

10. NEUROGENESIS AND DEVELOPMENT

P.10.1 Human embryonic vestibular nuclei

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Study was made in staged human embryos aged between 32 and 56 days (developmental stages 13 to 23). Embryos were from the Collection of the Department of Anatomy in Poznań. All embryos were sectioned serially and in some of them graphic reconstructions were made. The vestibular nuclei differentiate within the common afferent tract. In embryos at stage 13 this tract contains afferent fibers of cranial nerves 5, 7, 9, and 10. During further development the common afferent tract forms the solitary tract, vestibulospinal tract, and trigeminospinal tract. At stage 15 the sensory fibers of the vestibulocochlear ganglion join the common afferent tract. During stage 16 the cochlear and vestibular afferent fibers separate and the vestibular fibers enter the vestibulospinal part of the common afferent tract. The vestibular nuclei differentiate in embryos at stages 15 to 18. At stage 15 the lateral vestibular nucleus present well defined group of the large neurons. The superior vestibular nucleus appears in embryos at stage 16 and it consists of small irregular nerve cells. In embryos at stage 17 the formation of the groups of neurons which form the inferior vestibular nucleus are visible. At stage 18 the groups of the small loosely packed nerve cells form the medial vestibular nucleus. In the present study migration of neurons between vestibular nuclei are observed. The migration proceeds in two directions: anteriorly, from the lateral to the superior nucleus, and caudally from the inferior to the medial nucleus.

P.10.2 Role of adult neurogenesis in functional recovery and synaptic homeostasis after brain injury

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We have examined the issue of inherent ability of the brain tissue to heal itself after injury using transient global ischemia in rats as an experimental model. Transient global ischemia was induced with the 2VO method (Kee et al. 2001). The procedure resulted in delayed cell death in the CA1 field of the hippocampus while the dentate gyrus (DG) was spared. The rate of neurogenesis within the DG was enhanced during the period of 1-4 weeks following the ischemia. This was documented using standard immunohistochemical methods as shown previously (Kee et al. 2001). We now report on functional changes within the DG that are correlated with neurogenesis using electrophysiological recordings in the hippocampal slices from the same animals that were used for the immunohistochemical measurements. Synaptic strength and synaptic plasticity (LTP) in perforant path within the DG were reduced by 50% at 10 days after the ischemic injury but completely recovered at 35 days. A low dose gamma irradiation applied to the head selectively prevented the neurogenesis and the functional recovery. Similar experiments on sham-treated and on untreated control animals revealed a potent regulatory mechanism that maintains a balance between the synaptic strength, LTP, and neurogenesis. This research reveals novel mechanisms involved in brain recovery after injury.

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P.10.3 Similarities and differences in the timetable of the postnatal development of ventroposterolateral nucleus of the thalamus in the rat and rabbit - morphological and stereological study

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The aim of the present study is to compare the development of ventroposterolateral (VPL) nucleus of the thalamus in the rat and rabbit. Material consisted of 30 Wistar rats and 32 New Zealand rabbits aged from P0 to P180. Following stereological parameters were estimated for VPL: volume, numerical density and total number of cells. Taking the total number of cells into consideration, the development of VPL in both species ends in the third week of the postnatal life. The numerical density of VPL neurons decreases rapidly at the beginning of the postnatal life and stabilizes by about the third week. In both species, the gradual increase of the volume of VPL with simultaneous decrease of the neuronal density in the first week of postnatal life is caused mainly by changes of the neuropil volume. The total number of cells does not change remarkably during the first postnatal week; however, during the second week it decreases significantly, which is probably related to the naturally occurring cell death. From these observations we conclude that the most prominent qualitative and quantitative changes of VPL and its neurons undergo during the first two weeks of the postnatal life in both studied species, and point out that this period of time is the critical one for the morphological maturation of thalamocortical relay neurons.

P.10.4 The postnatal development and maturation of the basolateral complex of the rabbit amygdala

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The aim of the study was to evaluate the developmental and maturational changes of the rabbit basolateral complex (BLC) by means of stereological and histochemical methods. Material consisted of 45 brains of New Zealand rabbits, divided into nine age groups (P2 - P180). Following stereological parameters: the volume of the cerebral hemisphere and BLC, neuronal density and total number of neurons as well as volume of particular BLC nuclei were estimated. Additionally, developmental changes of acetylcholinesterase (AChE) activity in nuclei of BLC were examined. The volume of the cerebral hemisphere increases till 30th day of postnatal life, whereas volumes of BLC nuclei increase much longer - till 90th day. The density of neurons in all nuclei decreases till 30th day. The total number of neurons in the dorsolateral division of the lateral nucleus stabilizes the earliest - between 30th and 60th day, whereas in other nuclei (ventromedial division of the lateral nucleus, basomedial and basolateral nuclei) - between 60th and 90th day. The AChE activity is differentiated and changes in the development of BLC: it appears on P2, reaches maximum on P30 and decreases to the level of an adult animal on P60. In conclusion, we state that the stabilization of the AChE activity occurs one month earlier than the establishment of morphological changes in BLC what may indicate the role of the cholinergic system in BLC maturation.

P.10.5 Expression of calbindin D28k in dopaminergic neurons of ventral tegmental area in the development

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Neurons of the ventral tegmental area (VTA) are considered to be the main component of the brain award system. VTA is heterogeneous as far as its morphology, connections and content of neuroactive substances and CaBPs, including calbindin D28k (CB) concern. Our study utilized the immunohistochemistry methods to investigate: the distribution and morphology of CB- and TH-containing cells in VTA nuclei in the maturation process and to determine whether TH-positive cells also contain CB, if so how changes the range of colocalization during the development. Brains of 45 rats aged from P0 to P270 were studied. After fixation, brains were cut and stained for TH and CB with the immunohistochemical method. Both morphology and pattern of TH positive neurons do not change significantly in the studied postnatal period. These changes occur in the population of CB neurons reaching the maturity on P30. During the maturational process of CB positive cells, the number of CB neurons colocalizing with dopaminergic cells increases between the 2nd and 3rd month - when almost all CB positive cells colocalize with TH positive neurons. Our results may suggest that two populations of CB positive neurons are present in VTA. The first one - only CB positive - which dominates till P30, the second is CB/TH positive. CB in the former may play a role in the control of maturation of VTA, whereas in the latter is supposed to play rather a protective role for matured dopaminergic cells.

P.10.6 Time of generation of brain cells in the opossum *Monodelphis domestica*

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Pups of the gray short-tailed opossum are born at a stage of development comparable to embryonic stages in eutherians. We investigated the rate of cells' proliferation in the opossum brains in the postnatal period. Bromodeoxyuridine was injected i.p. (100 mg/kg) and the animals were killed 4 hours later. BrdU was detected immunohistochemically. Labeled cell nuclei were found in the vicinity of the ventricular zone. The most intense divisions lasted till the end of the sixth postnatal week. In the neocortex it peaked between the postnatal (P) days P5 and P20. Then the rate of cells' generation continuously decreased, starting from the frontal cortex, but lower numbers of labeled nuclei were still present until P35. The highest rate of proliferation in the dentate gyrus was found between P19 and P35. In the diencephalon labeled nuclei were found during most of the investigated period with hypothalamus showing the highest density of the BrdU-labeled cell nuclei at P14-19. The most intense proliferation in the cerebellum was present between P14 and P35. Labeled nuclei in the major fiber tracts, like fornix or anterior commissure were most frequent at P19. These results show that in the opossum the peak of the brain cells' generation occurs postnatally and that the cells are still proliferating at the time of eye opening (P34). In adult opossums cell divisions are present only in the ventricular zone of lateral ventricles and the subgranular layer in the dentate gyrus.

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P.10.7 The neuronal structure of the mamillary body in prenatal guinea pigs. Nissl and Golgi study

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The study was carried out on the mamillary bodies (Mbs) in prenatal guinea pigs - 43 days after conception. In this stage, the differentiating mamillary area has marked main nervous centres: the medial (Mm) and lateral (Ml) mamillary nuclei, and also the paramamillary nuclei: the supramamillary, tuberomamillary pars posterior and parafascicular nucleus (Sm, Tmp, Pfm). The length of Mbs is 0.9 mm, the width is 1.6 mm. Mm extends throughout the mamillary body, whereas Ml and Pfm are located only in its anterior segment. Tmp consists of several small cell groups. Mm is divided into 4 parts (posterior-Mmp, dorsal-Mmd, median-Mmn and ventrolateral-Mmv-l). Mmp forms the posterior segment of Mm, whereas the remaining parts were observed in its middle segment. The cells located in the vicinity of nervous bundles are better differentiated (Mmd, Mmv-l) than the other ones (Mmp, Mmn). In the studied area apoptotic bodies and also migrating cells were observed. In Golgi preparations there are also observed cells in various stages of differentiation, and generally their arrangement coincide with this observed in Nissl sections. The cells in the initial stage of differentiation were mainly observed at the ventral side of Mbs. Most of cells possess dendrites with enlargements and various appendages. The main routes of the mamillary body (the principal mamillary fascicle, fornix and mamillary peduncle) are well marked, and better visible in sagittal plane. The reconstructions of impregnated cells and the whole mamillary region were done.

P.10.8 The postnatal development of calcium binding proteins-immunoreactivity in the rabbit basolateral amygdala

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The basolateral complex (BL) of amygdala is a key structure of the limbic system. Nuclei of BL receive most sensory inputs and contribute massive excitatory projections to the other amygdaloid nuclei. Projection neurons of BL are influenced by intrinsic interneurons, among which populations of neurons containing calcium binding proteins (CaBPs): parvalbumin (PV), calbindin D28k (CB) and calretinin (CR) are distinguished. The aim of the present study was to characterize changes in the pattern of expression PV-, CB and CR-ir during postnatal period in rabbit BL. Brains of 36 rabbits, were fixed in paraformaldehyde, cut into coronal sections, stained immunohistochemically. The changes of CaBPs expression differentiated in the particular nuclei of BL. The basolateral nucleus reached the immunoreactivity characteristic for an adult animal the earliest - at the end of 1st month. At the end of 2nd month the stabilization was observed in the ventromedial part of the lateral nucleus and dorsal part of the basomedial nucleus. The dorsolateral part of the lateral nucleus and ventral part of the basomedial nucleus have reached the stabilization the latest - at the end of 3rd month. Differences in the developmental pattern of CaBPs immunoreactivity in BL support the theory of various origin of its parts and may contribute to better understanding of anatomical and functional organization of the amygdala.

P.10.9 Priming procedures accelerate differentiation of human neural progenitor cells

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It has been shown recently that stem cells (SC) isolated from embryonic and adult tissues could produce neural progenitors *in vitro*. These cells are able to differentiate into specific neuronal subtypes when transplanted into central neural system (NCS), however, up to now the process is inefficient and time consuming. The question arises if priming treatment could facilitate the generation of mature neural cells from SC. Materials and Methods. Human neural progenitor cells isolated from umbilical cord blood (HUCB-NP) were cultured in DMEM/F12 medium with LIF, EGF and FGF-2 or DMEM/F12 medium with 2% FBS and ITS (for priming). Then the cells were transfected with GFP marker gene and grown in the presence of 1) DMEM medium with 10% FBS and RA; 2) monolayer of rat embryo brain cells; 3) organotypic hippocampal slices of 7 days old rats. After 7, 14 and 21 days of culture HUCB-NP were analysed in confocal microscope using anti-nestin, MAP2, GFAP and GalC Abs and the influence of local environmental cues that dictate their fate choice were compared. Results and Conclusions. HUCB-NP propagated in priming medium revealed higher capability to differentiate into neurons, astrocytes and oligodendrocytes than their counterparts cultured in normal conditions. It seems that priming procedure initiates neuronal differentiation of HUCB-NP which could be beneficial in their transplantation into CNS.

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P.10.10 Prenatal depletion of catecholamines influences expression of the apoptotic signal proteins in the rat nonapeptidergic neurons on the 3rd and 15th postnatal days

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It has been shown that catecholamines (CA) may stimulate neurons' apoptosis as well as inhibit it. In our previous works we have shown that increase as well as decrease of the CA level in the brain leads to activation of proapoptotic protein caspase-9 expression in the nonapeptidergic neurons of the hypothalamic supraoptic and paraventricular nuclei of the adult rats, thus initiating the caspase-9-dependent pathway of apoptosis. The aim of the present work was to elucidate the role of the prenatal CA depletion in the initiation of apoptosis in the nonapeptidergic neurons of rats on the third and 15th postnatal days. The decrease of the CA level was provided by injection of the CA synthesis inhibitor α -MPT to female rats on the 13th to 20th days of pregnancy. In control, pregnant females were daily intraperitoneally injected with saline instead of drugs during the same gestation period. Prenatal chronic depletion of CA caused a significant increase of the proapoptotic protein caspase-9 in neurons of the supraoptic nucleus in both three days and 15 days old rats. Significant increase of the antiapoptotic protein bcl-2 expression was also observed. Therefore, the CA deficiency in the brain during last third of pregnancy leads not only to initiation of apoptosis in the neurons of supraoptic nucleus of pups, but also to activation of the antiapoptotic mechanisms. Prenatal CA deficiency also causes an increase of the tyrosine hydroxylase content in neu-

rons of the supraoptic nucleus of the pups. Thus, we suggest that apoptosis of neurons in the supraoptic nucleus is caused by high levels of CA in the postnatal period and not by the delayed effect of low CA levels during embryogenesis. We also observed a significant increase of caspase-9 content in the paraventricular nucleus neurons at the third and 15th days in rats subjected to prenatal CA synthesis blockade, in comparison to the control group. However, the increase of bcl-2 was observed only on the third day of postnatal life; at the 15th day bcl-2 levels decreased comparing to the control group. In the paraventricular nucleus, in contrast to the supraoptic nucleus, prenatal CA depletion did not lead to tyrosine hydroxylase expression in neurons. But at the third day the density of tyrosine hydroxylase containing fibers in the paraventricular nucleus was significantly higher in comparison to the control pups. We suggest that the caspase-9-dependent apoptosis pathway in the neurons of paraventricular nucleus is stimulated by the increased level of CA. Therefore, prenatal CA depletion leads to the compensative increase of CA level in the supraoptic and paraventricular nuclei and causes apoptosis initiation as well as activation of the protective antiapoptotic mechanisms in the nonapeptidergic neurons of rats during postnatal life.

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11. NEUROANATOMY

P.11.1 Localization of the S100B protein in the brain of shrews, *Sorex araneus* and *Sorex minutus*

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While investigating possible mechanisms of seasonal changes in the brain volume of the *Sorex* shrews (Insectivora), we studied the localization and type of cells expressing the S100B protein in the brains of two species of shrews (*S. araneus* and *S. minutus*). The calcium binding protein S100B is the most frequently expressed member of the S100 family in the brain. In the rat and mouse S100B is expressed in astrocytes and neurons. It may act as a trophic factor, stimulating differentiation of neurons and proliferation of astroglia. We used the technique of double immunofluorescent labeling for the S100B and either glial fibrillary acidic protein (GFAP) that is present in astrocytes or neuronal nuclear protein (NeuN) that marks nuclei of neurons. In the shrews' brains the S100B immunoreactive cells were found in the nucleus accumbens, septal nuclei, some thalamic nuclei, superior and inferior colliculi and in the lower brainstem. In the brainstem structures the majority of the S100B positive cells showed an overlapping labeling with NeuN, and therefore were neurons. Very strong immunostaining for S100B was observed in the cerebellar Purkinje cells, neurons of the gigantocellular reticular nucleus and the vestibular nucleus. Scarce S100B positive cells were found in the cortex and hippocampus of shrews' brain. Generally pattern of expression of the S100B in the brain structures was similar to that in the mouse, and there were practically no differences between *S. araneus* and *S. minutus*.

