the lower density of D_2 dopamine receptors in these brain regions. Animals receiving *icv* the antisense ODNs were treated with imipramine (10 mg/kg *ip*, 24, 5 and 1 h before test) and subsequently subjected to the forced swimming test. The obtained results indicate that imipramine indeed shorten the immobility time in animals subjected to Porsolt test, however it doesn't effect this parameter in the rats receiving *icv* antisense ODN directed against mRNA coding for D_2 dopamine receptor. *Icv* injection of random ODN sequence neither changed the binding parameters of [^{3}H]spiperone nor produced any alterations in the effects of imipramine in the rats subjected to Porsolt test.

The obtained results indicate the significant role of D_2 dopamine receptor in the studied behavioral effects of imipramine.

THE MOTIVATIONAL PROPERTIES OF SELECTIVE DOPAMINE D2/D3 RECEPTOR AGONISTS STUDIED WITH AN OPERANT PROCEDURE IN RATS

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Accumulating data provide clear evidence for a pivotal role of dopamine in reward-related processes and in incentive learning. The present series of experiments were undertaken in order to provide more evidence for an influence of some dopamine receptor agonists (D2/D3) on the incentive value of food in the standard operant schedule. The reinforcing ability of these compounds was assessed by their ability to reinstate nonreinforced lever pressing at the lever that previously delivered the pellets of food. We next tested whether treatment with D2/D3-like agents could modulate the reinforcing effects of "priming" food's pellets delivered during this nonreinforced period (extinction).

The D2/D3 agonists (7-OH-DPAT, apomorphine) induced dose-related increases in nonreinforced responding at the food-paired lever. Moreover, at lower doses, these compounds greatly enhanced the priming ability of some food's pellets given additionally during the period of extinction. This action was abolished by the pretreatment with D2-antagonist- raclopride but not by D1-antagonist- SCH 23390. Some D2/D3-specific neuroleptics (sulpiride, amisulpride) caused no effects in this operant procedure. These compounds, in a narrow range of low doses, can enhance the incentive value of food using a place conditioned procedure.

The present results provide evidence that some D2/D3-specific agonists have their intrinsic rewarding properties and they can enhance the rewarding value of the food as measured in an operant schedule.

EFFECT OF UNILATERAL BLOCKADE OF VTA DOPAMINE TRANSMISSION ON BEHAVIOR ELICITED FROM CONTRALATERAL VTA

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Unilateral electrolytic lesion of VTA facilitates feeding and locomotor responses to electrical stimulation of the contralateral VTA. This effect was found to be GABA-dependent. The objective of the present study was to verify the hypothesis that " the contralateral facilitation effect" is also dependent on dopaminergic transmission.

The experiments were done on male Wistar rats implanted with unilateral VTA cannula and contralateral VTA stimulating electrode. Locomotor response was elicited by electrical stimulation of VTA in a latency - frequency paradigm. The effects of D_1 receptors blockade with SCH 23-390 (0.0, 100, 1000 ng), D_2 receptors blockade with sulpiride (0.0, 1250, 2500 ng) and 6-OHDA (8 µg) lesion of VTA dopaminergic neurons was studied in the separate experiments. It was found that stimulation-induced locomotor response was facilitated by all treatments which manifested as a decrease of the reaction threshold and a leftward shift of the latency-frequency function. Decrease of the threshold expressed as a percentage of the preinjection baseline was dose-dependent in SCH 23-390 and sulpiride groups (SCH 23-390: 0.0 ng - 0.71±0.7 %; 100 ng 3.1±6.0 %; 1000 ng -19.5±3.8 %; sulpiride 0.0 ng 2.8±4.5 %; 1250 ng -18.2±5.8 %; 1000 ng -23.3±6.5 %). 6-OHDA lesion caused a decrease of the threshold by -20.6±3.7 %. The results indicate that "the contralateral facilitation effect" at the level of the ventral tegmental area is dependent on the dopaminergic transmission. It may involve a compensatory increase in dopamine relase in the intact hemisphere.

EFFECT OF CADMIUM APPLIED JOINTLY WITH DIAZEPAM TO PREGNANT RATS ON THE CENTRAL DOPAMINE (DA) RECEPTOR REACTIVITY AND GLUCOSE UPTAKE IN THE BRAIN AND PERIFERAL TISSUES OF OFFSPRINGS.

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Previously we have shown that ethanol (EtOH) applied togather with cadmium (Cd) to pregnant rats prevent some toxic effect of the metal and its deposit in the brain of offsprings. Some similarities in the molecular action on the central nervous system between EtOH and diazepam (DIAZ) are noticed. Therefore the reactivity of the central DA receptors, glucose uptake and Cd level was measured in rats prenatally exposured to Cd and DIAZ (separately or jointly). Wistar rats were given water solution of Cd ($CdCl_2$) 50 ppm ad libitum without or with daily injected DIAZ 0.5 mg/kg SC throughout pregnancy. Male adult offsprings were injected IP with the central DA agonists or antagonists (quinpirole, SKF 38393, haloperidol, SCH 23390) and tested for characteristic behavioral effects (yawning, oral activity, catalepsy). Beside 6-^{3}H-D-glucose 500 µCi/kg IP was injected and the rats were scarificied 15 min or 4 h later. CPM/100 mg wet tissue of the diserete parts the brain and peripheral organs was recorded. Beside the level of Cd in the different tissues of rats body was estimated.

It was shown that Cd and DIAZ modulate central DA receptors reactivity and glucose uptake in some examined tissues. Beside DIAZ modified the Cd deposit in the body of rats.

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NITRIC OXIDE (NO) AND CENTRAL EFFECTS OF THE DOPAMINE (DA) AGONISTS ON CIRCULATORY SYSTEM IN RATS. P. Nowak, R. Brus, A. Kasperska, R. Szkilnik. Department of Pharmacology, Silesian Academy of Medicine, 41-808 Zabrze, Poland.

Adult male Wistar rats were anesthetized with urethane (1.5 g/kg IP), implanted with intracranial cannula for intracerebroventricular (ICV) injection, and continously monitored for (a) mean arterial blood pressure via an indwelling catheter in the right carotid artery, (b) heart rate via an ECG lead II signal and (c) respiratory rate via a photoelectric transducer. All electronic inputs were connected to an IBM-AT-compatibile computer via a 14 bit I/0 card. To process and store data, a software program was developed and used. The L-NAME or L-Arginine were apply IV before or after ICV injection of dopamine (DA) or central DA receptor agonists.

L-NAME and L-Arginine by itself influenced on measured parameters and modulate central effects of DA receptor agonists.

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EFFECT OF THE AMINOACID L-ARGININE AND ITS ANALOG NITRO-L-ARGININE METHYL ESTER HCl (L-NAME) ON CENTRAL DOPAMINE (DA) RECEPTOR REACTIVITY. R. Brus, R. Szkilnik, A. Kasperska, J. Oświęcimska and R.M. Kostrzewa[1]. Department of Pharmacology, Silesian Academy of Medicine, 41-808 Zabrze, Poland and [1]Department of Pharmacology, Quillen College of Medicine, East Tennessee State University, Johnson City, TN 37614, USA.

Nitric oxide (NO), a novel intracellular messenger of mammalian brain, affects a variety of physiological and pathological functions. Previously we showed that NO modulates central DA D_3 receptor reactivity in rats. In the current study DA D_2 (SKF 38393 HCl, 10.0 mg/kg) and D_2 (quinpirole HCl, 0.2 mg/kg) receptor agonists or DA D_1 (SCH 23390 HCl, 0.5 mg/kg) and D_2 (haloperidol, 0.5 mg/kg) receptor antagonists were administered IP to male and female adult Wistar rats, 10 min after administering the NO donor L-arginine (300 mg/kg IP) or NO synthase inhibitor L-NAME (25 mg/kg IP). Controls received saline. The following behaviors were recorded: locomotor activity time, grooming time, catalepsy time and numbers of rearings. We observed that L-NAME attenuated SKF 38393-induced locomotor acitvity, grooming time and rearing in male and female rats, although the effect of L-NAME was more prominent in females. L-NAME also enhanced SCH 23390-induced catalepsy, while L-arginine was without effect. Quinpirole-induced locomotor activity was enhanced by both L-NAME and L-arginine. Only L-arginine increased quinpirole-induced rearing activity; only L-NAME increased haloperidol-induced catalepsy. In summary, L-NAME and L-arginine each enhanced behavioral expression of DA D_2 receptor mediated actions, while L-NAME suppressed DA D_1 receptor mediated actions. These findings on DA D_1 and D_2 receptor reactivity extend our previous report that NO has a modulatory role on central DA receptors. Supported by Silesian Academy of Medicine grant NN-1-30/97 (RB) and the Fogarty International Science Exchange Program with Poland (RMK).

EFFECT OF L-NAME AND L-ARGININE ON IRRITABILITY AND AGGRESSIVITY AFTER CENTRAL DOPAMINE AGONISTS APPLY IN 5,7-DHT AND 6-OHDA - NEONATALLY LESIONED RATS. A. Kasperska, J. Oświęcimska, R. Szkilnik, R. Brus. Department of Pharmacology, Silesian Academy of Medicine, 41-808 Zabrze, Poland.

Male Wistar 3 days old rats were injected intracerebroventricularly with neurotoxins 5,7-dihydroxy-tryptamine (5,7-DHT 50 μg) and 6-hydroxydopamine (6-OHDA 135 μg) or saline (10 μl). In adult animals the effects of nitric oxide synthase inhibitor (L-NAME 25 mg/kg), nitric oxide donor (L-Arginine 300 mg/kg) and the central dopamine D_1 and D_2 receptor agonsists (SKF 38393 0.3 mg/kg and Quinpirole 0.1 mg/kg) were apply intraperitoneally. The spontaneous irritability and aggressivity (induced by electrical stimulus 30V/0.8 mA coming from floor steel rods for 10 sec every 60 sec during 10 min) were recorded. Increase of irritability in neonatally 6-OHDA-lesioned rats and aggressivity in neonatally 5,7-DHT-lesioned rats were observed. L-NAME intensified irritability and aggressivity by itself and after SKF 38393 apply. L-Arginine was without effect.
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Catalepsy induced by nitric oxide synthase inhibitor: the role of dopaminergic D_1 and D_2 receptors.

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The aim of this research was to compare the cataleptogenic effect of nitric oxide synthase (NOS) inhibitor N-nitro-L-arginine (NNLA) administered alone and in combination with D_1 and D_2 receptor antagonists. Intraperitoneal injection of single doses of NNLA (0.1-10.0 mg/kg) produced dose-dependent and long-lasting (>8h) cataleptic-like effect. Single dose of nitric oxide (NO) donor, molsidomine, prevented the cataleptogenic effect of NNLA thus suggesting the critical role of NO synthesis blockade in NNLA catalepsy.

In addition we have demonstrated that catalepsy induced by D_2 receptor antagonist, haloperidol, was potentiated by low doses of NNLA. On the other hand this was not true for catalepsy induced by D_1 receptor antagonist SCH23390. This finding may indicate a specific role of D_2 receptor in NNLA-induced catalepsy

ADENOSINE - DOPAMINE INTERACTION AND CATALEPSY IN RATS

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Recently, functional interaction between adenosine A_2 and dopamine D_2 receptors has been postulated: adenosine receptors are thought to inhibit striatal dopaminergic functions. The central administration of adenosine A_2-agonist - CGS 21680 induced catalepsy in the rat (Ferre et al. 1991).
In the present experiments we evaluated other adenosine analogues if they could produce a catalepsy in experimental animals, and this action was compared to haloperidol catalepsy. Catalepsy in rats was assessed in six tests according to Simon et al. (1969). Agonists of A_1 receptors: R-PIA (0,1-0,2 mg/kg i.p.) and CPA (0,02-0,05 mg/kg i.p.) and agonists of A_2 receptors: NECA (0,05-0,1 mg/kg i.p.) and CGS21680 (10 and 20 μg/rat i.v.c.) induced catalepsy in rats. This action of all adenosine analogues and haloperidol (1mg/kg i.p.) was antagonized by nonselective antagonists of adenosine receptors - caffeine and theophylline (10 and 25 mg/kg). Selective A_1 receptor antagonist - CPT (3 and 6 mg/kg) did not change or even enhanced the action of all cataleptogenic substances, but DMPX - A_2 selective antagonist (3 and 6 mg/kg i.p.) reduced catalepsy.
Dopamine receptor agonists - amphetamine and apomorphine (1 and 2 mg/kg) decreased dose-dependently the cataleptogenic activity of adenosine analogues.
Our results support the suggestion about the interaction between striatal dopamine and adenosine and indicate that adenosine receptors may be involved in catalepsy of rats.

