1. NEUROPHARMACOLOGY

P.1.1 Influence of paxil on epinephrine levels in pregnant females rat brains and development rat foetuses

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Paxil (paroxetine hydrochloride) is an orally administered antidepressant drug. The aim of this experiment was to study the influence of paxil (paroxetine hydrochloride) on epinephrine levels in pregnant females brains and development rat foetuses. Pregnant females (10-12 animals in each group) were treated by gavage in doses 5.0, 10.0, 15.0 mg/kg body mass of paxil each day of gestation from 7 to 13. Controls were performed on three groups of pregnant females: UC – untreated control, TC - treated with water and Tween 80 by gavage in equal volume, ST – females receiving chlormetine hydrochloride as a standard teratogen. Pregnant females were euthanised and caesarean sections were performed on 21th day of gestation. The evaluation of birth defects of internal organs was carried out according to Wilson's technique in Barrow's and Taylor's modifications. Foetuses void of viscera were subjected to double stain of osseous and cartilaginous elements according Peter's method. Obtained brains of pregnant females were fixed in liquid nitrogen homogenised and tested. On the basis of this study it has been found out that paxil in all doses has not embryotoxic and teratogenic effects. A significant difference of epinephrine levels in pregnant female's brains after 15 mg/kg body mass was noted.

P.1.2 Kainate-induced superoxide and nitric oxide production in the rat brain

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We investigated the role of nitric oxide (NO) and superoxide in the neurotoxicity of the rat brain. Stimulation of glutamate receptors by kainic acid induces NO release, which in turn modulates glutamate transmission and also induces superoxide production, which may be one of mediators of excitotoxic neuronal injury in the central nervous system. We investigated superoxide production and the accumulation of nitrite, the stable metabolite of NO which was measured by the Griess reaction at different times (5 min, 15 min, 2 h, 48 h and 7 days) following kainate injection in the ipsi- and contraleteral hippocampus, striatum, cerebellum and forebrain cortex homogenates. We also investigated the effects of 7-nitroindazole (7-NI), a specific neuronal nitric oxide synthase inhibitor *in vivo*, on nitrite concentration after kainic acid injection (0.5 mg/ml, pH 7.2) unilaterally into the CA3 region of the rat hippocampus. 7-NI (100 μM) can effectively inhibit NO synthesis in rat brain after kainate-induced intrahippocampal neurotoxicity, suppressed nitrite accumulation and attenuated neuronal damage induced by glutamate receptor overactivity. Free radicals including superoxide are responsible for postlesional cytotoxicity. The increase of superoxide in distinct brain regions, which are functionally connected *via* afferents and efferents, suggests that these regions are affected by the injury.

P.1.3 The effect of pentylenetetrazol-induced kindling of seizures on *in vivo* striatal metabolism of dopamine and brain [3h]-citalopram binding

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Rats subjected to the repeated administration of an initially ineffective dose of PTZ (35 mg/kg, i.p.), gradually developed clonic-tonic convulsions. In PTZ pretreated animals there appeared an enhancement of striatal dopamine metabolism (an increase in the HVA/DA ratio). The concentration of serotonin metabolite, 5-HIAA, was also increased, indicating disinhibition of local serotonin neurons activity. The changes in the metabolism of monoamines occurred independently of a direct seizure activity, i.e. several days after the last episode of chemically-induced convulsions. In these rats a statistically significant and selective reduction in the binding of [3H]-citalopram, a marker of 5-HT transporters, was found in the CA3 field of the hippocampus (P=0.009), and a similar tendency, close to the significance level, in the dentate gyrus (P=0.05). This effect was accompanied by a loss of neurons and activation of microglia in the hippocampal formation. The present data suggest the important role of CA3-serotoninergic innervation and striatal dopamine, in pentylenetetrazol induced kindling of seizures, a model of temporal lobe epilepsy.

P.1.4 Antidepressant-like activity of magnesium in the forced swim test in mice

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The effect of acute and chronic treatment with magnesium, the N-methyl-D-aspartate glutamate receptor antagonists, was studied in the forced swim test in mice. Magnesium (hydroaspartate) in doses 20 and 30 mg/kg, reduced immobility time in the forced swim test. Moreover, combined treatment with magnesium and imipramine at their ineffective doses (10 and 15 mg/kg, respectively) induced a statistically significant effects in this test. The doses active in the forced swim test did not affect locomotor activity. Also chronic treatment with magnesium (14 days, 30 mg/kg/day) was active in this test. The antidepressant-like activity and lack of tolerance to this effect of magnesium suggest the potential antidepressant activity of this biometal in human depression and support the hypothesis that antagonism of the NMDA receptor is involved in the antidepressant action.

P.1.5 Anticonflict effect of diazepam and buspirone given concomitantly to the rat

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Anxiolytic effect of drugs acting through serotonergic 5-HT1a receptor (buspirone – BUSP) or GABA/BDZ receptor (benzodiazepines such as diazepam - DIA) can be not prompt enough (serotonergic drugs) or accompanied by adverse side effects such as tolerance, dependence or synergism with ethanol (benzodiazepines). The aim of the study was to investigate anticonflict action of subthreshold doses of DIA and BUSP given concomitantly to the rat, once or repeatedly for ten days. Experiments were performed on male Wistar rats (220 ± 20 g). BUSP (0.5 or 0.75 mg/kg) or DIA (0.25 or 1.75 mg/kg) have been injected intraperitoneally (i.p.) 30 min prior to each experiment. Anticonflict action of these drugs (given alone or concomitantly) was tested using Vogel conflict test. Motor performance and locomotor activity were tested with rota-rod and open field tests, respectively. The hypnotic action of ethanol (20%, i.p.) given together with BUSP or DIA was investigated. Anticonflict effect of BUSP 0.5 + DIA 0.25 was immediate and significant. Such mixture of drugs did not disturb motor performance and locomotor activity both after single and repeated administration. In comparison with DIA 1.75, BUSP 0.5 + DIA 0.25 potentiated the hypnotic effect of ethanol to the lesser extent. These results suggest that such mixture of anxiolytics may give therapeutic effect more rapid than following 5-HT1A agonists and devoid of at least some adverse side-effects characteristic for high benzodiazepine doses.

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P.1.6 Dopaminergic mechanism in the basal forebrain of the rat for production of species-specific 50-khz ultrasonic vocalizations

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Preliminary experiments have indicated that the mesolimbic dopamine system is involved in production of 50-kHz ultrasonic calls in adult rats. This type of calls has been observed in a number of social situations associated with appetitive behaviour of adult rats. Forty rats were stereotaxically implanted with chronic cannulae in several basal forebrain structures, including the nucleus accumbens and the anterior preoptic region. Direct intracerebral injections of 5-20 µg of amphetamine induced significantly higher number of 50 kHz vocalizations than injection of saline into the same brain sites. Vocalizations were identified sonographically and analyzed. The response was predominantly observed in the shell of the accumbens. Amphetamine also induced 50 kHz calls from the medial preoptic region but failed to induce the response from the dorsal striatum, the anterodorsal preoptic nucleus, and the anterior olfactory nucleus. The amphetamine-induced response was dose dependent with its peak dose between 7-10 µg. The sonographic analysis of vocalizations showed a species-specific pattern of the calls. The average single call duration of 34.6 ms and the average sound peak frequency of 58.3 kHz after amphetamine application did not differ from those parameters after vehicle injection (30.7 ms and 59.6 kHz).

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P.1.7 The influence of injections of haloperidol and 6-hydroxydopamine on behavior of snails and electrical characteristics of withdrawal interneurons

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The interest to study the mechanisms of dopaminergic transmission in the brain is caused by its well known role in the motor, cognitive and neuroendocrine functions, as well as a possible participation in pathogenesis of the nervous-psychic diseases. So we carried out the comparative study of the effects of 6-hydroxydopamine (6-OH-DA) and haloperidol (HAL) on the behavioral reactions of grape snails and parameters of the electrical activity of withdrawal interneurons. It was shown that injections of both HAL and 6-OH-DA decreased the snail's velocity of locomotion. The parameters of defensive reactions in these groups at the beginning and end of the experiment did not differ. During the influence both of HAL and 6-OH-DA after development of long-term sensitization (LTS) the velocity of locomotion also decreased, but the parameters of the defensive behavior, remarkably increased towards the end of LTS, while during influence of HAL and 6-OH-DA it did not change. In the groups of animals receiving injections of HAL or 6-OH-DA the procedure of LTS training did not changed the reactions of pneumostome to the test stimulation, i.e. - to LTS development. The measurements have shown that injections of the neurotoxin 6-OH-DA lead to depolarization of membrane of the withdrawal interneurons and decreased the threshold potential.

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P.1.8 Ammonia- and N-methyl-D-aspartate-induced accumulation of cyclic GMP and hydroxyl radicals in microdialysates of the rat striatum: protective effect of taurine

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Acute ammonia neurotoxicity caused by i.p. administration of ammonium salts is thought to be mediated by overactivation of N-methyl-D-aspartate (NMDA) receptors, with ensuing generation of free radicals and extracellular accumulation of cyclic GMP arising from stimulation of nitric oxide (NO) synthesis. In this study, infusion of ammonium chloride (effective extracellular concentration, efc – 5 mM) or NMDA (1 mM) into the striata of rats *via* microdialysis probes increased the contents of cyclic GMP and hydroxyl radicals in the microdialysates. Co-infusion of taurine (efc – 10 mM) virtually abolished both the ammonia- and NMDA-induced accumulation of cGMP. Taurine also attenuated accumulation of hydroxyl radicals evoked by either treatment. This result is the first evidence of a potential of taurine to attenuate the effects of NMDA receptor overactivation by ammonia *in vivo* and points to the inhibition of the NMDA receptor-mediated NO synthesis as a possible mechanism of its neuroprotective action. Taurine or its blood-brain-barrier penetrating analogues may be applied in treatment of ammonia-induced neurological deficits.

P.1.9 Estrogen receptor beta in rat hippocampus in kainic acid model

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Estrogens act as neuroprotectants, promote neuronal growth and regeneration and induce synapse formation. Estrogen receptors (ER), alpha and beta, are the object of a growing number of studies of brain function and dysfunction. The rat hippocampus has shown particular responsiveness to estrogens while expressing both ERs. Role of ERbeta in the hippocampus was studied using the kainic acid (KA) seizures model. This glutamate analog causes depolarization leading to convulsions, neurodegeneration in CA subfields and plasticity in DG. Underlying every process occurring in these cells are changes in gene expression. We have noted that mRNA of ERbeta increases as a result of the processes activated by KA. Immunohistochemistry has confirmed the increase in ERbeta signal in the CA and the hilus of DG after stimulation with KA, with a peri-nuclear localization. In double immunostaining, ERbeta signal co-localizes with neuronal, rather than glial, markers. Furthermore, extra-nuclear ERbeta was also noted in primary hippocampal cultures, where following stimulation with either KCl or glutamate, there was a focusing of the ERbeta signal outside the nucleus. The fact that expression of ERbeta is affected by KA-driven depolarization of neurons suggests a role for this protein in events occurring in stimulated neurons. Its extra-nuclear location would point to a function in processes other than the direct activation of gene transcription, such as coupling to second messengers for local signaling within neurons.

P.1.10 Effect of antidepressant drugs on hypothalamic--pituitary-adrenal axis activity – molecular mechanisms

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Antidepressants inhibit some changes evoked by glucocorticoids or stress. However, molecular basis of antidepressant action on glucocorticoid receptor function and CRH gene regulation have not been fully elucidated. We attempted to approach this problem using mouse fibroblast cells (L929) transfected with mouse mammary tumor virus – chloramphenicol acetyltransferase reporter plasmid (LMCAT cells). We found that classical and new generation antidepressants inhibited the corticosterone-induced reporter gene transcription in LMCAT cells in a concentration- and time-dependent manner. Mood stabilizers (lithium, carbamazepine and valproate) had similar, but weaker effect on this parameter. Further experiments were carried out on Neuro2A cells stably transfected with a human CRH (-663 to +124 bp) – CAT plasmid. Imipramine, amitriptyline, desipramine, fluoxetine and mianserin inhibited the basal activity of CRH gene promoter. Moreover, forskolin-induced reporter gene activity was suppressed by imipramine, amitryptyline and desipramine. Inhibition of the hCRH gene promotor activity and glucocorticoid receptor-mediated gene transcription may be a molecular mechanism by which antidepressant drugs attenuate HPA axis hyperactivity in depressed patients.

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P.1.11 The influence of pharmacotherapy in depressive patients

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The routine EEG of 71 (48 females and 23 males) untreated depressive patients and 106 (66 females and 40 males) depressive patients during pharmacotherapy were analysed. The number of EEG abnormalities in treated and untreated patients, as well as in males and females subgroups, were compared using CHI2 test. We found more EEG abnormalities treated female patients and significantly more (P<0.03) in untreated depressive females. We also found the greater increase of EEG abnormalities in males vs. females during antidepressive pharmacotherapy.

P.1.12 Chronic imipramine alters the responsiveness of rat hippocampal circuitry to the activation of 5-HT1A, 5-HT4 and 5-HT7 receptors

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Adaptive modifications of the serotonergic system have been suggested to form an important mechanism of therapeutic effectiveness of antidepressant treatments. Using *ex vivo* rat hippocampal slices, prepared 2 days after last administration of the drug, we investigated the effects of a tricyclic antidepressant drug, imipramine, administered repetitively for 14 days. Spontaneous epileptiform bursts were recorded from the CA1 area in nominally Mg^{2+}-free conditions. Bath serotonin (5-HT) application resulted in two opposite effects on the frequency of epileptiform activity. While the early, inhibitory effect was mediated by 5-HT1A receptors, the late, excitatory effect was mediated by the 5-HT4 and 5-HT7 receptors. Chronic imipramine treatment resulted in a strengthening of the inhibitory effect of 5-HT1A receptor activation and in an attenuation of the excitatory effects related to the activation of 5-HT4 and 5-HT7 receptors. The present data, obtained using recording of hippocampal network activity, show that chronic treatment with imipramine results in an overall enhancement of the inhibitory action of 5-HT on hippocampal circuitry, mediated by three 5-HT receptor subtypes.

P.1.13 NG-nitro L-arginine attenuated the behavioral but not biochemical effect in neonatal but not adult rats after of intracisternal injection of 5,7-dihydroxytryptamine

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In the present study we examined the nitric oxide synthase inhibitor NG-nitro L-arginine (L-NA) on behavioral and biochemical effect, after serotoninergic nerve depletion by intracisternal injection of the serotonin (5-HT) neurotoxin , 5,7-dihydroxytryptamine (5,7-DHT) in neonatal and adult male Wistar rats. One group of neonatal (age of 3 days) rats was injected with neurotoxin, while the second group of neonatal rats received 5,7-DHT and L-NA. In adult groups rats (age of 3 months) one group was injected with 5,7-DHT while the second group received 5,7-DHT and L-NA. Control groups (both neonatal and adult) were injected with saline. Afterwards, all rats were studied at the end of the 4th month of age, examined in the open field and force swimming tests, and to determine the distribution and density of [3H]citalopram binding sites in the brain was analyzed using quantitative autoradiography. Moreover, the concentrations of 5-HT and other neurotransmitters in CNS using HPLC method were studied. Our result showed that the L-NA injestion attenuated behavioral effect of 5,7-DHT lesion in neonatal but not adult groups rats only (i.e. the activity in forced swimming test). At the some time there was no L-NA effect on 5,7-DHT -induced changes in 5-HT concentration in the brain. In neonatal rats L-NA reversed the effect of 5,7-DHT on 5-HT transporter in the nucleus accumbens, striatum and ventral tegmental area but not in the hippocampus. Our results suggest that the diffusible chemical messenger nitric oxide is involved in neuronal plasticity in mesolimbic ventral tegmental area after the 5,7-DHT brain damage.