P.11.2 The death of the brain cells during life cycle of shrewsBiałoskorska K.¹, Djavadian R.¹, Taylor J.R.E.², Turlejski K.¹¹Nencki Institute of Experimental Biology, Warsaw, Poland;²University of Białystok, Białystok, Poland

The Soricid shrews' are born in early summer, decrease their size during autumn, winter and grow again in the spring. The reduction of their body size is accompanied by 20-30% reduction of the capacity and weight of brains (Dehnel 1949). About half of the loss is recovered in the spring. While searching for mechanisms of the Dehnel effect, we investigated the rate of brain cells' death and generation in the life cycle of the common shrew (*Sorex araneus*) and the pygmy shrew (*S. minutus*). The TUNEL-labeled nuclei were frequently found in the dentate gyrus of hippocampus and olfactory bulbs, where the rate of cell death seemed to parallel the rate of cells' generation. In other brain structures such nuclei were found only occasionally. Single labeled nuclei were visible in the neocortex and cerebellum of very young animals, during the first summer of their life. There was no wave of cell death in the autumn, or in any other age group. In the autumn, the number of visible nuclei in a series of every tenth section through the neocortex of a shrew varied from zero to two cells. We estimated from our results, that in the first six months of the shrew's life 1% or less of the volume of their neocortex and other brain structures could have been reduced by apoptosis. Therefore, except of the two structures in which brain cells are exchanged, the population of the brain cells is stable during shrews' life, in spite of the great oscillations in their brain volume.

P.11.3 Axonal branching of nucleus "k" neurons projecting to the cerebellar paramedian lobule

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Many aggregations of the brainstem neurons, including those of reticular core, send projections into the cerebellar cortex. Nucleus "k" lies in the pontine reticular formation ventrolaterally to the motor trigeminal nucleus and is divided into large medial (dorsal "k1" and ventral "k2") and small lateral ("k3") parts. A double fluorescent retrograde technique was used to examine whether neurons of nucleus "k" may project by way of axonal collaterals to the rostral (rPML) and caudal (cPML) parts of the rabbit paramedian lobule, known to be the face-forelimb and hindlimb receiving areas, respectively. As a result of unilateral injections of Fast Blue (FB) and Diamidino Yellow (DY) into cortex of rPML and cPML, respectively, FB or DY single labeled neurons were found in all subdivisions of nucleus "k" with a clear ipsilateral dominance. Distribution pattern of labeling suggests that certain topographical relationships exist between location of neurons in "k" subdivisions and their projection sites in rPML and cPML target regions. Moreover, small number of FB+DY double labeled cells were observed in "k1" and "k3" subdivisions. It indicates that some neurons of nucleus "k" may branch to supply both forelimb and hindlimb PML regions and, because of bidirectional connections of nucleus "k" with oculomotor complex, they could be involved in regulation of ocular movements during animal displacement.

P.11.4 The neuronal structure of the medial preoptic area in the guinea pig. Nissl and Golgi studies

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The aim of the study was to examine the neuronal structure of the medial preoptic area (MPA) in the guinea pig. The preparations were made by the Golgi impregnation and stained according to the Nissl method. In the MPA of guinea pig the following types of neurons were distinguished: 1) Bipolar neurons with rounded, oval or fusiform perikarya measuring 15-25 microns. From the opposite sides of the cell body there arise 2 thick, smooth dendritic trunks, most of them divide first time dichotomically near the soma or at longer distance and often once again at various places. Some dendrites may remain undivided. The dendritic branches have a wavy course, they are relatively long and some of them are observed even up to 350 microns. The bipolar neurons are the most numerous in the guinea pig MPA. 2) Multipolar neurons with perikarya from oval to quadrangular which measure 20-25 microns and have 3-4 primary dendrites. These dendrites spread out in all directions and are usually sparsely branched, much of them do not branch at all. 3) Triangular neurons (18-25 microns) have 3 dendritic trunks which arise conically from the perikaryon and divide dichotomically in the vicinity of the soma and sporadically once again after 50-60 microns of their course. The dendritic branches may give off thin ramifications. The triangular neurons are the least numerous in the MPA of the guinea pig. In all the types of neurons an axon arises either from the initial portion of the dendritic trunks or directly from the soma.

P.11.5 Neurons of the claustrum-endopiriform region projecting to the rat entorhinal cortex and some of their immunohistochemical properties

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Distribution of retrogradely labeled neurons in the claustrum (Cl) and endopiriform nucleus (EN) following injections of Fluoro Gold (FG) into the entorhinal cortex and their reciprocal relations with cells containing CaBPs were investigated. 2% saline solution of FG was injected iontophoretically into the entorhinal cortex of adult Wistar rats. Immunohistochemical methods were applied to visualize FG, parvalbumin, calbindin D28k, calretinin on fixed coronal sections. They were studied with BioRad μ Radiance confocal system. The anterior EC receives afferents mainly from Cl excluding its most rostral part and also from the caudal part of dorsal EN. More caudal injections resulted in labeling of the projecting zone within the ventral part of EN. The medio-posterior part of EC receives afferents from Cl. Neurons labeled after FG injections were intermingled with cells containing CaBPs. Among neurons containing calretinin and calbindin D28k only few showed colocalizations with retrograde dye. However, many PV immunoreactive terminals were observed on FG labeled neurons, especially in Cl and ventral EN. Present study, showing some differences in the distribution of CaBP immunoreactivity, support the hypothesis concerning distinct differences between Cl and EN observed also in their efferents.

P.11.6 The neuronal structure of the striatum in the fox: Golgi and Nissl studies

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The studies were carried out on telencephalons of adult fox. Five types of neurons were distinguished: 1) Medium-size spiny neurons are predominated in the striatum. Their perikarya have polygonal, triangular, rarer fusiform shape. Dendritic tree is strongly ramificated (2-10 dendrites). Spiny are seen on the primary dendrites, but their most concentration is observed on the terminal portions. They are differentiated as regards shape. An axon emerges a long conical elongation from the cell body. 2) Medium-size aspiny neurons of two kinds: the cells of first kind have 2-5 long primary dendrites, which have a relatively straight course. Dendritic trunks are smooth, however the dendritic branches may be covered with swellings. The neurons of the second kind have oval perikarya and 3-9 tortuous dendritic trunks. The bifurcations are placed near the soma making that the dendritic tree has a bushy form. The dendrites are covered with swellings. 3) Large poorly ramified neurons, which perikarya are multipolar, elongated and rounded. Dendrites may be smooth or possess irregular swellings. The single spines are rarely seen on their terminal portions. 4) Small, mostly rounded, spiny neurons with a long axon. They have 2-7 dendrites, which are usually covered with densely packed spines. 5) Rounded, small aspiny neurons of two kinds. The first is represented by the neurons with wavy, varicose dendrites (2-5), whereas the second one by the cells with tortuous, bifurcating near the cell body dendrites. Their swellings have a bead-like form.

P.11.7 Pattern of parvalbumin-immunoreactive neurons throughout cytoarchitectonic subdivisions of the perirhinal cortex in the canine brain

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Different morphological types of parvalbumin-immunoreactive neurons: multiform, bipolar and round were found in areas 35 and 36 of the perirhinal cortex. Areas 35 and 36 can be distinguished based on the dominant morphological types of parvalbumin-positive cells and characteristic distribution of immunoreactive neuropil in the cortical layers. Area 35 occupies the fundus of the posterior rhinal sulcus. Layer I of this area can be distinguished by the occurrence of a wide, dark band of immunoreactive axonal terminal plexus, which encompasses most of the layer. Layer II forms an unlabelled band. Single, large multiform cells and bipolar cells, with vertically arranged dendrites and small lightly stained round cells, are sparsely distributed in layer III. The characteristic feature of the layer is a radial pattern formed by dendrites of the bipolar neurons. Prominent layer V contains densely-packed, dark multiform cells with long dendrites, as well as small, bipolar cells with dendrites arranged in various orientations. Behind these cells, darkly stained neuropil form an arc around the fundus of the rhinal sulcus. Horizontally oriented bipolar cells and multiform cells are present in layer VI. The neuropil of the layer are relatively lightly labelled. Area 36 occupies the lateral bank of the posterior rhinal sulcus. In contrast to area 35, a narrow band of dark terminal plexus is seen in the superficial part of layer I. Similar to area 35, layer II of area 36 is also unlabelled. Layer III contains sparse, small, round and single bipolar cells. Layer V contains darkly stained bipolar cells arranged vertically into a radial pattern. Additionally, a wide, moderate dark boundary of neuropil is clearly visible in this layer. Single, small bipolar cells, oriented ver-

ically are present in layer VI. The distribution of parvalbumin-immunoreactive cells and neuropil together with Nissl staining can be used as valid and reliable criteria for subdividing the perirhinal cortex.

P.11.8 Electron microscopical studies on the mesencephalic termination areas of the rat vestibular nuclei

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The afferent fibers originating in the vestibular sense organs terminate in the superior, medial, lateral and descending vestibular nuclei of the brainstem. Numerous studies suggested functional differences between the individual nuclei that can be associated with their distinct connections. In a series of experiments we have injected Phaseolus vulgaris leucoagglutinin (PHA-L) into one of the vestibular nuclei of the rat and studied the anterograde connections of the secondary vestibular neurons. The main conclusion of these experiments is that there is a significant overlap in the termination areas of the vestibular nuclei, albeit individual differences exist both in the strength of the projection and in the termination area. One of the major differences was found at the level of the mesencephalon in the vestibulo-ocular connections: the oculomotor and trochlear nuclei received the densest projection from the superior vestibular nucleus (SVN), whereas the weakest connection was detected with the lateral vestibular nucleus. Earlier physiological studies in cat described the inhibitory nature of this connection, which is probably mediated through the GABAergic neurons. In our electron microscopical study we have found that the terminals of the SVN established symmetric synaptic contacts in the oculomotor nucleus. More than two third of the boutons (82.3%) terminated on dendrites, the rest of them established axosomatic contact. Most of the labeled terminals were GABA positive and few of them showed positivity for the glycine. Our findings support the results of the physiological experiments. In the mesencephalon the other termination area was found in the red nucleus from all of the vestibular nuclei. Its magnocellular part establish widespread connections with those structures that play an important role in the coordination of the motor activity, but the direct connection of the red nucleus with the vestibular nuclei was not described. In the magnocellular part of the red nucleus the PHA-L labeled boutons were in close contact with the perikarya and proximal dendrites. The terminals of the SVN origin established symmetrical axodendritic and axosomatic synapses approximately in equal number showing GABA positive reaction. Our results indicate that the SVN can modify the activity of the cerebello-rubral and cortico-rubral pathways exerting inhibitory action on the neurons of the red nucleus.

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12. NEUROIMMUNE BIOLOGY

P.12.1 Granulocytes participating in inflammatory reaction of fetal and newborn brains

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Morphologic features of inflammatory reactions in developing central nervous system appear since the half of pregnancy. The cells composing the infiltrations arise earlier during development but their presence in circulation and final localization in fully mature inflammatory reactions is prolonged in time. The aim of this work was to compare the picture of inflammatory infiltration in a group of fetal brains in bacterial infections and with aseptic lesions. It was found that numerous granulocytes appeared in bacterial infections, contrary to aseptic reactions. The young maturing cells and granulocytic reactions present the subsequent pictures in development of inflammatory reaction.

P.12.2 Interplay between circulating beta-endorphin (B-END) and NK cell activity (NKCC) in pigs under immobilization stress (IMB)

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Peripheral blood level of B-END, cortisol (COR) (both determined by RIA) and NKCC (Cr51 release assay) were measured in immobilized crossbred Pietrain male pigs divided into three genotype groups according to the molecular assay (PCR) for the mutation of the ryanodine receptor RyR1 (halothane) gene: nn-recessive homozygotes or stress-susceptible, Nn-heterozygotes or stress susceptibility gene carriers, and NN-dominant homozygotes or stress-resistant. All animals were fitted with a permanent jugular vein catheters for blood sampling. It was found that the mean plasma B-END level differed significantly between RyR1 genotypes; the highest level of B-END was observed in Nn pigs, especially in the early phase of stress; besides the late suppressive effects on NKCC, there was a highly significant rise of NKCC in the beginning of IMB. The most evident elevation of B-END level and NKCC was observed in stress gene carriers and the stress susceptible pigs, and those effects seemed to be independent of COR level. Positive correlation between B-END level and NKCC, especially in the early period of stress, revealed possibility of interdependence between those parameters. Paradoxically, the most intensive stress reaction was observed in heterozygous animals commonly considered as stress resistant.

P.12.3 Effect of endogenous and exogenous melatonin on experimental peritonitis in chickens

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Photoperiodic information, encoded in circadian pattern of melatonin (Mel) synthesis and release by the pineal gland, is subsequently perceived as a regulatory factor involved in the control of several physiological processes exhibiting diurnal and seasonal rhythmicity. The immunoregulatory and anti-oxidant activity of Mel is generally accepted. Previously, we have shown that exogenous Mel modulated the chicken inflammatory reaction in two complementary steps: first, Mel inhibited the development of inflammation, presumably thanks to its anti-oxidant properties, and next a pro-inflammatory influence was observed. This last effect seemed to be mediated *via* some mediators synthesized and secreted by the immune cells under influence of Mel. The experimental peritonitis was examined in the different seasons in chickens kept from hatch in constant, 12 h photoperiod. The development of inflammation seemed to be correlated with the seasonal changes in the diurnal pattern of activity of AA-NAT, a key enzyme in Mel biosynthesis. Moreover, exogenous Mel influenced various immune parameters in the same, treatment-dependent way: pre-treatment with Mel diminished the activity of both peritoneal leukocytes and splenocytes and this effect was reversed by endogenous opioid antagonist - naltrexone.

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P.12.4 Ultrastructural and enzymatic changes in early phase of experimental allergic encephalomyelitis and the effect of oral treatment with spinal cord proteins hydrolysis in Lewis rat

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Experimental allergic encephalomyelitis (EAE) is an autoimmune inflammatory diseases of the central nervous system that can be induced by immunization with myelin proteins. Ultrastructural changes in the early phase of experimental allergic encephalomyelitis (EAE) are following: carioskeletal damage with vesicular structures in carioplasm, compartmentation of endoplasmic reticulum, swollen cisterns of Golgi apparatus, increased activity of microglia and damage of axons with myelin sheets desorganisation. After pretreatment with spinal cord proteins hydrolysis (called hydrolysis) no change in carioskeletal proteins were found but many pores in nuclear envelope were observed. Also, there was seen number of collagen fibrils in the perivascular region. Determination of metalloproteinases activity MMP9 and MMP2 showed increased level both of them in EAE by 7 and 2 fold, respectively, in comparison to the control values. After oral pretreatment with hydrolysis, levels of both metalloproteinases dropped by 30% in comparison to the previous levels in EAE. In summary: above data of ultrastructural and enzymatic changes indicate reducing of inflammatory processes in early phase of EAE after oral pretreatment with spinal cord proteins hydrolysis.

13. NEURODEGENERATION

P.13.1 Effects of adenosine on neurodegenerative properties of mitochondrial toxin, 3-nitropropionic acid

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Increasing evidence suggests that an impairment of oxidative phosphorylation may underlie neuronal death in neurodegenerative diseases. The mitochondrial toxin, 3-nitropropionic acid (3-NPA) is an irreversible inhibitor of succinate dehydrogenase, an enzyme which is present in both Krebs cycle and the mitochondrial complex II. Chronic administration of relatively low doses or local application of 3-NPA were demonstrated to evoke selective neuronal loss within striatum and neurological symptoms resembling Huntington's disease. Adenosine, an endogenous neuromodulator, exerts potent neuroprotective effects *in vivo* and *in vitro*. The aim of the present study was to evaluate the effect of adenosine receptor agonists on the neurodegeneration evoked by 3-NPA. The studies were carried out on male Wistar rats. Striatal damage was induced by local stereotaxic injection of 3-NPA in the dose of 300 nmol. The extent of neurodegeneration was evaluated basing on the glutamate decarboxylase activity (GAD) measurement. The intrastriatal injections of both selective adenosine A1 receptor agonist, R-PIA (0.6 nmol), and non-selective adenosine A1/A2 agonist, 2-CADO (1.0 nmol), together with 3-NPA attenuated the decline of GAD's activity induced by this mitochondrial toxin. The GAD activity was increased from $2,836.0 \pm 172.0$ (68% of control) to $4,090.0 \pm 310.0$ (96% of control) ($P < 0.001$) and from $2,957.0 \pm 132.0$ (67% of control) to $3,827.0 \pm 215.0$ (90% of control) ($P < 0.01$), respectively. The co-administration of adenosine receptor antagonist, 8-(p-sulfophenyl)teophylline, together with adenosine receptor agonists reversed their protective effect. Obtained results indicate that diminished adenosinergic modulation may contribute to the development of neuronal loss following 3-NPA administration.