REF: 1. Ferre et al.: Neurosci. Lett., 1991, 130, 162-164.
2. Simon P. et al.: Therapie, 1969, 24, 985-995.

THE PARKINSONIAN-LIKE MUSCLE RIGIDITY INDUCED BY TETRAHYDROISOQUINOLINE (TIQ) IN RATS.

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1,2,3,4-Tetrahydroisoqinoline (TIQ) as well as the related compounds sansolinol and N-methyl-sansolinol are regarded as putative endogenous toxins which induce Parkinson's disease in humans. It has been shown that these substances evoke certain behavioural and biochemical symptoms similar to those found in parkinsonian patients and in monkeys and rats. Recently we have demonstrated that treatment with model substances inducing parkinsonian symptoms in animals, such as reserpine, haloperidol, and 6-hydroxydopamine (6-OHDA destroying ca. 90% of nigral dopamine cells) increase the muscle resistance and the EMG reflex response to passive movements in rats. The latter effects resemble those seen in parkinsonian patients. The aim of the present study was to find out whether TIQ injected acutely or chronically evokes muscle rigidity and causes degeneration of dopamine nigral cells. Muscle tension was measured as resistance (torque, mechanomyogram, MMG) of a rat's hind foot to passive flexion and extension. Simultaneously with the MMG, fine-wire electrodes recorded the electromyographic (EMG) response to movements. Degeneration of catecholamine cells was assessed by immunostaining of frontal brain slices for thyrosine hydroxylase. TIQ injected both acutely (50 mg/kg, ip) and chronically (50 mg/kg/day, 19 days, ip), as well as after a 3-day withdrawal period induced an increased torque and late EMG reflex responses to movements. Preliminary inspection of the brain slices immunostained for tyrosine hydroxylase did not reveal any marked neurodegeneration. Our preliminary results show that, like in other model substances (reserpine, haloperidol, 6-OHDA), TIQ induces parkinsonian-like muscle rigidity, however, its mechanism of action remaines unknown.

Poster session - Neuropharmacology

GABA - 5-HT SYSTEMS LINK IN ANXIETY CONTROL IN RATS.

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Serotonin (5-HT), one of the most widespread neurotransmitters, controls many functions of the brain. Its role in emotional behavior is well known and the drugs acting via 5-HT (5-HT_{1A} particularly) receptors were proposed as anxiolytic agents. Serotonin was also suggested as the neurotransmitter system playing important role in the anxiolytic action of wide class $GABA_A$-benzodiazepine ($GABA_A$-BDZ) receptor complex agonists. It was previously reported and confirmed by this study that inhibition of serotonin transmission can produce anxiolytic effects. Administration of *p*-chlorophenylalanine (*p*-CPA, 5-HT synthesis inhibitor) or 5,7 dihydroxytryptamine (5,7 DHT, 5-HT neurotoxin) produced pronounced disinhibition of rat behavior in Vogel's conflict test. As it was assessed by the biochemical method, after neurotoxin administration, 5-HT and its metabolite 5-HIAA levels were decreased in various brain structures to at least 20% of baseline values. Interestingly no influence of 5-HT depletion on rat behavior was observed in the Open Field Test. Such profile of action was similar to that observed after intraperitoneal administration of partial (bretazenil, Ro 19-8022) and selective BDZ-1 rec. subtype (abecamil, zolpidem) $GABA_A$-BDZ receptor complex agonists. Biochemical assessment of the effect of $GABA_A$-BDZ receptor full agonists (midazolam, diazepam), partial agonist (bretazenil), and selective BDZ-1 subtype agonists (abecamil, zolpidem), administered i.p. at the doses active in Vogel's test, revealed decrease of serotonin and 5-HIAA levels at least in hippocampus comparable to that observed after chemical lesions of 5-HT system. Moreover, as it was shown with use of autoradiography, serotonin depletion after i.p. administration of p-CPA caused significant decrease of 3[H]muscimol binding in limbic forebrain structures.

INTERACTION BETWEEN SEROTONINERGIC AND GABA-ERGIC NEURONS IN ADAPTIVE PROCESSES TO FEAR EVOKING STIMULI

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The aim of the present study was to analyse the role of interaction between central serotonergic (5-HT) and GABA-ergic neurones in adaptive processes that occur in a fear evoked reaction upon the repeated presentation of fearful stimuli in rats. For that purpose we used the rat neophobic reaction to a novel environment in the open field test, performed during consecutive 5 days (15 min long session a day). The motor activity and exploring behaviour was examined in the control and p-CPA pretreated animals (p-chlorophenylalanine, 150 mg/kg, 3 x daily). Additionally, in vitro serotonin concentration in the rat forebrain limbic structures and ^{3}H-muscimol binding (autoradiographic studies) was evaluated.

It was found that the population of rats could be divided into the groups of high (HR) and low responders (LR) on the basis of changes in their locomotor activity. High responders were characterized by enhanced motility during the first two days of the experiment. p-CPA pretreatment influenced the processses of habituation in the LR group only, runing up the activity scores to those of the HR. These behavioural changes were paralleled by a deep and selective serotonin depletion in the forebrain limbic structures. Complimentary autoradiographic studies revealed the clear [^{3}H] muscimol binding decrease in the dentate gyrus of the hippocampus in PCPA-administered rats, what would be explained as a receptor down-regulation of activated GABAergic pathways after the serotonergic lesion.

Triazolam Drug Levels Following Sublingually and Orally Administered **Premedication. C.W. Berthold and R.A. Dionne (NIDR, NIH, Bethesda, MD)** The clinical utility of orally administered anxiolytic drugs for surgical premedication is limited by delayed onset, a ceiling in peak efficacy, and prolonged recovery. Sublingual administration of triazolam 0.25 mg had demonstrated to result in greater reduction in intraoperatiave anxiety as well as less pain perception during oral surgery in comparison to oral triazolam and placebo (Berthold CW et al. 1994). The present study evaluated plasma drug levels in patients undergoing oral surgery following 0.25 sublingual triazolam or 0.25 mg oral triazolam administered one hour prior to surgery. Blood samples (6 ml) were collected via an IV line at 0, 15, 30, 45, 60, 75, 90, 150, 180, and 240 min following drug administration and analyzed by gas chromatography. No difference was seen between the two drug groups in the mean plasma triazlolam levels during the first 60 min following drug administration prior to the start of surgical procedure. Mean plasma levels during surgery and following surgery were higher ($P<0.05$) in the sublingual group in comparison to oral administration. These data support the previous results that sublingual administration results in greater plasma drug levels than oral administration leading to greater anxiloytic activity during dental procedures. No evidence was seen of faster onset or higher drug levels in the immediate postoperative period suggesting that a minimum of 60 min is needed to achieve the greater anxiolytic effects following the sublingual route of administration. Conversely, the greater plasma drug levels postoperatively did not result in any evidence of delayed recovery or greater psychomotor impairment. These results suggest that the sublingually administered benzodiazepine triazolam has clinical utility as an alternative to oral administration for those anxious patients in need of IV sedation for anxiolysis.

PARTICIPATION OF NITRIC OXIDE IN TH BENZODIAZEPINE WITHDRAWAL SYNDROME IN MICE AND RATS.

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Recent observations indicate that nitric oxide (NO) may play a role in the development of morphine tolerance and dependence. For example, attenuation of some signs of morphine withdrawal by Nω-nitro-L-arginine methyl ester (L-NAME) and Nω-nitro-L-arginine (L-NOARG), nonselective inhibitors of NO synthase, was demonstrated.

The aim of this paper was to estimate the influence of NO synthase inhibitors on some benzodiazepine (BZ) withdrawal signs in mice and rats. The influence of L-NAME and L-NOARG on the intensification of pentylenentetrazole (PTZ) seizures resulting from the chronic diazepam (DZ) administration was studied in mice. The effects of L-NOARG on the withdrawal signs (audiogenic seizures, hypermotility and weight loss) resulting from the long lasting influence the DZ, clonazepam (CZ), signopam (SG) and chlordiazepoxide (CDP) was tested in rats.

In rats, BZ dependence was obtained by subcutaneous (sc) implantation of 2 pellets containing 75 mg of BZ each for 3 weeks, and in mice, 1 pellet (a 75 mg of DZ) simultaneously with sc injection of the drug, for 2 weeks. Pellets were removed and about 72 h (mice) and 10 h (rats) later the studies were done (preceded by ip injection of 10 mg/kg of flumazenil, BZ receptor antagonist). Modification of mice response on the threshold dose (50-55mg/kg) of PTZ was studied during 1 h. The number of clonic and tonic seizures was noted in each mouse.

L-NAME (50, 100, 200 mg/kg) and L-NOARG (75 mg/kg) significantly decreased the number of clonic seizures and protected DZ-dependent mice against tonic seizures and death. Withdrawal signs in DZ-, CZ-, SG- and CDP-dependent rats manifested as: 1) a significant increase of locomotor activity measured in actometer for 30 min., 2) a significant decrease in body weight in 6 h and 12 h, and 3) audiogenic seizures (electric bell, 92 dB, 60s). L-NOARG (15 mg/kg) significantly decreased the hypermotility in CZ-dependent rats, inhibited a decrease in body weight in 12 h of withdrawal in CDP- and CZ-dependent rats and distinctly but nonsignificantly protected against audiogenic seizures.

The obtained results suggest that NO may be involved, at least in some part, in the BZ withrawal signs.

STUDIES ON THE INTERACTION OF ANTIDEPRESSANTS WITH ANTIEPILEPTICS IN FORCED SWIMMING MICE.

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The aim of the present study was to investigate the influence of acute or chronic treatment with antiepileptic drugs / phenytoin, valproate,carbamazepine,vigabatrin,lamotrigine/ on the antidepressants´/ imipramine, amitriptyline,maprotyline,mianserine,fluoxetine, fluvoxamine/ activity in Albino-Swiss mice in Porsolt´s forced swimming test.

Antiepileptic drug was always injected before the antidepressant and the observation begun 30 min. later. In chronic experiment both drugs were co-administered for 14 days and the forced swimming test was conducted 48 hours later.

It was shown that acute or prolonged treatment with antidepressants shortened the immobility time in swimming mice. The most of antiepileptics given in a single dose counteracted this effect of antidepressants. However in chronic experiment such interaction was observed only with some of antidepressant drugs.

The present results suggest that acute but also chronic treatment with antiepileptics could reduce the activity of some antidepressant drugs.

THE INFLUENCE OF ANTIDEPRESSANT TREATMENTS ON PROTEIN KINESES ACTIVITY IN RAT BRAIN

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Protein phosphorylation is a post-translational modification of proteins, that modulates the expression of specific functions of various proteins and is a major biochemical mechanism by which cells integrate extracellular signals and maintain their homeostasis, cellular functions and survival. The phosphorylation of substrate proteins by protein kinases play a key role in signal transduction and function of neurons. Protein kinases has been associated with several physiological and pathological states including depression. It has been observed that a chronic treatment of rats with antidepressants reduces cAMP-dependent protein kinase (protein kinase A - PKA) activity in soluble fraction whereas increases its activity in particulate fraction from rat brain frontal cortex and it has been proposed that antidepressant drugs act via modification of a cAMP dependent phosphorylation system. It has been also published that antidepressant drugs decrease the activity of protein kinase C (PKC) in soluble and particulate fractions from rat brain cerebral cortex and hippocampus. The aim of the present study was to investigate the effect of antidepressants and electroconvulsive treatment on the activity of (PKA), (PKC) and calcium/calmodulin dependent protein kinase II (CaM-KII) in the hippocampus and the frontal cortex of rat brain. The preliminary date indicate that while the activity of PKA or PKC was not changed by antidepressant treatment, chronic administration of imipramine decreased CaMKII activity (measured by the phosphorylation of hydroxyl groups of serine and threonine residues of synthetic substrate AK III by ^{32}P ATP) in soluble fractions together with translocation of the enzyme activity to the particulate fraction.