P.1.14 The effects of D1 receptor agonists and antagonist on the acquisition of socio-sexual experience in sexually naive male rats measured by precontact 50-kHz vocalizations (PV)

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Dopamine plays role in mediating the rewarding effects of stimuli. PV emitted by male during a 5-min period before introduction of female reflects the acquisition of sexual experience. Sexually naive rats do not vocalize or do it sporadically a few times only. From sessions third or fourth PV is usually intensive. Such vocalizations correspond to shortening of ejaculation latency (EL) and are related to rewarding value of sexual contact (Bialy M., Rydz M., Kaczmarek L. (2000) Behav Neurosci 114: 983-90). In sexually experienced rats partial D1 receptors agonist SKF 38393 did not influence on PV and EL (Beck J., Bialy M., Kostowski W. (2002) Physiol Behav 76: 91-7). In presented experiments role of D1 receptor agonist SKF-38393 (1 and 2.5 mg/kg), selective agonist SKF-82957 (3, 10, 30, 125 μg/kg), and selective antagonist SCH-23390 (2.5, 5 and 10 μg/kg) during acquisition of sexual experience were investigated. Males had 5 copulatory sessions each separated by 7-10 days period. Males did copulate either to first intromission after one ejaculation or stay 25 min with receptive female if did not ejaculate. Both agonists inhibit increase of PV. Ejaculation latencies were affected only by the both highest doses of SKF-82957. The D1 antagonist inhibits both PV and EL only at the highest dose. The results suggest that D1 receptors are involved in acquisition of socio-sexual experience.

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P.1.15 Characterization of receptors for VIP and PACAP in turkey cerebral cortex

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Vasoactive intestinal peptide (VIP) belongs to a superfamily of structurally related polypeptides, which also includes pituitary adenylate cyclase-activating polypeptide (PACAP), peptide histidine-isoleucine (PHI), peptide histidine-methionine (PHM), secretin, and glucagon. The primary structure of VIP is highly conserved among vertebrates. VIP shows 68% amino acid sequence homology with PACAP27. VIP is widely distributed in the brain and peripheral organs and exerts pleiotropic physiological functions. Various effects of VIP are mediated through interaction with two receptors types, VPAC1 and VPAC2, which recognize with a similar affinity both VIP and PACAP. PACAP can also bind to another receptor, PAC1, which expresses modest or weak affinity for VIP. In this study receptors for VIP and PACAP were characterized in turkey cerebral cortex (CCx) by *in vitro* binding technique, using [125I]-VIP as a ligand. The specific binding of [125I]-VIP to membranes of turkey CCx was found to be rapid, stable, saturable and of high affinity. The relative rank order of the tested peptides to inhibit [125I]-VIP binding to turkey CCx was: PACAP38 = PACAP27 ≥ mammalian VIP > chicken VIP >> PHI >> chicken VIP16-28, secretin (inactive). About 50% of [125I]-VIP binding sites in turkey CCx were sensitive to Gpp(NH)p. It is concluded that turkey CCx contains VPAC-type receptors.

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P.1.16 The effect of 17betaestradiol on angiotensin II-induced changes in tyrosine kinase activity in rat pituitary and pituitary tumor

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The angiotensinII is the multifunctional peptide, which can act *via* several membrane and cytosolic enzymes including protein tyrosine kinases. The best known receptors for AngII are the AT1 receptors present in the anterior pituitary. Tyrosine kinases are connected with receptors for extracellular modulators and are involved in many cell processes. The aim of our study was to investigate, if the 17betaestradiol, steroid hormone which plays important role in function of pituitary and has been shown to decrease AT1 binding sites, can change the activity of tyrosine kinase in presence of AngII in pituitary gland and pituitary tumors. The homogenates of pituitary and tumor were a source of enzymes. The PTKs activity was determined using the synthetic polymer polyGlu80,Tyr20 as the substrate and 32PgammaATP as the donor of phosphorus. The results showed that AngII alone had no effect on polyGluTyr phosphorylation by PTK in pituitary but stimulated this reaction in tumor cells. 17betaestradiol alone slightly increased the 32P incorporation to substrate in pituitary. The activity of PTK was decreased in presence of estradiol in tumor cells. The AngII and estradiol together treatment lead to inhibition of PTK activity in both homogenates, but the greater changes were observed in tumor.

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P.1.17 GABA medications and hypokinesia studies

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Patients that suffered cerebrospinal traumas in accidents frequently remain in the hypokinetic condition for long periods and the hypokinesia leads to impairment of the cerebral blood circulation. Therefore, investigations of the ways of improving cerebral blood circulation are of great interest and practical significance. We were investigating cerebral blood circulation in rats, the effect of changing the blood viscosity and the influence of various drugs on the functional condition of the rats. We investigated the influence of the GABA, Pyrolidon, Pyroglutamic acid and tokoferol (vit.E) injections and found that GABA injections led to the best results. Further studies showed that GABA and tokoferol (vit.E) in combination gave results even better than GABA itself.

P.1.18 Ginkgolide B preferentially blocks chloride channels formed by heteromeric glycine receptors in the hippocampal pyramidal neurons

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It has been found recently that the platelet activating factor antagonist ginkgolide B is a selective use-dependent blocker of glycine-gated chloride channels. In this study we have used various chloride channel blockers to get more insight into the mechanism of ginkgolide B blocking action. GABAA receptor antagonist picrotoxin is known to block alpha1 homomeric glycine (Gly) receptors, while being ineffective for heteromeric 3alpha/2beta glycine receptors. Studying pyramidal hippocampal neurons of rat, we found that the effect of picrotoxin depends on the age of the animals. Its blocking ability was characterized by IC50 = 140 μM and IC50 = 354 μM for 7- and 14- days old rats, respectively, indicating at a possibly increased contribution of heteromeric receptors. We have found that the blocking action of ginkgolide B is subjected to a more drastic change in the same range of ages: the IC50 value is decreased from 1.6 μM for 7-days old rats to 0.267 μM for 14-days old rats. When measured on the background of ginkgolide B (1 μM), IC50 for picrotoxin was μM. Taken together, these findings prompt that ginkgolide B might reveal more specific affinity to heteromeric Gly receptor-gated channels, than for homomeric ones.

P.1.19 The effect of nucleoside analog L-ribavirin on embryonic and adult astrocyte in culture

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Parkinson's disease (PD), one of the major neurodegenerative disorders, is characterized by the degeneration of dopaminergic neurons (DA) in the substantia nigra pars compacta (SNpc). Apart from this dramatic loss of DA neurons, it was found that SNpc is also the site of a specific glial reaction in PD, which could be either protective or detrimental to dopaminergic neurons. Given the significant role of astrocytes in maintaince, survival and death of DA neurons, in our work we aimed to determine the possible neuroprotective role of L-ribavirin on astrocyte function in complex glia/neuron interplay. L-ribavirin (1-b-L-ribofuranozil-1,2,4-triazol-3-karboksamid) is nucleoside analog with shown antiproliferative and antiinfalmmatory effect on astrocytes in rat models of brain trauma and multiple sclerosis. In our experiments we used mesencephalic astrocytes (EA) isolated from embryos (15-day gestation), and cortical astrocytes (AA) isolated from 2 days old Wistar rats. Cells at confluence were treated with trypsin-EDTA and placed in 96-well culture plates in different concentration (0.4 x 106 cells/ml, and 0.8 x 106 cells/ml) and treated with L-ribavirin (1-50 μM), 18 hours after plating. The level of MTT reduction, as quantitative assessment of metabolic activity, cellular viability and proliferation was determined 24 and 48 hours following the treatment with L-ribavirin and compared with control cells. Increase in metabolic activity was detected in embryonic astrocytes 24 hours after treatment. The incubation time with L-ribavirin didn't have influence on MTT reduction level. On the other hand, treatment of adult astrocytes with L-ribavirin didn't show effects on MTT reduction regardless of cell concentration and incubation time. Further experiments with BrdU test (quantitative immunoassay for cell proliferation) will determine whether increased level of MTT reduction is due to the effects of L-ribavirin on proliferation or metabolic activity of astrocytes.

P.1.20 The examination of functional state of vasopressinergic neurons and signal apoptotic proteins expression in nNOS-knockout mice

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The aim of current work was to investigate the role of NO in regulation of functional state of hypothalamic vasopressinergic neurons and expression of apoptotic proteins during dehydration and low catecholamines level in the brain. In all experimental groups the content of vasopressin in hypothalamic supraoptical and paraventricular nuclei was lower in wild type mice in comparison with knockout mice. Moreover, in knockout mice we observed high level of caspase-9, p53 and bcl-2 in comparison with wild type animals. These results can be explained by activation of vasopressinergic neurons in supraoptic and paraventricular nuclei in the absence of NO. Thus, our data confirm the hypothesis that NO plays a role of inhibitory factor in regulation of vasopressinergic neurons.The activation of vasopressinergic system evoked by dehydration increased caspase-9 and p53 levels in both nuclei in wild type mice and decreased that in knockout mice. Probably, the dehydration is a very strong stimulus for vasopressinergic system, which induces apoptosis of neurons, and NO plays a significant role in apoptosis initiation under specific activation of vasopressinergic system.The low catecholamines level in the brain decreased vasopressin content in both nuclei in wild type mice as well as in knockout animals. These data confirm our suggestion (previous data) about inhibitory action of catecholamines on vasopressin release from cell body. Moreover, the blockade of catecholamines synthesis induced caspase-9- and p53-dependent pathways of apoptosis in supraoptical and paraventricular nuclei. We suppose that catecholamines level play a critical role for cell survival. Basing on our data we also can suggest that activation of apoptosis under low catecholamines level probably doesn't depend on NO presence.Thus, we have shown that NO participates in regulation of hypothalamic vasopressinergic neurons function including initiation of apoptosis under specific stimulatory condition such as dehydration. Moreover, the low level of catecholamines influences on the functional state of hypothalamic vasopressinergic neurons independently of NO.

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P.1.21 Signal transduction mediated by cannabinoids

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The term „cannabinoids" refers to compounds of fairly different structures (e.g. plant-derived terpenoid delta-9-tetrahydrocannabinol, synthetic aminoalkylindole WIN 55,212-2 or endogenous eikosanoid 2-arachidonoyl-glycerol). These compounds share the ability to activate specific G-protein-coupled cannabinoid receptors. To date, two cannabinoid receptors have been cloned, having low overall amino acid sequence identity: CB_1 receptors are localized predominantly in the basal ganglia, hippocampus, cerebellum and cortex, while the CB_2 receptors are expressed by the cells of the immune system. Cannabinoid receptors are usually, though not exclusively, localized presynaptically. Endogenous ligands at these receptors (arachidonic acid derivatives anandamide, 2-arachidonoylglycerol and noladinether) are synthesised *de novo* on demand from the phospholipid precursors of the postsynaptic cell membrane and usually act as retrograde messengers reducing the probability of release of other neuromediators from the presynaptic neuron. Thus, the CB_1 and CB_2 receptors are mainly inhibitory, coupled to $G_{i/o}$ – proteins. The CB_1 receptor seems to be a constitutively active receptor that can sequester $G_{i/o}$-proteins and prevent other $G_{i/o}$-coupled receptors from signalling. Some authors, however, have recently suggested a state-dependent bidirectional coupling of CB_1-receptors since these can under certain conditions couple to G_s-protein-mediated pathway. Besides, CB_1 and not CB_2 receptors *via* G-protein activation but independently of cAMP inhibit voltage-dependent N-, P/Q- and L-type calcium channels and activate inwardly rectifying potassium channels (GIRK). Cannabinoids are also able to activate mitogen-activated protein kinase (MAPK) *via* a G-protein but not cAMP dependent mechanism and to mobilize intracellular calcium *via* activation of phospholipase C by a mechanism that is not fully clarified. Hence, the actions of cannabinoids are complex and much dependent on the conditions, e.g. pathological states. Moreover, a CB_1 receptor agonist specific coupling (agonist trafficking) has recently been demonstrated. This finding is extremely important because it opens the possibility of designing new drugs that would selectively target one of the intracellular pathways of CB_1 receptors and thus could at least partially separate the desired therapeutic effects (e.g. antidyskinetic or analgesic) from the side effects (e.g. euphoria or postural hypotension).

2. SYNAPTIC TRANSMITION AND EXCITABILITY

P.2.1 Modification of the ACh-induced current of active and dormant snail *Helix pomatia* by fast temperature changes

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Using single electrode voltage clamp method we have found that actylcholine (ACh) induces transient inward dose dependent current on the membrane of the identified *Helix pomatia* Br neuron. We analyzed the effects of fast cooling and heating as well as thermal acclimation on the ACh inward current. The experiments were conducted on active and dormant snails acclimated either to 20 and 70°C at least four weeks. The fill coefficient remained approximately 1 in all cases, which means that there is a single ACh binding site on the membrane. Fast temperature alteration induces binding affinity changes only in dormant snails. In that group of animals fast cooling increased binding affinity. After fast temperature alteration the decay of the response to ACh remained unchanged in all groups examined. After acclimation on 70°C the ACh inward current increased in both active and dormant snails. However, acclimation on 20°C decreased ACh inward current in active snails only. On both groups of snails, after acclimation on 70°C, binding affinity remained unchanged. Acclimation to 20°C increased binding affinity only in active snails. After acclimation to 70°C the decay was prolonged. After acclimation to 20°C, the decay of the response to ACh was prolonged only in dormant snails.

P.2.2 The effect of changes of intracellular Ca^{2+} on electrical characteristics of withdrawal interneurons of naive and learned snails

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We investigated the influence of the change of concentration of intracellular Ca^{2+} on the electrical characteristics of withdrawal interneurons of naive and learned grape snails. The tapping on the shell was used as a conditioned stimulus. Blowing the air stream in the hole of the pulmonary cavity was used as an unconditional stimulus. To increase the intracellular concentration of Ca^{2+} we used the solution of caffeine and to decrease the concentration we injected Ca chelator, EGTA. The analysis of electrical characteristics after the increase of intracellular Ca^{2+} concentration showed that the membrane potential of interneurons was not changed in neither naive nor learned snails. However, with the increase of intracellular Ca^{2+} concentration the threshold of generation of action potentials of the withdrawal interneurons markedly decreased in both groups: from 20 mV in a normal solution to 16 mV in the solution with caffeine in a group of naive snails and from 17.2 mV to 13 mV in the learned snails. After decreasing of the intracellular Ca^{2+} concentration the membrane potential was not changed in neither group, but the threshold potentials of the withdrawal interneurons reliably increased in naive snails and decreased in the learned snails.

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P.2.3 Voltage dependence of gabaergic transmission

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Fast GABAergic synaptic transmission in the adult brain is mediated by ionotropic GABAA receptors. Although these receptors are activated by ligand, it has been reported that their kinetics can be modulated by the membrane voltage. However, the mechanism of such modulation has not been described in details. Since the membrane potential in neurons is known to vary over a wide range, it seems interesting to explore GABAA receptor modulation by this factor. For this purpose, current responses to ultrafast GABA applications were recorded at membrane voltage ranging from -70 to +70 mV. Using this technique the agonist can be applied within less than 70 μs enabling us to describe the GABAA receptor kinetics with resolution adequate to the time scale of synaptic currents. We have found that the membrane depolarization enhances the rate and extent of receptor desensitization. The recovery process in the paired-pulse protocol has been slowed down by increase in membrane potential. We have also observed that current to voltage relationship shows a rectification (smaller slope) at positive membrane potentials. These observations are consistent with an enhancement of desensitization at increasing membrane potentials. We conclude that physiologically occurring changes in the membrane potential may significantly affect the GABAergic synaptic transmission.