P.13.2 Postural stability in parkinsonians

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Parkinsonism is a common neurological disorder; it affects about 2% in the elderly groups. The diagnosis of Parkinson's disease (PD) is based on the presence of two or more of the major symptoms: tremor, rigidity, akinesia and postural instability. PD patients are not able to use peripheral and visual information in a normal manner in motor tasks. The pathological tremor present in these individuals acts as noise and prevents them from performing normally. Group of twenty subjects (age range 62-81): 10 elderly and 10 PDs participated in the experiment. Postural sway was recorded by a force platform (PROMED, Poland) via the 12 bit A/D interface (sampling frequency 20 Hz) in eyes open and eyes closed conditions. Analysis of the data involved comparison of sway fractal dimension (Df, Higuchi algorithm) in order to describe changes in the postural stability. The results from this study clearly support a difference between groups. The mean Df of sway in the elderly was at the level 1.61 ± 0.1 whereas in the parkinsonians 1.81 ± 0.14 . The difference of the fractal dimension reached the level of significance only for the antero-posterior direction ($t = -3.29$, $P < 0.01$). The increased Df may result in the decline of sensory acuity due to decreased signal-to-noise ratio. The increased noise may affect also speed of other neuronal processes involved in the recovery program and thus further limit dynamic postural stability in Parkinson's disease.

P.13.3 Phosphatidylinositol transfer protein altered during aging and Parkinson's disease (PD)

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Phosphatidylinositol transfer proteins (PI-TPs) are responsible for the transport of phosphatidylinositol (PI) and other phospholipids to the membranes and they are involved in lipid signaling. The aim of this study was to investigate the effect of aging and PD on the PI-TPalpha level in different parts of the brain. Moreover, the effect of Abeta 1-40 and non-Abeta component (NAC) peptides on PI-TPalpha level in adult and aged brain slices was investigated. Aged animals (24 and 36 months old), adult (4 months old) and mouse model of PD were used for these studies. Mice C57/BL received three injections of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) at 2 h intervals in a total dose of 40 mg/kg and after 3, 7, 14 days they were used for the studies. Brain slices were incubated with aggregated Abeta 1-40 or NAC for 4 h at 37°C. The level of PI-TPalpha was determined using immunochemistry methods. PI-TPalpha level decreased in brain of 36 months old by 20% comparing to adult. In animal model of PD, PI-TPalpha level decreased exclusively in striatum 3, 7, 14 days after MPTP by 15, 31, 36%, respectively, compared to control. Among the investigated neurotoxic peptides only NAC affected PI-TPalpha. Our results suggest that alteration of PI-TPalpha level in aged brain and PD may disturb phosphoinositides transport and signaling.

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P.13.4 Poly(ADP-ribose) polymerase and Nitric Oxide Synthase prevent energy failure and oxidative damage evoked by lipopolysaccharide

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The expression of inducible isoform of Nitric Oxide Synthase (iNOS) plays important role in endotoxic shock. However, little is known about the constitutive isoform(s) of NOS (cNOS) that may also enhance NO release, leading to DNA damage and overactivation of Poly(ADP-ribose) Polymerase (PARP). The aim of our study was to analyze the role of cNOS and PARP-1 in the mouse brain during endotoxic shock. Mice C57BL/6 and 129/sv wilde type and PARP-1 knock-out were injected i.p. with lipopolysaccharide (LPS; 1-10 mg/kg b.w.) alone or together with NOS inhibitors: 7-nitroindazol (7-NI; 25 mg/kg b.w.) and NG-nitro-L-arginine (NNLA; 30 mg/kg b.w.) or with PARP-1 inhibitor, 3-aminobenzamide (3-AB; 30 mg/kg b.w.) i.v. DNA integrity, carbonyl group, thiobarbituric acid reactive substances were determined during 48 h after LPS injection. Our data showed that systemic injection of LPS significantly enhances oxidative damage to macromolecules in the brain. Enhancement of protein and lipid oxidation, significantly depend on the dose of LPS and the time of inflammation. Pharmacological and genetic inactivation of PARP-1 as well as NOS inhibition protected brain macromolecules against oxidative damage evoked by LPS injection. Depending on the intensity of inflammation, inhibition of NOS or PARP-1 may have a neuroprotective effect.

P.13.5 Homocysteine modulates expression of APP immunoreactivity in the rat brain

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Hyperhomocysteinemia is an independent risk factor in Alzheimer's disease. Our recent studies demonstrated that group I metabotropic glutamate receptors (mGluRs) and NMDA receptors cooperatively mediate HCY-evoked neurotoxicity. These receptors are known to modulate differentially beta amyloid precursor protein (APP) expression and processing. Here we examined the effects of intrahippocampal injections of HCY in adult and 7 days old rats on the immunoreactivity of several APP epitopes, evaluated at 0.5 h, 1 h and 3 h after injection. Microdialysis of the adult rat hippocampus demonstrated that ^{45}Ca release induced by 5 mM D,L-HCY is strongly inhibited by 1 mM LY367385, which confirms the stimulatory effect of HCY on group I mGluRs in the rat hippocampus. As determined by Western blotting, unilateral intracerebral injection of HCY (0.5 μmol) resulted in the progressive increase in the immunoreaction with antibodies against several N-terminal and C-terminal domains of APP for up to 3 h. Only the immunoreaction with antibody specific to amyloid epitope of APP (613 – 620 amino acid) which increased after 1 h, significantly decreased 3 h after injection. These data suggest that HCY induces enhancement of the expression of APP with delayed activation of its processing *via* non-amyloidogenic pathway. We will present data concerning developmental aspect of these changes and the effects of group I mGluRs and NMDA receptor antagonists. Supported by grant BPZ-KBN-002/CD/P05.

P.13.6 Tau protein pathology accompanies reduced axonal transport in cholinergic neurons of aged rats

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In the present study we confirmed relationship between age-induced disturbance in cholinergic phenotype of BF cholinergic neurons and their impairment in retrograde axonal transport. We evaluated the expression level of proteins associated with microtubule apparatus, Tau 231 and respective kinase Cdk2 and characterized the distribution of these proteins in the cellular compartments of neurons in BF and hippocampus (HP) of young and aged rats. These findings were correlated with the expression of P-TrkA and NGF in the cortex and HP as well as with the retrograde labelling of BF cholinergic neurons following injections of fluorogold into neocortex and HP. After injection of tracer the number of retrogradely labelled neurons in BF areas was significantly lower in aged rats. The number of ChAT-immunoreactive neurons was also significantly lower. Furthermore, we observed a lower density of P-TrkA in the cortex and HP of aged rats. The results of NGF staining in neocortex showed no differences between young and aged rats. However, the consistent phenotypic loss was observed in BF neurons of aged rats. A weak staining for Tau 231 restricted to axonal structures was observed in the brain sections of young rats. In contrast, the data observed in aged rats demonstrated the presence of Tau 404 isoform mainly in the somatodendritic compartment. The immunohistochemical results also showed higher expression level of Cdk2 kinase in aged rats. The results demonstrate that certain basal forebrain cholinergic neurons in aged rats lose their ability to sustain retrograde transport of fluorescent tracer. These changes coincide with altered compartmentalization of tau isoform in neurons of BF and hippocampus. We suggest that degenerative changes in cholinergic neurons of aging rats may be explained by a failure of retrograde axonal transport of target-derived factor induced in turn by a tau-dependent deterioration in the cytoskeleton.

P.13.7 MOG immunization protects dopaminergic neurons against MPTP induced damage

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The immunological system is suggested to play an important role in the pathological processes of the CNS. Immune cells are involved in various degenerative processes where they may help neurons to preserve or aggravate the injury. In order to investigate an influence of autoimmune reaction on neuronal injury we immunized C57Bl mice, 2 and 8-10 months old, with MOG, prior to intoxication with MPTP that produces nigrostriatal neurons damage. MOG immunization induced autoimmunological reaction with clinical features of allergic encephalomyelitis. Nigrostriatal damage was evaluated by a measurement of dopamine content in striatum (HPLC), TH protein expression (immunoblotting) in striatum and in the substantia nigra (immunochemia). However, mice immunized with MOG revealed the features of EAE they showed less neuronal damage after MPTP administration. Dopamine depletion in mice injected only with MPTP declined by 60% and in mice immunized with MOG prior to intoxication was about 19% less than in mice receiving only MPTP. The decrease of TH protein expression in striatum was also about 15% smaller and the number of TH positive cells fallen less by 16% in the group receiving MOG and MPTP. There was no difference between young (2 months old) and old (8-10 months) mice. In conclusion, the autoimmune reaction induced with MOG protected dopaminergic neurons of the SN against MPTP intoxication.

P.13.8 Hypothermia and MK-801 fail to inhibit induction of ischemic tolerance in brain *in vivo*

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Tolerance to brain ischemia may be induced by preceding the injurious insult with sublethal one, or with hyperthermia. Literature data indicate that hypothermia and inhibition of NMDA receptors with MK-801 (known to produce postischemic hypothermia) both inhibit induction of ischemic tolerance. In this study we examined relations between inhibition of NMDA receptors, brain temperature and induction of ischemic tolerance in brain. The tolerance to injurious 3-min global forebrain ischaemia was induced 48 h earlier by preconditioning 2-min ischemia in the Mongolian gerbils heated or spontaneously regulating brain temperature, which was measured with telemetric equipment. Loss of CA1 pyramidal neurones was assessed 14 days after 3-min ischemia. Our experiments demonstrated that ischemic preconditioning reduces neurodegeneration from about 75% to 20-50% in normothermic gerbils, but failed to induce tolerance in heated (moderately hyperthermic) animals. Application of MK-801, 3 mg/kg injected i.p. 1 h before 2-min ischemia recovered tolerance in heated animals, and failed to influence induction of ischemic tolerance in the unheated gerbils, in spite of developed hypothermia. These data do not confirm previous reports showing inhibition by hypothermia and MK-801 of the induction of ischemic tolerance in Mongolian gerbils. Moreover, hyperthermia exacerbates 2-min ischemia and cancels its preconditioning features.

P.13.9 Homocysteine fails to exacerbate brain damage in 7 days old rats submitted to hypoxia-ischemia

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Hyperhomocysteinemia is a recognised risk factor in atherosclerosis, CNS ischemia and disease of Alzheimer, which points to a direct homocysteine (HCY) neurotoxicity. Recently we demonstrated that HCY neurotoxicity is mediated cooperatively by group I mGluRs and NMDA receptors. It has been suggested (Lipton et al. 1997) that hyperhomocysteinemia might exacerbate excitotoxicity involved in pathogenesis of ischemic brain damage in the NMDA receptor-mediated process. In this study hypoxia/ischemia (HI) in 7 days old (PND7) rats was induced by unilateral carotid ligation and 65 min hypoxia. We investigated whether i.p. injection of D,L-HCY (83 μ moles/kg b.w.) 30 min before the insult, influences the brain damage evaluated after 14 days by estimation of deficit of weight of the ipsilateral brain hemisphere. HCY concentration in the serum of PND7 rats (2.07 μ M) increased to 3.93 μ M, upon induction of HI, and to 5.07 μ M after HI. A weight deficit of the ipsilateral hemisphere in control and HCY treated rats was 37.7% and 40.9%, respectively, (N.S.). These results demonstrate that twofold increase in homocysteinemia does not exacerbate brain damage induced by HI in PND7 rats. This negative result may be related among other causes to inadequate rise in HCY levels, to weak affinity of HCY to NMDA receptors and/or to a known neuroprotective effect of group I mGluRs agonists in PND7 rats.

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P.13.10 Treatment with selective cyclooxygenase 2 inhibitor rofecoxib in mice model of Parkinson's disease

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Cyclooxygenase (COX) 1 and 2 are indicated as a factors playing important role in many neurological disorders. 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is a neurotoxin which causes neuropathological, behavioral and biochemical changes that closely mimics Parkinson's disease. We investigated the expression of COX1 and COX2, prostaglandins (PGs) production, dopamine (DA) depletion in stria of C57Bl/6 mice after MPTP administration. We also examined if therapy with rofecoxib a selective COX2 inhibitor after MPTP intoxication would cause changes in COX expression, tyrosine hydroxylase (TH) protein expression, PGs production and striatal DA concentration. We observed overexpression of COX2 protein and transcript reaching maximum level at 7th day after MPTP and lasted until 21st day with cox-1 expression not altered. PG production raised significantly only 24 hours after MPTP and thereafter returned to the level of a control group. Changes in prostaglandins production do not correspond to changes in COX2 expression. Rofecoxib (10 mg/kg) treatment being started 24 h after MPTP and lasted 14 days did not influence striatal TH expression and decreased DA concentration. These results together suggest that COX2 may be involved in MPTP induced neuronal degeneration and regeneration.

P.13.11 Progenitors are more vulnerable to neurodegeneration and neuroprotection than mature oligodendrocytes

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Oligodendrocytes are the neural cells particularly vulnerable for the cytotoxic effect evoked by different neurodegenerative processes such as ischemic insult, hypoxia or hypoglykemia. The mechanisms underlying observed effects are still poorly understood due to the sophisticated process of oligodendrocytes differentiation and maturation. To address this problem, the cells of two distinct stages of differentiation i.e. progenitors (O-2A) and mature, myelinating oligodendrocytes (MBP+) were selected for investigation of the effects of different apoptogenic factors (H_2O_2 , hypoglykemia) *in vitro*. Primary cultures obtained from the brain hemispheres of 18-days old Wistar rat embryos kept for 10 days in culture served for the establishing pure oligodendrocyte culture (the "shake-off" method by McCarthy and de Vellis, 1980). Oligodendrocytes were cultured in DMEM with addition of insulin, transferrin and sodium selenite. Cytotoxic influence of selected apoptotic factors as well as neuroprotective effects of CsA were estimated by immunochemical detection. The obtained data indicate that progenitors are at least two-fold more sensitive both to cytotoxicity and to neuroprotection. The investigation is being carried on in the aim of their potential clinical application.

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P.13.12 Assessment of inflammatory process on acute phase of ischemic stroke based on examination of selected markers

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Inflammatory factors influence on circulation, especially in penumbra, during acute phase of stroke, and may enhance area of ischemic region. We examined 51 patients - 36 patients in early stage of stroke and 15 patients with other noninflammatory diseases as a control group. Patients with stroke were divided into two groups: 15 patients with infection before the stroke and 21 without any infectious process before stroke. We analyzed parameters of inflammation: activity of chitotriosidase, level of C-reactive proteins (CRP), immunoglobulin IgG, number of white body cells (WBC), and level of fibrinogen. We observed statistically significant different ($P < 0.05$) in the activity of majority parameters of inflammation (CRP, WBC, activity of chitotriosidase), between the control group or group without infection vs. group with infection. Increase activity of chitotriosidase, which is marker of activation of macrophages, during the brain infarct damage suggests important role of active inflammatory process in pathogenesis of stroke. It was significantly more active in the group of patients with infection before stroke, what may also suggest important role of infection as a risk factor in pathogenesis of stroke. Continuation of this study may be important for better understanding of role of immunological process during the acute phase of stroke.