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COMPARISON OF FLUOXETINE AND TIANEPTINE ON COGNITIVE BEHAVIOUR IN RATS.

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The authors compared the behavioural effects of single administration of fluoxetine (selective serotonin reuptake inhibitor - SSRI) and tianeptine (serotonin reuptake enhancer). Male, Wistar rats were used in this study. A dose of 10 mg/kg i.p. for fluoxetine and 5 mg/kg p.o. for tianeptine was chosen, because this doses had no sedative effect. In the forced swimming test tianeptine caused prolongation (through statistically insignificant) of immobility time, in comparison with controls. The difference was, however, significantly higher, compared with the fluoxetine group, because the later drug caused a shortening (though not significant against control group) of immobility time. Unexpected the joint administration of these two drugs of opposition action mechanism, resulted in strongest shortening of immobility time, significant in comparison both with tianeptine and fluoxetine given alone. A different pattern was observed in the anxiolytic two compartment test: both antidepressants showed anxiolytic effects after separate administration, while after joint application the anxiolytic effects were entirely abolished. In memory test (labyrinth) tianeptine had no effect, while fluoxetine caused a very marked improvement of memory. Joint administration of tianeptine and fluoxetine resulted in slight worsening of memory exponents, in comparison with fluoxetine alone. The differences of results, are probably caused by the opposite molecular mechanisms of the two antidepressants.

BEHAVIORAL CHARACTERISTICS OF SM-13496, A NOVEL ATYPICAL ANTIPSYCHOTIC AGENT.

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Schizophrenia is a heterogenous disorder that displays diverse symptoms including the positive symptoms (e.g., hallucination and delusion), negative symptoms (e.g., apathy and social withdrawal) and dysphoric mood disturbances (e.g., anxiety and depression). In the present study, we studied the behavioral characteristics of SM-13496, a novel antipsychotic candidate which has a high affinity both for dopamine D_2 and 5-HT_2 receptors. Oral administration of SM-13496 markedly inhibited the behaviors induced by dopamine agonists (e.g., methamphetamine and apomorphine) and the conditioned avoidance response. The potency of the D_2-blocking action of SM-13496 was equal to 1/5 the potency of haloperidol or risperidone. In addition to the antipsychotic activity, SM-13496 showed anxiolytic and mood stabilizing actions. SM-13496, unlike conventional antipsychotics, significantly increased the punished water licking in the Vogel's conflict test and inhibited the conditioned fear stress-induced freezing in rats (MED=3 to 10 mg/kg, p.o.). Furthermore, SM-13496 neither induced catalepsy by itself nor potentiated catalepsy induced by haloperidol at dosed up to 1000 mg/kg. These findings suggest that SM-13496 is a novel antipsychotic agent with a broad clinical spectrum and minimal extrapyramidal side effects.

CHRONIC TREATMENT WITH NEUROLEPTICS ALTERS THE LEVEL OF NMDA RECEPTOR SUBUNITS IN THE RAT BRAIN: AN IN SITU HYBRYDIZATION STUDY

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It has been assumed that an abnormal balance between the dopamine and glutamate systems especially in cortical structures, may be involved in the pathophysiology of schizophrenia, and that neuroleptics interact with the central glutamatergic system. Recently we have shown, using a receptor autoradiography, that chronic treatments with neuroleptics influence the binding of radioligands to the NMDA receptor complex in the cerebral cortex of rats.

The aim of the present study was to investigate the influence of chronic treatments with haloperidol and clozapine on the level of the mRNA encoding NMDAR1, NMDAR2A-B subunits of NMDA receptors in cortical and subcortical structures using in an situ hybridization method. The drugs were given to a animals in drinking water for 3 months: haloperidol in a dose of 1 mg/kg/day, and clozapine in a dose of 30 mg/kg/day. On day 5 of the withdrawal, the rats were killed by decapitation. The oligonucleotide probes (cDNA), corresponding to the sequence encoding the NMDAR1, NMDAR2A-B subunits of NMDA receptors (NEN Du Pont), were labelled with [^{35}S]dATP (NEN Du Pont) using terminal transferase. Haloperidol significantly lowered the level of the mRNA encoding NMDAR1 subunit of NMDA receptors in the dorso-lateral part of the striatum, and frontal and parietal cotrices. On the other hand, chronic treatment with clozapine raised the level of the mRNA encoding NMDAR1 subunit of NMDA receptors in the ventro-lateral striatum, nucleus accumbens and insular cortex of rats.

The obtained results suggest that some of the long-term effects of typical and atypical antipsychotics may be the mediated by glutamatergic system, and that these drugs influence both the ligand binding to NMDA receptors and the level of the mRNA encoding NMDAR1 subunit of NMDA receptors.

DIFFERENT EFFECT OF Ca^{2+} CHANNEL BLOCKADE ON AMPHETAMINE HYPERSENSITIVITY CAUSED BY CHRONIC NEUROLEPTICS TREATMENT.

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Chronic administration of several neuroleptic, such as haloperidol, leads to development of hypersensitivity of the central dopaminergic system, resulting in tardive dyskinesia. Following our earlier results, showing that the Ca^{2+} channel blocker nifedipine, nimodipine and verapamil antagonizes the hyperresponsiveness to apomorphine induced by chronic administration of haloperidol, we investigated now how co-administration of various Ca^{2+} channel blockers with neuroleptics would affect the responses to amphetamine during the period of withdrawal and neuroleptic-induced changes in the density of cortical α-1 adrenoceptors. The experiments were carried out on male Wistar rats (200-250 g) that for 14 consecutive days received neuroleptics: haloperidol (1 mg/kg ip) or pimozide (4 mg/kg ip); the neuroleptic injections were preceded 20 min. earlier with Ca^{2+} channel blockers: nifedipine (5 mg/kg ip), nimodipine (5 mg/kg ip) or verapamil (10 mg/kg ip). Amphetamine, 0.7 mg/kg ip, was administered 24 h, 8 days, and 3 and 5 weeks after the last neuroleptic dose and the animals were immediately after tested for locomotor activity. Other animals received no amphetamine but were killed 24 h or 3 weeks after the end of neuroleptic treatment and binding of [^{3}H]prazosin to homogenates of cerebral cortex was studied. Chronic administration of both neuroleptics resulted in hyperresponsiveness to amphetamine, which was observed even after 5 weeks after withdrawal. Co-administration of nifedipine resulted in potentiation of the response to amphetamine, while co-administration of other Ca^{2+} channel blockers, nimodipine and verapamil, attenuated the response. Chronic administration of the neuroleptics did not affect the binding parameters of cortical α-1 adrenoceptors, but in rats receiving the neuroleptics with nifedipine, the density of [^{3}H]prazosin binding sites was increased by 25-30%. In contrast, co-administration of verapamil did not affect the α-1 adrenoceptor density. The results demonstrate that the interaction of various Ca^{2+}channel blockers with chronically administered drugs may depend both on their general propensity to block Ca^{2+} channel influx and on their individual properties and confirm that most Ca^{2+} channel blockers may attenuate the development of dopaminergic supersensitivity after chronic neuroleptic treatment.

IMPACT of SEROTONIN (5-HT)$_{1A}$ RECEPTOR STIMULATION on the LOCOMOTOR and DISCRIMINATIVE EFFECTS of AMPHETAMINE and COCAINE in RATS

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It is currently accepted that the dopamine (DA) system is implicated in several psychiatric disorders, including those associated with the abuse of drugs. An increasing number of reports have recently indicated that 5-HT$_{1A}$ receptors may influence the DA activity; however, their role has not been clearly definied. In the present study we investigated the impact of 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT), a 5-HT$_{1A}$ receptor agonist, on the locomotor and discriminative stimulus effects evoked by the indirect DA agonists amphetamine and cocaine.

The experiments were carried out on adult male Wistar rats kept under standard laboratory conditions (locomotor experiments) or on a food-restricted diet (discrimination procedure). The motor hyperactivity evoked by amphetamine (0.5 mg/kg; i.p.; -15 min) or cocaine (5 mg/kg; i.p.; -15 min) was measured individually in animal perspex boxes (with light beams and photocells) and was recorded for 60 min. In the discrimination procedure, separate groups of animals were trained to distinguish between amphetamine (0.5 mg/kg, i.p.; -15 min) and saline (2 ml/kg; -15 min), or cocaine (5 mg/kg; i.p.; -15 min) and saline (2 ml/kg; -15 min) during 15-min sessions on the fixed ratio 10 schedule of reinforcement (further details in: *Filip and Przegaliński, 1997, Pol. J. Pharmacol., 49, in press*). 8-OH-DPAT (i.p.) or (S)-N-tert-butyl-3-[4-(2-methoxyphenyl)piperazin-1-yl]-2-phenylpropanamide (WAY 100135, a selective 5-HT$_{1A}$ receptor antagonist; s.c.), was administered at 30 or 60 min before the tests, respectively.

Amphetamine and cocaine produced significant increases in the locomotor activity of rats by about 200 and 183%, respectively. 8-OH-DPAT (0.125-0.5 mg/kg) dose-dependently attenuated the effects of these drugs. WAY 100135 (10 mg/kg) did not change the hyperactivity induced by amphetamine or cocaine, but - when administered prior to 8-OH-DPAT - it reduced the inhibitory effect of 8-OH-DPAT on the hyperlocomotion evoked by either psychostimulant. At the dose range used, neither 8-OH-DPAT nor WAY 100135 affected the locomotion of untreated rats.

When given in the training doses, both amphetamine and cocaine induced a high level discrimination, i.e. 100 and 90%, respectively. 8-OH-DPAT (0.125-0.5 mg/kg) neither substituted for the training drugs nor antagonized the stimulus effects of amphetamine (0.5 mg/kg) or cocaine (5 mg/kg). When given in combination with amphetamine (0.025-0.5 mg/kg) or cocaine (0.25-5 mg/kg), 8-OH-DPAT (0.5 mg/kg) did not change the dose-response curves of these psychostimulants.

Our results indicate that increases in the locomotor activity, but not discriminative stimulus effects, both induced by amphetamine and cocaine in rats, are affected by stimulation of 5-HT$_{1A}$ receptors. In agreement with other authors' reports, the present study supports the hypothesis that different neurotransmitter systems modulate the psychostimulant-evoked effects.

BEHAVIORAL EVIDENCE FOR THE INVOLVEMENT OF SEROTONERGIC NEUROTRANSMISSION IN PSYCHOTOMIMETIC EFFECTS OF DIZOCILPINE

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The biochemical data indicate that non-competitive antagonists of NMDA receptor like phencyclidine or dizocilpine may influence the biochemical parameters characteristic of serotonergic neurotransmission. Therefore it was of interest to investigate whether behavioral effects of dizocilpine (locomotor hyperactivity, disruption of the delayed alternation task and the sensorimotor gating). which might be indicative of its psychotomimetic properties, are blocked by antagonists of serotonergic receptors of 5-HT$_{1A}$ and 5-HT$_2$ subtype. It was found that dizocilpine produced dose-dependent enhancement of locomotor activity, the reduction of prepulse-induced inhibition of the acoustic startle response and dose-dependently increased the number of errors in the delayed alternation test. All these effects of dizocilpine were significantly attenuated by the 5-HT$_{1A}$ receptor antagonist WAY 100,135 but not by ritanserin, the 5-HT$_{2A}$ receptor antagonist. Positive impact of ritanserine on the effects of dizocilpine has been seen in the prepulse-induced inhibition of the acoustic startle response only.

The obtained results point to the important role of the 5-HT receptors in the psychotomimetic effects of dizocilpine and suggest that blockade of the 5-HT$_{1A}$ receptors might have some positive therapeutic effects in the treatment of psychotic states. They are in line with the data indicating the involvement of serotonin in experimental models of schizophrenia.