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P.2.4 Voltage-dependent Ca^{++} currents in cardiac dorsal root ganglion neurons and in cutaneous vasoconstrictor sympathetic neurons in rats

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Kinetic and pharmacological properties of voltage-gated Ca^{++} currents have been tested in anatomically defined cardiac dorsal root ganglion (DRG) and cutaneous vasoconstrictor (VC) sympathetic neurons. Cardiac DRG neurons were significantly larger than VC neurons, their medium cell membrane capacitances were 45.2 and 12.7 pF, respectively. N, P/Q and L type of Ca^{++} currents were present and T type Ca^{++} currents was absent in cardiac DRG and cutaneous vasoconstrictor neurons. Maximum Ca^{++} current was significantly larger in cardiac DRG than in cutaneous VC neurons (-3.8 and -0.93 nA, respectively) as well as calcium current density (102 pA/pF and 77 pA/pF, respectively). Activation thresholds of Ca^{++} currents in cardiac DRG and in cutaneous VC neurons were not different. V1/2 and slope factors of activation in cardiac DRG and in cutaneous VC were not significantly different. V1/2 for inactivation in cardiac DRG was significantly shifted towards more hyperpolarized membrane potential than in cutaneous VC neurons (-49.0 and -34.7 mV, respectively). N type Ca^{++} current density was significantly larger in cutaneous vasoconstrictor neurons than in cardiac DRG neurons. P/Q type calcium current is larger in cardiac DRG neurons than in cutaneous vasoconstrictor neurons (36 and 25%, respectively). We conclude that kinetic and pharmacological properties of voltage gated Ca^{++} in cardiac DRG and in cutaneous vasoconstrictor are different.

P.2.5 Voltage-dependent K^+ currents in rat cardiac dorsal root ganglion (DRG) neurons

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Properties of voltage-gated K^+ currents were tested in cardiac DRG neurons in rats. The neurons were labelled using a fluorescent tracer Fast-Blue injected into the pericardial sack. Three types of K^+ currents were identified: 1) IAs - slowly activating and slowly time-dependently inactivating current, with V1/2 of activation -18 mV and current density at +30 mV equal to 164 pA/pF, V1/2 of inactivation at -84 mV. 2) IAf current - fast activating and fast time-dependently inactivating current, with V1/2 of activation +2 mV and current density at +30 mV equal to 180 pA/pF, V1/2 of inactivation -26 mV. At resting membrane potential IAs current was inactivated, while IAf current - available for activation. The IAs current recovered faster from inactivation than IAf current. 4-AP (10 mM) and TEA (100 mM) produced 98% and 92% reductions of IAf current, respectively, and 27% and 66% of IAs current, respectively. 3) The IK current did not inactivate over time. Its V1/2 of activation was -11 mV and its current density was 67 pA/pF. This current was inhibited by 95% (100 mM of TEA), while 4-AP (10 mM) produced its 23% reduction. We suggest that at hyperpolarized membrane potentials the fast reactivating IAs current limits the action potential firing rate of cardiac DRG neurons. At depolarised membrane potentials the IAf K^+ current, the reactivation of which is very slow, does not oppose the firing rate of cardiac DRG neurons.

P.2.6 Changes in extracellular pH affect recombinant GABAARs

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GABAA receptors are heteropentamers and their kinetics and pharmacology depend on their subunit composition. It has been shown that GABAARs are modulated by protons and that this effect depends on receptor subtype but the mechanisms of such differential modulation remain unknown. In the present study we have examined the effect of extracellular changes in pH on recombinant alpha1beta2gamma2 (a1b2g2) and alpha1beta2 (a1b2) GABAARs. We recorded the current responses to ultrarapid GABA applications. We found that in the case of a1b2g2 receptors, increase in proton concentration decrease the amplitude as well as increase the rate and extent of desensitisation. This is qualitatively similar to what we observed for neuronal GABAARs. Proton effects on a1b2 receptors were qualitatively different than those on a1b2g2. In particular, while acidic pH enhances the responses of a1b2g2, the a1b2 receptors are inhibited. Both a1b2g2 and a1b2 receptors were inhibited at basic pH. Moreover, the a1b2 receptors are much more sensitive to variations in pH. Thus, increase in pH by 0.2 above pH 7.2, caused 50% reduction of a1b2 receptor-mediated currents while increase in pH by 1 was needed to inhibit a1b2g2 receptors to the same extent. We conclude that protons differentially modulate receptors containing or lacking g2 subunit and that physiologically occurring pH changes strongly modulate GABAA receptors.

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P.2.7 Voltage-dependent modulation of N-type calcium channels in hippocampal neurons by intracellular sodium ions

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Sodium ions act as carriers for depolarizing currents in neuronal activity, but may serve as second messengers. For instance, cytoplasmic Na^+ directly activates G protein-gated K^+ channels (Sui et al. 1996) and regulates activity of a principal subtype of glutamate receptors - NMDA receptors (Yu and Salter 1998). Present study demonstrates influence of sodium ions on N-type hippocampal calcium channels. Our results show that increase of intracellular sodium leads to voltage-dependent (VD) inhibition of N-type calcium channel activity. *Vice versa*, decrease of sodium concentration provides increase of calcium channels activity. Patch clamp studies of whole-cell N-type calcium currents were performed on acute isolated (Kiskin et al. 1990) hippocampal CA1 pyramidal neurons of rats. Stimulation of Na^+ channels with a burst of short depolarizing pulses was used for elevation of intracellular Na^+ concentration. Na^+ influx resulted in inhibition of N-type calcium channels, which was partially withdrawn with conditioning prepulse. Blockade of sodium channels by tetrodotoxin (1 μM) or substitution of Na^+ for non-permeable ion in extracellular saline, completely eliminates both effects. Intracellular perfusion with Na^+-containing saline led to appearance VD facilitation of calcium current which was completely withdrawn by 50 μM of specific free Gbetagamma chelator QEHA peptide. Intracellular sodium led to slowing of the activation kinetics of calcium current. The VD modulation led to changes only on rising branch of calcium current IV curve. Our data demonstrate that Na^+ ions can participate in the regulation of calcium influx. On the basis of characteristic voltage-dependence of

the observed phenomenon and diminish of the modulation by Gbetagamma scavenger, we suggest that sodium-induced Ca^{2+} channel inhibition is mediated by G-proteins.

P.2.8 Effect of stimulus intensities on evoked inhibitory postsynaptic currents in single synapses of hippocampal neurons

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Inhibitory postsynaptic stimulus-evoked currents were studied using the whole-cell technique on cultured rat hippocampal neurones. Currents arose in response to gradual activation of neurotransmitter release by direct extracellular electrical stimulation of a single presynaptic bouton by short (3 ms) depolarization pulses. Stimulus-evoked inhibitory postsynaptic currents (IPSC) fluctuated with regard to discrete aliquot values of their peak amplitudes, and assumed as superimposition of statistically independent quantal events. In experiments on a single presynaptic terminal, linear increase of the stimulating pulse amplitude caused different behaviour of IPSC. In a range of small intensities of stimulating pulses, the amplitudes of evoked IPSC reached some maximal value. A further linear increase of stimulating pulse intensity resulted in the reversible decrease of the amplitudes of evoked IPSC, thus amplitudes with high quantal value were decreased. Accordingly, average amplitudes of evoked IPSCs had bell-shaped dependence on intensity of stimulating voltage. These studies demonstrated that each event of neurotransmitter release does not depend on the previous event and occurs only at definite, most probable times, but linear increase of stimulating pulse amplitude causes non-linear (bell-shaped) modulation of synaptical quantal release.

P.2.9 Functional inhibitory synapses between spinal cord and dorsal root ganglion neurons in co-culture

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In our experiments isolated neurons from rat dorsal root ganglia (DRG) and spinal cord (SC) were co-cultured for about 20 days. Identified SC neuron was used as a presynaptic cell and DRG neuron - as a postsynaptic one for recording evoked postsynaptic currents. Local extracellular stimulation of SC neuron was carried out after establishing whole-cell configuration between recording pipette and the DRG neuron. Postsynaptic currents were in fact recorded in 9% of investigated DRG neurones. These postsynaptic currents showed average latency between the beginning of the stimulus and the beginning of the current of 4.7 ± 0.29 ms, average time to peak of 3.1 ± 0.14 ms and average time constant of decay of 17.53 ± 1.04 ms. The amplitude of evoked postsynaptic currents depended on membrane potential and reversed at potential close to equilibrium potential for given inside and outside concentrations of Cl^- (-19 mV) calculated by the Nernst equation. Complete and reversible block of evoked postsynaptic currents was observed after the application of 20 mM bicuculline. We conclude that evoked postsynaptic currents are result of the opening of GABA-receptor Cl^--channel. The results seem to be the first direct prove that spinal cord neurons can form functional inhibitory synapses on dorsal root ganglion neurons.

3. BIOCHEMISTRY OF ADDICTION

P.3.1 Differential reactivity of the mesolimbic DA-system of AA and ANA rats to NMDA: a dual probe microdialysis study

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Direct activation of the A10 dopamine (DA) neurons, which has been hypothesised to play a role in the reinforcement from abused drugs, may be caused by glutamate. In the present study we investigated the effect of perfusion of the Ventral Tegmental Area (VTA) with N-methyl-D-aspartate (NMDA) on the extracellular levels of DA and its metabolites in the Nucleus Accumbens (N. Acc.) and of male alcohol preferring AA (Alko Alcohol) and alcohol avoiding ANA (Alko Non-Alcohol) rats using *in vivo* dual probe reverse microdialysis (MD). MD cannula was stereotaxically implanted above the shell of N. Acc. (A/P 1.7, L 1.2, H -6.5 mm) and VTA (A/P -5.6, L 2.2 -11°, H -8.5 mm) under halothane anaesthesia. Probes (CMA/12, CMA Microdialysis, Sweden, 2 mm and 1 mm, respectively) were inserted 6 days after the surgery and perfused at a flow rate of 1.4 ml/min. Samples were collected from the N. Acc. of awake rats every 15 min. After baseline period of 90 min 0.5 mM NMDA was applied into VTA *via* reverse MD for 15 min. Dialysate samples were immediately frozen. The concentrations of monoamines in the dialysate were assayed with high performance liquid chromatography with electrochemical detection. The levels of DA and its metabolites in N. Acc. were significantly increased (immediately for DA and at 30 min for DOPAC and HVA after application of NMDA) in both AA and ANA rats ($n = 18$, $P<0.05$). The increase in the release of DA was significantly higher in AA than ANA rats (184% and 122% of basal level, respectively). The levels of the metabolites were, however, similar. The level of DOPAC was 116% and 121% of the basal, and the level of HVA 117% and 119% of the basal in AA and ANA rats, respectively. The results obtained suggest greater reactivity of the mesolimbic DA pathway in AA rats than in ANA rats to glutamatergic stimulation through NMDA receptors in the VTA. The differential sensitivity to NMDA may contribute to the difference in alcohol intake among the two rat lines.

P.3.2 Molsidomine but not NG-nitro L-arginine inhibits spontaneous ethanol drinking in rats

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Nitric oxide (NO) plays an important role in physiological and pathological processes in the central nervous system, like: learning and memory, neurodegeneration, pain and addictive disorders. Experiments on animals and clinical studies show that ethanol influences significantly nitric oxide synthase (NOS) expression. Decreased expression of neuronal NOS (nNOS) was demonstrated in cultures of cerebellum cells and was shown to be reversed by L-arginine administration. It was also proven that nNOS expression increased only during initial phase of ethanol consumption and subsequently decreased. Aim: We studied effects of an indirect NO donor- molsidomine (MOL) and a NOS inhibitor- NG-nitro L-arginine (L-NA) given subchronically on spontaneous drinking of ethanol and sucrose solution in rats, in free-choice two-bottle paradigm. Both MOL and L-NA were given i.p. once a day at following doses:

MOL- 1.0-50.0 mg/kg and L-NA- 0.1-1.0 mg/kg. Results: MOL significantly decreased ethanol consumption and its preference in a dose dependent manner and this effect augmented proportionally to treatment duration. On the other hand L-NA failed to influence spontaneous ethanol drinking. Conclusion: our result indicates the role of NO in the mechanism of ethanol intake and addiction.

P.3.3 Changes in serotonin and dopamine levels in rats exposed to cocaine or saline – associated context after 30-day abstinence

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In this experiment rats were allowed to self administer cocaine at the dose 0.3 mg/kg/inj (FR 5) .The control group received in the same time the saline injection (yoked procedure). After 30-day abstinence halves of the rats from both groups were placed again in the experimental cages. Then rats were decapitated and several brain structures were removed and submitted to the monoamines measurement using the HPLC system. In the rats exposed to the experimental context the concentrations in the striatum of dopamine and its metabolites (DOPAC and HVA), as well as serotonin and 5-HIAA, were significantly higher. No differences were found in others tested structures. Surprisingly, the exposure to experimental environment evoked similar changes in "cocaine" and "yoked" rats. The results from biochemical tests do not reflect the differences in observed behavior. The "cocaine" group but not the "yoked" group placed in experimental cages showed drug seeking behavior. On the other hand, both groups showed very similar changes in monoamine neurotransmission. Thus, the neural base of behavior associated with dependence development seems to be more complicated and more discrete changes in neural networks associated with memory, emotive-motivational and locomotor systems are responsible for addiction associated behavior.

P.3.4 Decreased ethanol drinking by WHP rats treated with naltrexone

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Among animal models of addiction, genetically selected lines of rats with well-established phenotype of excessive and low ethanol (EtOH) consumption are of particular importance. The voluntary EtOH consumption, at least 5 g/kg/24 h, is a necessary condition for an animal model of alcoholism. In our laboratory the alcohol-preferring WHP (Warsaw High Preferring) and alcohol non-preferring WLP (Warsaw Low Preferring) lines of rats have been developed. Rats of WHP line consume approximately about 10 g/kg/24 h of EtOH when given ad libitum access to food, water and a 10% (v/v) ethanol solution (two bottle test). In the same conditions the rats of WLP line consume less than 1 g/kg/24 h of EtOH according with the criteria for non-preferring alcohol lines. WHP rats were treated with opioid antagonist naltrexone (1.0, 2.5, and 5.0 mg/kg) daily for 4 consecutive days and EtOH intake was assessed using the limited access paradigm. The results showed dose-related suppression of EtOH intake with maximal effect of 5 mg/kg dose occurred on third and fourth day of treatment. Our results support the hypothesis that opioid receptors mediate reinforcing effect of ethanol.

P.3.5 Effect of cannabinoids on thyroid parafollicular (C) cells activity in rats

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The interactions of marijuana and its principal psychoactive constituent, Delta-9-tetrahydrocannabinol, with neuroendocrine systems has been recently reported. The aim of the present study was to evaluate a single i.p. injection of a stable analogue of the endogenous cannabinoid, anandaminde - R-(+)-Methanandamide (2.5 mg/kg) and CP 55,940 (0.25 mg/kg), an egzogenous agonist of CB1 receptor, on calcitonin (CT), TSH, total and ionated calcium plasma level, as well as, on CT immunoreactivity of thyroid parafollicular cells. Four hours after both cannabinoids injection significant decrease of CT and TSH plasma levels, without changes in calcium concentrations was observed. These changes were accompanied by enhancement of CT immunoreactivity, evaluated with avidin-biotin peroxidase complex method by means of rabbit's antibodies against CT, observed in parafollicular cells. In thyroids taken from cannabinoids treated rats the majority of follicles, particularly located peripherally, had large size and low epithelium. Moreover, dilatation of blood-vessels was observed in comparison to the control thyroid glands. Reported changes were similar in R-(+)-Methanandamide and CP 55,940 treated rats. This is the first evidence that a single injection of cannabinoids: R-(+)-Methanandamide and CP 55,940 significantly decreases CT plasma concentration.

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P.3.6 Effects of chronic morphine on the alpha subunits of G(i/o) and G(s) protein mRNAs expression in rat prefrontal cortex

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The development of tolerance to and dependence on morphine and other chronically administered psychoactive drugs involve changes in genes expression. G-proteins (GP) that are heterotrimers composed of alpha, beta and gamma subunits couple extracellular signal receptors with their intracellular effectors. Among GP, the G(s) and G(i/o) families are coupled to adenylyl cyclase in a stimulatory and inhibitory manner, respectively. We aimed to asses the effect of chronic treatment with morphine (in increasing doses of 7.6-50 mg/kg, 2 x, 14 days, i.p.) on the expression of Galpha(s), Galpha(o), Galpha(i1) and Galpha(i2) mRNAs in rat prefrontal cortex, measured in 2 and 48 h after the last dose of the drug. Total RNA was reverse transcribed to cDNA, then specific sequences encoding given alpha subunits were amplified by the multiplex RT-PCR, followed by an enzymatic restriction analysis to distinguish G(i1) and G(i2) mRNAs. While no change was found after 2 h in any genes tested, the 48 h of withdrawal period significantly increased the expression of G(i1) and of G(o) and did not affect the G(i2) and G(s) mRNAs. Our data showing the elevated expression of inhibitory GP in the prefrontal cortex after 48 h withdrawal of morphine suggest that an attenuation of cAMP pathway in this brain area is involved in a cellular mechanism of the development of morphine dependence.