P.13.13 Age- and sex-dependent differences in the inducible nitric oxide synthase mRNA expression in a murine model of Parkinson's disease

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Nitric oxide (NO) is involved in several aspects of brain function, from development to learning and memory. Under conditions of excessive NO formation it can act as a neurotoxin and may contribute to the pathogenesis of several neurodegenerative disorders including Parkinson's disease (PD). The neurodegeneration may result from inflammation-induced proliferation of microglia and reactive macrophages, production proinflammatory cytokines, expression of inducible nitric oxide synthase (iNOS). We investigated the influence of age and gender on iNOS gene expression (measured by RT-PCR) in the striatum of male and female C57Bl/6 mice (3 and 12 months old) in a murine model of PD induced by 1-methyl-4-phenyl-1,2,3,6 tetrahydropyridine. In young and old male mice iNOS mRNA expression rapidly increased and was higher than in controls as early as at 6 h after MPTP intoxication; peaked at 24 h, next - significantly decreased at 3rd day, and was observed till 14th day. In young and old female mice iNOS mRNA expression was observed later than in males: it started from 24 h. We observed differences in the expression of iNOS mRNA in the young animals in comparison to the aged ones. In aged mice, the expression of iNOS was higher than in young at the every time points after MPTP intoxication.

P.13.14 Pergolide mesilate, dopaminergic D2 receptor agonist, enhances serum antioxidant enzymes activity in Parkinson's disease

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Recent studies indicated that free radicals are involved in the pathogenesis of Parkinson's disease (PD). The aim of our study was to investigate the effect of PD treatment on the concentration of free radicals (FR), cGMP, glutathione and the activity of superoxide dismutase (SOD) and catalase in the serum of patients. The study was carried out using 17 age matched controls, 16 PD patients treated with L-DOPA and 11 PD patients treated together with L-DOPA and pergolide mesilate (PM). Free radicals, lipid peroxidation, other compounds and enzymes activity were measured using ELISA, spectrofluorometric or spectrophotometric methods. Our data indicated that FR concentration increased significantly in the patients treated with L-DOPA alone. TBARS were not changed in both PD treated groups. However, PD patients treated with L-DOPA and also with L-DOPA+PM characterized significantly higher cGMP level in serum and higher activities of SOD and catalase comparing to control. Glutathione concentration significantly decreased in serum of PD patients treated with L-DOPA but it was maintained close to control in L-DOPA+PM treated patients. These data indicated that L-DOPA+PM is more efficient therapy comparing to L-DOPA alone in maintaining of antioxidative defense in PD patients.

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14. MOLECULAR BASIS OF BRAIN FUNCTIONS

P.14.1 Prolonged homocysteine neurotoxicity *in vitro* is mediated cooperatively by NMDA receptors and group I metabotropic glutamate receptors

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Our recent *in vivo* and *in vitro* studies demonstrated that homocysteine (HCY) applied at millimolar concentrations induces mobilisation of intracellular calcium and acute neurodegeneration, which is cooperatively mediated by group I metabotropic glutamate receptors (mGluRs) and NMDA receptors. Here we investigated whether a prolonged HCY neurotoxicity evoked by pathophysiologically relevant concentrations of HCY also depends on group I mGluRs. Cerebellar granule cells were exposed for 3 days to 750-1,000 μ M D,L-HCY, then neuronal death was evaluated using propidium iodide staining. The results of these experiments demonstrated that HCY at micromolar concentrations induces a significant neurodegeneration. At lower HCY concentrations this effect was potentiated by 50 μ M glycine, which points to partial involvement of NMDA receptors in this process. Neurotoxicity of 250 μ M HCY was only slightly inhibited by NMDA receptor antagonist 0.5 μ M MK-801, or by antagonists of mGluR1 and mGluR5, 25 μ M LY367385 and MPEP, respectively, when the antagonists were given separately. A complete neuroprotection was achieved upon simultaneous application of the antagonists of both, group I mGluRs and NMDA receptors. These results indicate that a prolonged HCY neurotoxicity is mediated cooperatively by NMDA receptors and by group I mGluRs, with involvement of both, mGluR1 and mGluR5 subtypes.

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P.14.2 Expression of mRNA's for glutamine transporters in malignant human brain tumors

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In neoplastic tissues glutamine (Gln) is a key growth factor and Gln uptake is rate limiting for Gln utilization. Recently, a number of cell membrane proteins capable of Gln transport have been cloned, but their expression in human CNS tumors has not been studied. Here we used the RT-PCR technique to analyse mRNA expression for Gln transporters in postoperative samples of human malignant glial tumors. Tumor samples derived from malignant gliomas WHO grade III (astrocytoma anaplasticum) to IV (glioblastoma) showed an overexpression of SN1, a protein that is relatively most enriched in the CNS, and ASCT2, a protein that is normally overexpressed in peripheral tumors, the expression amounting to 3.5- and 2.5-fold that measured in control brain tissue. Messenger RNA's for other Gln transporters (SN2, ATA1) showed similar expression in control and tumor samples. The expression of SN1, but not of ASCT2 or any other mRNA, decreased significantly upon cultivating the tumor tissue in a dissociated culture *in vitro*. The results suggest a specific role of SN1 in the metabolism of glia-derived human tumors, and strict requirements for SN1 expression that are only met *in situ*.

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P.14.3 Tetraethylammonium-induced long-term potentiation in rat motor cortex involves activation of extracellular signal-regulated kinase cascade

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Brief application of tetraethylammonium (TEA), a potassium channel blocker, resulted in long-term potentiation (TEA-LTP) of field potentials evoked in layer V intralaminar connections in adult rat motor cortex *in vitro*. In standard slice incubation conditions, 2 hours after TEA application, the magnitude of responses was increased by $60 \pm 16\%$. Preincubation of slices in $50 \mu\text{M}$ PD 98059, a specific inhibitor of mitogen-activated protein kinase kinase, resulted in only transient TEA-induced response increase and after 2 hours the magnitude of responses returned to baseline values ($3 \pm 6\%$ increase). A statistically significant increase in active (phosphorylated) forms of extracellular signal-regulated kinases (ERK1 and ERK2) was immunodetected at 30 minutes after TEA-LTP induction using Western blotting technique. These results indicate that the activation of the ERK signaling cascade is necessary for induction of a persistent, NMDA receptor – independent form of synaptic plasticity in the motor cortex.

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P.14.4 Elevation of free radical production in cultured C6 cells by long-term ammonia treatment: Modulation by antioxidants

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Oxidative stress is thought to be one of the mechanisms underlying ammonia neurotoxicity. A recent study has demonstrated that acute treatment of cultured astrocytes with 1-10 mM ammonium chloride (ammonia) produces a transient increase of free radicals, which peaks within a few minutes (Murthy et al. 2001, *J Neurosci Res* 66: 282). However, long-term hyperammonemia *in vivo* is the condition that induces profound, cell-specific morphological and metabolic changes in astrocytes. In this study we compared free radical accumulation in an astrocytoma cell line (C6) grown in control conditions and treated with 10 mM ammonium chloride (ammonia) for 24 h, by measuring fluorescence of cells preloaded with the fluorescent dye 5-(and 6-)carboxy-2',7'-dichlorodihydrofluorescein diacetate (DCFDA). DCFDA fluorescence was found to be increased in ammonia-treated cells, and the cells often showed an increased short-lived response to an additional 45 min treatment with 10 mM ammonia. Co-treatment with ascorbic acid for 24 h prevented free radical formation by ammonia and largely decreased the free radical level in non-treated cells. A slight reduction of free radical formation was noted with taurine, but not with glutathione. We propose that free radical production may be a major trigger of astrocytic dysfunction and damage in chronic hyperammonemias and advocate attempts to treat the condition with non-thiol free radical scavengers.

P.14.5 Changes of neuronal and astroglial expression of I κ B inhibitory protein of NF κ B transcription factor following devascularization of rat cerebral cortex

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Regulation of nuclear factor- κ B (NF κ B) pathway in the pathology of the central nervous system is extensively studied. A prerequisite of NF κ B activation and its access to the nucleus is a degradation of I κ B inhibitory molecule. Our preliminary data indicate that enhancement of NF κ B p50 and p65 subunit expression following devascularization of cerebral cortex was rarely accompanied by their nuclear presence. This study aimed to test whether the lack of NF κ B nuclear translocation is due to changes of I κ B protein level. Immunohistochemical study of I κ B localization was carried out in devascularized cortex of the adult rat. One, three and seven days post lesion were analyzed. Starting from the first postlesion day, the number of I κ B IR neurons was drastically reduced. Only few neurons found in close proximity of the injury showed stronger I κ B IR than in controls. In contrast, the number of p50/p65 IR neurons increased in the same area. Extensive astrogliosis following devascularization was accompanied by I κ B induction in numerous astrocytes. This region was also rich in p50/p65 expressing astrocytes, but in most cells both proteins were present in the cytoplasm only. Our findings indicate that the lack of nuclear presence of NF κ B in neurons and astrocytes following devascularization is controlled by different mechanisms.

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P.14.6 Transition metal ions strongly inhibit phospholipase C activity by reactive oxygen species in rat brain cortex

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Highly reactive transition metals, such as copper (Cu^{2+}) and iron (Fe^{3+}) that play an obligatory role in generating reactive oxygen species (ROS) are implicated in the pathogenesis of Alzheimer's disease (AD) and have been found in high concentrations in amyloid plaques. These ion metals can mediate and potentiate Abeta toxicity. The aim of this study was to investigate the effect of Cu^{2+} and Fe^{3+} on phosphoinositide degradation by phospholipase C (PLC) that is altered in AD. PLC activity was determined in synaptic plasma membranes (SPM) from rat brain cortex using radiolabeled phospholipids as a substrate. Exposure of SPM to CuSO_4 resulted in a concentration-dependent inhibition of PLC hydrolysed phosphatidylinositol (PI-PLC). CuSO_4 at $50 \mu\text{M}$ concentration alone and in combination with $25 \mu\text{M}$ Abeta 25-35 decreased PI-PLC activity by about 60% and at $100 \mu\text{M}$ concentration by 83%. Instead of ROS generation FeCl_2 alone and in the presence of Abeta had no effect on PI-PLC activity. However, PLC degraded phosphatidylinositol-4,5-bisphosphate (PIP2-PLC) was inhibited through CuSO_4 and FeCl_2 at $25 \mu\text{M}$ concentration by 60% and 75%, respectively. These data indicated that PIP2-PLC is more susceptible than PI-PLC to ROS generated through redox active metal ions. Our study presented that transition metal ions through inhibition of PLC may be responsible for alteration of lipid mediators concentration and for disturbance of signaling processes.

P.14.7 Different age-related changes of Poly(ADP-ribose) polymerase-1 expression and function in rat brain parts

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The nuclear enzyme Poly(ADP-ribose) polymerase-1 [PARP-1; E.C.2.4.2.30] is activated in response to DNA breaks and regulates DNA repair and gene expression. Its maximal activity correlates with mammalian life span. However, in case of massive DNA damage PARP-1 can cause energy depletion and cell death. In our previous experiments we observed 50% decrease of PARP basal activity exclusively in aged hippocampus while there was no change in cerebral cortex or cerebellum. The aim of the present study is to investigate age-related changes of maximal activity and expression of PARP-1 protein and the level of Poly(ADP-ribose) in different brain parts. PARP-1 immunoreactivity in old brains declines by 20% in hippocampus, cortex and cerebellum and by 50% in striatum. Moreover, PARP in aged hippocampus is unresponsive to cytotoxic stimuli (NMDA and Amyloid beta) or genotoxins (MNNG) that activate it in adult tissue. However, the level of Poly(ADP-ribose) is higher in aged brain suggesting that a change in PAR catabolism could influence activity of the enzyme. Our results indicate that specific activity of this enzyme important for DNA repair is differently altered by aging in particular parts of the brain. This may result from covalent modification of PARP-1. The lack of activation of the enzyme in aged hippocampus may have great impact on the vulnerability of this brain part to cytotoxic or mutagenic factors.

P.14.8 HCS is a unique hybrid protein

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A new gene that encodes the HCS2 protein in *Helix Lucorum* was cloned. It is the first example of a protein where a neuropeptide and calcium-binding structure are present in the same precursor protein. It contains 4 neuropeptides with amino acid consensus YPRX (where X is leucine, isoleucine, valine or proline) at the C-terminal that corresponds to the calcium-binding domain EF-hand. The calcium-binding capacity of the HCS2 protein was shown by 3 independent methods: 1) mobility shift assay; 2) overlay blotting; 3) equilibrium microdialysis. The dissociation constant of the HCS2 neuropeptide was measured by equilibrium microdialysis and the value proved to be $6,71E10^{-5}$. We produced rabbit's antibodies for all those neuropeptides. Immunocytochemistry with the C-terminal calcium-binding domain antibodies showed that this protein is expressed in four command neurons of the parietal ganglion (PPa2, PPa3, LPa2 and LPa3.). In the juvenile snails this protein was also expressed in several small cells of the pleural ganglion. Additionally, Western-blotting showed expression of the HCS2 in neurons of the parietal ganglion

P.14.9 AP-1 protein modulators of transcription

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Activating Protein (AP-1) is a dimer of Jun (c-Jun, JunB, JunD) or Jun and Fos (c-Fos, delta FosB, FosB, Fra-1, Fra-2) proteins. Its function in neural plasticity has been widely investigated in different brain structures. It has been shown that its composition is crucial for its action as transcription factor. Moreover, its action may be modulated by interactions with other proteins which either create protein complexes or introduce post-translational modifications. Unfortunately, there is still very little known about proteins which interact with AP-1 complex as an entity. In our work we try to identify new AP-1 interacting proteins by the means of SOS-Recruitment System. Using recombinant protein consisted of c-Fos-JunB bound to hSOS and cDNA library derived from rat visual cortex 2 h after behavioural training of two-way active avoidance, we have isolated several clones with genes from cDNA library, which potentially encode proteins interacting

P.14.10 The role of microtubule dynamic instability in neurite outgrowth

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Microtubules (MTs) are structural components of neurites and play an important role in their development. Dynamic instability of MTs (i.e., the irregular switching between MT polymerization and depolymerization) was shown to be necessary for neurite guidance, although its connection with saltatory neuron outgrowth is unclear. The growth cone is a small compartment at the tip of a neurite, where tubulin concentration fluctuations could influence MT dynamic instability. Furthermore, MTs in growth cones are tightly packed, which makes it possible that tubulin gradients can further change MT dynamics. By means of Monte Carlo computational models we study MT dynamic instability in a small compartment and its connection to neurite outgrowth dynamics. We find that in a small compartment a single MT may show non-exponential transition times, a feature that has been observed both in non-neural cells and *in vitro*. Non-exponential transition distributions were attributed to some kind of MT memory, which makes the probability of transition of an MT increase with time. In our model, this memory emerges by changes in tubulin concentration in the compartment. Such MT behaviour could be very important for the effective exploratory behaviour that both MTs show inside the cell and growth cones in tissue. We also show that transition frequencies are higher (or MT is more dynamic) in closed compartment than in media with constant tubulin concentration. This shows the possibility of modulating the dynamics of MTs in closed compartments not only by MT associated proteins, but also by limiting or increasing the amount of available free tubulin. The dynamics of a simulated MT population in a closed compartment will be compared to neurite outgrowth dynamics. The effect of motor proteins on MTs might be necessary to account for all the aspects of dynamic instability and growth cone dynamics.