THE EFFECTS OF NONCOMPETETIVE ANTAGONIST OF NMDA RECEPTORS ON THE DENSITY OF 5-HT$_{1A}$ AND 5-HT$_{2A}$ SEROTONIN RECEPTORS IN THE RAT BRAIN

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The present study investigated the impact of dizocilpine (0.4 mg/kg), a noncompetitive NMDA receptor antagonist, on the density of serotonergic receptors of the 5-HT$_{1A}$ and 5-HT$_{2A}$ subtype and on the metabolism of serotonin. The binding of [^{3}H]8-OHDPAT to 5-HT$_{1A}$ serotonin receptors, was increased after dizocilpine, as was shown by autoradiographic studies in the frontal, cingulate and part of enthorinal cortex, subregions of the hippocampus and raphe nuclei. The above receptor changes were observed at 2 and, in some brain regions, at 24 hours after dizocilpine. The impact of dizocilpine on the density 5-HT$_{2A}$ receptors ([^{3}H]ketanserin as a ligand) measured in various subregions of the cortex and striatum was less consistent, showing both decrease, increase and no effect, dependently on time after administration or selected brain region. Dizocilpine increased the concentration of the serotonin metabolite 5-HIAA in the prefrontal cortex and hippocampus, respectively, at 2 and 3 or only 3 hours after administration, being without effect on the level of serotonin. In the dorsal raphe nucleus, dizocilpine decreased the level of serotonin without significant effects on the level of 5-HIAA (0.5 hour after administration), or increased the level of 5-HIAA without alterations in the concentration of serotonin (3 hours after administration). It is concluded that single administration of dizocilpine may alter the density of serotonergic 5-HT$_{1A}$ and 5-HT$_{2A}$ receptors and in consequence may influence the function of the central nervous system associated with activation of various serotonergic receptors.

Mediation by nitric oxide of the carbachol-induced corticosterone secretion

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The presence of nitric oxide synthase the enzyme responsible for nitric oxide (NO) formation has been found in the hypothalamic structures and the pituitary gland. Nitric oxide modulates the release of CRH from the rat hypothalamus in vitro. This suggests a role for NO in regulating secretion of ACTH from the pituitary corticotrops and corticosterone from the adrenal cortex. The role of endogenous NO in the hypothalamo-pituitary-adrenocortical (HPA) response to cholinergic stimulation in vivo is not clear. The purpose of the present experiment was to elucidate the role of endogenous NO in the HPA response to central cholinergic stimulation in conscious rats. L-arginine, a NO donor, given in larger doses (120-150 mg/kg ip) 15 min prior to icv carbachol markedly diminished the carbachol-induced rise in corticosterone secretion. Systemic pretreatment with nitric oxide synthase inhibitor, Nω-nitro-L-arginine methyl ester (L-NAME) significantly raised the carbachol-elicited corticosterone response and addition of L-arginine completely blocked the effect of L-NAME. A similar increase in the carbachol-induced corticosterone response was caused by an icv pretreatment with L-NAME, indicating a central site of interaction of NO with cholinergic stimulation of the HPA response. L-NAME is a weak inhibitor of neuronal NOS itself and must first be de-estrified to Nω-nitro-L-arginine (L-NNA) to potently inhibit a NOS. Systemic and icv pretreatment with L-NNA augmented more effectively the carbachol-induced rise in corticosterone secretion than pretreatment with L-NAME by either route. These results indicate a significant involvement of endogenous NO in the HPA response to central cholinergic, muscarinic receptor stimulation. They also suggest the importance of hypothalamic NO in this mediation.

REVERSAL OF BEHAVIORAL EFFECTS OF PENTYLENETETRAZOLE BY THE NEUROACTIVE STEROID GANAXOLONE

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Neuroactive steroids are naturally-occurring or synthetically-derived compounds with anticonvulsant, anesthetic, anxiolytic, analgesic and hypnotic properties. The major site of neuronal activity appears to be with specific steroid-sensitive sites on the $GABA_A$receptor/chloride ionophore complex. Ganaxolone (3α-hydroxy-3β-methyl-5 -pregnan-20-one) is a synthetic neuroactive steroid protected from metabolic attack of the 3 position. The prior observation that ganaxolone appeared to reverse the marked behavioral changes induced by the convulsant pentylenetetrazole (PTZ), was systematically studied in the present study. A model to quantify PTZ-induced behaviors is described and used to evaluate ganaxolone in comparison with the anticonvulsants valproate, ethosuximide, clonazepam, diazepam and phenobarbital. All compounds were compared using dose equivalents based upon their respective ED_{50} values in preventing convulsions induced by 70 mg/kg PTZ. The ED_{50} dose and lower doses of ganaxolone prevented the observed behavioral effects of PTZ as well as its depressant effects on locomotor activity and rearing of mice. In contrast, the other anticonvulsants, if effective, were much less potent. Most strikingly, most of the other anticonvulsants were incapable of preventing all of the behavioral effects of PTZ. Only phenobarbital prevented all behavioral effects of PTZ but only at doses 4- to 8-times the anticonvulsant ED_{50}. Rather than normalizing behavior like ganaxolone, however, phenobarbital resulted in supranormal behavioral responses (e.g., increases in activity). Repeated administration of PTZ did not decrease the protective efficacy of ganaxolone. The results document the unique pharmacological profile of ganaxolone and suggest additional potential benefits from its use as an antiepileptic. Further, as behavioral effects of PTZ have been used to model anxiety and anxiety associated with withdrawal from drugs of abuse, ganaxolone may find additional therapeutic application in those areas.

INTERACTION OF ADENOSINE AND AT II AGENTS ON SEIZURE THRESHOLD IN MICE

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PTZ (10 mg/cm^3) was infuced into the lateral tail vein at a rate of 0.005 cm^3 /s. The seizure threshold for each consecutive phase (excitation, clonus and tonic seizure) was expressed as cm^3 of the infused solution. AT II (1 μg) and CPA (1 μg) significantly increased the PTZ seizure threshold while their respective antagonists DuP753 (100μg)), PD123319 (5μg) and 8-p-SPT (100μg) decreased the threshold. Pretreatment of DuP753 as well as PD123319 antagonized the effect of CPA. Adenosine A_1 receptor antagonist 8-p-SPT also blocked the increasing effect of AT II on PTZ seizute threshold. Combination of ineffective dose of AT II (o.1 μg) and CPA (1 μg) lead to pronounced increase of the PTZ threshold i.e. AT II potentiates the effect of CPA.

Taken together, the results show close interaction between both AT II receptor subtypes (AT_1 and AT_2) and adenosine A_1 receptor on the regulation of the seizure threshold.

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Differentiation of analgesic and thermoregulatory mechanisms in mice selectively bred for magnitude of swim stress-induced analgesia (SSIA)

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Swiss-Webster mice were selectively bred for high and for low magnitude of analgesia produced by 3 min swim at 20°C, as assessed by a hot plate test (56°C). SSIA in the high analgesia (HA) line is partially reversed by naloxone or naltrexone, and therefore is qualified as mixed opioid/nonopioid, whereas SSIA in the low analgesia (LA) line is resistant to naloxone, and so is nonopioid in nature. Swimming in 20°C or cooler water causes a greater hypothermia in HA than in LA mice. However if the two lines are given different swim parameters (water temperature/swim duration) so as to produce equal fall in core temperature, the magnitude of SSIA is always much higher in the HA than in the LA line. Swim produced hypothermia was not altered by naloxone in any line.

Five min swimming at 20°C was found to produce about 20% higher maximum oxygen consumption in LA than in HA mice. This difference was paralleled by a lower and higher hypothermia in the respective line. Fifteen min exposure to 79% helium/21% oxygen (helox) mixture at -5 to 0°C produced a pronounced hypothermia that did not differ between the lines. At -2.5°C ambient temperature in helox LA mice displayed significantly higher maximum oxygen consumption than the concurrent line.

Fifteen min exposure to ambient cold (-5°C to 0°C) in helox produced analgesia (assessed with a hot plate test) whose magnitude, as that of SSIA, markedly differed between the lines. This analgesia was not merely due to possible stressful action of the helox exposure itself, because with increasing ambient temperature up to +20°C the magnitude of hypothermia and analgesia decreased to very low values. The helox produced analgesia was partially antagonized by 10 mg/kg of naltrexone hydrochloride in HA but not in LA mice. Neither was the hypothermizing effect of helox exposure or maximum oxygen consumption in helox modified by naltrexone.

It is suggested that selective bidirectional breeding of mice for divergent magnitude of SSIA has also modified the animals' metabolic responsivity to ambient cold. The two traits are possibly controlled by a common genetic mechanism. The hypothermizing effect of swimming, rather than the nonthermal component of swim stress appears essential in producing SSIA in the selected mouse lines. Whereas the breeding strategy has affected both the opioid and the nonopioid pain inhibitory system(s), the selection produced thermoregulatory and metabolic changes depend on a nonopioid mechanism.

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EFFECTS OF SEROTONIN (5-HT_1) RECEPTOR AGONISTS AND NITRIC OXIDE ON NEUROGENIC BLOOD FLOW RESPONSES IN THE DURA MATER OF THE RAT

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Severe headaches such as migraine pain are thought to result from the neurogenic inflammation of the meninges, in particular the dura mater encephali. Hyperemia, an important element of neurogenic inflammation, can experimentally be elicited in the rat dura mater through local electrical stimulation, while the dural arterial flow and its changes are monitored using laser Doppler flowmetry. Reproducible increases in arterial flow were evoked by electrical stimulation (0.5 ms pulses of 7.5-15V at 5-10 Hz for 30 s) close to the sagittal superior sinus in anesthetized rats. These responses could be blocked by local anesthetics and dose-dependently inhibited by topical administration of the calcitonin gene-related peptide antagonist $CGRP_{8-37}$ but not by the NK_1 antagonist RP 67580. The serotonin (5-HT_1) receptor agonists sumatriptan (5-HT_{1D} agonist) and CP 93,129 (5-HT_{1B} agonist) attenuated the basal flow and the stimulated flow increases at high doses by 20%. The nitric oxide (NO) synthase inhibitor L-NAME decreased the basal flow by 30% and the stimulated flow by 50% after systemic but not after local administration. We conclude that the stimulated dural arterial flow is neurogenically mediated by the release of neuropeptides from perivascular trigeminal afferent nerve fibres. The main neuropeptide involved is probably CGRP but not substance P. 5-HT_1 receptor agonists, which have been shown to block neurogenic plasma extravasation, inhibit the stimulated flow only at high doses. NO that is presumably released from endothelial cells seems to be tonically involved in the dural arterial flow.

EFFECT OF IBMX ON MELATONIN PRODUCTION IN EMBRYONIC CHICK PINEAL CELLS

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Melatonin production in pineal glands and pineal cells isolated from 19-day-old chick embryos is rhythmic - it is high at night and low during the day *in vitro*. Cyclic AMP is involved in control of melatonin synthesis in the chick. Agents increasing cyclic AMP levels, such as an inhibitor of phosphodiesterase 3-isobutyl-1-methyl- xanthine (IBMX), acutely increase melatonin output in postnatal period.

In the pilot study the effects of IBMX in two concentrations (5×10^{-4} and 5×10^{-5} mol.l^{-1}) on melatonin production in pineal glands isolated from 19-day-old chick embryos were tested during both the dark and light phases of the LD (12:12) cycle. For the subsequent experiments only the higher dose was used, since only this one was effective. The effects of 3, 6, 9 and 12 hour treatment with IBMX on melatonin production of cultured pineal cells were measured. IBMX was supplemented to the medium at the end of the dark phase of the LD cycle, or subjective dark phase of DD or LL, respectively. After the treatment, melatonin production of pineal cells without IBMX was measured for next 3, 6, 9 and 12 hours.

IBMX stimulated melatonin production immediately after the treatment in cultured pineal cells in both DD and LL conditions. There was a stimulatory post-effect of IBMX on melatonin production under LD and LL conditions. Melatonin response to IBMX was affected by the length of treatment. Our data suggest that IBMX increases melatonin synthesis in cultured pineal cells isolated from 19-day-old chick embryos.