P.3.7 Endothelial NOS knockout mice present significantly lower alcohol preference than the wild type

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Introduction: Nitric oxide is a major messenger molecule with multiple function in the cardiovascular, immune and nervous system. NO is generated by three synthases: endothelial (eNOS), neuronal (nNOS) and inducible (iNOS). Several experiments on animals and clinical studies show that ethanol is able to increase eNOS activity and influence activity of nNOS and iNOS in the brain after single and chronic administration. Aim: We studied ethanol effects in endothelial NOS knock-out mice (KO[NOS-3]) vs. wild type (C57BL6/J) on locomotion, hypnotic and hypothermic effects of ethanol, and on spontaneous drinking of ethanol and saccharin in free-choice two-bottles paradigm. Results: In the open field test, administration of single dose of the ethanol (1.0 mg/kg i.p.) resulted in similar significant increase in the motor activity in both endothelial NOS knock-out mice and wild type animals. Also, no difference in hypnotic and hypothermic effects was observed after administration ethanol in the higher dose of 5.0 mg/kg i.p. Interestingly, the spontaneous intake of ethanol solutions (2-12% v/v), (free-choice two-bottles paradigm) was significantly lower in eNOS knock-out mice in comparison with a wild type. On the other hand, no difference between groups, in the spontaneous saccharine intake was observed. Conclusion: Our results suggests that eNOS may play an important role in the chronic alcohol intake and addiction. On the other hand, eNOS is rather not involved in acute effects of ethanol.

P.3.8 The influence of ifenprodil on ethanol withdrawal syndrome

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The anxiolytic effects of NR2B antagonist, ifenprodil during ethanol withdrawal was assessed in Wistar rats. Anxiety was measured by the elevated plus maze test. Male rats were exposed to ethanol according to the method described by Adams et al. (1995) for 14 days (20% w/v ethanol was given *per os* at doses increasing from 3 to 5 g/kg). Behavioral testing took place 24 h after withdrawal of ethanol. Ifenprodil significantly reversed the anxiety-like effect induced by withdrawal from ethanol. This drug increases the percentage of open arm time and more significantly increases the percentage of open arm entries. These effects of ifenprodil were lower then those obtained after injection of elenium, benzodiazepine receptor agonist. Furthermore, the influence of ifenprodil on ethanol withdrawal seizures was examined. Dependence on ethanol was induced according to Adams method (1995) for 7 days. Ethanol withdrawal seizures (audiogenic seizures) were induced by electric bell 10-12 h after the last ethanol injection. Ifenprodil significantly inhibited ethanol withdrawal seizures. The results suggest that NR2B subunit of MNDA receptor is involved in the ethanol withdrawal syndrome and drugs acting on this subunit may be a potent advantage in the treatment of ethanol withdrawal syndrome without inducing side-effects characteristic for competitive and non-competitive NMDA receptor antagonists. Adams et al. (1995) Clin Exp Res, 19: 195-199.

P.3.9 Behavioural correlates of ethanol intake in WHP and WLP rats

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In our laboratory the alcohol-preferring WHP (Warsaw High Preferring) and alcohol-non-preferring WLP (Warsaw Low Preferring) lines of rats have been developed. WHP rats consume about 10 g/kg/24 h of EtOH when given food, water and 10% (v/v) ethanol (two bottle test) ad lib. and WLP rats consume less than 1 g/kg/24 h of EtOH. The EtOH/water ratio was higher than 2 in WHP animals what indicates their preference for ethanol. The amount of water consumption by WLP rats was about 80-90 ml/kg/24 h while WHP drunk about 10 ml/kg/24 h of water. There are no differences between male and female WHP rats in ethanol drinking. For 4 days of drinking assessment male and female WHP rats consumed 10 g/kg/24 h of ethanol. The phenotype of WHP and WLP rats has constant characteristic as the exposition of animals to increasing concentrations (2-10%) of ethanol failed to change profile of ethanol intake. WHP rats showed increased locomotor activity in open field test after i.p. injection of 0.5 g/kg ethanol. WHP rats crossed about 3,000 inches of distance during 30 min and WLP rats less than 2,000 inches. This result indicates a positive correlation between high ethanol preference and ethanol-induced locomotor stimulation. After i.p. dose of 5.0 g/kg ethanol there are two times longer time of sleep in WHP than WLP rats.

4. CYTOKINES AND BRAIN PATHOLOGY

P.4.1 Effects of LPS and Interleukin-1 on operant behavior

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Bacterial lipopolysaccharide (LPS) and interleukin-1 (IL-1) produce anorexia in rodents. Mice were trained to receive a reinforcement of 20 mg of sweetened milk. Under a fixed ratio (FR 1) schedule the rate of responding was increased by overnight food deprivation and decreased by restraint immediately before the operant session. Injections of IL-1 (100 ng, i.p.) depressed responding in non-deprived mice but not in the deprived animals. D-fenfluramine (FEN, 20 mg, i.p.) decreased responding in both non-deprived and deprived mice. Under a progressive ratio (PR 5) schedule overnight fasting or FEN affected responding but IL-1 did not. Food-deprived rats were trained to receive 45 mg food pellets under variable interval 2.5 min schedule (randomized intertrial intervals from 13 to 360 s; pellets available under FR 1 during 8 presentations of 30 s long acoustic or visual stimulus; during the intertrial intervals one pellet available every 150 s). LPS (1 µg, i.p.) decreased responding during the intertrial intervals but had little effect on the responding and number of reinforcements when food availability was signaled by the stimuli. The results indicate that impairment in operant performance is not a significant factor in the LPS- and IL-1-induced anorexia. It is concluded that neither LPS nor IL-1 affected motivation to feed.

P.4.2 Apoptosis and cells synthesizing key proinflamatory cytokines in injured human brain areas

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The acute inflammatory response is regarded as entirely beneficial host response to injury. However, the persistent accumulation of inflammatory cells in the tissue necrotic area may be risk to surrounding tissue and may lead to chronic inflammation. The aim of the study was to select the injured brain areas that were infiltrated by cells containing the key proinflammatory cytokines and to detect in these areas apoptosis as a suspected mechanism for clearance of the cells. Studies were performed on the autopsy brains of human fetuses and newborns affected by perinatal ischemia and asphyxia. The intracellular expression of interleukin-15 and -18 (IL-15, IL-18), tumor necrosis factor alpha (TNF-alpha), and cyclooxygenase-2 (COX-2) proteins were detected by means of immunohistochemistry using specific antibodies. The presence of DNA fragmentation, typical of apoptosis, was detected using a TUNEL assay. In addition tissue sections were examined in electron microscope. In the tissue stained by cresyl violet the intensive karyorrhexis in the injured brain areas was found. Numerous leukocytes scattered in these areas were immunopositive to IL-15, IL-18 and COX-2 antibodies. Some of them demonstrated characteristic ultrastructural features of apoptotic cells. The results lead to the conclusion that in the injured brain of human fetuses and newborns apoptosis/karyorrhexis is an effective mechanism of elimination of cells producing proinflammatory cytokines.

P.4.3 Neurotrophin receptor TrkBFL, TrkBTK- and TrkB-like proteins in the spinal cord: lessons from mRNA and protein expression patterns

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In the spinal cord (SC), neurotrophin receptors TrkB were reported in motoneurons and glia, but detailed characteristics of their localization are lacking. We studied TrkB full length (FL) receptor expression in the lumbar SC of the adult rat using *in situ* hybridization (radiolabeled RNA probes for TrkB tyrosine kinase domain) and immunohistochemistry (polyclonal antibodies (Ab) against TrkBFL). TrkB mRNA was found in large neurons of the ventral horn, in smaller neurons and presumably non-neuronal cells in the spinal gray (SG), and in sparse cells in the white matter (WM). Distribution of the protein(s) detected with anti-TrkBFL Ab corresponded to that of TrkB mRNA in the SG, however, the number of TrkB immunoreactive (IR) oligodendrocytes exceeded that of small cells expressing mRNA. Also in the WM, more TrkBFL-IR than TrkB mRNA-expressing cells was found. Cross-reactivity with the truncated form (TK^-) was excluded, as $TrkBTK^-$-IR was absent from large neurons and oligodendroglia. Also Western blotting with anti-TrkBFL Ab revealed protein bands of 145, 125 and 80 kDa (differently glycosylated forms of TrkBFL and/or TrkBFL-like protein), different to a 95 kDa band recognized by anti-TrkBTK Ab. Our data document high level of TrkBFL mRNA and protein expression in several classes of spinal cells, and suggest that oligodendrocytes may express, additionally, TrkBFL-like protein, different to $TrkBTK^-$.

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P.4.4 TNF-alpha G-308A gene polymorphism and the clinical course of stroke

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TNF-alpha is a proinflammatory cytokine that has been implicated in the pathogenesis and course of cerebral infarct. A high interindividual variation of TNF levels has been observed in humans. G-308A substitution in the promoter region of the TNF-alpha gene has a direct effect on TNF-alpha gene regulation and may be responsible for the association of TNF2 with high TNF phenotype. To examine, whether the TNF-alpha G-308A polymorphism is associated with the clinical course of cerebral infarction (CI), we typed TNF-alpha genotypes (PCR-RFLP method) in 301 patients with CI. Distribution of genotypes was: G/G (70.99%), G/A (27.10%), A/A (1.9%). Allele frequencies: allele G: 63.5%; allele A: 36.5%. As compared with G/G genotype carriage, the carriage of genotype G/A was associated with significantly worse neurological and functional outcome measured using the Scandinavian Stroke Scale (SSS), Barthel Stroke Scale (BSS) and Rankin Scale (RS) at entry (SSS, P=0.007; BSS, P=0.03) and at discharge (BSS, P=0.04; RS, P=0.02). The carriage of genotype G/A was associated with higher 30-days mortality rate (18.31%) as compared with the carriage of G/G genotype (13.44%), however, observed difference was not statistically significant. Results of our study suggest that genetic variation at the TNF-alpha locus is a genetic factor that influences the clinical course of the disease.

P.4.5 The influence of age and gender on the TNFa mRNA expression in murine model of Parkinson's disease (PD)

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A male preponderance of PD suggests a link between gonadal hormone (such as estrogens) levels and PD. Neuroinflammatory have been linked with PD pathogenesis. Immunosuppressive prosperities of estrogens were documented. Aging is associated with increased levels of proinflammatory cytokines that may enhance the brain's susceptibility to neurodegeneration. We investigated the influence of age and gender on TNF gene expression in a murine model of PD induced by MPTP intoxication. TNF mRNA was measured by RT-PCR in the striatum of male and female, young and aged mice after 6 h; 1, 3, 7, 14, 21 days post MPTP injection. In young and aged male mice the TNF showed similar expression pattern. TNF was increased already at 6 h after MPTP injection and peaked at 1-day time point. The second increased of TNF was detected at the 7th day post intoxication. This increase was more prominent in aged than in young male. The pattern of expression of TNF in young and aged female mice differed from this observed in male mice. In female mice the significant increase of TNF was 24 h after intoxication. The elevation was observed at 3-day time point. The expression of TNF had diminished at 7th day but not yet recovered to control level. There is an age and gender-dependent difference in the TNF gene expression profiles in striatum by MPTP injection.

P.4.6 Effect of methylprednisolne on immunological parameters in multiple sclerosis patients during relapse

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Intravenous application of glucocorticosteroids is the most common therapeutic method used in multiple sclerosis (MS) relapse. Their mechanism of action still remains not fully known. The aim of our study was to determine immunological changes in peripheral blood (PB) of MS patients before and after 7 days from starting intravenously given methylprednisolone (MP). Eighteen patients with clinically defined MS at age 35.18 ± 11.07 years (range 21-50) were qualified. We have used two-color cytometry to count PB cells of following phenotypes: CD3-CD19+, CD3+CD19-, CD3+CD4+, CD3+CD8+, CD3-CD56+16+, CD3+IL-6, CD19+IL-6, CD14+IL-6, CD45+IL-6, CD3+IL-8, CD19+IL-8, CD14+IL-8, CD45+IL-8. Increased percentage of B cells, decreased percentage of cytotoxic T cells and NK cells was noted after 7 days from starting MP treatment comparing to the baseline. There was also significantly lower level of T lymphocytes and monocytes secreting IL-8. IL-8 seems to play important role in facilitating migration of inflammatory cells to the central nervous system (CNS). Published in the literature *post mortem* studies of MS patients brains revealed increased expression of receptors for IL-8. One of postulated MP mechanism of action is blood-brain-barrier repair. Found in our study changes of IL-8 secreting cells seem to confirm this effect.

P.4.7 Amitryptyline vs. venlafaxine in the TNF-alpha production in experimental model of depression in rats

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Based on the previous original observation (Maes et al. 1999), cytokines (CK) might be involved in the pathophysiology of depression. However, the cellular interactions at the brain sites of CK production and action are not fully understood (Konsman et al. 2002). At the experimental level CK induce anhedonia in the form of a decreased preference for saccharin solution in rats (Dantzer et al.1999). Moreover, chronic antidepressant (AD) treatment attenuates the behavioral effects of CK. The present study was designed to examine the effects of amitryptyline (AMI) and venlafaxine (VX) in rats subjected or not to chronic mild stress (CMS) on the TNF-alpha production after stimulation by lipopolisaccharide (LPS). On the basis of sucrose intakes in the final baseline test, the animals were divided in the two groups. One group was subjected for 7 weeks to CMS procedure. Control animals (CT) were housed in a separate room. On the basis of their sucrose intake scores following 3 weeks of stress 3 subgroups were treated with AMI or VX (both 10 mg/kg/2×daily i.p.) or vehicle. After the 4 weeks treatments with AMI and VX each group was divided into matched subgroups and examined for LPS induced (100 μg/kg i.p.) sucrose consumption before blood samples taken for TNF-alpha estimations. AMI treatment, but not VX, significantly attenuated of LPS induced TNF-alpha production. In conclusion: suppression of proinflammatory CK production is not a property of VX activity.

5. BRAIN OSCILLATIONS AND RHYTHMS

P.5.1 Short duty cycle destabilizes anti-phase oscillations; they can be restabilized by gap junctions

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Mutually-inhibitory pacemaker neurons with duty cycle close to 50% operate as a half-center oscillator (anti-phase coordination), even in the presence of weak to modest gap junctional coupling. For electrical coupling strength above a critical value synchronization occurs. But, as shown here with modeling studies, the effects of electrical coupling depend critically on a cell's duty cycle. Instead of oscillating either in-phase or anti-phase, model cells with short duty cycle express additional rhythmic patterns, and different transitions between them, depending on electrical coupling strength. For weak or no electrical coupling, cells do not oscillate in anti-phase but instead exhibit almost in-phase activity. Strengthening this weak coupling leads to stable anti-phase activity. With yet stronger coupling strength stable in-phase (synchrony) emerges but it coexists with the anti-phase pattern. Thus, the network shows bistability for an intermediate range of coupling strength. For sufficiently strong electrical coupling synchrony is the network's only attracting rhythmic state. Our results, numerical and analytical (phase plane treatment), are based on a minimal but biophysically-motivated pacemaker model and illustrations for an Hodgkin-Huxley model suggest that some results for short-duty cycle may extend to spiking patterns.