P.14.11 Study of the new adhesive proteins by methods of eye tissue culture

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We studied the adhesive proteins of cell microenvironment in the neural retina and retinal pigment epithelium in the eye of higher and lower vertebrates. We also attempted to determine their role in the mechanisms of maintenance of the retinal structure and functional state. Novel adhesive proteins were obtained from neural retina and retinal pigment epithelium of the bovine eye, and these proteins differ from others known and described earlier in the literature. They have distinct physico-chemical properties and demonstrate various biological effects. It is likely that the proteins we found constitute a new family of proteins showing biological activity in extremely low concentrations (10^{-12} M). Furthermore, in accordance with our data, these adhesive proteins show a tendency to form intermolecular aggregates in solutions. In the study of the biological effects of adhesive proteins we used our own methods of eye tissue culture. Three types of methods of eye tissue culture were investigated. The first method is the newt's (*Pleurodelis waltl*) neural retina culture. The retina was obtained by surgical detachment from the retinal pigment epithelium. The second method is the culture of posterior segment of the newt's eye, obtained after removal of the anterior chamber, vitreous and neural retina. The third method is the culture of the posterior segment of the newt's eye obtained after removal of the eye's anterior chamber and vitreous, but maintaining contact with the neural retina and pigment epithelium. Adhesive proteins were added to the culture medium in extremely low quantities. After culturing for 43-48 hours, tissues were fixed and cut for microscope slides. Evaluation of the state of intercellular adhesive interactions was carried out by the methods of morphological analysis and the cell survival was evaluated by vital staining. Stabilization of the retinal pigment epithelium cell's differentiation was evaluated by morphological analysis. Proteins were analyzed to show the maintenance of intercellular interactions, retinal cells' survival and stabilization of the retinal pigment epithelium cells' differentiation. Effects of the adhesive proteins were more expressed in the model of the posterior segment of the eye, rather than in the isolated neural retina culture. Our data show an important role of the analyzed proteins in regulation of cells' differentiation and morphogenesis. Our adhesive proteins, their physico-chemical properties, biological effects and active concentrations are different from those of for the already known adhesive proteins.

P.14.12 The mammalian Kruppel-like Zn-finger DNA-binding proteins are capable of interacting with triplet repeats (GCC)n within regulatory areas of genes attended by functioning of CNS

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The functional role of triplet nucleotide repeats (GCC)_n, which are found within regulatory areas of a lot of mammalian genes predominantly attended by processes of development and functioning of central nervous system, such as genes of dopamine receptors, calcineurin B, neuronal voltage-dependent calcium channels, very

low density lipoprotein receptor (vldl-r) and other, is not practically investigated. However, the expansion of such repeats in different regions of human genome is a cause of a number of severe inherited diseases. For example, expansion of GCC repeats in the 5'-untranslated region of human FMR-1 gene results in "fragile X-chromosome syndrome" (X-chromosome linked mental retardation). DNA-binding proteins specifically interacting with (GCC)_n repeats may be involved in stabilization/destabilization of the triplet repeats and in transcription regulation of GCC-triplets containing genes. To date only one (GCC)_n-binding protein – CGGBP-20 has been described. Earlier we have found and characterized the nucleotide sequences (GCC)_n as cis-acting elements (GCC-elements) of transcription regulation in the gene promoter of mouse ribosomal protein L32 (rpL32) and in the 5'-untranslated region of human very low density lipoprotein receptor gene (vldl-r). In the present work DNA-binding proteins specifically interacting with GCC-element of rpL32 promoter were isolated from human hepatoma cell line HepG2 by consequent preparative EMSA and three stages of affinity chromatography at specific (immobilized oligonucleotide copy of GCC-containing composite cis-acting element within rpL32 promoter) and non-specific (binding site for human lactoferrin) sorbents and identified by MALDI-TOFF assay. Sequence analysis of identified genes revealed they contain Kruppel-like Zn-finger DNA-binding domains. The coding regions for two from identified genes (flj14011 and xm65119) were cloned by RT-PCR and mammalian and bacterial expression vectors for those genes were constructed. It was shown in co-transfection experiments, that FLJ14011 protein represses the activity of rpL32 promoter. DNA-binding properties of FLJ14011 and XM65119 proteins are currently under investigation. In parallel experiments it was found out, that the other Kruppel-like transcription factor ZF5 highly homologous to FLJ14011 and XM65119 proteins is capable of binding with GCC-element of rpL32 gene. Therefore, obtained data allow us to describe a novel family of (GCC)_n-binding proteins containing Kruppel-like Zn-finger DNA-binding domains and acting as transcriptional regulators.

15. LEARNING AND MEMORY

P.15.1 Nicotine-induced place preference and behavioral sensitization is affected by the calcium channel blockers

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The present experiments were undertaken to investigate the effects of some L-type voltage-dependent calcium channel antagonists on nicotine-induced locomotor sensitization and conditioned place preference in rodents. Repeated daily injections of nicotine produced progressively large increases in locomotor activity in mice especially to a subsequent systemic nicotine challenge. Pretreatment with a dihydropyridine nimodipine, a phenylalkylamine verapamil and a benzothiazepine diltiazem significantly blocked the acquisition of nicotine-induced locomotor sensitization in a dose-dependent fashion. When mice were injected of these compounds prior to the challenge dose of nicotine, the expression of locomotor sensitization was also attenuated. In the place preference paradigm, nicotine produced a place preference to the initially less-preferred compartment paired with its injections during conditioning. Pretreatment with nimodipine, verapamil and diltiazem at the dose inactive in producing the place conditioning completely blocked the establishment of nicotine-induced place conditioning in

rats. These results suggest that both locomotor stimulant and rewarding effects of nicotine are calcium-dependent. The results are discussed in the context of functional association of nicotine with neuronal calcium ions and calcium channels especially in connection with neuroadaptive long-lasting changes in brain structure and function after prolonged nicotine administration that ultimately lead to dependence.

P.15.2 Dopamine D1 receptors are involved in the mediation of procognitive effects of angiotensin IV and angiotensin II(3-7)
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Intracerebroventricular (i.c.v.) angiotensin IV (Ang IV) and Ang II(3-7) were previously found to enhance learning of conditioned avoidance responses (CARs), facilitate recall of a passive avoidance (PA), and improve object recognition (OR) in rats. Since dopaminergic system is crucial for the cognitive processes in this study I sought the dopamine mediation of these effects using SCH 23390 as a selective D1 receptor antagonist. Male Wistar rats (180-200 g), pretreated with SCH 23390 (0.05 mg/kg i.p.), were 1 h later given Ang IV or Ang II(3-7) (1 nmol i.c.v.) and then tested in the above cognitive paradigms as well as in the open field and elevated plus maze to control for the unspecific, respectively motor and emotional, effects of our treatments on the memory tests. Both, Ang IV and Ang II(3-7) effectively enhanced learning of CARs ($P < 0.05$), recall of PA ($P < 0.001$), and improved OR ($P < 0.001$). Pretreatment with SCH 23390 abolished all these procognitive effects of both peptides. SCH 23390, Ang IV, and Ang II(3-7), given at the same doses and routes as in the cognitive tests, did not significantly influence crossings, rearings and bar approaches in the open field and number of entries to the open arms of the elevated plus maze making thus major contribution of the unspecific effects of our treatments to the results of the memory tests improbable. In conclusion, these results indicate that the functional dopaminergic D1 receptors are necessary for the Ang IV and Ang II(3-7) procognitive effects to occur.

P.15.3 The investigation of membrane mechanisms of behaviour plasticity during learning: role of Ca ions and serotonin
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The studies of cellular (membrane) mechanisms of associative learning and long-term memory attracts the attention of neurobiologists. Serotonin is a well known neurotransmitter and neuromodulator. However, Ca^{2+} ions play also an important role in the plasticity. Therefore, the main purpose of our study was to analyze the role of serotonin and Ca^{2+} ions in the mechanisms of associative learning in the grape snails. It was shown that after injection of the neurotoxic analogue of serotonin, the 5,6- dihydroxytryptamine (5,6-DHT), the learning ability of the grape snails is destroyed for two weeks and afterwards it recovers. We described earlier the decrease of the membrane and threshold potentials of withdrawal interneurons during the defensive reflex conditioning. We found now that injection of the 5,6-DHT prevents the decrease. It was shown that the curve of dependence of the change of threshold potential in the learned snails on the increasing extracellular concentrations of Ca^{2+} is bell-shaped, while in the naive snails the change is linear. It was also found that the decrease of intracellular concentration of Ca^{2+} by the Ca^{2+} chelator EGTA led to an increase of excitability of neurons of the learned snails, while the excitability was decreased in the naive snails. These

results demonstrate the participation of membrane structures of the neuron in the mechanisms of long-term memory.
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P.15.4 The changes of electrical characteristics of withdrawal interneurons of snails after chronic injections of haloperidol
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Recently, the therapeutic action of the antipsychotics is connected with the depletion of either dopamine or serotonin. The long-term influence of haloperidol (HAL) on the model molluscs leads to a significant decrease of the level of dopamine (Baker et al. 1995, Sakharov et al. 1996). Therefore, the main task of our paper was to study functions of the identified neurons and to determine the influence of HAL on their electrical parameters. In the experiments we used the grape snails *Helix lucorum*. HAL was injected daily during 7 days, in the dose of 1 mg/kg into the internal cavity in the volume of 0.1 ml. All injections were produced in the region of sinus node of the snail. The measurements of electrical characteristics of withdrawal interneurons showed that the injections of HAL led to hyperpolarization of neurons number 2 and 3 of the left and right parietal ganglions. It was shown that the resting potential is changed to -69 ± 0.4 mV in comparison with -58 ± 1 mV in the control snails. After the injection of HAL the value of the threshold potential also increases for 3 mV. Thus, it was found that the chronic application of HAL leads to the increase of threshold of generation of the action potentials and to hyperpolarization of membrane potential of the withdrawal interneurons in the grape snails.
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P.15.5 Anisomycin-induced inhibition of memory to environmental conditioning in snail during reactivation
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Recent experimental data demonstrate the instability of memory in certain phases, which shows the need of detailed analysis of the processes of reactivation of long-term memory. Therefore we made a study of the influence of protein synthesis inhibition by anisomycin on the consolidation of memory after learning. We used grape snails *Helix lucorum*. The environmental reflex conditioning (ER) was made in standard conditions. Snails ($n = 10$) placed on a ball floating in water, were given five electrical shocks a day (1-4 mA, 1 s, 50 Hz) for five days. Testing the behavioral reactions in response to tactile stimulation of legs on the ball and on flat surfaces showed the presence of the ER conditioning. One day after the testing a session of "reminders" of ER was conducted, which consisted of placing the snails for 20 minutes on the ball. After reminders the snails were injected with anisomycin (control snails were injected with the saline solution). Later testing showed that the injection of anisomycin led to forgetting of the ER conditioning. Thereby, it was shown that the ER could be destroyed by the use of protein synthesis inhibitor during reactivation of the memory.
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P.15.6 Portocaval shunt and brain reward system

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In subjects with chronic liver dysfunction brain aminergic systems are greatly altered. High voluntary alcohol consumption is observed in rats with portocaval anastomosis (PCA) that serve as a relevant disease model. To check whether the brain reward system undergoes any changes after PCA, voluntary alcohol intake after DA receptor blocker was tested and *ex vivo* D1/D2 receptor binding studies were performed in PCA and sham operated rats, 3-4 mo after the surgery. Sulpiride, 5 mg/kg i.p. daily, was given for 4 consecutive days to PCA ($n = 8$) and SHAM ($n = 7$) rats, each placed individually in metabolic cage with free access to 10% ethanol, water and feed. Consumption of fluids and feed was recorded daily. Three days control period with no drug preceded the treatment. CCx membranes and [N-methyl-³H]SCH23390, conc. 0.06-15 nM, with (+)-Butaclamol, 1 μ M as a cold ligand, or [³H]Spiperone, conc. 0.02-8 nM, with 10 μ M S(-)-Sulpiride were used to characterize D1 and D2 receptors, respectively. RESULTS. In contrast to PCA operation, sulpiride treatment did not modify voluntary alcohol, water or feed consumption by either group. Saturation binding data: D1 receptors, PCA: $K_d = 0.903 \pm 0.27$ nM; $B_{max} = 72.86 \pm 7.05$ fmol/mg protein vs. Sham: $K_d = 1.01 \pm 0.17$ nM, $B_{max} = 87.96 \pm 4.85$ fmol/mg protein; D2 receptors, PCA: $K_d = 0.415 \pm 0.301$ nM, $B_{max} = 9,523 \pm 2.26$ fmol/mg protein vs. Sham: $K_d = 0.447 \pm 0.241$ nM, $B_{max} = 18.23 \pm 4.51$ fmol/mg protein. D2 receptor density decrease corresponds well with previously found DA system activation and indicates compensatory change.

P.15.7 Effect of bacterial endotoxin (LPS) on the acoustic startle responses and the open-field behavior in mice

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Administration of laboratory rodents with the lipopolysaccharide (LPS) induces sickness behavior that involves robust changes in locomotor activity. However, distinguishing between ambulatory and investigatory components of total locomotion has received little attention. The presented study investigated effects of LPS on the acoustic startle reaction (ASR) and on locomotor activity in the open field (OF) in mice. Control groups were injected with saline (SAL/SAL), indomethacin (IND/SAL) and both (IND/LPS). Two hours after injections ASR amplitude were measured and next mice were videotaped in the OF in red light during one 5 min session. Locomotor activity as numbers of crossing (ambulation) in the peripheral part of the OF and rearing (investigatory activity) were counted for each of one minute interval. No difference in the ASR magnitude has been found between the groups. In the OF all animals displayed decrease ambulation during the last 2 min of exploration, whereas rearing responses increased after first minute of exposition to the OF. However, the LPS mice, contrary to other groups, displayed lower level of rearing responses. These results show that in mice rearing behavior is more sensitive than ambulation to changes induced by LPS. Furthermore, because investigatory activity may reflect cognitive processes, it may be tentatively concluded that LPS affects cognition.

P.15.8 Investigatory and ambulatory behavior in object recognition and open field tests in rats with late relapse hepatic encephalopathy (HE) in the thioacetamide model

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Rats treated with 2 i.p. doses of 250 mg/kg of thioacetamide at 24 h intervals develop liver injury and changes in brain morphology and metabolism typical of HE, which after transient recovery show a relapse 60 days later. These late relapse HE rats were tested for investigatory behavior and locomotor activity using object recognition (OR) and open field (OF) tests. OR tests included habituation, spatial and object novelty (NO) recognition on circular field. Day and night OF sessions comprised evaluation of the number of crossings in the three parts of the OF and number and duration of contact with NO in last two sessions. HE rats presented a lower number and shorter duration of contact with displaced object than control rats in the OR. While the number of contacts with NO was equal in control and HE rats, the contact duration in HE rats was shorter. Control rats showed increased number of crossings in the peripheral part of OF after the first session, whereas HE rats displayed a constant pattern of activity. At the beginning of the first session HE rats spent more time on the internal part of OF than control rats. These results suggest that high level of ambulation in HE rats reflected disturbances in simple locomotory responding and/or in the attention processes.

P.15.9 Instrumental approach and departure reactions in male rats tested with estrous female

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It has been observed during the testing of the instrumental sexual responses that male rats, after the copulatory contact with female, tend to return spontaneously to the start compartment. We have elaborated the instrumental response aimed to return to the start compartment. The apparatus consisted of two compartments ("start compartment" and "goal compartment") connected by opaque guillotine door. Both compartments were equipped with bars. During the test, male rat was placed in the start compartment whereas the incentive estrous female was tethered in the goal compartment. After the instrumental response (5 bar presses) has been accomplished, the door separating him from the goal compartment was opened, thus enabling the male to contact with the female. The subsequent opening the door enabling the male to return to the start compartment, was possible only after accomplishing the second instrumental response. This response consisted of 2 bar presses and occurred only during short intervals between respective mount bouts. The analysis of this instrumental departure response may give some information about the role of drive satisfaction (or hypothetical "antidrive") and/or opponent processes in sexual preparatory and consummatory activities.

P.15.10 Amygdalar heterogeneity in rats: quantitative, not qualitative, differences in c-Fos expression evoked by different motivational states

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It is known that plastic changes related to acquisition of fear responses occur mainly in the lateral and basolateral nuclei of the amygdala. However, no data regarding pattern of c-Fos expression evoked by conditioning of the alimentary response in different subdivisions of the amygdala have been available so far. The aim of the present study was to compare c-Fos activation resulted from defensive and alimentary learning procedures. The CER and/or instrumental appetitive responses were trained in the same Skinner box apparatus. Male Long-Evans rats were divided into four control (C1-4) and two experimental groups (E1-2). c-Fos expression was analyzed: in home-cage controls (C1), after one daily session of stimulus habituation (C2), after the first day of alimentary (E1) and defensive CER training (E2), after the 10th day of alimentary training (C3) and after the 10th day of defensive training (C4). In the cortical nuclei conditioning of the appetitive response evoked intense immunostaining, while learning of the defensive reaction resulted in more moderate expression. An increase of c-Fos expression in the medial part of the basolateral nucleus was related to both the alimentary and CER training. However, more considerable increment was evoked by conditioning of defensive response.