EFFECT OF KB-2796 ON PHOSPHOLIPIDS AND LIPID PEROXIDATION IN RABBIT ISCHEMIC SPINAL CORD

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The effect of a calcium channel blocker, (KB-2796) on ischemia/reperfusion-induced changes of phospholipids (sphingomyeline-SM, phosphatidylcholine-PC, phospahidylserine-PS, diacylglycerol and phosphatidic acid-DG+PA, phosphatidylethanolamine-PE), phosphatidyl inositol-PI, and lipid peroxidation was investigated. Spinal cord ischemia was induced by abdominal aorta ligation just below left renal artery. KB-2796 (20mg/kg, i.p.) was administered either a/ 10min before 30min ischemia, b/ 10min before ischemia and 60min of reperfusion, or, c/ immediately after ischemia. Calcium blocker administered before ischemia/reperfusion was shown to inhibit increase in lipid peroxidation products in homogenates of L_4-S_1 segments of spinal cord subjected to ischemia/reperfusion to the level close to controls. Significant decrease of SM and DG+PA levels was detected when KB-2796 was applied before ischemia. KB-2796 reduced postischemic changes in SM, PC and PI when given 10 min before ischemia/reperfusion, and, SM, PC and DG+PA when administered immediately after ischemia. The present data indicate, that KB-2796 can efficiently influence ischemia/reperfusion-induced alterations of some membrane-bound phospholipids and products of lipid peroxidation.

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The new line of rats selectively bred for high ethanol consumption: Biochemical and behavioral studies.

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Selectively breading of Wistar rats for the voluntary ethanol (EtOH) consumption is being carried out. Eleven generation have been achieved of (EtOH) Warsaw High Preferring (WHP) (absolute EtOH intake 5.0-11.0 g/kg/24h and EtOH Warsaw Low Preferring (WLP) lines (less than 1.0 g/kg/24h EtOH intake). The mean duration of sleep induced by 5.0 g/kg EtOH I.P. was 162.2 min and 184.6 min in WHP and WLP rats respectively ($p<0.05$) but no difference in the tolerance was noted. Administration of low and moderate doses of EtOH (0.25 and 0.5 g/kg I. P.) produced similar reduction in locomotor activity in WHP and WLP rats. The blood EtOH concentration after i.p. administration of 2.0 g/kg EtOH reached significantly higher values in WLP as compared with WHP rats. Biochemical study has revealed marked differences between two lines in the brain monoamines concentration in striatal 5HT, 5HIAA, dopamine and DOPAC as compared with WLP rats but the opposite effect was observed in the septal area. In general, the results show a marked differences between two lines in the EtOH bioavailability and the concentration of brain neurotransmitters.

5,7-DIHYDROXYTRYPTAMINE LESION DOES NOT AFFECT ETHANOL-INDUCED DISCRIMINATIVE AND AVERSIVE STIMULUS EFFECTS IN THE RAT.

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Both animal and human studies indicate an important role of brain serotonergic pathways in ethanol drinking behavior. Generally, an inverse relationship between EtOH drinking and central serotonergic function has been postulated. In the present study, we examined the effect of 5,7-DHT lesion on ethanol-induced discriminative stimulus effects and taste and place aversion conditioning. Central biochemical analysis revealed that 5,7-DHT (250 μg per rat) produced marked and selective depletion of 5HT in the hippocampal formation and the limbic forebrain. Ethanol-induced discriminative stimulus control was unaffected by the lesion. Similarly, neither ethanol-induced conditioned taste aversion nor conditioned place aversion was influenced by 5,7-DHT administration. These results suggest that central 5HT neurons are not primarily involved in the discriminative and aversive stimulus effects of EtOH in the rat. Therefore, it appears that 5HT agonists and antagonists may influence EtOH drinking through other mechanisms then affecting its aversive and discriminative stimulus effects.

DOES OPIATE RECEPTOR BLOCKING ACTUALLY REDUCE ALCOHOL DRINKING

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In rats liver damage leads to an increased voluntary alcohol intake. Experimental and clinical data indicating that opioid antagonists reduce alcohol consumption prompted us to examine whether chronically given naloxone could modify enhanced preference to alcohol in animals with hepatic insufficiency. Wistar rats with thioacetamide-induced liver cirrhosis and their untreated counterparts were used. During experiment rats were housed individually in metabolic cages with 12-hour light-dark cycle (lights off 7.00 p.m.). They were offered a choice between 10% ethanol solution and tap water and had also free access to food. The amounts of consumed fluids and food as well as excreted urine volume were monitored daily. During first 3 days of experiment when animals have not received any drug, the mean amount of alcohol consumed per day per kg of body weight was 7.41±1.34 (n=8) for TAA-cirrhotic vs 0.57±0.91 (n=6) for control rats. The corresponding daily urine excretion was 20.71±6.78 ml and 12.44±3.44 ml, respectively. Naloxone, 10 mg/kg b.w., was given by s.c. injection at 7.00 p.m. for 10 consecutive days. The results show that upon treatment TAA rats tended to take less alcohol. However, there was a significant decrease in total fluid intake. The reduction in consumed fluids appears to be primarily due to decreased water ingestion (by 45%). In both groups, TAA and control, naloxone significantly suppressed urine excretion - by 50% (10.59±2.31 ml) and 30% (8.25±1.53 ml), respectively. In control animals no further differences were found in response to naloxone treatment. In conclusion, present study indicates that chronic naloxone decreases total fluid intake whilst per cent contribution of consumed ethanol remains constant throughout the examined period. The antidiuretic properties of naloxone have also been noted by others.

DOPAMINE D-3 (AUTO) RECEPTORS IN MEDIATION OF THE REINFORCING EFFECTS OF ETHANOL

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It seems convincing that ethanol shares with other addictive drugs the ability to enhance the mesolimbic dopamine (DA) neurotransmission. Release of DA in dopaminergic neurons projecting to the striatum and n.accumbens is controlled by negative feedback action of presynaptic receptors including D-3 (auto) receptors. Throughout the experiments on rats it was observed that the taking of ethanol causes the changes in the density and the affinity of D-3 sites in the striatum. Using the radioligand binding technique it was found that a minor DA metabolite salsolinol (SAL, a product of condensation between DA and pyruvic acid or acetaldehyde), which endogenous level being influenced by ethanol, impacts on interaction of DA with autoreceptors. The ability of SAL to displace ^{3}H-DA from D-3 binding sites is characterized by value of ED_{25} to be appr. 0.1 μM. It was also observed *in vivo* that the interaction of DA with D-3 receptors was significantly decreased following the SAL i.p. injection, 20 mg/kg: B_{max} were 45±1, 85±12 and 108±2 after 30, 60 and 120 min, accordingly, vs 144±6 fmoles/mg of protein in control. These own data are in a good agreement with the recent findings that R-SAL locally administered into the brain with a microdialysis probe exerts a marked and repetitive stimulatory influence on DA release in various brain regions. The diminution of control of DA release via the inhibition of D-3 receptors with SAL is considered to explain the stimulatory effect of ethanol on DA overflow in the striatum and n.accumbens as a proposed mechanism for the reinforcing properties of ethanol..

ICS205-930 a 5-HT_3 RECEPTOR ANTAGONIST AND VOLUNTARY ALCOHOL INTAKE BY SHUNTED RATS.

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Rats with portocaval anastomosis (PCA) exhibit increased preference for alcohol (Fogel et al. 1991; 1997). It has been hypothesized that drugs of abuse produce reward through a stimulation of dopaminergic transmission (Di Chiara & Imperato, 1986) and that permissive role in drug-induced stimulation of dopaminergic firing plays serotonergic transmission via 5-HT_3 receptors (Carboni et al. 1989). The studies were conducted to check whether ICS 205-930, 5HT_3 selective antagonist, could reduce the increased voluntary alcohol intake when administered to PCA rats. PCA and sham operated Wistar rats 1 and 2 months following the surgery were used. Animals were caged individually and had free access to tap water and 10% ethyl alcohol. ICS 205-930 was given for 6 days(100 μg/kg i.p.,twice daily).Treatment was proceeded and followed by 3 days control periods. Neither during the treatment nor during the subsequent 3 days significant changes either in alcohol or the total volume of consumed/24 h fluids occur, suggesting that in PCA rats 5HT_3 receptors may not be involved in the development of an increased preference for alcohol.

References:
Fogel et al. Agents Actions, 1991, 33, 150
Fogel et al. Alcohol. Clin Exp Res, 1997, *in press*
Di Chiara & Imperato. J Pharmacol Exp Ther 1986, 239, 219
Carboni et al.. Eur J Pharmacol 1989, 164, 515

Poster session - Glia

Characterisation of the **NFAT** (Nuclear Factor of Activated T cells) transcription factor from glioma cells

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The NFAT (Nuclear Factor of Activated T cells) transcription factor is essential for cytokine production during the immune response. This transcription factor consists of cytoplasmic protein belonging to NFAT/Rel family and AP-1 complex. The formation of active transcription factor is inhibited by the immunosuppressive drug - cyclosporin A (CsA), which prevents dephosphorylation of NFAT protein and its migration to the nucleus.

In our studies, using Western blot analysis as well as immunofluorescence detection, we demonstrated the presence of the cytoplasmic component of NFAT - NFATC1, in rat C6 glioma cells. Gel-shift assay revealed the presence of NFAT DNA binding activity in nuclear extracts from proliferating glioma cells and its disappearance after CsA treatment. Another consequence of CsA treatment were changes in the migration of NFATC1 protein in the SDS-gel. These changes could reflect transition of NFATC1 from dephosphorylated to phosphorylated, nonactive form.

Treatment of these cells with CsA induced apoptotic cell death which was manifested by cell body shrinkage and loss of extensions, condensation of chromatin, nuclear deformation and DNA fragmentation. We found a correlation between loss of NFAT activity and apoptotic cell death.

Our results demonstrate for the first time the presence of T cell specific transcription factor - NFAT in astrocyte-derived cells and suggest the possible involvement of this factor in the regulation of cell survival.

TEMPORAL PROFILES OF ASTROGLIAL NERVE GROWTH FACTOR AND INTERLEUKIN-1BETA IMMUNOREACTIVITIES IN THE HIPPOCAMPUS AFTER TRANSIENT GLOBAL CEREBRAL ISCHEMIA IN RATS

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It has been demonstrated that treatment with nerve growth factor (NGF) can ammeliorate delayed neuronal death after ischemia, however the role that endogenous pool of NGF plays in ischemia is not clear. Several recent studies report that astrocytes have the ability to produce NGF under a variety of pathological conditions.This opens a possibility that astroglial neurotrophic activity may play a role in protecting neurons against degeneration, also in ischemia. On the other hand, the action of a known inducer of NGF, a proinflammatory cytokine - interleukin 1-beta(IL-1beta) which is induced by ischemic insult, was proposed to result also in neuronal degeneration. The aim of our study was to investigate the effect of transient complete cerebral ischemia induced by cardiac arrest upon the changes and cellular distribution of the NGF and IL-1beta immunoreactivities in the hippocampus 1,3,7 and 14 days after recirculation. We also aimed to study the relation between these changes and the time course and extent of degeneration of pyramidal neurons in CA1 layer, known to be especially vulnerable to ischemic insult.Immunoreactivities of NGF, IL-1beta, and glial fibrillary acidic protein (GFAP)-an astrocytic marker were investigated on free floating sections using a standard avidin-biotin procedure. Microglial reaction was monitored by lectin binding.Increased staining and number of astrocytes were detected already after 1 day in all layers. Later on the reaction became more intense. The strongest reaction was observed at 14th day, especially in the CA1 sector. The increase in IL-1beta in non neuronal cells was visible already at first day after resuscitation, and that of NGF followed, being well expressed at the 3rd day. The strongest intensification of both reactions was observed at 14th day being most evident in CA1 sector. Both regional and temporal profiles of NGF and IL-1beta resemble closely that of GFAP changes pointing to their astroglial origin. In addition, the double immunostaining technique allowed to exclude microglia as a source of both parameters. No clear cut relations were observed between the extent of degeneration of pyramidal cells in CA1 layer and intensification of NGF and IL-1beta reaction in astrocytes suggesting a delicate balance between neuroprotective and neurodegenerative actions of NGF and IL-1beta, respectively.

NONOVERLAPPING PATTERNS OF EARLY Jun AND c-Fos NEURONAL AND ASTROGLIAL ACTIVATION FOLLOWING NEOCORTICAL INFARCTION.