P.5.2 The effects of light on the action potential of *Helix pomatia* photosensitive neurons

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Some time ago we described photosensitive neurons in *Helix pomatia* subesophageal ganglia which react on onset of light with membrane depolarization. In the present work we analyzed the effects of light on the action potential of *Helix pomatia* photosensitive neurons. The experiments were carried out on the isolated subesophageal ganglionic complex of *Helix pomatia*. Single photosensitive neurons were impelled by a K-citrate filled microelectrode (R 3-5 MΩ). A single voltage-clamp apparatus was used for current and voltage clamp measurements. Illuminating the cell for 10-20 s, followed by 1 min darkness tested the effect of light. In a group of photosensitive neurons in the left parietal ganglion, the onset of light prolongs significantly (by about 40%) the duration of action potential. The broadening of the action potential after onset of light was found to be due to its calcium component and could not be induced after blocking the Ca^{2+} channels by Cd^{2+} and in absence of Ca^{2+} in the medium. The onset of light induced constantly an enhancement of the calcium current. The prolongation of the action potential was time and temperature dependent suggesting involvement of some second messenger in the light induced modulation of voltage-gated channels.

P.5.3 Reductions in gamma band EEG and cognitive performance associated with post-concussive symptoms

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Traumatic brain injury (TBI) can result in a number of cognitive deficits including difficulties in attention, memory and speed of information processing. Gamma band (30-100 Hz) EEG has been associated with perceptual binding and higher cognitive activity. The present study investigated whether gamma is specifically reduced in individuals who have sustained a TBI, and how such a reduction may relate to cognitive performance levels. Thirty-six subjects (23 with mild/moderate TBI; 13 controls) completed a perceptual discrimination task. EEG was recorded up to 100 Hz and analyzed for four scalp electrode sites (Fz, Cz, Pz, Oz). Amplitudes were calculated for seven frequency bands (1-4, 4-8, 8-11, 12-14, 15-30, 30-55 and 70-100 Hz). As hypothesized, TBI was significantly associated with reduced levels of 30-55, and 70-110 Hz activity at these midline sites, with the largest difference at Oz. In addition, reduced gamma activity at Oz was significantly correlated with self-report measures of perceived inability to think clearly. Both the theoretical and clinical relevance of the present findings are discussed.

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P.5.4 The effect of intraseptal injection of atropine on carbachol-induced hippocampal theta rhythm in cats

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Hippocampal formation (HPC) theta field activity depends on the integrity of cholinergic input from the medial septal/vertical limb of diagonal band of Broca (MS/vDBB). It has been histochemically demonstrated that approximately 50% of the fibers forming septo-hippocampal projection are cholinergic. Cholinergic nature of HPC theta was well documented both *in vivo* and *in vitro*. In the previous study we demonstrated that intrahippocampal microinjections of carbachol induced well-synchronized long lasting episodes of theta activity in freely moving cats. This effect was observed at least for 60 min. In the present study intrahippocampal injection of carbachol was pretreated by intraseptal injection of atropine. Microinjections of cholinergic antagonist into MS/vDBB resulted in diminishing the amplitude, power and density of carbachol-induced hippocampal theta activity during 30 to 40 min postatropine followed by total inhibition of theta rhythm. The participation of the medial septal cholinergic input in generation of hippocampal theta field activity in cats is discussed.

P.5.5 The effect of gap junction blockage on theta-like activity recorded from hippocampal formation slices

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Current evidence strongly suggests that direct physical coupling between neurons *via* gap junctions is an important mode of intercellular communication. It was earlier demonstrated that gap-junctional communication underlines the neural mechanisms of oscillations and synchrony. Since our first demonstration of cholinergically induced theta rhythm in hippocampal formation (HPC) *in vitro* this experimental model has been successfully used in a number of investigations concerning physiology and pharmacology of this EEG pattern. In a present study we investigated the role of gap junctions in maintenance of carbachol-induced theta recorded in the HPC *in vitro*. Cholinergically induced hippocampal theta field potential and single unit activity was recorded from CA3c, CA1 and dentate area in a presence of 100 μM carbenoxolone or quinine (gap junction blockers). Forty-minute continuous perfusion with these agents resulted in abolishment of both theta-field and related unit discharges. Involvement of electrical coupling in production of *in vitro* theta oscillations is discussed.

P.5.6 Discharge patterns of cholinegically activated cells during theta rhythm recorded in hippocampal formation slices

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Since our initial demonstration of carbachol (CCH) induced theta-like activity in hippocampal formation (HPC) slices we have carried out a number of experiments to validate this *in vitro* model to study central mechanism responsible for theta production. In a present study we focused on cellular mechanisms responsible for production of theta in completely isolated HPC. We characterize a single unit discharge patterns of cells activated during CCH induced theta-like activity. In addition to typical related and nonrelated cells which were earlier described *in vivo* we discovered a novel type of theta related cells. These cells were found to discharge precisely in the beginning and at the end of each theta epoch. On the basis of these findings we suggest that isolated HPC is capable not only of producing theta oscillations but also of gating mechanism which determines time duration of each theta epochs and intervals between theta epochs.

P.5.7 Posterior hypothalamic GABAA mediation of hippocampal theta rhythm in the freely moving cats

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The production of the hippocampal formation theta rhythm depends on the relationship of a number of structures localized on the different levels of the central nervous system. It was shown that tonic inputs from reticular formation are transmitted to the limbic cortex *via* the posterior hypothalamic region (PH). Recently we have demonstrated that cholinergic (M1) system localized in PH area was actively involved in mechanism responsible for generation of hippocampal theta oscillations in freely moving cats. In a present study we analyzed the effect of activation and inhibition of PH GABAA-ergic system on hippocamal theta in this species. The following GABAA-ergic agents were administrated into the PH area: muscimol (GABAA receptors agonist) and bicuculine (GABAA receptors antagonist). The injection of muscimol elicited suppression of hippocampal theta. The total blocking effect was observed for 5 hours. After 24 hours amplitude and power of theta recovered to the control level. In contrast to muscimol, bicuculine injections into PH increase only power of hippocampal theta. Results obtained in the present study indicated that GABAA receptors localized in the posterior hypothalamus area are engaged in the generation of hippocampal theta field activity in the freely moving cats. Furthermore, these results supported earlier suggestion that generation of hippocampal theta depends on the dynamic interaction between cholinergic and GABA-ergic systems.

P.5.8 The effects of chronic imipramine administration on hippocampal synaptic plasticity

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It has been established that hippocampus is involved in affective disorders and hippocampal synaptic plasticity may be implicated in pathophysiology of stress-derived disorders such as depression. We examined the effects of chronic administration of a tricyclic antidepressant, imipramine, on various forms of synaptic plasticity in the hippocampal slices. Rats were treated twice daily with imipramine (10 mg/kg p.o., for 14 days) or water. Two days after the last imipramine administration field excitatory postsynaptic potentials (fEPSPs) were recorded in stratum radiatum of CA1 area. Long-term potentiation (LTP) was induced by tetanic stimulation (2 trains of 1 s, 100 Hz, spaced 5 min apart). Depotentiation was induced by low frequency stimulation (LFS, 1 Hz, 900 impulses) 30 min after LTP induction. In a separate set of slices long-term depression (LTD) was induced by LFS. Repeated imipramine administration didn't change the magnitude either of LTP or LTD, however the amount of depotentation was attenuated. While in control experiments depotentation reduced the initial slope of fEPSP by 77%, in imipramine treated animals the amount of depotentiation was 42%. The results indicate that chronic administration of imipramine didn't impair the range of hippocampal synaptic plasticity, but it rather strengthened the maintenance of LTP by the attenuation of depotentiation.

P.5.9 Dynamics of beta (attentional) activity following flash stimulus

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We have previously demonstrated that experimental situations requiring attentive visual behavior are accompanied by enhanced power of local field potentials (LFP) in beta frequency range (16-24 Hz) as recorded in visual thalamic and cortical sites. This beta activity appeared after presentation of preparatory flash stimulus, which signaled the delayed visual cue. Here we analyzed in detail the dynamic of these attention-related signals. It appeared that the flash preparatory stimulus elevated the LFP power spectrum in wide frequency range from 10 to 30 Hz, with strong peak in the beta range, during 200-1,000 ms period after the flash stimulus offset. This strong initial burst was followed by secondary oscillatory events at the beta range which appeared repetitively with the average rate of 0.2-0.3 Hz and gradually ceased in amplitude throughout the 10 s interstimuli period. These bursts are most evident in visual cortical area 18, but appear also in area 17, lateral geniculate nucleus and lateral-posterior pulvinar complex. They are especially prominent in recordings from the representation of the central visual field. The consecutive secondary bursts were correlated with the typical LFP events representing eye movements. Such correlation suggests that attention bursts of beta activity are required for gaining the visual processing with each new fixation blink.

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P.5.10 Cortical modulation of spontaneous neuronal activity in the cat's dorsal lateral geniculate and perigeniculate nuclei

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We have previously shown that in cats with pretrigeminal brainstem transections inactivation of visual cortex by cooling results in changes of receptive fields in neurons of the lateral geniculate (LGNd) and perigeniculate (PGN) nuclei. Here we report the effect of elimination of cortical feedback on spontaneous activity of single units in LGNd and PGN. The reversible inactivation of areas 17 and 18 of the visual cortex were obtained by cooling the cortical surface to the temperature of 5-10°C. Such procedure resulted in changes (by at least 20%) in neuronal spontaneous firing rate of 48% of LGNd cells; the decrease being more frequent (61.5%) then increase (38.5%). Elimination of cortical feedback had even greater effect on the PGN, where 93% of cells changed their spontaneous activity. We observed increase of spontaneous firing rate for 69% of PGN cells and decrease for 31% of cells. Cortical inactivation changed also pattern of the bursting activity of thalamic neurons. In a half of LGNd and two-thirds of PGN cells cooling of the visual cortex resulted in statistically significant increase of interspike intervals in spontaneously occurring bursts. Our results indicate that corticofugal feedback influences not only visual responsiveness of thalamic neurons, but also frequency and pattern of their spontaneous firing, presumably by tonic modulation of their membrane potential.

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P.5.11 Beta-frequency, attention-dependent coupling between local field potentials recorded in the cat's lateral geniculate and cortical representations of the central visual field

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We have previously proposed that enhanced beta (16-24 Hz) local field potential (LFP) activity in the primary visual cortex (VCx) and lateral geniculate nucleus (LGN) may be an electrophysiological correlate of the attentional mechanism that increases the gain of afferent visual information. In this study we calculated correlation between amplitude envelopes of band-pass filtered LFP signals recorded simultaneously in the LGN and VCx representations of the central visual field (within 5 deg from area centralis), during visual and auditory attentive situations. The analysis was done separately for theta (3-8 Hz), alpha (8-13 Hz), beta (16-24 Hz) and gamma (30-45 Hz) frequency bands. Mean correlation obtained for ten LGN-VCx recording pairs in three cats did not differ between the visually and the auditory attentive trials when calculated for: theta, alpha or gamma frequencies. The only difference was found for the beta band where correlations obtained for the visual trials were two times higher than those calculated during the auditory attentive task. This attention-related coupling emerged from synchronized amplitude fluctuations of beta oscillatory activity within the cortico-thalamic circuits involved in central vision. Thus, beta activity might be specifically important for cortico-thalamic functional relation during visually attentive processing.

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P.5.12 Reconstructed half-center network of biological oscillators needs electrical coupling to express anti-phase patterns

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Our theoretical findings indicate that, in spite of traditional view, anti-phase activity does not naturally arise as a consequence of reciprocal inhibition. Indeed, half-center oscillator expresses almost-in-phase instead of anti-phase activity, unless electrical coupling is present. In order to test these findings we isolated two identical cells from two lobster stomatogastric ganglia (STG) using standard procedures. Then both inhibitory and electrical synapses were introduced between these two cells through dynamic clamp system, which enable us to control synaptic strengths. After isolation, and under muscarinic stimulation, these isolated cells continuously express oscillations of short duty cycle with similar frequencies. When coupled solely by reciprocal inhibition, they oscillate almost in phase. Further adding electrical coupling led to a stable anti-phase activity pattern. Thus, counter-intuitively (and in accordance with theory), electrical coupling may promote anti-phase oscillations in networks of inhibitory neurons.

P.5.13 Network from spikes: a computational framework for identification of neuronal interactions from spike trains

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The paper presents a computational approach for fitting a spiking neural network model to the temporal pattern of spikes in a given reference spike train. Each neuron in the model network is represented as controlled oscillator, whose firing times are under influence of spikes generated by the other neurons in the network. The strength of interaction between each pair of neurons is characterized by a single parameter representing the weight of putative synaptic connection. The optimal weights and spontaneous firing intervals of the neurons are determined by an iterative optimisation procedure. The proposed approach is applicable for identification of univariate or multivariate spike trains arising when, respectively, the activity of one or more of individual neurons are recorded.

P.5.14 Relationships between shared input and excitatory connection as shown by correlated discharge of renal neurons

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Shared input is frequently occurring type of neural coordination found in the cortex and medulla. It appears in an isolated form or may be combined with other types of functional connectivity. We tried to assess the relations between shared input accompanied by excitatory connection in spike trains of renal neurons whose resting discharge is generated by medullary neurons. Results were obtained from anaesthetized rabbits. The activity of single units was isolated from renal nerve recordings by means of computer program. The cross-correlation analysis showed that the amplitude of excitatory connection accompanying shared input was 10.1 ± 4.7 imp/s while that of isolated excitatory connection was significantly higher and reached 21 ± 5.2 imp/s ($x \pm$ SEM.; $n = 93$ and 20, respectively; $P<0.001$). The relationship between the amplitude of excitatory connection and its shift from time zero was significant when it was combined with shared input ($r = 0.387$; $P<0.001$) and non-significant when it appeared as a pure type of coordination. These data indicate clear cut relationships between combined types of neural coordination. The depressing effect of shared input on the size of excitatory connection supports the view that the former is of inhibitory character.

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P.5.15 Age-dependence of light-regulated noradrenergic activity in the chick pineal gland

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The avian pineal gland is innervated by sympathetic fibres originating from the superior cervical ganglia. In the chicken, these nerves play a role in generating the circadian rhythm of melatonin. In our studies we analyzed the effect of age on dynamic of sympathetic input to the chicken pineal. In 1- and 2-week old chicks, kept under 12 light: 12 dark cycle, concentration of pineal noradrenaline (NA) did not exhibit significant day-night changes. On the contrary, in 4- and 8-week old birds pineal NA levels were markedly higher during the night compared to the day-time values. In 4-week old animals kept under constant darkness (DD) the rhythmic pattern of changes in pineal NA persisted for one day, with higher values during the subjective dark phase than during the subjective light phase, but it disappeared on the second day of DD. Exposure of 4- and 8-week old chicks to light (30 min, 150 lux) at night markedly decreased the pineal NA content by 45%, while it was ineffective in 1- and 2-week old birds. It is suggested that the light-driven rhythmic oscillation in NA-ergic input to the chick pineal gland progressively develops within the first month of the animal's life, a feature which may influence the impact of retinal illumination on the pineal melatonin-synthesizing activity.

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P.5.16 PACAP38- and cVIP-evoked increase in cyclic AMP production in the chick cerebral cortex: a role of protein kinase C

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Pituitary adenylate cyclase-activating polypeptide (PACAP) and vasoactive intestinal peptide (VIP) are members of a superfamily of polypeptides, which also includes glucagon, secretin and GHRH. PACAP exists in two biologically active forms, PACAP38 and PACAP27, consisting of 38 and 27 amino acids, respectively. The diverse biological actions of PACAP and VIP are mediated via specific receptors: PAC1 receptor shows selective affinity for both forms of PACAP, whereas VPAC1 and VPAC2 receptors are equally sensitive to PACAP38, PACAP27 and VIP. Both forms of PACAP and VIP (the chicken form; cVIP) have been found to (1) stimulate cAMP production and (2) activate protein kinase C (PKC) in the chick cerebral cortex. The presence of PAC1 and VPAC receptors in the chick cortical membranes has been demonstrated. In this study we examined a role of PKC in the stimulatory action of PACAP and cVIP on cAMP production in the chick cerebrum. The PACAP38-activated cAMP formation was not affected by PKC activators (PDB and PMA) and inhibitors (staurosporine and NPC-15437). On the other hand, cVIP-evoked increase in cAMP procudtion was significantly decreased by PDB and PMA, and increased by staurosporine. It is suggested that in the chick cerebral cortex cAMP response mediated by VPAC- receptors, but not by PAC1 receptors, is regulated by the PKC signaling pathway.