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P.15.11 The response to low stress novelty in RHA and RLA rats – behavioral and electrophysiological approach

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Genetically selected Roman rat sublimes RHA (high emotional reactivity) and RLA (low emotional reactivity) differ in emotional reactivity measured in various tests. The aim of this experiment was to study exploratory behavior in novel, but non-stressful situation in rats of both lines. In order to reduce emotional responsiveness and control the level of novelty the animals were habituated for first 11 sessions to the experimental chamber consisting of start and tunnel zones. On the 12th session the novelty was introduced into one of the tunnel zones and subsequent two sessions were conducted with the chamber being turned into the experimental mode. For the analysis the selected behavioral measures of spontaneous activity were video-recorded. The RHA subjects significantly increased the investigatory, object oriented activity, while the RLA rats showed no response. In attempt to recognize the possible neurophysiological mechanisms underlying the behavioral differences between the sublimes, LFP activity was recorded from the hippocampus, since the hippocampal theta rhythm has been found to reflect the emotional state of animal performing motor reactions. For technical reasons the experimental design had to be simplified, what did not changed the behavioral results. The comparison of total power spectrum of the last habituation trial to the first trial after introducing novelty showed increased amplitude of theta band in RLA and the decrease in RHA rats.

P.15.12 Response to novelty and subsequent habituation in the familiarized conditions in three strains of rats

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The purpose of this study was to investigate the response to novelty in three strains of rats (BN, WAG, LEWIS) under non-stressful conditions. To reduce fear, a procedure of repetitive placing in the experimental chamber consisting of start, and two tunnel zones was used. Each animal was placed daily in the experimental chamber for a 6 min period. The first eleven sessions were the habituation sessions. In the twelfth session, the novelty was introduced into the screen and tunnel zones. The subsequent ten sessions were conducted under novelty conditions. Behavioral activities such as walking, object contacts, time spent in given zones, and entering the tunnels was measured. All the comparisons were made for 2 three-minute intervals, using a three-factor MANOVA, involving 2(sex) * 3(strain) * 14(3-min interval). In all subjects, experimental manipulation (introducing the novelty) resulted in an increase in time spent in the experimental tunnel zone and decrease in time spent in the start and control tunnel zones. However, significant strain differences were observed. All subjects spent more time inside the experimental tunnels after introducing the novelty into the chamber. Significant strain differences were observed in the course of habituation. All subjects responded with decreased walking. Rats of all three strains climbed on the tunnels more after the introduction of the novelty. The males showed clear habituation in this activity, while females persisted throughout all sessions. The sex and strain differences obtained in this study may be attributed to mechanisms specific to exploration, therefore the low stress testing environment seems to be most suitable for studies on novelty related, cognitive behavior mechanisms.

P.15.13 Energizing effects of stimulus modality in habituation process

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It has been shown that visual and/or auditory cues exert non-homogeneous effects on behavior that was observed during habituation and further learning procedures. The present study was designed to provide information about unconditional effects of the visual and auditory cues on shuttle activity in rats. The assumption was that a manner of responding during shuttle-box training was determined by the purely energizing attribute of the warning stimulus, besides its signaling and motivational properties. The shuttle activity and the rate of rearing responses during four habituation sessions (about 9 min each) were studied in 46 male Moll-Wistar rats. The rules established by the Ethical Committee of Animal Research of the Nencki Institute were strictly followed. The subjects were randomly divided into four groups. A base rate of responding was measured in two control groups (12 ss each) to background stimuli: light (illumination of the shuttle-box apparatus equal to 105 lx) in Group L, and/or darkness (10 lx) in Group D. In two experimental groups: NL (12 ss) and ND (10 ss) each of four habituation sessions consisted of twenty 5-s presentations of the auditory cue (white noise, 70 dB). The background stimulus was light in Group NL and darkness in Group ND. The frequency of crossing from one to another compartment was markedly enhanced by noise, regardless of the background stimulus. There was no difference in the shuttle activity between base-rate control groups. However, more rearing responses were seen in Group D than in Group L.

P.15.14 Auditory recognition impairment which follows removal of tonotopic auditory cortices is not specific to acoustical properties of stimuli or to sound source class

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Four dogs were trained on auditory recognition task with trial-unique stimuli. Following lesions to auditory tonotopic cortices (medial and anterior ectosylvian areas), performance on the task was heavily impaired. In order to assess whether the impairment was related to acoustical properties of stimuli, all sounds were described with 60 acoustical parameters. Eleven factors were extracted with Principal Components Analysis. For each stimulus pair used in the task, 11 sums and 11 differences of pair members' factor scores were calculated and their correlation coefficients with performance level to respective pairs were computed. Effect of the tonotopic fields lesions did not depend selectively on any acoustical factor analyzed. This result contrasts that obtained following lesions to the non-tonotopic auditory fields (Kuśmirek et al. 2001, *Acta Neurobiol Exp* 61: 225). Additionally, no effect of sound source class (e.g., animal, machine, musical instrument) on the task performance was found following any lesion. The data provide further evidence for functional dissociation of auditory cortical areas in the dog. Our results suggest that the auditory cortex operates on the level of acoustical features rather than sound sources.

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P.15.15 Entorhinal/perirhinal cortex lesions in the dog disrupt object recognition while visuospatial function remains intact

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To examine the role of medial temporal lobe structures in object recognition and visuospatial working memory, beagle dogs were trained daily on both an object recognition task and a complex delayed non-matching to position task. The subjects then received bilateral hippocampal or entorhinal/perirhinal (rhinal) cortex lesions, which were made by aspiration and confirmed by post-operative structural MRI's. The rhinal cortex lesions severely disrupted performance on the object recognition task. One animal was unable to relearn the task, and the other could not perform accurately at delays longer than 5 seconds. The rhinal cortex lesions did not affect visuospatial function. The hippocampal lesion did not disrupt reacquisition or performance at short delays on either task. These results demonstrate that object recognition requires an intact rhinal cortex in the dog, and they establish a dissociation in the role of the rhinal cortex in object recognition and visuospatial function.

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P.15.16 Role of the mmp-9/timp-1 complex in prefrontal cortex synaptic plasticity in freely moving rat

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Long term potentiation- or depression-like (LTP- or LTD-) mechanisms in hippocampo-prefrontal cortex (PFC) synapses may play an important role in the storage and processing of cognitive information. Describing the molecular framework of plasticity at synapses seems to be an important task in understanding mechanisms of many mental disorders, such as schizophrenia and depression. Among the most promising proteins supposed to play a role in maintenance of neuronal plasticity are the matrix metalloproteinase-9 (MMP-9) and its natural inhibitor tissue inhibitor of matrix metalloproteinases (timp-1) complex engaged in extracellular matrix reorganization and shown to be activated in brain by neural activity and learning procedures. To check whether mmp9/timp-1 complex plays a role in plastic changes in hippocampo-PFC LTP model, we induced timp-1 gene overexpression in the PFC using adenoviral vector transfer. Two weeks later electrodes were implanted into the PFC and hippocampus and after one week recovery LTP study was performed. It was possible to observe stable LTP for 6 hours in PFC of freely moving rats after high frequency stimulation of hippocampus in control animals. In contrary, timp-1 overexpression in PFC disrupted LTP recorded for one hour after its induction and caused its decay to baseline. This report shows the importance of reorganization of the extracellular matrix in induction and maintenance of plastic changes in PFC.

P.15.17 R-(+)-Methanandamide alters cognitive processes in rats

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The purpose of the present study was to evaluate the influence of the stable analogue of endogenous cannabinoid anandamide - R-(+)-Methanandamide on recognition memory in rats. Thus, the effect of R-(+)-Methanandamide on recognition memory was assessed in an "object recognition" test based on discrimination between a familiar (A) and a new object (B) presented 60 min later. Because cannabinoids in higher doses can produce motor inhibition and anxiogenic-like effect, the influence of R-(+)-Methanandamide on psychomotor activity and its anxiogenic property was evaluated in an "open field" test and in "elevated plus maze" test, respectively. R-(+)-Methanandamide at both used doses: 0.25 and 2.5 mg/kg, given 15 min before presenting familiar object A, significantly attenuated recognition memory, measured by the difference in exploration of object B and a duplicate of object A, as compared to the control group. None of the parameters evaluated in an open field, performed immediately after "object recognition" test: ambulation, rearings, bar approaches and grooming was changed by R-(+)-Methanandamide. Moreover, there were no differences in performance between R-(+)-Methanandamide injected and control rats in "elevated plus" test. There is the first report that the stable analogue of endogenous cannabinoid anandamide - R-(+)-Methanandamide exerts disruptive effect on recognition memory in rats.

The study was supported by AMB grant No. 3-24794.

P.15.18 Effect of CP 55,940 on recognition memory in rats

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Cannabinoids are known to attenuate learning and memory in humans. In animals, disruptive effect of cannabinoids on memory was shown in behavioural tests based on conditioning. The purpose of the present study was to determine, whether potent CB1 receptor agonist, CP 55,940, affects recognition memory in rats. The influence of CP 55,940 on recognition memory was evaluated in an "object recognition" test, based on discrimination between a familiar object-A and a new object-B presented 1 h later. Because cannabinoids at higher doses can produce motor inhibition and anxiogenic-like effect, the influence of CP 55,940 on psychomotor activity and its anxiogenic property was evaluated in an open field and in "elevated plus maze" test, respectively. CP 55,940 at the i.p. doses: 0.025 and 0.25 mg/kg, given 15 min before, and at the higher dose given immediately after presenting object A, significantly attenuated recognition memory, measured by the difference in exploration of object B and a duplicate of object A. CP 55,940 at the higher dose significantly attenuated ambulation, rearings and bar approaches and in both doses also time of grooming evaluated in an open field, performed immediately after "object recognition" test. There were no differences in performance between CP 55,940 injected and control rats in "elevated plus maze" test. There is the first evidence that CB1 receptor agonist, CP 55,940 impairs recognition memory in rats. The study was supported by AMB grant No. 3-24610.

P.15.19 CPPG influence behavioral effects of phaclofen

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The role of CPPG, an antagonist of group III metabotropic glutamate receptors (III mGluRs) in certain behavioral effects of phaclofen, an antagonist of GABA-B receptor, was assessed. Phaclofen, given intracerebroventricularly (i.c.v.) at the dose of 0.5 microgram per rat, did not change activity of rats in open field test, was ineffective in the passive avoidance tests, and it decreased the number of entries into open arms, shortened the time spent in open arms in the "elevated plus" maze, measuring anxiety. CPPG given i.c.v. at doses 1.0; 10.0 μ M per rat, did not influence motility in open field test, retrieval but it impaired consolidation in the passive avoidance situation. In the "elevated plus" maze CPPG used at both doses produced anxiogenic effect, it decreased the number of entries into open arms, and shortened the time spent in open arms. Coadministration of phaclofen and CPPG enhanced the crossings in the open field test and effect of phaclofen on consolidation, but impaired on retrieval in the passive avoidance test, did not change activity of phaclofen in "elevated plus" maze. Summary, CPPG the antagonist of group III mGluR, modulates phaclofen activity, which is reflected by producing beneficial effect on consolidation and unprofitable effect on retrieval of passive avoidance situation and hyperlocomotion obtained in open field test.

P.15.20 Topography of c-Fos expression in the cortical and hippocampal neurons of 129sv and c57Bl/6 mice during fear conditioning

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Rostro-caudal patterns of c-Fos expression in the motor (M1 and M2), cingulate cortex (Cg1, RSA; PrL, Cg2, RSG), hippocampus (CA1, CA2, CA3) and dentate gyrus were analyzed in 129sv and c57Bl/6 mice after single trial fear conditioning. Mice were exposed to context and tone followed by an electric footshock (footshock group - FG) or were exposed to context only (context group - CG). Control mice were taken from a home cage (HG). In the HG the level of c-Fos expression was uniformly low and significantly differed from FG and CG in all studied brain regions, except rostral parts of M1, M2 and caudal parts of RSA and RSG. Distribution of c-Fos positive neurons was uneven in the cingulate cortex and variable (in respect to strains and groups) in hippocampus. The number of c-Fos positive neurons increased in the motor cortex of FG and CG in rostro-caudal direction. A higher level of c-Fos expression in FG as compared to CG and 129sv as compared to c57Bl/6 mice was observed in the rostral part of CA1, medial part of M2 and caudate parts of the cingulate cortex and dentate gyrus. This differential distribution of c-Fos positive neurons suggests uneven participation of the analyzed brain regions in the process of learning and can indicate their functional heterogeneity.

P.15.21 Effect of sensory training upon signal transmission in the barrel cortex of mice

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Vibrissal barrel cortex is often used as an experimental model of experience-induced plasticity. Three conditioning sessions that paired stimulation of a row of whiskers with a tail shock were used. This paradigm caused an expansion of cortical representation of the trained row, when plastic changes were mapped using 2-deoxyglucose. However, details of the synaptic transmission within barrel field and their modifiability after training were not investigated. A current-source density (CSD) analysis was carried out in cortical *in vitro* slices, which were obtained from naive and trained mice. Slices were cut orthogonally to rows of barrels and were incubated in standard conditions. Electrical stimuli applied in the middle of barrel column in layer IV evoked field potential in adjacent column. We tested CSD between trained and untrained barrel rows and untrained-untrained rows in the same slices. Preliminary data indicate that three main current sinks between barrel columns exist; long latency current sinks in the neighboring column were present in supra- and infragranular layers and short latency in layer IV. No significant differences were found in CSD between naive and trained mice. Current experiments are aimed at determining training-induced modifications in short-term plasticity of synaptic transmission between barrels.

P.15.22 Impaired barrel cortex plasticity in presymptomatic Huntington's disease transgenic mice

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Huntington's disease (HD) is an autosomal dominant genetically transmitted neurodegenerative disorder. The neuropathology in HD is a selective neuronal cell death in several brain regions including cortex. There are specific deficits in several forms of learning and memory in HD transgenic mice and these cognitive impairments are attributed to the dysfunction of neurons rather than cell death. Although changes in synaptic plasticity were shown within the hippocampus and striatum of HD transgenic mice, there are no studies considering cortical plasticity abnormalities in HD. We have shown recently that R6/1 HD mice show deficits in cortical plasticity following sensory deprivation and this is accompanied by a severe impairment in the discrimination learning ability. In this study the effect of classical conditioning training on barrel cortex plasticity in presymptomatic R6/1 HD mice was investigated with 2-deoxyglucose (2DG) brain mapping. The training consisted of row B of vibrissae stimulation paired with alimentary reward. In HD negative mice, cortical representation of "trained" row B of vibrissae was enlarged after training in all layers of the barrel cortex, while in HD positive mice there was no row B enlargement. Our results suggest that R6/1 HD transgenic mice show deficits in plasticity of sensory cerebral cortex.

P.15.23 How does the learning affect GABAA receptor subunits mRNA expression in cerebral cortex of mice?

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GABAA receptors belong to ligand-gated ion channels family. They are pentameric, heteromeric assemblies and are permeable to chloride ions. 18 different subunits of these receptors were identified. In the somatosensory cortex the most prevalent subunits are: alpha1, beta2 and gamma2. We showed that classical conditioning, in which stimulation of a row of vibrissae was paired with a tail shock, results in changes in cortical inhibitory system. GABAA alpha1 mRNA is upregulated in layer IV of cortical representation of trained row of vibrissae. In this study we examined the mRNA expression level of beta2 and gamma2 subunits of GABAAR. For *in situ* hybridization, 35S-labelled oligonucleotides were used as antisense probes. The effects were examined 1 h, 24 h and 5 days after the training, which lasted for 3 days. There are no changes observed in beta2 subunit mRNA level. Expression of mRNA of gamma2 subunit increased 5 days after the training, but in contrast to other elements of gabaergic system we investigated, the change was observed in layers II/III. This indicates that learning-dependent regulation of GABAA receptor phenotype occurs in specific cortical sites with different latency.