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Multiple studies implicate inducible transcription factor c-Jun in apoptotic neuronal death; less numerous studies present evidence for its role in regenerative processes. JunB has also been proposed to participate in neuronal death. In the present study we were interested in discovering weather two different neuronal populations that die after devascularizing infarction of the neocortex: cortical neurons, degenerating via an unknown mechanism, and thalamic neurons dying presumably via apoptosis (Soriano et al., Neuroreport 7, 1993) are induced to express and translocate Jun proteins. In order to verify the possibility that Jun induction is accompanied by activation of Fos family of proteins, c-Fos expression patterns were investigated. 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. c-Jun/AP-1 and c-Fos(4)X polyclonal antibodies (1:300, Santa-Cruz) and ABC Vectastain kit were used. Under normal physiological conditions (control rats) differential, partially overlapping patterns of Jun and c-Fos neuronal immunoreactivity (IR), and exclusively Jun astroglial IR was detected. In the neocortex Jun and c-Fos were localized within neuronal perikarya and proximal dendrites with the most extensive labeling of pyramidal (layer V) neurons. In the subcortical structures Jun IR was restricted to striatal magnocellular, septal, and reticular but not thalamic neurons, while weak c-Fos IR was widely distributed. Injury caused strong, perikaryonal, but also nuclear Jun IR induction within infarcted area. On the contrary, no c-Fos IR induction within infarction occured. In the remaining cortex no Jun IR induction was found, while intensification of c-Fos perikaryonal staining at 0.5h was followed by nuclear induction 1.5h postlesion. At 3h and 6h, most cortical areas were recruited for c-Fos nuclear expression. No neuronal changes in the contralateral cortex and subcortical structures were detected, but enhancement of Jun IR in the cytoplasm of astroglial perikarya and processes within lesion proximity was discovered. Results suggest Jun but not Fos involvement in immediate postinjury responses in neurons destined to die within infarcted area and point to the possibility of AP-1 Jun homodimeric complexes formation in these neurons. Experiments aimed to establish later Jun and Fos responses are in progress. Supported by the KBN grant to Nencki Institute and KBN grant #1030 to M.Skup.

INTRACEREBRAL INJECTION OF INTERFERON GAMMA SUPRESS THE ASTROCYTE PROLIFERATION IN THE BRAIN OF ONE-WEEK-OLD RAT. A DOSE-DEPENDENT ACTION.

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Interferon gamma (IFNγ) is well known as a key factor inducing inflammatory changes in the injured brain. However, *in vitro* investigations show that this cytokine may elicit various behaviour of cells at different developmental stages or derived from different species. The present study examines the influence of IFNγ on the astrocyte proliferation in the rat brain injured within the early period of postnatal development.

Six-day-old male rats received a mechanical lesion in the left cerebral hemisphere which was followed by immediate injection of 1μl of recombinant murine IFNγ into the lesion site. Three concentrations of IFNγ were applied: 5, 50 or 500 U/μl. One or 2 days after the injury the rats were injected with ^{3}H-thymidine and sacrificed 4 hours after the injection. Thereafter, brain sections were immunostained for glial fibrillary acidic protein (GFAP), subjected to autoradiography and examined microscopically to record numbers of GFAP-immunopositive astrocytes labeled with ^{3}H-thymidine. In the IFNγ-injected rats, a statistically significant decrease in the intensity of reactive astrocyte proliferation was revealed. On day 1 after injury the intensity of astrocyte proliferation showed strictly dose-dependent changes. On day 2, however, this dependence was less expressed. Relations between the astrocyte reactivity and the developmental stage when the brain was injured and IFNγ-injected are discussed. The results represent the first *in vivo* evidence of a dose-dependent action of IFNγ on the astrocyte proliferation in response to injury.

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THE PROLIFERATIVE RESPONSE OF MICROGLIA TO INJURY IN THE RAT BRAIN AT DIFFERENT STAGES OF POSTNATAL DEVELOPMENT

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Reactive transformations and proliferation of microglia are frequently reported as the earliest phenomena characteristic to the process of posttraumatic scar formation in the injured central nervous system. However, age-dependent changes in the spatiotemporal pattern of injury-induced microglial proliferation are still a weakly explored research area. To examine the development of microglial reactivity, a mechanical injury was inflicted to the left cerebral hemisphere in rats of four age groups: newborns, 6, 14 and 30 day-old. Proliferating cells were labeled with [^{3}H]thymidine injected at different time intervals following the injury. Brain sections were processed for lectin BSI-B4 histochemistry and subjected to autoradiography. During microscopical observations autoradiographically- and lectin-labeled macrophages and three morphological types of microglial cells were distinguished. Numbers and and locations of the cell types within the injury area were recorded separately . Profiles of injury-induced changes in the total number of proliferating lectin-labeled cells as well as changes in their distribution in different age groups were analysed and compared with each other.
The results suggest that the microglial proliferative response to injury cannot be regarded as proportional to the developmental progress of the brain but it displays quantitative and qualitative features typical to different developmental stages.

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Reaction of microglial cells and lymphocytes after 1-methyl-4-phenyl-1,2,3,6-tetrahydropiridine intoxication in mice.

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1-methyl-4-phenyl-1,2,3,6-tetrahydropiridine (MPTP) intoxication leads to a quite selective damage of the dopaminergic neurons in the nigrostriatal pathways in several species including mice. In the previous study we observed microglial and astroglial reaction from the first day after MPTP treatment. Glial response to any insult of the central nervous system (CNS) is a common reaction observed in many brain pathologies and research models. They seem to be involved in the pathogenesis of many processes, for example, antigen presentation to the lymphocytes in SM and its model EAE, production of β-amyloid in Altzheimer disease. In the present study we examine the activation markers of microglia and lymphcytes presence to investigate glial and immunocompetent cells engagement in the neurodegeneration following MPTP treatment.

Brains' sections were stained for CR3 and FA11 as the activation markers of microglia, for MHC class II antigens, GFAP (a marker of astrocytes) and TH (a marker of the dopaminergic cells). T-lymphocytes were estimated using antibodies to CD3, CD4 and CD8 antigens.

Significant decrease in the number of dopaminergic neurons in SN was observed from the day 4 to the end of the study. The activation of microglia was seen from the 1st day in the striatum and from the 2nd day in SN. The reaction achieved the maximum on 2 and 3 day and than diminished. There were observed typical morphological changes of the cells: larger cell's bodies, shorter processes and increase of CR3 and FA11 staining. MHC class II positive microglia and astrocytes were observed on days 3 and 7 to the end of the study. Lymphocytes infiltration started from the 2nd day following MPTP administration and was seen till 14th day. The majority of the T-cells there were CD8+ cells but CD4+ cells were always present too.

The early activation of microglia after MPTP intoxication suggests some role of this cells in the pathogenesis of neuronal damage. The presence of MHC class II antigens on the glial cells and the lymphocytes infiltration may indicate some immunological surveillance during this process.

Poster session - Electroencephalography and evoked potentials

CHOLINERGIC-GABAergic MODULATION OF SCN EVOKED FIELD POTENTIALS IN THE RAT IGL.

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The intergeniculate leaflet (IGL) is an important component of the mammalian circadian system which integrate photic and nonphotic information to modify pacemaker function. Arousal-related processes form nonspecific systems can enhance the neuronal responses to light of ventral LGN cells including the IGL. Our previous experiments indicated that the amplitude of the SCN evoked potentials in IGL is significantly reduced after laterodorsal tegmental nucleus (LDTg) stimulation. In the present study we have investigated the effects of muscarinic agonist and anticholinestarses on the SCN evoked field potentials in the IGL. The experiments were conducted in anesthetized rats. IGL responses were evoked by electrical stimulation of the SCN. Additional, stimulation electrodes were inserted into the LDTg to induce cholinergic influence on the IGL activation. The evoked potentials was significantly reduced after LDTg stimulation. The same effect was observed if we administered muscarinic agonist and anticholinestarses instead LDTg stimulation. But in this case the inhibitory effect was more express. These findings confirm our previous results and suggest a strong neuronal integration between LDTg and IGL. Because nearly all, of the neurons in the IGL are GABA-producing and GABA is the principal neurotransmitter of the circadian system we suggest that this cholinergic inhibitory effect is mediated by GABAergic neurons located in IGL. However, we can not to exclude that after LDTg stimulation we activate GABAergic neurons presented also in LDTg.

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PEDUNCULOPONTINE TEGMENTAL NUCLEUS IS INVOLVED IN GENERATION OF HIPPOCAMPAL THETA RHYTHM

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It is well documented that the pedunculopontine tegmental nucleus (PPN) is involved in various waking-sleep phenomena including cortical desynchronization, REM-sleep generation and PGO spikes. Recently, it was found (Vertes et al., 1993) that cholinergic stimulation of PPN evoked theta activity in the hippocampus in rats. The objective of the present experiment was to assess the relative importance of PPN in generation of theta rhythm by studying the effect of PPN inactivation on sensory stimulation-elicited theta.

The experiment was done on urethane anaesthetized male Wistar rats implanted with hippocampal recording electrode and unilateral PPN cannula. Fairly robust theta activity was elicited by tail-pinch in urethanized rats. Then, 1μl of 20% procaine was injected through the PPN cannula and tail stimulation was applied again in 10 min intervals. It was found that sensory-elicited theta completely disappeared for up to 30 min after intra-PPN procaine injections. It recovered after the drug washed out. The effect was anatomically specific and confined to the PPN area.

The results indicate that the PPN may be one of the key brainstem structures regulating hippocampal theta activity.

THE EFFECT OF PROCAINE INJECTED INTO THE MEDIAL SEPTAL AREA ON THE HIPPOCAMPAL CARBACHOL-INDUCED RHYTHMICAL ACTIVITY.

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In the earlier study we demonstrated for the first time that intrahippocampal injections of carbachol (CCH) in the cat produced two distinguished patterns of well synchronized rhythmical activity. One - slow theta rhythm (3-6 Hz) and second - rhythmical activity (7-15 Hz). The pharmacological profile of these two CCH-induced patterns reveals that both are M-1 cholinergic (locally antagonized by atropine, pirenzepine but not gallamine).

The present investigation was undertaken to assess the role of the medial septal area (MS) in the generation of CCH-induced hippocampal EEG activity. Microinjection of procaine (local anesthetic) bilaterally into MS abolished slow theta rhythm. It was without any effect on the fast hippocampal activity. These results revealed very pronounced difference between two patterns of rhythmical activity obtained by CCH injections into the hippocampal formation in the cat.

Neuronal mechanisms responsible for these two distinct CCH-induced EEG patterns are discussed.

EFFECT OF PROCAINE INJECTIONS INTO HYPOTHALAMUS POSTERIOR ON THE SPONTANEOUS THETA RHYTHM RECORDED IN THE FREELY MOVING CAT.

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Theta rhythm is the largest, well synchronized and most prominent waveform generated in the mammalian limbic system. The main structures involved in the production of this activity are hippocampal formation (HPC) and entorhinal cortex (EC). As we demonstrated in previous experiments theta rhythm can be also recorded from the posterior hypothalamic area (Hpt) in the cat. The hypothalamic region actively involved in production of spontaneous theta in the cat has recently been mapped in our laboratory.

In the present study we extended our earlier observations concerning Hpt as a region not only involved in transmission of tonic and phasic pulses from the brain stem, but also actively generating local theta activity. The aim of experiments described here was to examine the effect of procaine injections into Hpt on theta rhythm recorded simultaneously from HPC and Hpt in freely moving cats. We found that procaine administrated to Hpt completely abolished theta in HPC and Hpt. This effect was reversed after 10 - 15 min. The role of Hpt in neuronal system which originates in the brain stem and responsible for the production of theta in the limbic cortex is discussed.

THE EFFECT OF PROCAINE INJECTION TO HIPPOCAMPAL FORMATION AND ENTORHINAL CORTEX ON THETA RHYTHM RECORDED FROM THESE STRUCURES.

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It is well established that the hippocampal formation (HPC) and entorhinal cortex (EC) are capable of independent generation of theta rhytm in the mammalian brain. In the present study we examined the effect of HPC or EC administration of the local anesthetic - procaine on spontaneous theta rhythm locally recorded in the freely moving cat.

We found that the intrahippocampal injection of this local anesthetic resulted in abolishing of theta rhythm, both in HPC and EC. This effect was found to be reversible: recovery of theta was usually observed after 15 - 30 min. from the injection. Administration of the procaine into EC abolished theta rhythm only recorded in this structure (time course of recovery did not exceed 30 min). However, the same procedure had moderate effect on HPC rhythmical activity: it diminished only amplitude of theta recorded in this structure.