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P.5.17 Effects of selected analogs of PACAP and VIP on the chicken VIP-stimulated cyclic AMP formation in chick brain

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The chicken vasoactive intestinal peptide (cVIP) and pituitary adenylate cyclase-activating polypeptide (PACAP27) potently stimulated cyclic AMP production in the chicken cerebral cortical slices. Mammalian VIP (mVIP) showed some activity only at the highest tested dose. Other tested peptides, i.e., PACAP6-27, cVIP6-28, mVIP6-28, or hybrid compounds, i.e., neurotensin6-11cVIP7-28 (NT-cVIP) and neurotensin6-11mVIP7-28 (NT-mVIP) did not show any significant agonistic activity. Thirty min preincubation of cortical slices with PACAP6-27, NT-cVIP or NT-mVIP competitively antagonized the cyclic AMP formation evoked by cVIP, with the truncated form of PACAP being the best antagonist. Preincubation of slices with mVIP6-28 also produced a significant inhibition of the cVIP-induced increase in cyclic AMP production, however its action was independent on the concentration of cVIP. In contrast to mVIP6-28, cVIP6-28 showed no antagonistic activity against the full-length peptide. In parallel experiments, 30-min pretreatment of cortical slices with PACAP6-27 significantly antagonized the PACAP27-evoked increase in cAMP formation; while mVIP6-28 or NT-mVIP hybrid were ineffective. It is concluded that in the chick brain, PACAP and cVIP stimulate cyclic AMP biosynthesis via PAC1 and VPAC type receptor, respectively, and PACAP6-27 seems to be the most potent PACAP/VIP receptor-nonselective antagonist. Unlike truncated PACAP, the tested NT-VIP hybrid peptides may represent the VPAC type receptor-selective blocking activity.

P.5.18 Influence of systemic administration of 5-HT1A/7 receptor agonist on the neuronal activity of the rat intergeniculate leaflet

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Systemically applied 5-HT1A/7 receptor agonist 8-hydroxy-2-[di-n-propyloamino]-tetralin (8-OH-DPAT) induces phase advance of rodent circadian clocks during the mid-subjective day[1]. This chronobiological property of 8-OH-DPAT depends on the intact intergeniculate leaflet of the lateral geniculate nucleus (IGL)[2], the source of neuropeptide-Y input to suprachiasmatic nucleus (SCN). The present study is aimed to determine the influence of intraperitoneal (i.p.) or intraventricular (i.c.v.) application of 8-OH-DPAT on the activity of IGL neurons. After injection of 8-OH-DPAT, there was observed significant increase of neuronal firing in IGL, however oscillatory nature of this activity was preserved at the same time. Such increase was observed after electrical destruction of dorsal raphe nucleus[3], source of 5-HT innervation of IGL and also there is data showing that systemic administration of 8-OH-DPAT reduces 5-HT release from the raphe originating terminals[4]. These results suggest that increase of IGL neuronal firing is triggered by disinhibition of cells caused by reduced 5-HT release.

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P.5.19 Melatonin synthesis in the chick pineal gland: regulation by UV-A light

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Melatonin (MEL) is a neurohormone produced by pineal glands and retinas of various vertebrates in a daily or circadian rhythm generated by an endogenous clock, with high levels at night. MEL rhythm is reflected by oscillations in the activity of serotonin N-acetyltransferase (AA-NAT; a key regulatory enzyme in MEL synthesis). Light, the most important environmental factor regulating MEL production, suppresses night-time AA-NAT activity and MEL level, and resets the oscillator generating the rhythm. We have recently demonstrated that, in addition to visible light, UV-A regulates MEL synthesis in pineal glands of chick and rat, and in the chick retina. The aim of this work was two-fold: to examine whether the chick pineal gland is directly sensitive to UV-A, and analyze a role of the retina in the suppressive action of UV-A on pineal MEL synthesis. Exposure of chicks, with either eyes or heads covered with black, opaque tape, to UV-A light (lambdamax = 365 nm; intensity of 10 $\mu W/cm^2$) at night significantly decreased pineal AA-NAT activity and MEL content. In tissue-culture studies, UV-A pulse applied to dark-adapted chick pineal glands reduced, in a time-dependent manner, AA-NAT activity. These results indicate that MEL synthesis in the chick pineal is regulated by UV-A in two ways: direct and indirect, involving photosensitive pinealocytes and the retinal signal, respectively.

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P.5.20 Visualization of daily changes in the L2 neuron morphology in the first optic neuropil of *Drosophila melanogaster*

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EM studies have shown that in *D. melanogaster* the axons of two classes of the lamina monopolar neurones that receive photic information from the photoreceptors, L1 and L2, swell and shrink in a rhythmic, circadian manner (Pyza and Meinertzhagen 1999), and therefore seem to be a good model to study the mechanisms of neuronal plasticity regulated by a circadian system. The transgenic line of Drosophila, in which L2 monopolar cell expresses GFP reporter protein (21D-GAL4xUAS-GFP) allows visualizing of oscillations in the morphology of the whole cell with a confocal microscopy. In our experiment, flies reared under a light/dark regime (LD12:12) were decapitated at five hours of the day (ZT0, ZT1, ZT4, ZT13, ZT16; ZT0 - beginning of the day, ZT12 - beginning of the night), and processed for confocal microscopy. The obtained results showed that apart from the axons, also other parts of the L2 cell display changes in the morphology over the period of 24 hours. There are visible changes of the axons' dendritic tree diameter, which is the largest at ZT4 and ZT13, and apparent changes of the cell nuclei size. The nuclei are the largest at ZT1 and ZT4, in females and males respectively, while they are the smallest at ZT16 in both sexes. The observed changes clearly indicate the cyclic remodelling of the structure of the L2 neuron by a circadian clock and certain differences between sexes in this respect.

P.5.21 Effects of neonatal asphyxia under various thermal conditions on circadian rhythm of temperature and activity in juvenile and adults rats

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Neurological and behavioral disturbances induced by neonatal asphyxia are observed during the whole life span. One of the postanoxic effects is disturbed emotionality, exhibited under stressful conditions as motor hyperactivity. However, there are no data concerning motor activity of the postanoxic rats under nonstressful conditions, i.e. in their home cages. Therefore we used a new-generation of biotelemetry device, implanted intra-abdominally, to record circadian changes (at L:D 12:12) in body temperature and spontaneous motor activity of juvenile (1.5 months old) and adult (4 and 12 months old) rats left undisturbed in their home cages. Neonatal rats were exposed to a critical anoxia while their body temperature was maintained for 3 h at different levels of: 33°C (normal body temperature of newborn rats), 37°C ("incubator" group) and 39°C ("high incubator" group). Juvenile and adult rats, irrespective of neonatal oxygen and thermal conditions, exhibited clear-cut circadian oscillations of body temperature and motor activity. Neither body temperature nor motor activity were significantly disturbed by neonatal asphyxia or neonatal body temperature. In conclusion, neonatal asphyxia does not seem to influence circadian rhythms of spontaneous motor activity and body temperature in rats.

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P.5.22 Spontaneous and glutamate inducted activity of intergeniculate leaflet neurons - *in vitro* studies

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The intergeniculate leaflet (IGL) of the lateral geniculate nucleus is responsible for the adjustment of the mammalian circadian rhythms to the non-photic cues. This thalamic structure receives a bilateral and overlapping retinal projection which arises from all parts of the retina[1]. In the majority (but not all) of retinal ganglion cells, glutamate functions as an excitatory neurotransmitter. The effect of L-glutamate and N-methyl-D-aspartate (NMDA) on the discharge activity of neurons in this structure was studied in our laboratory. We have observed that in the standard incubation fluid IGL neurons can display at least three types of firing pattern: irregular - with a wide variety of firing rates, tonic - with very stable level of activity, phasic (slow bursting) activity - with intermittent silent periods. Application of L-glutamate induced biphasic response, i.e. an initial transient excitation succeeded by an inhibition, or only an inhibitory response. Application of NMDA induced either an excitatory response (in majority of the examined neurons), or an inhibition. These results are the first electrophysiological demonstration of the neuronal activity of IGL *in vitro*. Our data are consistent with the prevalence of a glutamatergic input to the visual and the circadian system. References: [1]Hickey T.L., Spear P.D. (1976) Exp. Brain Res. 24: 523-529.

P.5.23 Opposing gradients of striatal spindles and gamma-oscillations in freely-moving rats

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To investigate the natural dynamics of cortex-basal ganglia neural circuits, we performed simultaneous recordings of single units and local field potentials from multiple striatal regions of freely moving rats. Local field potentials (LFPs) in striatum displayed prominent alpha/spindle oscillations (8-12 Hz) during both early phases of sleep and episodes of awake immobility. Striatal spindles had an earlier and more synchronous onset throughout the striatum than in cortical EEG, and were also more prolonged and reliable. The intensity of oscillation was strongest in lateral, caudal and dorsal regions of striatum, that are involved in acquisition of habits and motor skills. In these areas we found large numbers of fast-spiking cells that were tightly and rapidly entrained to these alpha oscillations. By contrast, in cognitive regions of striatum (medial, rostral and ventral) there were few fast-spiking units and field potentials instead showed prominent gamma-band oscillations (30-80 Hz) when animals were awake. Our results show that different striatal regions have distinct oscillatory modes, and suggest that the network of electrically coupled, fast-spiking interneurons can promote synchronous activity in the striatum. The resulting striatal spindles may be important for the consolidation of skills and habit learning.

P.6.2 The investigation of the serotonin role in long-term sensitization in snails: changes of electrical characteristics of withdrawal interneurons after influence of haloperidol and 5,6-dihydroxytryptamine

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Studies of the functional role of serotonin show its important role in the regulation of activity of the central nervous system, as well as in the mechanisms of learning and memory. The most important method for the study of functions of the serotonergic system in the activity of the nervous system and the plasticity of behavior is the use of neurotoxic analogues of serotonin, the 5,6- and 5,7-dihydroxytryptamine (5,6- and 5,7-DHT). It is well known that they cause specific degeneration of the serotonergic axons and thereby, create a deficit of serotonin. It was shown that serotonin is a key transmitter in the development of long-term sensitization (LTS). We conducted electrophysiological investigations of the influence of 5,6-DHT on the development LTS in the grape snails *Helix lucorum*. Our results show that the injection of 5,6-DHT blocked the development of LTS. We found earlier that the injection induces depolarization shift on the membrane of withdrawal interneurons and decreases the threshold potential. The main result of the presented study is that the injection of 5,6-DHT prevents a decrease of membrane and threshold potentials of the withdrawal interneurons, after the LTS shown by us earlier. This indicates an intercoupling between the plastic modifications of behavior and the properties of withdrawal interneurons on the level of neuronal membrane.

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6. SENSORY SYSTEM

P.6.1 Does the important stimulus inhibit the irrelevant activity in the brain cortex?

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The hypothesis that the advent of important information inhibited irrelevant cortical activity was tested using event-related potentials in humans[1]. Two blinks of light of different colors were presented in random order. The subjects were asked to count one of them mentally. Counted (target) stimuli evoked the P300 potential. Cortical responsiveness after target and non-target stimuli was evaluated by measuring the responses to additional probing blinks of light. Our previous experiments showed that responses to probes were inhibited after target presentation. This inhibition, however, differed in the frontal and posterior cortical fields[2]. In the present experiment we manipulated the level of irrelevant cortical activation by adding film projection in the background. Subjects were asked to ignore the probes and the film. The results showed that the inhibitory effect of the film was reduced in responses to target stimuli but only in primary and secondary cortical fields. In frontal fields the effect of the film was enhanced by targets. Thus, in the lower level visual fields the results confirmed the assumption that cortical activity produced by irrelevant film should be inhibited after the advent of relevant targets. In frontal regions, however, more complex relations were indicated. [1]Elbert T., Rockstroh B. (1987) J Psychophysiol 4: 317-333; [2]Michalski A. (2001) Acta Neurobiol Exp 61: 93-104.

P.6.3 Multiphoton imaging of dendritic spine motility in the developing mouse visual cortex *in vivo*

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Cortical dendritic spines are highly motile postsynaptic structures onto which most excitatory synapses are formed. It has been postulated that spine dynamics might reflect synaptic plasticity of cortical neurons. To test this hypothesis, we have investigated spine dynamics during the critical period in mouse visual cortex *in vivo* with and without sensory deprivation. The motility of dendritic spines on the apical tuft of layer 5 neurons was assayed by time-lapse two-photon microscopy. Spines were motile at the ages examined (P21-P42), although motility decreased between P21 and P28 and then remained stable through P42. Binocular deprivation upregulated spine motility during the peak of the critical period (P28), without affecting average spine length, class distribution or density. Deprivation at the start of the critical period had no effect on spine motility, while continued deprivation through the end of the critical period appeared to reduce spine motility slightly. We conclude that spine motility might be involved in critical period plasticity and that reduction of activity during the critical period enhances spine dynamics.

P.6.4 Cortical influence on the local field potentials evoked in the rat thalamus by the vibrissa stimulation

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It is widely agreed that the projection from deep layers of the cerebral cortex to the thalamus is crucial for thalamic information processing. Cortico-thalamo-cortical loop has been proposed to be involved in the mechanisms underlying phenomena of habituation, sensitization and attention. In order to identify the dynamics within the loop we studied cortical modulation of the local field potentials evoked (EP) in thalamic somatosensory nuclei (POm and VPM) by vibrissa stimulation in rats anaesthetized with urethan. We manipulated a level of the barrel cortex activation by means of electrical stimulation of the deep cortical layers and surface cooling or xylocaine (Xyl) application. The shortest latency negative wave of thalamic EP was insensitive to cortical manipulations. The longer latency components, followed by oscillations in the alpha frequency range, were enhanced when the moderate inactivation disinhibited the deep cortical layers, and were attenuated during cortical blockade. The stimulation of the cortex produced thalamic EP that matched those long latency components of vibrissa-evoked response that were modulated by cooling and Xyl. All effects, especially putative inhibitory ones, were more pronounced in VPM than in POm. We conclude that the earliest thalamic EP component represents peripheral input and the later ones correspond to the activity of cortico-thalamic and cortico-reticulo-thalamic projections.

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P.6.5 Extrageniculate visual pathways in the feline brain

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This presentation summarizes our work concerning the morphological and physiological properties of an extrageniculate visual pathway in the feline brain. It starts at the intermediate and deep layers of the superior colliculus (SC), its thalamic relay nucleus is in the suprageniculate nucleus (SG) and its cortical projection spreads the whole extent of the anterior ectosylvian sulcus (AES) including the insular cortex (IVA). It is our novel finding that the SG projects also to the dorsolateral part of the caudate body. Further we were able to describe neurons in the fastigial nucleus of the cerebellum that provide bifurcating axons to SG and SC on both sides of the brain. The receptive fields are extremly large consistently included the area centralis and extended practically to the entire visual field of the corresponding eye. The most delicate finding was the absolute absence of retinotopic organization. The neurons were primarily sensitive to very small stimuli moving very rapidly in a specific direction in their huge receptive field. The same receptive field properties have been found in neurons along AES including in IVA, in the SG and in the dorsolateral part of the caudate nucleus. These morphological and physiological data support the notion that this extrageniculo--extrastriate system plays some delicate behavioral role.

P.6.6 Spatiotemporal frequency response profiles of single neurons in the cat's superior colliculus

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The spatial and temporal visual sensitivity of single neurons from the superficial layers of the superior colliculus were studied by means of extracellular recordings in halothane-anesthetized cats. Responses of neurons were recorded during stimulation with sinusoidal gratings moving in a preferred and null direction and the spatiotemporal frequency response profiles of the neurons were mapped out. All tested neurons were responsive to very low spatial frequencies with peak sensitivity in a range of 0.05-0.15 cycles/degree. Spatiotemporal frequency response profiles reveal existence of two distinct populations of collicular cells - temporal frequency tuned and speed tuned. Temporal frequency tuned cells were sensitive to a particular temporal frequency of moving grating in a wide range of spatial frequencies tested. Speed tuned cells responded selectively to particular combination of spatial and temporal frequencies, that is, to a certain speed of stimulus movement. Regions of peak sensitivity for these cells were located along oblique lines in spatiotemporal frequency planes which represented constant velocity. The existence of speed tuned cells has been reported only in MT of primates and in pretectal nucleus of the optic tract in wallaby (Clifford and Ibbotson 2003). Our results indicate that speed tuned cells are present also in feline superior colliculus.