P.15.24 Learning-induced expression of GAD67 in synaptic terminals of the barrel cortex

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Classical conditioning involving stimulation of facial vibrissae produces an expansion of the cortical representation of the row of vibrissae used in the training, in layer IV of the barrel field. Functional reorganization of Sml cortex is accompanied by increased density of small GABAergic cells, and 50% increase of GAD67-IR neurons in the hollows of the trained row. In the present study, GAD67-IR in synaptic terminals was examined. Optical disector was used to quantify GAD67-IR synaptic terminals in barrel hollow. Results from one trained (CS+UCS) one control (CS only) and one naive mouse were quantified. The most notable observation was the increase of numerical density of GAD67-IR puncta in the hollow in deep part of lamina IV in the trained barrel compared to the unstimulated side, and both control groups. After training, numerical density of GAD67-IR puncta was $3.07 \times 10 \text{ exp.}7 \text{ mm}^3$. In comparison, in control barrel on the unstimulated side, was $1.82 \times 10 \text{ exp.}7 \text{ mm}^3$. In the corresponding barrel of CS only group numerical density of GAD67-IR puncta was $1.05 \times 10 \text{ exp.}7 \text{ mm}^3$, and in control barrel on unstimulated side, it was $1.15 \times 10 \text{ exp.}7 \text{ mm}^3$. In the barrel of naive mice at the bottom of the barrel numerical density of GAD67-IR puncta was $1.04 \times 10 \text{ exp.}7 \text{ mm}^3$. These results suggest involvement of GAD67-IR terminals in the phenomenon of learning dependent cortical plasticity.

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P.15.25 PSD95 in learning-dependent cortical plasticity

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PSD95 is a postsynaptic protein directly associated with NMDA receptor subunits NR2A and NR2B and thus implicated in synaptic transmission and plasticity. We investigated the involvement of PSD95 protein in NMDA receptor dependent, learning induced expansion of representational maps in somatosensory cortex of adult mice. Plasticity was induced in the barrel cortex, part of somatosensory cortex where inputs from facial vibrissae are represented. PSD95 mRNA and protein level were examined in the barrel cortex after a three days long classical conditioning training, in which activation of facial vibrissae was linked to an aversive stimulus. In subcellular fraction enriched in postsynaptic densities from the barrel cortex, it was estimated by Western blotting that the PSD95 protein level increased after the 3 days-long training by about 50%. After the first training session, level of PSD95 was unchanged. Changes in mRNA level were checked 1 hour, 1 day and 5 days after the training, by *in situ* hybridisation method with antisense oligonucleotide as a probe. We found no changes in mRNA expression level at any of the examined time points. The results suggest a role of PSD95 in maintenance, but not in induction of plastic changes. Elevation of PSD95 protein level in synapses after training is not due to increase in its mRNA transcription.

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P.15.26 Agmatine modulates the effect of acamprosate on short-term memory in chronically ethanol treated outbred rats

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A multiple acamprosate (AC) treatment has no negative effect on short-term memory in chronically ethanol treated rats (Szulc et al. 2002). However, co-administration of a single polyamine ligand, such as arcaine or spermidine, with AC was found to impair short-term memory (Mikolajczak et al. 2002). The aim of this study was to evaluate the interaction of agmatine (AG), another polyamine ligand, and AC on social recognition in the model of experimental alcoholism. The study was performed using outbred ethanol preferring (PR), non-preferring (NP) and control Wistar rats. After 3 months of ethanol intake the animals were treated with AC (500 mg/kg/day, p.o.) for 21 consecutive days and single AG (20 mg/kg, i.p.) before the short-term memory evaluation in social recognition test (Thor and Holloway 1982). Ethanol intake impaired short-term memory in NP rats, but there were no differences between PR and control animals. AC improved memory in control animals, yet the drug did not affect social recognition in both ethanol-treated groups. AG administration did not impair short-term memory in any of the investigated animals. However, co-administration of AG in a single dose with multiple AC doses produced worse results in PR and control rats. It may, therefore, be concluded that the effect of repeated AC administration can be changed by the presence of AG, so the results obtained should be approached carefully.

P.15.27 Temporal dynamics of memory consolidation in the prefrontal cortex: NMDA receptors in the early phase, beta adrenergic receptors in the late phase

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The NMDA receptors antagonist, APV and the beta adrenergic receptor antagonist, timolol, induce amnesia when injected (i.c.v.) respectively 5 minutes or 2 hours after odour discrimination training, suggesting that NMDA receptors are involved in the early phase and beta receptors in the later phase of memory formation. Studies of region-specific c-fos immunoreactivity after the odour learning revealed a spectacular increase in c-fos activity in trained rats compared to yoked controls, specifically in the prelimbic cortex (PLC), suggesting that this might be the effect of action of the i.c.v. injections. To test this, rats were implanted with cannula just above the PLC and trained in the odour task. They were injected with APV, 5 min after the last trial, or with timolol 5 min or 2 h post training. APV applied into PLC 5 min after training induced amnesia 48 h later, while timolol had no effect when treatment was 5 min post training. Timolol 2 h after training induced amnesia. Release of noradrenaline (NE) in the PLC during the post-training period was monitored by *in vivo* microdialysis. A significant increase in release of NE in the PLC was seen 2 h after the training, precisely the time window when timolol injection is effective in inducing amnesia.

P.15.28 Neuronal activity in the rat's medial frontal cortex during odor learning task

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To study the processes of memory consolidation we use a foraging task where rats quickly associate one of three odors with a reward. Significant c-FOS increase after the learning session suggested that the medial prelimbic cortex (mFC) was activated when the rat performed the task. To examine the dynamics of its activation we run the experiment on the group of rats with chronic microelectrodes implanted into mFC. Over 75% of the cell recorded in mFC changed their firing rate: around 30% was tonically excited during whole session; some cells responded with excitation (14%) or inhibition (14%) when the rat was placed into the experimental box and around 20% increased their firing in the start-box during inter-trial intervals. The effects can be related to many aspects of changing environment yet we suppose that the activation observed specifically during inter-trial intervals could be involved in processes of memorization of experiences from preceding trial. Indeed, significant increase of cells' firing rate during this period was followed by shortening of the latency of rat's behavioral response. Such an activation was no longer observed after the rat achieved asymptotic performance. Our preliminary results confirmed that cells in prelimbic area of the medial frontal cortex are involved in complex mechanisms underlying memorization of odor-reward association. Study supported by PAS-CNRS joint grant, and Fyssen Foundation fellowship for EK

P.15.29 Engagement of medial frontal cortex and locus coeruleus during olfactory learning: Responses to changing odor-reward contingencies

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Different lines of evidence suggest that the noradrenergic nucleus locus coeruleus (LC) and the medial prefrontal cortex (mFC) are engaged during olfactory learning. To explore neuromodulatory influences of LC on mFC, we recorded unit activity simultaneously from LC and mFC during multiple sessions of olfactory discrimination learning, extinction and reversal. The onset of each trial was signaled by a preparatory signal, a light that persisted for the duration of the trial. All LC neurons were phasically excited by the preparatory signal, while 35% of mFCx units were tonically inhibited by the light until the end of the trial. There was little initial response in LC or mFC to odors *per se*, but LC neurons responded vigorously to primary reward (chocolate milk) in early learning trials, during reversal, or when novel odors were introduced. This response rapidly switched to the CS+, well before the odor-reward contingency learning was expressed behaviorally as go - no go responding. Many mFCx neurons (25%) showed an anticipatory response to reward. Some mFCx units (15%) signaled specific outcomes of the task (error or correct responses). Although no direct interaction between LC and mFCx could be discerned, we emphasize that LC changes always preceded MPFC both within and between trials, suggesting that LC could play a permissive or promoting role in the responses of that population of mFC neurons showing response flexibility during changing task contingencies.

P.15.30 Memory and anxiety-related behaviour in adult and aged rats: effects of neonatal asphyxia, body temperature and chelation of iron

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Newborn mammals, showing reduced physiological body temperature, might be protected against iron-mediated, delayed neurotoxicity of perinatal asphyxia. Therefore, we decided to study effects of (1) body temperature, maintained for 3 h at physiological level of 33°C or elevated to 37°C (incubator group) or 39°C (high incubator group) and (2) iron chelation with deferoxamine, in newborn rats exposed to a critical anoxia, on behavioral responses in adulthood and aging. At 4, 12, and 24 months of age both postanoxic and control rats were subjected to a passive avoidance task and were given three trials of 5 min duration each on an "elevated plus" maze (for testing the anxiety-like behaviors). Both the passive avoidance and the anxiety level were unaffected in rats exposed to anoxia at physiological body temperature of 33°C. Both responses, however, were weakened in animals exposed to anoxia at 39°C. Deferoxamine injected postanoxia reversed the disturbances. In conclusion, physiologically reduced neonatal body temperature can provide a protection against iron-mediated postanoxic disturbances of stress responses in adult and aged mammals. In contrast, high incubation results in the delayed postanoxic disturbances.

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P.15.31 Carbamazepine may disrupt short term memory processes in humans

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The influence of antinociceptive medication on cognitive processes is well known. This study was aimed at searching for a possible modulation of putative anticognitive effects of the non-steroid antiinflammatory drugs (NSAID) and fentanyl by the carbamazepine, used as an adjuvant in the analgetic treatment. There were 4 groups of 6-9 patients receiving: 1) NSAID, 2) Transdermal (TTS) fentanyl, 3) TTS fentanyl plus submucosal fentanyl, 4) TTS fentanyl plus NSAID and carbamazepine. All subjects received similar treatment before and after analgesia. On the 5th and 30th day after starting analgetic drugs, all patients underwent a battery of the following cognitive tests: 1) Rey Verbal Learning Test (RVLT), 2) Wechsler Digits Recall Test (WDRT), 3) Beck Depression Inventory (BDI), 4) Hopkins Symptoms Check List (HSCL). Eleven drug-free healthy volunteers (control) were evaluated with the same battery of psychological tests. Only patients receiving carbamazepine together with fentanyl and NSAID were impaired in verbal learning. Their scores on the two short-term memory tests (WDRT and RVLT) were lower ($P < 0.05$) comparing to the control. Patients from all groups were emotionally undisturbed (as assessed by BDI and HSC). In conclusion, carbamazepine as an adjuvant in the analgetic therapy may adversely interfere with the short-term memory processes.

P.15.32 Synaptic plasticity depending on Ca^{2+} concentration in a model neuron

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We present a model of single neuron (Matlab/Simulink environment), enabling analysis of changes of a synaptic transmission (conductance) which depends on signals conducted by a synapse. The analysis was focused on the influence of dynamic changes of Ca^{2+} concentration, caused by NMDA-induced Ca^{2+} -current, on the postsynaptic activity of the cell. The basic idea for dynamic regulation of synaptic conductance is that the electrical activity of a neuron plays a feedback role in regulating its currents. At the first stage we used mathematical function which simulated changes of synaptic conductance induced by spiking activity of neuron. This function did not represent any specific, physiological mechanism, but general facilitation and depression effects, similar to LTP or LTD. Next, we build a model neuron with NMDA and AMPA receptors. This enabled modeling of molecular mechanisms of the postsynaptic plasticity, based on the observation that LTP correlates with phosphorylation/dephosphorylation of sites on the GluR1 subunit of AMPA type glutamate receptor. We simulated activation of NMDA receptor, an increase of intracellular Ca^{2+} in the postsynaptic cell and molecular processes inside the cell. We demonstrated that experimentally observed plasticity could be accounted for by activity-dependent regulation of composition and function of NR2A, NR2B subunits. The model has proven to be promising, as it showed that the dynamics of changes of postsynaptic potential amplitude is alike to animal data.

P.15.33 Lack of effects of Ang IV on the acquisition of the water maze task and microsomal ryanodine channel function

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In the present study we investigated the effect of angiotensin IV on the acquisition of spatial task by rats and on Ca^{2+} transport in microsomal membranes isolated from rat hippocampus, the brain structure essential for spatial memory. Wistar rats injected intracerebroventricularly with 1 nmol of angiotensin IV or saline, were subjected to the water maze training using hidden (learning) or visible (nonlearning) escape platform. Rats showed overall good acquisition of the task and mean escape latency decreased from 55 seconds to less than 10 seconds during 5-day training. Learning significantly increased [3H]ryanodine binding to microsomal ryanodine receptors and markedly decreased both receptor affinity for the ligand and microsomal Ca^{2+} uptake. Angiotensin IV was without effect on the rate of acquisition of the spatial task but increased (by 47%) maximal ryanodine binding in hippocampal microsomes of the learning rats. The peptide, however, did not affect decreased net Ca^{2+} uptake in rats subject to learning procedure. Since microsomal Ca^{2+} -ATPase activity was similar in all tested groups, the lower net Ca^{2+} uptake in learning rats could be attributed to elevated expression of ryanodine receptors and resulting increased Ca^{2+} release. Our results do not support the view about predominant role of angiotensin IV in acquisition of spatial memory, although they cannot exclude the possible modulatory effect of the peptide.

P.15.34 Analysis of individual variation in spatial reference and working memory under allothetic and idiothetic orientation cues in rat

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The present study was designed to examine which kind of memory: reference or working, better correlates with individual variation in rats spatial learning abilities tested under different navigation requirements. To answer this question, two groups of rats were trained to an arbitrary performance criterion in a partially baited 12-arm radial maze where entries to six semi-randomly chosen unbaited arms were scored as reference memory errors (RMEs), while re-entries to baited arms were scored as working memory errors (WMEs). One group of rats was trained in the presence of distant visuo-spatial (allothetic) cues belonging to the experimental room. In the second group, rats were trained to search for food in the radial maze surrounded by dark plain curtains to eliminate distal cues. The latter rats in their navigation had to rely mainly on movement-generated (idiothetic) cues. 10 days after reaching criterion performance, all animals were examined for memory retention. The present experiment confirmed the facilitating effect of the presence of distal visual cues on place learning in rats. Task-dependent (between-group) differences in the rate of learning were attributed to differences in the frequency of reference memory errors. In order to assess the within-group variation in spatial learning, rats from both groups were classified as poor (number of choices to the acquisition criterion more than group mean + 3 SEM), good (number of choices to the acquisition criterion less than group mean - 3 SEM), and the remaining intermediate learners. Within-group variation in the rate of task acquisition reflected significant individual variation in the frequency of working but not reference memory errors. These results were looked upon from the evolutionary perspective.

P.15.35 The effects of 5,6-dihydroxytryptamine injection on the defensive reflex conditioning in *Helix lucorum*

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Serotonin is involved in regulation of such important forms of behavior of molluscs, as defensive behavior, food behavior, locomotion, reproduction, circadian rhythms. In the last years there were many experiments where the neurotoxins: 5,6- and 5,7-dihydroxytryptamine (5,6- and 5,7 DHT) that destroy the serotonergic terminals were used. It is known that depletion of serotonin by 5,7-DHT inhibits elaboration of the conditioned defensive reflex of food aversion (Balaban at al. 1986). We have conducted the analysis of the role of serotonin in learning at the level of electric characteristics of the snails' identified neurons that were conditioned for the defensive reflex. The snails were injected twice with 5,6-DHT (dose 15 mg/kg, with addition of ascorbic acid) and then they were conditioned. Tapping on the shell was used as a conditioned stimulus and a light air blow into the pneumostome was used as an unconditioned stimulus. It was shown that snails that were trained for one week after the first 5,6-DHT-injection did not elaborate the conditioned reflex. If the snails were trained for 2 weeks after the first injection of neurotoxin, they learned but slower than the control group injected with the saline solution (SS). The electrical characteristics of withdrawal interneurons were recorded after the behavioral experiments. Three mV depolarization and 2.5 mV decrease of the threshold potential was observed in the 5,6-DHT-injected snails. These changes

persisted for three weeks after injection of 5,6-DHT. Such membrane and threshold potentials were not present in the 5,6-DHT-injected snails that were conditioned for either one or two weeks after the first injection of 5,6-DHT. Thus, the injection of 5,6-DHT that depleted serotonin in the nervous system lead to depolarization and, consequently, to the increase of excitability of the withdrawal interneurons in the snail. In the present study we showed that conditioning of the defensive reflex in the 5,6-DHT-injected snails is not accompanied by further decrease of the membrane and threshold potentials of neurons as compared to animals that were only injected with 5,6-DHT.