INTRASEPTAL MICROINFUSIONS OF GABA AGONISTS: EFFECTS ON HIPPOCAMPAL THETA FIELD ACTIVITY.

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It is generally agreed that theta activity (RSA) occuring in the hippocampal formation (HPC) and other limbic structures depends on the activity of cholinergic and GABA-ergic neurons localized in the medial septum.

The effect of intraseptal microinjections of the GABA-A and GABA-B agonists (muscimol and 3-aminoprophyl (methyl) phosphine acid = SKF-97541, respectively) on spontaneous and sensory stimulation-induced HPC theta activity was investigated in freely-moving cats. The injections of muscimol or SKF-97541 into the medial septum/vertical limb of diagonal band of Brocca (MS/vDDB) resulted in decrease of HPC theta activity amplitude and power. The frequency of RSA was not affected by this experimental procedure.

The obtained results provided further evidence of medial septal GABA-A and GABA-B receptors involvement in the production of RSA in the hippocampal formation of the cat.

COMPARATIVE ANALYSIS OF EPILEPTIFORM ACTIVITY DEVELOPED IN THREE IN VITRO MODELS OF EPILEPSY

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A large number of data indicates that a shift in the balance of excitatory and inhibitory processes may result in seizure activity in the neocortex. Although basic phenotypes of the seizures are more or less similar in various forms of epileptic activity, the underlying mechanisms can be very different. In these experiments, epileptic neuronal activity was modelled in neocortical slices. Spontaneous and evoked epileptiform discharges developing in Mg^{++}-free solution, in the presence of 4-aminopyridine (60 μM) and bicucullin (60 μM), respectively, were analysed using extracellular micro-electrophysiology. The latency of the first spontaneous epileptiform event as well as the duration of a particular discharge were the longest in the case of Mg^{++}-free model. These discharges were more complex in appearance than those developing after aminopiridine or bicucullin application. Stimulating the corpus callosum at 0.1 Hz, the amplitude of the evoked responses was bigger, their duration was shorter and the number of afterdischarges was smaller compared to spontaneous epileptiform events. The effects of the non-competitive AMPA antagonist GYKI 53784 (20-80 μM) were also investigated in the seizure models. It decreased both the duration of the spontaneous epileptiform discharges and the frequency of them. It also decreased the amplitude and duration of the evoked responses, and proved to be the most effective inhibitor in the Mg^{++}-free model.

LATERAL HYPOTHALAMIC HYPOSOMNIA: EFFECT OF ELECTROLYTIC AND CYTOTOXIC LESIONS AND MUSCIMOL INJECTIONS. J. Orzeł-Gryglewska, A. Nowacka, W. Trojniar, J. Tokarski, Department of Animal Physiology, University of Gdańsk, Poland.

An increase in waking time with a reduction of slow-wave sleep (SWS) and paradoxical sleep (PS) (hyposomnia) was found after bilateral electrolytic lesions of the lateral hypothalamus (LH). The aim of the study was to evaluate a possible anatomical substrate of LH hyposomnia by comparing the effects of total LH damage by means of electrolytic lesion, selective (cytotoxic) destruction of LH neuronal pericaria by means of ibotenic acid injections and transient inhibition of LH neurons of this area by microinjection of muscimol. Four groups of male Wistar rats were used: I - subjected to the bilateral electrolytic lesions (anodal current, 2 mA, 15-20 s) of the tuberal part of LH; II - sham lesioned; III - injected with ibotenic acid (bilaterally, 0.3 μg/0.5 μl); and IV - injected with muscimol alone (bilaterally, 50 ng/0.5 μl) and muscimol (25 ng/0.5 μl) in combination with bicuculline (50 ng/0.5 μl). Hippocampal and cortical EEG was recorded during one-hour morning sessions before and after the lesions in groups I, II and III and for 6 hours after injection in group IV. Percentage amounts of waking, SWS and PS were calculated for each group. The increase in waking time by about 22% ($p \leq 0.001$), with a simultaneous reduction of SWS (by 17%, $p \leq 0.001$) and PS (by 5%, $p \leq 0.001$) was found in the first week after electrolytic lesion. In the ibotenic and sham-lesioned rats respective changes were meaningless (-3.4% W, 3.0% SWS, 0.4% PS in group III, and -3.6% W, 2.9% SWS, 0.7% PS in group II). Muscimol injection caused an increase in waking time by 18% ($p \leq 0.001$) and a decrease in SWS by 13% ($p \leq 0.001$) and PS by 5% ($p \leq 0.001$). This effect was blocked by bicuculline (7.0% W, -4.2% SWS, -2.8% PS). The results suggest, that LH hyposomnia can be attributed to the damage of neuronal fibres passing this area rather then the intrahypothalamic neurons and suggest participation of GABA-ergic systems of LH in sleep-waking regulation.

A preliminary study of quantitative EEG spectral analysis in young schizophrenic patients treated with a neuroleptic drug.

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The results of some research on quantitative spectral EEG analysis in schizophrenic patients were contradictory untill now. For example, Nagase et al. have recently reported that 12 neuroleptic-naive schizophrenic patients revealed decreased alpha2 power as compared to healthy controls (Biological Psychiatry, 1996, 40, 6, 452-456). The goal of our preliminary study was to compare quantitative EEG spectral analysis in young schizophrenic patients treated with a neuroleptic drug with this of healthy controls.

Ten schizophrenic patients, 20-35 years old, with an exacerbation of psychosis according to ICD 10 criteria, were enrolled into the study. The patients underwent psychometric assession with PANSS, CGI, GI and computer-assisted 16-channel EEG recording was performed in waking state with eyes closed at the beginning of the treatment and after 3-4 weeks of the treatment with one neuroleptic drug. Eight 4-second epochs with no evident artifact was choosen for FFT. A statistical analysis of QEEG was performed using non-parametric methods.

The patients revealed some differences as compared to matched controls in all bands except for alpha2. The differences were quite similar at the beginning and after several weeks of the treatment. The differences were most pronounced in the frontal areas and in the left hemisphere. The alpha2/alpha1 ratio was calculated for the patients and controls; the ratio showed no significant differences between groups.There were no dissimilarities in patients group between early and later stage of the treatment.

The results obtained show some discrepancies from these above cited. A small number of subjects in both studies might be resposible for this difference, or previous treatment of the patients in this study can be regarded as an important factor influencing QEEG results.

THE EFFECT OF BIOFEEDBACK ON P300 EVENT-RELATED POTENTIALS IN HUMANS.
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It is generally assumed that P300 component of event related potential (ERP) represents the advanced level of information processing that can be subjected to the conscious, voluntary control. Recent studies revealed that, using feedback technique it was relatively easy to reduce the P300 amplitude but enlarging it was extremely difficult (1). The aim of my experiment was to test the hypothesis that this asymmetry was due to the superposition of two processes: conditioning that produced symmetrical effects of up- and downtraining and the drain on processing resources of the brain. It was shown that such drain always reduced the P300 amplitude (2). ERPs were recorded from Fz, Cz and Pz electrodes in 14 subjects. Red and yellow light flashes were used as stimuli and subjects were asked to count mentally one of them (odd-ball procedure). Spoken words were used as a feedback to increase the amount of necessary processing. The analysis was focused on the initial stages of training when processing of feedback information engaged the large resources but the effects of learning were small. When compared with the no-feedback condition, downtraining produced the instantaneous reduction of P300 component that confirmed the drain hypothesis. But uptraining did not produce the significant changes, indicating the presence of other process that compensated the effects of drain and was fully efficient even at the very initial stages of learning.

1. Sommer, W. and Schweinberger, S. Biol. Psychol. 33 (1992) 37-49
2. Kramer, A.,Sirevaag, E. and Brune, R. Human Factors 29 (1987) 145-160.

NOVEL STATISTICAL ANALYSIS OF EEG DATA.

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The new parametric statistical Curve Discrimination (CD) test was developed in order to determine if two continuous functions differ. The test can be applied to two experimental, or one experimental and one theoretical functions. It may also be used globally to decide if two functions differ, or locally, to find regions of divergence of functions.

The test relies on the difference between cumulated values of two series of discrete representations of functions. The known probability distribution of the value of each function at each point sets the probability distribution of tested difference as a convolution of component distributions. Based on this relation, one can calculate the probability that two functions differ (confidence level).

The CD test was applied to the EEG data of seven rats under classical conditioning procedure. The test was applied to FFT courses averaged separately from two classes of FFTs. These FFT classes corresponded to two different experimental periods: obtained before and after the reinforcing stimulus. For each FFT class and each frequency the mean value and its standard error were calculated. Frequencies at which data of two classes differ were found by local CD test.

The CD test proved it's sensitivity and discriminative power. For each animal, the test revealed local spectral power changes at ß frequency band. We consider the CD test as more trustworthy than concurrent nonparametric tests.

PORTABLE, COMPUTER PAPERLESS SYSTEM FOR HUMAN SLEEP ANALYSIS*

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In this work our own portable, paperless system for continuous monitoring and analyzing of human polysomnogram was constructed. A polygraphic amplifier with an 12 bit analog-to-digital converter was placed in a Medelec headbox (DG-32). The headbox is connected to a portable IBM-PC computer through our own interface. Records from 8 (optionally from 16) derivations with a sample frequency of 120 cps/channel were employed. Our own software allows polygraphic data to be presented on-line on a screen and storage on a computer hard disc. Data can be stored and transmitted on the portable, MO disc or CD disc. This software with modular structure allows for fully automatic analysis of a human sleep, and operates classical multichannel amplitude-frequency analysis (2 EEG channels, 2 EOG channels, EMG, ECG, Respirogram and Motor Activity channels) and spectral EEG analysis (Fast Fourier Transform). It also draws hypnograms and presents results in a multi-faceted way.
This system was tested in all-night physiological sleep records of on 35 human subjects of both sexes, aged 19-26. An agreement of 85.7% was obtained in comparison between visual (1) and computer hypnograms.

References:
1. Rechtschaffen A. and Kales A. (Eds) US GPO., Washington,1968.

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Poster session - Varia

VENTILATORY RESPONSE TO INTRACAROTID SEROTONIN CHALLENGE IN CATS

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Evidence suggests that intracarotid injection of serotonin (5HT) in cats produces reflex apnoea, bradycardia and hypotension without subsequent respiratory stimulation. To gain further insight into peripheral mechanism regulating the respiratory response to 5HT, we were investigating the ventilatory effects of intracarotid 5HT challenge and looked at the contribution of vagal afferents to this chemoreflex.
Experiments were done with 25 anaesthetized, spontaneously breathing cats. Tidal volume was measured at tracheostomy. Serotonin was administered into common carotid artery (0.05 mg/kg) in the intact animals and after subsequent division of the vagi. Fourteen cats presented short-lived expiratory apnoea of 3.24±0.6s and 4.5±0.8s (mean ±S.E.M.) while intact and following vagotomy, respectively. In post-serotonin breathing minute ventilation rose significantly ($P<0.01$) throughout 60s after the challenge, which was mainly due to increased respiratory rate ($P<0.001$). Tidal volume tended to augment, but did not achieve statistical significance. Following vagotomy the ventilatory changes were less intense and of shorter duration.
The outcome of these findings indicates variable apnoeic spells and enhanced ventilation after intracarotid 5HT challenge, which may be associated with excitation of peripheral chemoreceptors, as opposed to long expiratory arrests and depressed ventilation evoked by 5HT given to the pulmonary circulation.