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P.6.7 Decrease of alpha retinal ganglion cell density after binocular visual deprivation

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Visual deprivation alters the normal development of higher brain centres. The effects of visual deprivation previously were reported primarily in the Y pathway. These included a decrease in the number of Y-like cells in the dorsal lateral geniculate nucleus and reduction in the size and density of retinogeniculate Y axonal arbors. A decrease in the density of parafoveal ganglion cells has also been reported after long term visual deprivation in the monkey. In this study we investigated the effects of patterned vision deprivation on the cat's Y retinal ganglion cells, as a model of congenital cataract in children. The distribution of large (alpha, physiological Y) retinal ganglion cells were investigated in two retinas following binocular deprivation (BD) and in two controls. The BD cats were deprived by covering their eyes with double-thickness linen masks from eye opening until 6 months of age. Retinas were taken *post mortem*, flat-mounted and stained with cresyl violet. Alpha cells were counted within sample box 0.6 mm^2 every 0.77 mm. Our results indicate that deprivation reduced the density of alpha ganglion cells by about 18% in central retina. No changes were detected in other regions of BD cats' retinas as compared to control. The decrease of alpha cells density in central retina may contribute to deficits in functioning of Y pathway following visual deprivation.

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P.6.8 Global shape perception in cats early deprived of pattern vision

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We showed earlier that motion detection of a random dot pattern in binocularly deprived cats (BD) was impaired (Burnat et al. 2002). In the present study we examined global shape perception in 4 BD and 4 control (C) cats. Cats learned to discriminate a square *versus* a horizontally oriented rectangle with constant stimuli method. Daily session consisted of 4 blocks with 20 trials. The thresholds of the aspect ratio of the rectangle sides were calculated for each cat, taking into account only blocks with 70% or more correct responses. The BD cats showed higher aspect ratio threshold than the C cats. In addition, thresholds of the BD cats were significantly higher for large size stimuli (sides of the square - 60 and 80 pixels) compared with small size (20 and 40 pixels). Moreover, when orientation of the rectangle was changed to vertical the BD cats could perform the task only with the largest aspect ratio of the rectangle. We conclude that the BD cats do not attend to the global shape of the stimuli. Instead, they choose a local cue to discriminate, and are not able to modify it. Probably, normal pattern stimulation during development of visual system is needed for the proper use of the global features of the stimuli, e.g. aspect ratio of sides of the rectangle. This conclusion is confirmed in patients after early removal of congenital cataract (Lewis et al. 2002). Supported by a Polish - Flemish grant No. BIL99/29.

P.6.9 Processing of motion stimuli defined by luminance, flicker, and opposed motion in primate areas MT and MST

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An earlier investigation[1] suggested, that motion-sensitive neurons in the middle temporal area (MT) of monkey extrastriate visual cortex encode motion, invariant of specific stimulus parameters. The neuronal responses obtained by second-order stimuli defined by flickering objects moving over a stationary background with identical mean luminance were similar to the responses obtained by moving luminance contrast. In our experiment we used different types of second-order motion stimuli to examine whether motion processing in MT and the adjacent middle superior temporal area (MST) is invariant even for second-order stimuli lacking flicker-motion. Because motion stimuli with stationary background always contain a flicker-motion component, we used a dynamic random dot background to obtain second order stimuli free of flicker-motion. The three stimuli we used were a Fourier first-order motion, flicker-defined object motion, and a Theta-motion stimulus in which the directions of retinal-image-motion and object-motion are opposed. Using a static background the directional selectivity of MT and MST neurons is generally higher, compared to a presentation on dynamic background. Though half of the MT-cells and approximately three quarters of the MST-cells answered to the flicker-defined stimulus, the responses to the object motion component of the Theta stimulus was weak or absent. Our results suggest that the directional selectivity of single neurons from areas MT and MST is dependent on specific stimulus-background segregation cues such as luminance or temporal structure. However, it cannot account for the perception of motion-stimuli defined by opposed motion such as the Theta-motion stimulus. [1]Albright T.D. (1992) Science 255: 1141-1143.

7. MOTOR SYSTEM

P.7.1 The tetanic depression in fast motor units of the cat gastrocnemius muscle

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The tetanic depression found in the rat muscle is a factor influencing the tension of motor units. This phenomenon was visible in tetani evoked at two frequencies of stimulation: the lower frequency immediately followed by higher frequency of stimulation. The ability of muscle fibres to generate the tension at higher frequency of stimulation was limited. However, it was not clear if this effect occurred only in the rat muscle or it concerns all mammals. In this study the tetanic depression was investigated in motor units of the medial gastrocnemius muscle in deeply anaesthetized cats. In 70 motor units three patterns of tetani were analysed: at 30 Hz, at 20 and 30 Hz and again at 30 Hz. In all units the tension generated within 30 Hz part of the middle tetanus was lower than the tension generated by the same constant frequency of stimulation immediately earlier or later (the tetanic depression). The same result was obtained at frequencies 40 Hz, 25 and 40 Hz and 40 Hz. The depression amounted to 14.5% for fast fatigable units and 16.5% for fast resistant units in 30 Hz tetani, whereas 6.2% for FF and 7.3% for FR units in 40 Hz tetani. The results proove that a tetanic depression exists in the cat muscle. Its intensity depends on the firing rate of a motoneuron and on the fusion of tetanus. In slow motor units the tetanic depression was not observed. The presence of a tetanic depression in several species suggests that this phenomenon may also affect motor control processes in humans.

P.7.2 Different effects of locomotor exercise on neurotransmitter level in the upper and lower lumbar segments of the spinal cord

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Locomotor exercise leads to an induction of neurotrophins[1], regulatory proteins having an impact on neurotransmission and plasticity. Our aim was to test how the exercise influences neurotransmitter content in the lumbar spinal cord. Seven male Wistar rats walked on a treadmill about 1,000 m daily at a speed 20-25 cm/s. Daily exercise consisted of three 20-min sessions separated by about 1 hour rest. The rats were decapitated after 4 weeks of walking exercise. Five rats that were never trained served as controls. Amino acids: glutamate (Glu), aspartate (Asp), glycine (Gly), gamma-aminobutyric acid (GABA) and monoamines: noradrenaline (NA) and serotonin (5-HT) levels were measured in whole tissue homogenates of L1/L2 and L3-L5 spinal cord segments using HPLC. Exercise caused an increase of neurotransmitter levels in L1/L2 segments. The strongest and significant effect appeared for GABA and NA, which increased by about 17.5% and 13.7% over the control levels, respectively. In contrast, in L3-L5 segments all neurotransmitters decreased. Significant decrease was observed for Asp (9.9%), Gly (11.6%) and GABA (9.4%). Our results show that locomotor exercise causes higher requirements for all tested neurotransmitters at the upper lumbar segments, where central pattern generator might be located, than at the lower segments with the hindlimb motoneuron pool. [1]Skup et al. (2002) Exp Neurol 176: 289-307.

P.7.3 Anticipatory postural adjustment *versus* electro-mechanical delay

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Electro-mechanical delay (EMD) is a time between the onset of the electromyographic record of the activity of the muscle, and the onset of the releasing by this muscle its mechanical force. For the volitional contraction of the skeletal muscles, the EMD amounts the 30 to 60 ms. When the goal is to restore the balance of the human body, the other than that underlied the EMD biomechanical phenomena are functioning. Namely these are the neural processes, the postural responses. Besides the volitional the automatic postural responses (APR) are functioning. Following a displacement of the body from vertical, the muscles of the leg and trunk quickly contract to return the center of gravity (COG) to a position of equilibrium. The earliest of these APR appears within 120 to 150 ms after the displacement. The method for quantification of the APR is the Dynamic Posturography (Nashner and Peters 1990).The anticipatory postural adjustment (APA) as well as the APR allow to control the balance and keep the COG over the base of support. The APA, however, occur 20 to 50 ms before the actual disturbance. If a balance disturbance is predicted, the body will respond in advance by developing a "postural set" to counteract the coming forces. The method of quantification of the APA is widely accepted since pioneer work of Belenkii et al. According to the method described in 1967 by Belenkii et al., the APA examinations were performed in 7 healthy persons, 7 patients with Parkinson's disease and 5 post-stroke patients. The APA was recorded most often in the healthy subjects, whereas in the patients, this phenomenon was most often absent.

P.7.4 Dynamics of functional connections' changes in rat's limbic-motor integration system, and its relation to emotional and/or behavioural aspects

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The research is an attempt to examine interactions between neuronal assemblies of n. accumbens, basolateral n. amygdala, v. subiculum, and subpallidal area during various behaviors, in view of selectively distributed oscillatory systems of the brain. The LFP were recorded from the above structures, in rats in various emotional states and behavioral situations. For analysis we used a new method: direct Directed Transfer Function and full frequency Directed Transfer Function, which enabled determination, whether the flow is direct or not, and the direction, intensity and frequency band of information flow. Results show that flows between structures significantly change, depending on emotional and motor aspects of task performed by an animal, and on the LFP rhythm carrying the interaction. We presume that such results reflect interactions between functional neuronal assemblies active during a given task. The patterns changed after lesion of basolateral n. amygdala. The most sensitive to the damage was information flow from n. accumbens to subpallidal area. The most active after lesion was flow from subpallidal area to v. subiculum, which seems to participate in compensation of the lack of basolateral n. amygdala in the limbic-motor integration system. The study seems to provide a support in favor of the theory of functionally diversified neuronal assemblies, as well as the theory of frequency diversified carriers of information.

P.7.5 Two methods of microsurgical peripheral nerve reconstruction - comparison of functional and morphologic outcome

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The outcome of peripheral nerves transection is still not satisfactory despite of general capacity of peripheral nerves to regenerate and great progress of microsurgery. Routine technique of surgical nerve transection is to cut it perpendicularly. The same approach is used when the nerve grafting is performed. The purpose of this study was to determine whether the oblique transection of the proximal stump of the sciatic nerve influences the regeneration features when the nerve is directly re-sutured or grafted. The sciatic nerves of 20 rats were transected at an angle of 30°. In ten animals the nerve was rejoined directly. In the other 10 rats the 10-mm-long gap was performed and obliquely cut nerve graft was used to bridge the gap. In control 20 animals the sciatic nerves were transected, joined and/or grafted at a right angle. On twelve-weeks' follow-up the animals were observed for autotomy and gait pattern (foot-print test). The histological evaluation of regeneration included: (1) GAP-43 staining for growth-cones; (2) S-100 and GFAP labeling for Schwann cells; (3) electron microscopy for myelination; (4) H&E and Masson trichrome staining to visualize potential neuromas at the nerve proximal stumps. We found that the oblique transection, joining and/or grafting of peripheral nerves presents advantages in both functional and histologic features of regeneration when compared to perpendicular one.

P.7.6 Changes in motor unit action potentials in spinalized rats

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The influence of complete and partial lesions of the spinal cord on motor unit action potentials (MUAP) was studied in the rat medial gastrocnemius muscle. Experiments were performed on 192 motor units in 3 groups of animals: (1) four weeks after the complete section at the level of Th9 segment, (2) four weeks after a hemisection on the side of the muscle investigated, at the Th9 level, (3) the control group with the spinal cord intact. After the complete section, the significant decrease in amplitudes of MUAP was found in FF and FR units (mean values: 0.49 and 0.21 mV, respectively), in comparison to intact animals (1.27 mV and 0.51 mV, respectively). A decrease of amplitudes after the hemisection was smaller, the mean values amounted to 0.74 mV in FF and 0.32 mV in FR units. However, no changes were found for MUAP amplitudes in S motor units of all groups. On the other hand, differences in the time course of MUAPs between the intact and spinalized animals were insignificant for all types of motor units. The observed decrease in MUAP amplitudes accompanied a parallel decrease in tension of fast motor units. It is hypothesized that changes concern mainly fast units due to their higher sensibility to the lack of descending input to motoneurones after a cordotomy. The results indicate that MUAP amplitude is the factor important in evaluating electrical activity of muscles after spinal cord lesions of various extents.

P.7.7 Hypoglossal nerve activity during picrotoxin seizure

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Epileptic seizures induce disorganization of respiration by unknown neural mechanism. The present study tests the hypothesis that one of the elements of respiratory disturbances during seizure is enhanced activity of upper airways and lack of synchronization between the activity of the respiratory nerves. For this study a picrotoxin animal model of epilepsy was used. It is based upon the importance of reduced GABA-mediated inhibition in epileptogenesis. Anesthetized, tracheotomized, vagotomized cats were paralyzed and artificially ventilated. Phrenic and hypoglossal nerve activities, blood pressure and blood gases were recorded. Picrotoxin in increasing dose from 0.3 mg/kg to 1.2 mg/kg was administered intravenously. Picrotoxin at lower doses triggered short lasting bursts of very high amplitude superimposed on hypoglossal respiratory activity and corresponding to it transient phrenic inhibition and subsequent excitation of low amplitude. In expiration a short lasting excitation occurred in both nervous outputs. Irregular respiratory pattern accompanied these effects. Higher doses of picrotoxin evoked repeated discharges overlaying phasic hypoglossal activity and evident depression of phrenic activity. At the same time high frequency oscillation of the phrenic activity decreased while becoming evident in the hypoglossal activity. These features clearly manifest desynchronization of the hypoglossal and phrenic activity that may lead to inadequate lung ventilation during seizure.

P.7.8 Tachyphylaxis and long-lasting impairment of locomotion after intrathecal injection of serotoninergic and noradrenergic antagonists in intact rats

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In intact adult rats (n = 10) an alpha-2 noradrenergic (NA) antagonist yohimbine (Y) and serotoninergic (5-HT) antagonist cyproheptadine (C) were administered intrathecally at the L1/L2 level to study their effects on unrestrained locomotion. Both drugs induced strong, dose-dependent effects on locomotion in rats. The high and medium doses of Y (200, 100 μg/20 μl) and of C (300, 150 μg/20 μl) elicited transient complete paraplegia in the hindlimbs, which was followed by a gradual return of the ability to support the hindquarters and then locomotion with poor balance. These effects could last, depending on a dose, up to 30 min. Small doses of Y (50 μl/20 μl) and of C (100 μg/20 μl) were followed by transient instability of gait or limb abduction only. It was also found that repetitive injections every 2nd or 3rd day of the same drug at high and medium doses often caused tachyphylaxis which usually led to a lack of reaction to the third injection. However, the high doses of these drugs could induce impairment of locomotor movements lasting up to several days. These results show that both noradrenergic and serotoninergic systems are involved in the control of locomotion in intact rats. Their antagonist may, however, easily affect neuronal networks involved in this control.

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P.7.9 Neuroma formation after oblique transection of peripheral nerve

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Neuroma formation often occurs at the proximal end of transected nerve, complicating the healing after gap-injuries or nerve biopsies. Most of such neuromas cause therapy-resistant neuropathic pain. The purpose of this study was to determine whether the oblique transection of the proximal stump of the sciatic nerve can prevent neuroma formation. The sciatic nerves of 10 rats were transected unilaterally at an angle of 30° and the peripheral segments of the nerves were removed. In control 10 animals the sciatic nerves were transected at a right angle. Twenty weeks after surgery the nerves were re-exposed and collected. Presence of neuromas was determined by a two board-certified pathologists on the basis of the histological (H&E and Masson trichrome staining) and immunohistochemical (neurofilaments, Schwann cells and laminin) evaluations. We found that the oblique transection of peripheral nerves, contrary to perpendicular one, is rarely followed by neuromas development.