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16. PAIN AND ANALGESIA

P.16.1 N-terminal nociceptin/orphanin FQ fragment inhibits morphine withdrawal

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Nociceptin/orphanin FQ (N/OFQ) is a natural agonist of the NOP (ORL1, OP4) receptor. It blocks the antinociceptive effect of morphine and inhibits morphine withdrawal syndrome. Nociceptin *in vitro* is cleaved to the N-terminal hexapeptide. This fragment shows a bi-phasic effect after i.c.v. administration, causing antinociception followed by hyperalgesia. The analgesic effect is blocked by naloxone, suggesting involvement of the opioid system. Based on this finding, in the present study we tested the N-terminal nociceptin cleavage product and its two derivatives for their ability to inhibit morphine withdrawal syndrome, elicited by naloxone injection to morphine-dependent rats. The natural sequence, Phe-Gly-Gly-Phe-Thr-Gly, attenuated morphine withdrawal syndrome. Similar but weaker effect was observed for the peptide with replaced N-terminal phenylalanine with tyrosine (Tyr-Gly-Gly-Phe-Thr-Gly), which is a mandatory residue for binding to the opioid receptors. Earlier reports indicated that replacement of the C-terminal glycine by alanine (Phe-Gly-Gly-Phe-Thr-Ala) causes loss of hyperalgesic activity of such hexapeptide. In present study this sequence also failed to inhibit morphine withdrawal. In summary, modifications of N/OFQ(1-6) at either end caused a dramatic loss of its biological activity in pain transmission and morphine dependence.

P.16.2 Study of pharmacological effects of new imidazo-pyrimidine derivatives: E2, E5, F2, F3, G3 and G4 with putative central activity in mice

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The investigated compounds, synthesized by condensation reaction of 1-aryl-2-aminoimidazolines-2 with respective malonic acid derivatives, contain in their structure pharmacophoric moieties responsible specifically for the affinity toward opioid (μ) receptors (hydrophobic and pi-pi interactions, acceptor hydrogen bonds) and serotonin (5-HT₂) receptors (hydrophobic interactions, pattern of the acceptor hydrogen bonds). That's why these compounds were studied in behavioral test commonly used to predict a potential influence on central nervous system (CNS) in mice (after their i.p. or s.c. administration). All tested imidazo-pyrimidine derivatives from the presented groups, E, F and G, are characterized by low toxicity (LD₅₀ over 1,430 mg/kg, except G3 derivative) and slight influence on motor coordina-

tion of mice assessed in the rota-rod test. The depressive influence on spontaneous locomotor activity was observed only after administration of derivatives of F series (F2 and F3), but body temperature was long-lasting decreased by compound shown as E5 and G4. Amphetamine hyperactivity was not changed by all tested compounds. The potent antinociceptive activity of derivatives series F and G, but not E, was observed in the writhing test in mice. However compound shown as F3 significantly but in the short-lasting way prolonged response to thermal stimuli studied in the tail-flick test. The strong antinociceptive effect of G4 compound evaluated in the writhing test was completely blocked by naloxone, suggesting some connection with endogenous opioid system. The head-twitch responses to 5-HTP were significantly decreased only by derivatives F group. Significant anticonvulsive property demonstrated only E5 derivative. The results seem to suggest some connection of derivatives group G with opioid and group F with 5-HTP endogenous systems.

P.16.3 Acquisition of the two-way avoidance and acoustic startle responses in mice selected for post-stress analgesia

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The research was accepted by the Nencki Ethics Committee. The experiment was conducted with 43 adult male mice. Two groups has been selectively bred for high- (Group N, 13 ss) and low analgesia (Group W, 15 ss), induced by 3-min swimming in 20°C water. The control Group K consisted of 15 ss. There were two stages: the shuttle-box avoidance training, and testing of the acoustic startle reaction (ASR). The avoidance training consisted of 6 daily sessions (50 trials each). The trial started with a white noise CS onset. Five seconds later the scrambled foot-shock (0.8 mA, US) activated the grid-floor. Running to the opposite compartment within 5 s of the CS immediately terminated its action and precluded the US (avoidance response). Similar reaction after the shock onset terminated the CS and US (escape). The intertrial intervals were 15-25 s duration. ASR amplitudes were measured after the avoidance training. Mice were exposed to 20, 110-dB acoustic pulses. No between-group differences were found in the avoidance acquisition. For N subjects fast shuttle-box responding corresponded with higher amplitudes of ASR. The longest latencies of instrumental responses were seen in groups W and K. However, the US onset provoked marked shortening of the escape latencies for W subjects, in contrast to K mice. The lowest ASR amplitudes were observed in Group W.

17. PSYCHOPHYSIOLOGY AND NEUROPSYCHOLOGY

P.17.1 ERP indices of source memory deficits after mild head injury

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Reduced cognitive efficiency is reported by 10-25% of individuals experiencing a mild head injury (MHI). To examine the neurocognitive basis of these post-concussive complaints we recruited 19 young adults with persistent cognitive complaint despite minimal loss of consciousness (0-20 min) and 13 age- and education-matched controls. Participants were asked to read a list of words and subsequently select them out of a test list which included these study words, new words (foils), and new words repeated once after a lag of 6 items (familiar lures). Non-injured individuals were very good at selecting study words and correctly rejecting both foils and familiar lures. Injured individuals performed similarly with respect to study words and foils but were twice as likely to select the lures. ERP data indicate that controls did not produce the positive amplitude shift usually associated with recognized words when these words were not part of the target category. For the MHI group, the target study words and the non-target lures elicited an ERP positivity of similar amplitude even on trials during which the non-target lures were correctly rejected. Thus, those in the MHI group were less efficient in their ability to disengage from the processing of salient distractors, a pattern of performance consistent with the commonly reported cognitive sequelae of mild concussive injury. We conclude that the problem stems from changes in the ability to efficiently allocate intentional resources and mirrors the neurocognitive changes associated with normal aging.

P.17.2 The effect of congenital deafness on the production of temporal intervals

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The production of temporal intervals is one of the duration judgment methods. It involves translation between experienced duration and conventional time units (e.g. seconds, minutes). The aim of the present study was to compare the accuracy of time production in normal hearing adolescents and in congenitally deaf, who displayed disturbed articulation and communicated using sign language. We tested 16 deaf individuals (8 male, 8 female), aged between 16-19 years and 16 normal hearing subjects, matched for sex and age. Subjects were asked to produce the duration ranged from 1 to 6 s. The intervals were filled with presentation of a visual stimulus (a green rectangle). The data showed that although all subjects produced relatively accurately the applied durations, in deaf individuals, comparing to controls, the shortest standard was over-estimated whereas the longer ones (above 3 s) were under-estimated. These results indicate that both groups are skilled in using the conventional time units. Nevertheless, in deaf subjects the over-estimation may be related to limitations in the working memory effectiveness. On the other hand, factors like impatience or inability to delay a response can result in under-estimation of longer intervals.

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P.17.3 The effect of experimental procedure and chronological aging on the auditory order threshold

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The existing evidence suggests that the temporal order of two acoustic stimuli can be properly recognized if their onsets are separated by a temporal gap of at least 40 ms. The present study showed that this auditory order threshold (OT) is independent of the applied experimental procedure (Exp.1), however, it changes with chronological aging (Exp.2). In Exp.1 we compared the OT in 20 right-handed subjects, aged 19-25 years, using inter- or intrahemispheric stimulation. Subjects identified the order of two 15 ms tones, presented with the inter-stimulus-interval (ISI) from 10 to 1,000 ms. In the interhemispheric task, two tones of 300 Hz were presented one to each ear, whereas in the intrahemispheric task two different tones of 300 and 3,000 Hz were presented binaurally. The results suggest that independently of the kind of stimulation the criterion of 75% of correct responses can be reached for ISI longer than 40 ms. This supports the view that the common neural mechanism underlies the judgment of temporal order, independently of the procedure applied. In Exp.2, eight centenarians were tested with the interhemispheric stimulation. The results showed the prolongation of the OT (nearly 4 times, so up to above 150 ms) in comparison with the younger subjects (Exp.1). To conclude, the neural mechanism responsible for OT seems to undergo the remodeling as the result of the biological aging.

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P.17.4 Event-related potentials for new and repeated words: a divided visual field study

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The ultimate goal of electrophysiological studies of human memory is to know the locus, nature and function of neural activity during encoding, storage, and retrieval of complex cognitive information. The specific aim of the present study was to test whether the hemisphere involved in encoding of verbal information is a critical factor influencing consecutive stages of memory formation. Event related potentials (ERPs) to unilaterally displayed verbal stimuli were recorded from symmetrical sites over the left (LH) and the right hemisphere (RH), i.e., the hemisphere contralateral and ipsilateral to the visual hemifield of stimulus presentation. ERPs recordings for new and repeated words were compared. Subject's task was irrelevant to electrophysiological effects of interest. EEG recording started 100 ms before the exposure of the stimulus and lasted 1,100 ms. The preliminary results for frontal recording sites revealed that direct stimulation of the LH hemisphere caused memory-related modulation of ERPs by word repetition ("ERPs repetition effect") both in the LH and in the RH whereas direct stimulation of the RH resulted in the lack of that effect in both hemispheres. Our results suggest that the emergence of ERPs repetition effect depends on the hemisphere which performs initial stages of encoding of verbal information.

P.17.5 The perception of the temporal order in cochlear implant users for auditory and visual stimuli

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Temporal order judgement (TOJ) is a measure of the minimum time interval between two successive stimuli required to correctly report their order. Experimental studies show that this value lies around 20-80 ms and is remarkably invariant for different sensory modalities. The aim of this study was to investigate whether impairments in auditory comprehension are accompanied by disorders in TOJ in cochlear implant users. In Experiment 1 we tested 12 postlingually deaf monochannel implant recipients and 12 normal-hearing subjects. The task was to identify the order of two 15 ms tones (300 and 3,000 Hz) presented in the free field with inter-stimulus-intervals (ISI) from 10 to 500 ms. In Experiment 2, 5 patients and 5 controls reported the order of two diodes (red and green) presented with ISIs mentioned above. Implant users needed longer (between 80-150 ms) ISI to correctly report the order of two tones than normal-hearing subjects (between 40-80 ms). Moreover, patients performed significantly poorer for shorter ISIs (10 and 20 ms) than controls. Similar effects were observed for the visual experiment. We postulate that in implant recipients difficulties in TOJ can be related to poor auditory comprehension, independently of the modality. It supports the theory postulating the central basis of temporal order perception. Supported by the KBN grant No. PO5B 10 119.

P.17.6 Memories for general and specific information are mediated by distinct regions of the prefrontal cortex

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Memories for general and specific visual information were investigated in 24 patients with unilateral prefrontal lesions and 10 normal control subjects. Lesions were limited to small areas within either the ventromedial (posterior part of the gyrus rectus) or dorsolateral (Brodmann's area 46/9) cortices. The Rey-Osterrieth Complex Figure test was used. Participants were asked to first copy a figure and then, after the 3-minute delay, reproduce it from memory without prior warning. In the copy task, all participants achieved similar scores. In the memory task, patients with right ventromedial and left dorsolateral lesions performed worse than the control subjects, whereas patients with left ventromedial and right dorsolateral lesions were not impaired. Right ventromedial prefrontal lesions caused the impairment of memory for the overall structure of the figure with preserved recall of details. Left dorsolateral prefrontal lesions resulted in the opposite pattern: simplification and loss of details with preserved recall of the overall structure of the figure. The data shows that memories for general and specific information are mediated by distinct regions of the prefrontal cortex, the former being supported by the right ventromedial and the later by the left dorsolateral cortex.

P.17.7 Sex differences in brain control of prosody

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Conventional wisdom suggests that women are more “emotional” than men. Does this mean that women express their emotions more fully than men? Or, do women experience more or stronger emotion than men? Substantial body of research has demonstrated that women are able to better understand emotional expression from face and gestures. Little is known whether similar sex differences exist in the ability to understand emotional expression in language (prosody). Prosodic cues communicate to the listener, among others, the affective disposition of the speaker (e.g., whether the speaker is angry or happy). There is now a growing number of studies, which suggests a dominant role of the right hemisphere in decoding affective prosody. The present study addressed two questions. First, we examined whether women were better than men in comprehension of emotional cues in voice. Second, we studied if lesions to different parts of the right hemisphere may differentially affect the prosody processing. Fifty two patients with damage to frontal, temporo-parietal or subcortical parts of the right hemisphere (RH) and 26 normal controls (C) made identification of emotions expressed by intonation in natural and pseudo sentences. The results showed that RH damaged subjects had decreased comprehension of emotional prosody. We also found that frontal RH damaged women had more impaired comprehension than frontal RH damaged men. However, subcortical lesions led to stronger impairment in men. This study revealed sex differences in brain organization of prosodic functions. The results provide further evidence for the notions that women are more sensitive to emotional signals than are men.

P.17.8 Reaction latency to negative vs. positive suboptimal (50 vs. 75 ms) semantic primes

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The larger program of studies aims to investigate the influence of implicit affect on explicit judgements - using the affective subliminal priming paradigm (Murphy and Zajonc 1993). In two reported studies data analysis is related to reaction latencies to target stimulus not primed or primed with negative or positive words exposed suboptimally. **METHOD.** Participants (Study I $n = 75$, Study II $n = 69$) were presented with a computer task. They were required to assess how far a particular target neutral stimulus - Chinese hexagram “representing human traits” - is relevant to the self. Some hexagrams were not primed, and some were primed with negative or positive words. Target stimuli (36 hexagrams rotated randomly) appeared on the computer screen optimally (1,000 ms). 20 of them were suboptimally primed (50 ms in Study I and 75 ms in Study II) by visually backwardly masked (postmasking) suboptimal stimuli (negative and positive adjectives). **RESULTS.** Mean reaction latencies to neutral stimuli (1) were not different in Study I and Study II in case of no prime; (2) were shorter in condition of shorter prime exposure duration (50 ms - Study I) than in condition of longer duration (75 ms - Study II); (3) in both studies (Study I and Study II) reaction latencies were shorter when primed by negative than by positive stimuli. The results are **DISCUSSED** in terms of sovereignty of positive and negative affective regulatory systems (“positive-negative asymmetry”) which may result in differentiation of reaction latencies.

P.17.9 Auditory temporal order thresholds in children: age effects and reliability of measurementBerwanger D.¹, Wittmann M.², von Suchodoletz W.¹¹Institute of Child and Adolescent Psychiatry andPsychotherapy; ²Generation Research Program Bad Tölz,

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Background: The auditory temporal order threshold is defined as the shortest time interval necessary between two acoustic events for a person to be able to identify the correct temporal order. There are numerous publications which suggest that the ability to detect temporal order of two sensory events separated by a time interval of some tens of milliseconds is associated with language competence. However, their reliability has not been sufficiently proven so far. **Methods:** We determined the auditory temporal order threshold of 108 children. To evaluate the retest-reliability and the stability, 54 children were tested twice; 20 of them after one week and 34 after four months. To calculate the order threshold we used the adaptive algorithm YAAP. **Results:** Ninety-six of 108 children were tested successfully. The threshold decreased with age. The reliability of temporal order threshold assessment over a period of one week is only moderate, and its stability over a time interval of four months is low. **Conclusions:** The results of our study show that the auditory order threshold task is too difficult even for many normally developed children, before the age of 7. The test-retest reliability suggests that the auditory order threshold measurement in children has to be considered with caution. A high temporal-order threshold does not necessarily confirm a diagnosis of temporal-processing disabilities.