TIME COURSE OF CHANGES IN SPONTANEOUS DISCHARGE IN RENAL POSTGANGLIONIC FIBRES FOLLOWING TRANSECTION OF THE AORTIC NERVES

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The rate of the ongoing discharge and distribution of its interspike-intervals in sympathetic postganglionic fibres was recently assessed 10 to 20 min after baroreceptor denervation (Wójcik et al., 1996). The observed changes probably underlie the alterations of the resting arterial pressure which were observed at different times following exclusion of the arterial baroreceptors. We have studied the time course of changes in the pattern of the ongoing discharge in sympathetic postganglionic fibres during first hour after transection of the aortic nerves. In rabbits anaesthetized with urethane + chloralose the ongoing discharge was recorded in single renal sympathetic neurons. The rate of discharge was measured and interspike-interval histograms were compiled in control conditions and then 6 times during 10 min intervals starting just after bilateral section of the aortic nerves. In control conditons the mean rate of discharge was 1.9 ± 0.1 spikes/s ($x \pm S.E.$; $n = 6$) and the spread of a histogram, which is difference between the longest and shortest interspike-intervals, was 1780 ± 95 ms. Severing the aortic nerves produced linear increase in the discharge frequency which 50-60 min after transection reached 4.9 ± 1.4 spikes/s ($P < 0.05$). The spread of a histogram decreased and in 50-60 min interval it amounted to 944 ± 145 ms ($P < 0.01$). Since transection of the aortic nerves did not affect the shortest interspike-intervals, these data suggest that the acceleration of the ongoing discharge occurs via decrease of the longest intervals.

PARALLEL INVESTIGATION OF CHOLINE TRANSPORT AND OF SYNAPTOSOMAL FLUORESCENCE UNDER pH-CHANGES CONDITIONS

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The choline transport accompaning of cholinergic neuronal activity was studied in brain rats synaptosomal fraction in the presence and in the absence of Na^+ ions. As a results of the neurotransmission in synapse the variations of pH are occur, therefore an investigation at different pH values was performed. We are assumed that under these conditions the changes of the choline transport may be due to either total membrane structural transitions or specific reply of the cholinergic structures. In order to elucidate it we studied intrinsic tryptophan synaptosomal fluorescence under variations of pH.

The results obtained showed the pH-evoked differences in the Na^+-dependent and Na^+- independent components of choline transport. It is assumed that pH-region from 6 to 7 is most important for regulation of cholinergic synaptic function, because here the complicated interpositions of curves of the two processes of choline uptake was revealed. At the same time we did not observe any significant pH-induced membrane transitions by the method of intrinsic synaptosomal membrane fluorescence in this pH region. We concluded that the pH-evoked choline transport changes may be due to specific cholinergic structures alterations.

HYPOTHALAMIC NEURONS SET TO NORMAL BRAIN TEMPERATURE: TRUE CENTRAL THERMODETECTORS?

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Extracellular single-unit recordings in slices of the rat hypothalamic thermoregulatory center revealed that warm-activated and cold-activated brain neurons can be divided to units sensitive to the temperature level and units sensitive to the rate of temperature change.

It was found that temperature behaviour of: (i) temperature-insensitive neurons, (ii) neurons sensitive to the rate of temperature change, and (iii) neurons with linear temperature-firing rate dependence is determined not only by their inherent properties but also by influences of synaptic network.

In contrast, both the firing rate and thermosensitivity of neurons which were excited upon reaching the temperature close to normal brain temperature during warming (threshold warm-sensitive units, 15 of 87 cells tested) or cooling (threshold cold-sensitive units, 4 cells) did not change in low calcium (0.2 mM)/high magnesium (8 mM) and in adenosine-containing (0.1 mM) media, suggesting these cells are inherently but not synaptically thermosensitive.

Threshold cold-sensitive neurons did not respond to picrotoxin (0.1 mM) but were inhibited by GABA (0.01 mM) and baclofen (0.01 mM), while threshold warm-sensitive neurons were not affected by all these substances.

The data allow to suggest that anterior hypothalamic warm-sensitive neurons set to threshold temperature close to normal brain temperature are central thermodetectors whose inherent thermosensitivity may control the brain and body temperature in a narrow-band range.

THE STUDY OF MELATONIN RELEASE FROM PINEAL GLAND OF THE DOMESTIC PIG USING A PERFUSION CULTURE TECHNIQUE.

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Our knowledge of the mechanisms controlling synthesis and secretion of melatonin (MEL) by the mammalian pineal gland arises, mainly, from the investigation performed on the rat. In other species, the regulation of pinealocytes function does not always seem to conform to the rat model. The domestic pig pineal gland differs markedly by its morphology and physiology from that of the majority of other mammalian species. The aim of the present work was to develop the perfusion culture technique of pig pineals for the study of regulation of MEL secretion. Pineals of 4-month-old pigs were removed immediately after animals slaughter and divided into four parts, which were mounted in separate perfusion chambers (volume 0.5ml). The chambers were perfused at a flow rate of 0.2 ml/min with medium 199 (gased with 95% O_2 / 5% CO_2). The medium and chambers were maintained at 37°C. Medium fractions were collected every 5 or 10 minutes. After 14 hours of perfusion the culture was terminated and the explants were fixed for electron microscopy. MEL concentration in the medium fractions was measured by validated direct RIA. The MEL release decreased rapidly during first two hours of perfusion (from 322.8±45.4 to 94.1±15.1 pg/min/mg wet tissue, n=15) and then slowly up to 3-4 hour of culture. After this period the concentration of MEL in fractions remained relatively stable up to the end of perfusion. The mean amount of released MEL between 4 and 4.5 hour of perfusion was considered as basal level (69.7±9.1 pg/min/mg wet tissue, n=15). To check the effect of norepinephrine (NE), this neurotransmitter was introduced to the medium in selected chambers for 30, 60, 120 min starting at 4.5 hour of perfusion. The MEL release increased to the maximal level within 20 minutes of perfusion with NE and this effect was dose dependent (150-200% increase at 10^{-6}M; 250-300% increase at 10^{-5}M). The concentration of MEL in medium decreased immediately after NE was removed from the medium. The electron microscopic study revelated that the majority of pinealocytes showed no signs of damage. The results of present study showed that 1) this method of culture is useful in the investigations of pig pineal gland activity; 2) the time course of MEL secretion from the pig pineal *in vitro* after stimulation with NE is markedly different from that in the rat and similar to the one described in the sheep. Supported by grant KBN 5 P06D 004 08.

Constancy of taste preferences in cats with different early taste experience

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It is known that sensory stimulation in the early period of life effects strongly the development of the visual system (see Żernicki 1991, Brain Res. Rev. 16: 1-13). This system represents sensory systems for stimuli devoided of motivational component. In the present work the effect of early taste experience on the taste system having motivational component was studied.

Eight cats from 4 litters were used. During the first 6 months of life they were fed with milk and either Whiskas with beef (B cats: 2 litters, 4 subjects) or Whiskas with tuna (T cats: 2 litters, 4 subjects). Both standarized foods were from cans produced by Master Foods. Beef was used in B cats and tuna was used in T cats. Then cats were trained in visual discrimination. After the criterion performance achievement, the same task was repeated using the second food, i.e. tuna in B cats and beef in T cats. Altogether the food was changed six times.

Two main results were obtained: (1) Beef was often refused by both B and T cats in all stages of training; (2) Discrimination learning was comparable in both B and T cats.

The results show that the taste motivation in cats does not depend on early beef or tuna experience. Thus, the taste motivation system appears to be somewhat more resistant than the visual system to the influence of early experience.

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THE ACTIVITIES OF PROTEIN TYROSINE KINASES IN HOMOGENATES AND SUBCELLULAR FRACTIONS OF PARIETAL LOBE AND GLIOMA OF HUMAN BRAIN

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Recent data indicate that protein tyrosine kinases (PTKs), a family of proteins wich include membrane-spanning receptor as well as nonreceptor PTKs, participate in the signalling processes for many factors, such as growth factors and cytokines.

We have studied PTKs activities in homogenates and subcellular fractions from glioma and adjacent tissue of human brain in order to compare their localization in the cell.

The material for our experiments was obtained from patients operated on at the Department of Neurosurgery and Chair of Oncology of Medical University of Łódź. We have determined the tyrosine-specific protein kinase activity using a tyrosine-containing synthetic peptide as an exogenous substrate (poly Glu^{80}, Tyr^{20}). Enzyme activities were expressed as pmoles P_i incorporated to the substrate per 1 mg of protein per 1 min.

Our data have proved that in subcellular fractions of glioma, especially in the cytosolic one, the activities of PTKs were much more higher than in parallel subcellular fractions of parietal lobes of human brain.

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Analysis of narrative discourse in developmental dyslexia

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The newest definition of developmental dyslexia (published in 1994 by the Orton Dyslexia Society) says, that it's a specific difficulty in reading and writing caused by disturbances in language development. Author's research concentrates on analysis of narrative discourse as an indicator of language development.

Children were asked to tell stories. Formal structure and episodic structure of the stories creating by dyslexic children and good readers were compared.

Each story, in order to play a communicative function, has to have a defined structure. Elements of the proper structure should answer questions: who?, what?, when?, where? and should be correctly placed in time (Shugar, Bokus 1988). The formal structure is a semantic pattern of creation of text. Data analysis indicates that level of agreement formal structure of the pattern and children's formal structure is lower in dyslexic group, than in good readers group.

The other indicator of narrative competence is episodic strucure of stories. Text discribing motivation of a heroe's activity, activity itself and the final effect, has an episodic structure. Data analysis indicates, that dyslexic children used significantly less often episodic structure of the story.

Concluding, a narrative discourse of dyslexic children is less communicative and includes less information. It is suggested it is an indicator of lower level of language development among these children.

Hemispheric activation disturbances in paranoid schizophrenia and unipolar depression measured by conjugate lateral eye movement.

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Conjugate lateral eye eye movement (CLEM) in response to various stimuli is an index of activation of left or right cerebral hemisphere. Under normal conditions, healthy persons show increased activation of left hemisphere in response to cognitive stimuli and increased activation of right hemisphere in response to emotional and spatial stimuli.

Using CLEM methodology, the study was performed in 16 patients with paranoid schizophrenia (8 male, 8 female) during exacerbation and improvement of the illness; in 16 patients with depression in the course of unipolar affective illness (8 male, 8 female) during acute episode and in remission as well as in 16 healthy control persons (8 male, 8 female).

In patients with schizophrenia during exacerbation of the illness, the features of excersive activation of left hemisphere were found. On improvement, there was a decrease of left hemisphere activation and the increase of right hemisphere activation.

The findings obtained in patients with depression during acute episode may be interpreted as lowered activation of both left and right hemisphere. Such changes persisted also into remission period.

The results point to differences between schizophrenic and depressed patients concerning hemispheric activation. They also suggest that such alterations may be state-dependent in paranoid schizophrenia and trait - dependent in unipolar depression.

THE MUSCLE RESTING POTENTIAL IN HONEY BEE. THE CONTRIBUTION OF POTASSIUM IONS.

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In some insect species changing the external potassium concentration causes changes of the resting membrane potential (RP) according to the Nernst equation. The resting membrane potential of the muscle cell in other insects varies with the change of external potassium concentration with a considerable divergence from the slope of 58 mV. Membrane potential (E_m) is very different from equilibrium potential for potassium ions (E_K). The aim of the present study was to examine the time dependence of the RP in dorso-ventral muscles of honey bee and to determine the role of potassium ions in the genesis of this potential. Experiments were performed on indirect flight muscles of honey bee workers (*Apis mellifera* L.) in situ. The preparations were "soaked" in standard or modified salines for 1,5 h, and RP of several cells in each preparation was measured. The conventional microelectrode technique was used. The standard physiological saline composition based on the analysis of ionic content of the honey bee's hemolymph was (in mmol/l): NaCl - 47,1; KCl - 27,1; $CaCl_2$ - 8,9; $MgCl_2$ - 0,5; glucose - 111.1; phosphate buffer pH=7,3. The concentration of potassium ions (0, 10, 50 mmol/l) was varied by changing the solution rapidly and the subsequent (within 1,5 h) changes of the RP were recorded. The average value of muscle RP measured just after the equilibration time was -24,2±0,5 mV in standard saline and did not vary with time. Both K-free and K deficient (10 mmol/l) saline caused a significant ($p<0,001$), developing with time hyperpolarization of 4-10 mV. Almost doubled physiological external [K^+] - 50 mmol/l resulted in a significant ($p<0,001$), developing with time depolarization of 9-17 mV. Obtained values of the RP for K concentrations lower than control were far from the predicted from the Nernst equation. The results might imply that the RP in studied muscles is the most probably determined by K^+ ions only in part. It is obvious that other ions contribute to the muscle RP in bee muscle. The complex nature of these fenomena requires further investigations.