P.7.10 Repetitive stimulation of the mesolimbic system influences locomotor response to novelty in rats

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The mesolimbic system undergoes plastic changes in response to its multiple activation which involve morphology of the dopaminergic neurons and dynamics of the neurotransmitter release. In the present experiment we studied whether two-week daily electrical stimulation of this system at the level of the ventral tegmental area (VTA), and/or its daily activation by environmental stimuli would change reactivity to novelty in rats bred in standard laboratory conditions. It was found that in comparison with two groups of animals kept in the vivarium throughout the experimental period (one implanted with VTA electrodes and the other naive control), that rats which were VTA stimulated showed increased locomotor response during subsequent exposure to novelty on the 1st , 8th and 15th day after termination of the stimulation procedure. Increased reactivity to novelty was also observed in animals non-stimulated electrically but moved every day from the vivarium to the experimental room for the time of electrical stimulation of the experimental group (exposure to environmental stimuli) but behavioral sensitization effect in these animals quickly habituated. The results point to prolonged behavioral consequences of multiple stimulation of the mesolimbic system, also by environmental stimuli.

P.7.11 Development of the muscle spindles in internal lingual muscles in human fetuses

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Nerve supply to tongue muscles has provided topic for several studies. Nevertheless, no detailed data are available on number, distribution and development of muscle spindles in internal muscles of the tongue. Muscle spindles acting as proprioceptors manifest variable numbers in inner muscles of the tongue. They are parallel to the muscle fibers and are attached to them. They exhibit two types of innervation: an afferent and an efferent one. Studies were conducted on internal muscles of the tongue in human fetuses aging 10 to 27 weeks. Fetal age was estimated by crown-rump length. Muscle spindles were evaluated and scored in serial sections made in frontal, horizontal and sagittal planes. In the fetus of the 14th fetal week the spindles were evaluated in cross-sections of the entire head, made in the frontal plane. Five to ten μm thick sections were stained with hematoxylin and eosin, according to Mallory, impregnated with silver protargol according to Bodian or with silver nitrate according to Holmes. The first muscle spindles (four in number) were observed in fetuses of 12th week. Subsequently, 8 fibers were seen in the 13th week, 23 in 15th week, 30 in 16th week, 12 in 17th week, 26 in 18th week, 30 in 19th week, 23 in 20th week, 17 in 25th week and 21 in 27th week. The results demonstrated that number of muscle spindles did not increase linearly with age to16th week. The most numerous and the least fibers were noted in the vertical muscle and in the superior longitudinal muscle, respectively.

P.7.12 Riluzole improves muscle recovery of slow but not fast muscles after sciatic nerve crush in newborn rats

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The aim of our study was to investigate the effect of Riluzole treatment (that blocks voltage-activated Na and Ca channels and inhibits presynaptic glutamate release) on the recovery of muscle properties after sciatic crush in newborn rats. The nerve injury at birth causes many motoneurons to soleus (Sol) and extensor digitorum longus (EDL) muscles to die. In Sol only 4.3 ± 0.9 (n = 6) motor units (MU) remain while 19.9 ± 2.3 (n = 7) remain in EDL. This loss of MU results in a significant reduction in force of both muscles. Treatment with Riluzole (16 mg/kg/day i.p. 14 days after injury) resulted in significant reduction of the effects of nerve injury in Sol but not in EDL muscles. The MU number of the reinervated Sol muscle increased (9.5 ± 1.5; n = 8), while in EDL muscle the MU number remained at the same level (19.1 ± 1.8; n = 10). The maximal tetanic tension of the reinervated Sol muscle also increased (25.5 ± 2.6 g vs. 15.3 ± 1.5 g), while in EDL muscle the maximal tetanic tension remains at the same level in treated and untreated animals (60.5 ± 6.5 g vs. 59.1 ± 4.5 g). These results indicate that Riluzole treatment improved the Sol but not the EDL muscle. The injured Sol muscles followed by Riluzole treatment weighed more were stronger and possessed bigger number of MU. In contrast, in EDL muscle no such effect was obtained. Further experiments are needed to explain these discrepancies.

8. NEUROPHYSIOLOGY OF APPETITE

P.8.1 Brainstem auditory evoked potential and visual evoked potential in anorexia and bulimia. Pilot study

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Young females diagnosed as eating disorders were examined with neurophysiological methods. The patients with anorexia (26) and bulimia (16) performed visual evoked potentials, brainstem auditory evoked potentials and routine EEG. There were found no pathological BAEP in both diagnostic groups. Two patients from 26 (7.6%) with anorexia as well as six patients from 16 (37.5%) with bulimia had abnormal VEP. In 10 (62.5%) bulimic and 19 (73.1%) anorectic patients we found pathological routine EEG. All 8 patients with abnormal VEP were within a group with pathological routine EEG. Our data provide to conclusion that in anorexia nervosa cortical bioelectrical activity is disturbed, while in bulimia the dysfunction concerns subcortical centers.

P.8.2 Effect of noradrenaline administration into the hypothalamic paraventricular nucleus on feeding-like responses in the rat

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Male rats displaying reliable ingestive response to the PVN injections of NA (40 nM) on a standard chow, were subsequently habituated to either a high energy density (14.5 kJ/g), carbohydrate rich diet (HC diet) or low energy density (7.3 kJ/g), carbohydrate free, protein rich diet (LP diet) until their daily energy intakes were equalized. Rats injected with NA ingested the same amounts of both diets and thus animals habituated to the LP diet consumed only half the total energy of those maintained on the HC diet. NA was then remotely and continuously delivered into the PVN using the microdialysis probe (40 nM during 2 min). About 10 s after the onset of NA delivery, the rats started to chew on inedible pieces of cork placed in the experimental cage and 2 min later they went asleep. After a 5 min break, the same pattern of behavior could be induced with the next NA delivery. It is concluded that the nutritional composition of the diets does not interact with the NA feeding response. The failure of NA administration to increase feeding in terms of energy intake and, on the other hand, its robust chewing stimulating effects, suggests that the primary role of the NA system of the PVN may not be controlling the carbohydrate and energy intake during feeding but rather gating a behavioral response that under appropriate circumstances could lead to ingestion.

P.8.3 The changes of routine EEG during therapy of patients with anorexia and bulimia

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Although there are few neurophysiological studies on patients with eating disorders, the subject is still unexplored. Our study concerns the intensity of changes in EEG and their alteration during the restoration in anorectic and bulimic patients. Examined group consisted of 32 females of mean age of 17 years: 22 with anorexia nervosa (A) and 10 with bulimia (B). All patients underwent EEG twice: by admission to hospital and after improving of nutrition state. Eight subjects have been also recorded by discharge. In A group 18 (81.8%) patients had abnormal EEG by admission; 16 (72.7%) patients had severe EEG changes like spikes, epileptiform discharges or general changes of background activity. During hospitalization and clinical improvement, the pattern of EEG in this group did not change. Moreover, abnormalities in EEG maintained in five subjects, who underwent EEG by discharge. In group B we found abnormal EEG in 8 patients by admission, but not as severe as A group. There were neither background activity changes nor epileptiform discharges. The second EEG recordings revealed abnormal EEG only in 5 patients; in 3 ones EEG improved into normal. Conclusion: A great number of females with anorectic type of eating disorder present severe abnormalities in EEG that do not change during clinical improvement. This finding supports the hypothesis of endogenous factors playing role in pathogenesis of eating disorders. Otherwise, in the patients with anorexia the pathology of EEG activity is also influenced by metabolic disturbances.

P.9.2 Parafollicular cells of thyroid gland in rats with experimental model of hyperthyroidism

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Parafollicular (C) cells belong to disperse neuroendocrine cells of APUD system. As the essential indicator of C cells activity calcitonin (CT) has been proposed. The purpose of this study was CT immunohistochemical detection and estimation of CT and TSH plasma concentration in rats with hyperthyroidism. Experimental model of hyperthyroidism was produced in male Wistar rats by i.p. injection of L-thyroxine at the dose of 40 μg/kg daily over 30 days. All rats were thyreoidectomized. The thyroids from experimental rats showed differences in size of follicles. The majority of follicles had large size with a low epithelium and was fulfilled with the colloid. These follicles demonstrated the presence of a few C cells, which were less immunoreactive for CT in comparison to the control group. In contrast, the smaller follicles, with higher epithelium were accompanied by higher number of C cells, within stronger CT immunoreactivity. In experimental rats plasma TSH and CT concentration was significantly reduced. The differences in immunoreactivity of C cells, dependent on their localization, in rats with hyperthyroidism confirm that co-localization of follicular and parafollicular cells is not accidental, and may play an important role in mutual relation between them.

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9. PHYSIOLOGY AND METABOLISM

P.9.1 Short-term modulation of respiration during intermittent hypercapnia

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This study examined the effects of intermittent hypercapnia (IH) on respiration. The experiments were performed in anesthetized, vagotomized, and ventilated rabbits that were exposed to a series of hypercapnic episodes (5% or 10% CO_2 in air) for 5 min, separated by 6 min recovery on air. Respiration was assessed from the phrenic nerve neurogram. Minute phrenic output (MPO) increased during all hypercapnic episodes, due chiefly to the volume component contribution, but the first response was the strongest with subsequent attenuation of responses. The magnitude of the sixth hypercapnic peak was significantly lower than that of the first one for the 5% CO_2 stimulus ($P<0.05$). During recovery on air, arterial CO_2 tension always returned to the baseline levels, while MPO did not, remaining at a steadily increasing level following each hypercapnic episode. The recovery MPO level after the sixth episode increased appreciably to 165 ± 22.5 % (SEM) of the beginning baseline value for 5% CO_2 and to 155 ± 34.8% for 10% CO_2. The results indicate that the increase of MPO started from a higher level following each hypercapnic episode and that the peak responses to hypercapnia gradually weakened with consecutive episodes, yielding in effect a smaller magnitude of response. The study showed the presence of a short-term modulatory effect of intermittent hypercapnia on respiration, which is depressant in character.

P.9.3 Altered expression of blood pressure regulating genes in the brain of rats with renovascular hypertension

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Previous studies from our department revealed altered expression of vasopressin (VR) mRNA receptors in the brain stem of rats with renin transgenic (TGR) hypertension. No significant changes were found in expression of mineralocorticoid receptors (MR) mRNA and nitric oxide synthase (bNOS) mRNA in the brain of TGR rats. The purpose of the present study was to determine expression of bNOS, V1aR and MR mRNA in the preoptic, diencephalic, mesencephalopontine, medullary and cerebellar regions of SD rats with renovascular (2K,1C) hypertension produced by clipping the renal artery and in their sham operated controls (C). The blood pressure was monitored by noninvasive techniques. The brains were harvested 4 weeks after surgery when all 2K,1C rats developed hypertension. PCR method was applied for quantitative analysis of mRNA expression. 2K,1C rats had higher expression of bNOS mRNA and V1aR mRNA in the preoptic region and lower expression of MR mRNA in the mesencephalopontine and medullary regions than C rats. No significant differences in expression of bNOS, V1aR and MR mRNA were found in the other brain regions. The study provides evidence that renovascular hypertension causes significant alterations in expression in the brain of mRNA of the three genes involved in central blood pressure control. The results of the present and previous studies argues for differential regulation of expression of V1aR, MR and bNOS mRNA in different types of hypertension.

P.9.4 Role of central V1 receptors in cardiovascular regulation in cardiac failure

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Growing number of studies reveals changes of activity of the central vasopressinergic system in several experimental models of hypertension. The present investigation was aimed at elucidating whether function of the brain vasopressinergic system is also affected during the heart failure produced by ligation of the left coronary artery (LCL). The study was performed on conscious Sprague-Dawley (SD) rats which were subjected either to LCL or to sham surgery. All rats were implanted with the left cerebral ventricle (LCV) cannula for LCV infusions, with arterial catheter for blood pressure (MAP) measurements and with electrodes for electrocardiogram (ECG) analysis. Four weeks after LCL constriction or sham surgery the rats were subjected to LCV infusions of (1) artificial cerebrospinal fluid (aCSF), (2) vasopressin (AVP) and (3) V1 receptors antagonist (V1ANT). The size of infarct was verified *post mortem*. Rats with infarct size smaller than 25% of left ventricle area were excluded from the LCL group. LCV infusion of aCSF and AVP did not influence MAP in LCL and sham rats whereas V1ANT caused significant decrease of MAP in LCL but not in sham rats. LCV administration of aCSF, AVP and V1ANT did not influence ECG in sham rats. LCL elicited prolongation of QRS intervals in ECG. The study provides evidence for increased function of the brain V1 receptors in regulation of blood pressure in rats with the post-infarct myocardial failure.

P.9.5 Oxygen consumption by *Periplaneta americana* under influence of chosen pyrethroids

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Insecticides from pyrethroids group are widely used. They modify the action of voltage dependent sodium channels in insects' nervous system leading to changes in excitability - finishing with insect's death. Their action depends in blockade in open position of sodium channels leading to disorders in flowing of sodium ions causing disturbances in action potentials and nervous impulses conductibility. These compounds can influence other vital processes changing general organism vitality. Their efficacy depends in ambient temperature. Metabolism is necessary for maintaining every vital process. Insecticides, causing increase of motor activity, contribute to rise requirement for oxygen; poisoning nervous tissue they lead to deregulation steerage process of metabolism rate. They are removed from organism in enzymatic processes dependant on oxygen consumption. Our aim was shadowing the influence of bifenthryne and deltamethrine on oxygen consumption in different ambient temperatures (15°C, 25°C, 35°C). Studies were made on 15 individuals (males) in each group. Every individual was examined in 5 experimental models: control (K), toxication by respective pesticide (P) – instant effect and in 3 groups of detoxication (D1-D3). Pesticide was given in a drop on the upper surface of the abdomen. Deltametrhin was used in 0.79 ng dose contained in a 5.71 µl of solution (0.90 ng/1 g body weight) and in bifenthryne: 36.76 ng, 5.88 µl (40.24 ng/1 g body weight) – respectively. Rates of oxygen consumption were studied in presence of absorber of CO_2 in hermetic test-tubes. Studies were made in 2.5 hour periods in all variants. Next results were obtained. In control oxygen consumption was dependant on temperature. Pesticides caused increase of this parameter adequate to ambient temperature. Detoxication was observed (returns to control). Influences of ambient temperature, pesticides and time on oxygen consumption were visible.

P.9.6 Influence of pirimicarb (carbamates) on the chosen parameters in frog (*Rana temporaria*)

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Insects are one of the factors threatening plant cultures, therefore various methods of fight with them are used. The most popular are various chemical insecticides among which carbamates play an important role. Some of the accidental receivers of these chemicals are frogs. Carbamates block the activity of cholinesterase, the enzyme catalyzing hydrolysis of acetylcholine, which leads to increased levels of acetylcholine in the nervous system, causing poisoning and death. Our aim was to show the influence of pirimicarb on the motor activity and temperature preferences in the frog. Studies were carried out on 12 individuals. The following models were used: control (K), intoxication (P) by pesticide – instant effect, detoxication I (D1) – 2 days after (P), detoxication II (D2) – after next 5 days and detoxication III (D3) – 5 days after D2. Results were recorded and analyzed with a computer method. Pirimicarb was given in 1 drop on the upper surface of the trunk skin in a dose of 4.48 µg of active substance contained in 4.65 µl of solution, giving the dose of 0.13 µg/1 g body weight. Each frog was kept in all experimental models for 1 minute in order to assure identical conditions of penetration of pesticide and similar stress-maker terms. Parameters in all models were studied in thermal gradient; temperature preference by thermocouples + infrared; motor activity by ultrasound. Each frog was studied in 5 successive models in 50 minutes periods. Analyzing thermal preferences, obtained results in (P) can be divided into 3 groups: increase of the preferred temperature by 5-15°C; lack of influence on the preferred temperature; decrease of the preferred temperature by 5-10°C. Yet in all individuals (especially in D1 and partly in D2) decrease of the preferred temperature by 10-15°C (first group) took place and by 0-5°C in remaining groups. In D3 in the majority of individuals we observed the increase of the preferred temperature i.e. return to norm (K). Second parameter, i.e. motor activity – in all 3 groups it was observed a decrease of activity (P) and slow return to norm between D1–D3, i.e. increase of motor activity. Process of detoxication in successive time sectors was visible.